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THE EFFECT OF DECREASED DIETARY CRUDE PROTEIN ON BUFFERING CAPACITY AND INTERMEDIARY METABOLISM OF WEANED PIGS

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ABSTRACT

This study was conducted to investigate the influence of diets with different crude protein content on dietary buffering capacity and biochemical parameters in pigs. 12 weaned piglets, average body weight of piglets in the experimental group was at the beginning about 9.3±0.7kg and 9.5±0.6kg in the control group. The control diet contained 209.4 g/kg crude protein and the experimental diet contained 176.0 g/kg. The experimental diet was supplemented with lysine, methionine and threonine to achieve a more ideal amino acid pattern. The buffering capacity was calculated from pH values and acid-binding capacity (ABC). In the present study was used pH = 4.0 as titration endpoint. Blood collection for determination of biochemical parameters was performed 4 times at weekly intervals in the control and experimental group 4 – 5 hours after feeding from *sinus ophtalmicus*. The decrease of protein content in the diet was manifested by decrease of the dietary buffering capacity (BUF4) (48 mEq *vs.* 67mEq), decrease of concentrations of blood urea (3.66mmol/l *vs.* 5.84mmol/l, average concentrations) ($P<0.001$), which means the increase of biological value in the feed mixture. The other biochemical indicators (total protein, albumin, glucose, total lipid and aspartate aminotransferase showed no significant dependence on the performed changes in the feeding of piglets during the experiment.

Keywords: amino acids; buffering capacity; metabolism; protein; urea

INTRODUCTION

Because of the increased availability of crystalline amino acids (AA) Lysine, Methionine, Threonine including the 'new' amino acids Isoleucine and Valine, and the continual need to improve the utilization of nutrients to reduce the impact of livestock production on the environment, there is always a need to more fully understand amino acid nutrition of non-ruminants (Kerr, 2006). Dietary supplementation of crystalline lysine for pigs can significantly increase body muscle protein accretion, which may be due to a greater increase in the rate of protein biosynthesis rather than that of protein degradation (Liao et al. 2015). In addition, nutritional studies have shown that dietary supplementation with several AA modulates gene expression, enhances growth of the small intestine and skeletal muscle, or reduces excessive body fat (Duan et al. 2016). Reduction of dietary crude protein (CP) could limit the growth performance of growing pigs, but a low-protein diet supplemented with deficient amino acids could reduce the excretion of nitrogen into the environment without affecting weight gain (Figueroa et.al., 2002; Lynch et al. 2007). The reduction of protein concentration and also buffering capacity have a great importance of weaned pigs. Due to the higher buffering capacity of the diets, the pH of the stomach content increases, thereby disrupting digestion and protein utilization after weaning (Blank et al. 1999). Supplements of synthetic amino acids to animal diets are important not only on nutritional and economic aspects, but also on environmental aspects. It is concluded that the supplementation of limited amounts of synthetic amino acids to diets for swine could spare 2 to 3 percentage units of dietary protein and substantially reduce nutrient excretion, especially nitrogen (Han and Lee, 2000). Meta-analysis of Sajeev et al. (2018) confirms that CP in animal diets and emissions of ammonia show a clear relationship. The meta-analysis revealed mean ammonia reduction of 17 ± 6% per %-point CP for cattle and 11 ± 6% for pigs. Most studies indicated that reduction of dietary CP could effectively decrease the nitrogen emission (Toledo et al., 2014).

This fact motivated us to determine the effects of reducing the dietary CP content from 20.94 to 17.60% on buffering capacity of diet and serum biochemical parameters.

MATERIAL AND METHODS

The experiment was conducted with 12 crossbred piglets (Slovakian White x Landrace), with an initial average body weight (BW) 9.5±0.6 in the control and 9.3±0.7 kg in the experimental group and weaning at 28 days of age. At weaning, piglets were divided into two groups (6 pigs in one group). The treatments contained equal number of females (2) and castrated males

(4). The experimental period lasted 28 days. The same ingredients for control and experimental group were used in experiment. Diets were formulated based on corn, wheat, barley, soybean meal, vitamin + mineral premix, salt. The animals were divided into two groups according to the two different crude protein levels of diet (20.94% and 17.60%) with different soybean meal concentration in diets (30 *vs*. 19.5%). Addition of limiting amino acids (AA) (Lys, Met, Thr) was used in experimental diet. Feed and water were allowed on an *ad libitum* basis. The feed composition of diets used in experiment and their nutrient content are shown in Table 1 and 2.

The experiment was carried out in the stall of Institute of Animal Nutrition and Dietetics at the University of Veterinary Medicine and Pharmacy in Kosice in compliance with the EU regulations concerning the protection of experimental animals.

Table 1 Ingredients (%) of diets used in experiment

Ingredients	Control diet	Experimental diet
Corn	35.00	38.00
Wheat	13.30	14.00
Barley	18.50	24.74
Soybean meal, CP 48%	30.00	19.50
Mineral-vitamin premix	3.00	3.00
Salt	0.20	0.20
L-Lysine HCl 78%	0.00	0.32
DL-Methionine	0.00	0.14
L-Threonine 98%	0.00	0.10

Diets were analysed for dry mater (DM), crude protein (CP), crude fibre (CF), ether extract (EE) and ash by the AOAC (2001). Nitrogen free extract (NFE) was mathematically calculated from previous parameters. Amino acids content and Metabolizable Energy (ME) in

both diets was calculated according to the program for formulation of diets for pigs from energy values and AA composition of feeds and addition of synthetic amino acids.

All feed samples were ground through a 2mm screen using a laboratory hammer mill and were stored in air-tight jars at room temperature until analysis. The procedure of Lawlor et al. (2005) was used to determine pH and acid-binding capacity (ABC). In the present study was used pH = 4.0 as titration endpoint. All pH measurements were made using a laboratory pH meter (Consort C 830, Belgium). A 0.5g sample of feed was suspended in 50ml of distilled and de-ionised water and continuously stirred with a magnetic stirrer.

Table 2 Chemical composition (g/kg, as fed basis, as dry matter basis) of diets containing different levels of crude protein for piglets

Parameters	Control diet		Experimental diet	
DM g/kg	880,40	1000,00	881,20	1000,00
CP g/kg	209,40	237,84	176,00	189,72
EE g/kg	23,30	25,46	24,00	27,24
CF g/kg	39,03	44,33	37,80	42,89
Ash g/kg	57,80	65,65	55,40	62,87
NFE g/kg	550,87	625,58	588,00	667,26
ME MJ/kg	13,30	15,11	13,20	14,98
Lys g/kg	13,45	15,27	13,50	15,32
Thr g/kg	8,50	9,65	8,60	9,76
Met+cys g/kg	6,40	7,27	6,60	7,49
BUF 4 mEq	67		48	

CP – crude protein; EE – etheric extract; CF – crude fibre; NFE – nitrogen free extract; ME – Metabolizable Energy, Lys – lysine; Thr – threonine; Met+cys – methionine+cysteine; BUF – buffering capacity

Titrations were performed by addition of acid (0.1N HCl) in variable increments. Initial pH and all further readings taken during the titration were recorded after equilibration for three minutes. ABC was calculated as the amount of acid in milliequivalents (meq) required to lower the pH of 1kg of sample to (a) pH 4.0 (ABC). The buffering capacity (BUF) was calculated by dividing the ABC by the total change in pH units [from initial pH to the final pH of 4.0 (BUF-4)]. BUF expresses the amount of acid required to produce a unit change in the pH of a feed sample.

Blood collection for determination of biochemical parameters was performed 4 times at weekly intervals in the control and experimental group, 4 – 5 hours after feeding from *sinus ophthalmic*. Biochemical parameters in blood serum (total proteins, albumin, urea, glucose, total lipids, AST-aspartate aminotransferase) were determined using a fully automatic random access benchtop analyser Ellipse (Italy).

All data were reported as the mean ± SD (standard deviation). The differences between means were determined according to the unpaired t-test using Graph-Pad Prism statistical program (Graph Prism software, USA). By conventional criteria, differences ($P<0.05$; $P<0.01$; $P<0.001$) were considered to be statistically significant.

RESULTS AND DISCUSION

The metabolic variables in blood serum analysed of the study are shown in Table 3.

Table 3 Effect of different dietary CP content on biochemical parameters of piglets

Parameters	Control group				Experimental group			
Week	1.	2.	3.	4.	1.	2.	3.	4.
Total protein, g/l	56.80 ±2.84	53.70 ±2.97	57.20 ±3.20	54.60 ±3.30	54.50 ±1.89	56.20 ±2.89	57.30 ±3.5 2	56.60 ±1.73
Urea, mmol/l	5.11[a] ±0.12	5.85[a] ±0.45	6.68[a] ±0.30	5.72[a] ±0.48	3.70[b] ±0.31	3.11[b] ±0.20	3.63[b] ±0.25	3.51[b] ±0.38

Albumin, g/l	34.40 ±1.19	36.40 ±1.77	33.50 ±1.03	32.20 ±2.10	36.20 ±2.33	34.50 ±1.30	33.49 ±2.41	33.30 ±2.14
Glucose, mmol/l	5.52 ±0.53	5.45 ±0.25	5.31 ±0.45	5.26 ±0.89	5.6 5 ±1.87	6.18 ±0.69	6.23 ±2.46	5.45 ±0.37
Total lipids, g/l	1.78 ±0.18	1.64 ±0.49	1.94 ±0.27	1.85 ±0.22	1.79 ±0.87	1.69 ±0.49	1.87 ±0.39	1.69 ±0.22
AST, µkat/l	0.29 ±0,03	0.35 ±0.07	0.31 ±0.04	0.24 ±0.04	0.28 ±0.02	0.39 ±0.03	0.41 ±0.05	0.24 ±0.02

[ab]Significant differences (P<0.001); AST – aspartate aminotransferase

The first part of our experiment was performed to investigate the effect of feeding low crude protein diet to piglets on the dietary buffering capacity containing different levels of crude protein for piglets. The decrease in the dietary CP content was manifested by decrease of the buffering capacity (BUF4) 48 mEq and 67mEq (Table 2). The metabolic variables in blood serum analysed in this study are shown in Table 3. No significant differences between groups in serum total protein and albumin were observed. Average concentrations of total protein and albumin for all four weeks experimental period were slightly higher in experimental group (56.15 g/l and 34.37 g/l, respectively) compared to control group (55.57 g/l and 34.12 g/l, respectively). Urea as an important indicator of protein nutrition showed marked changes. During the whole experiment, serum urea concentration was significant lower (P<0.001) in pigs fed with low CP diet supplemented with essential amino acids (Lys, Met, Thr) compared to which consumed a higher CP diet. Reducing the dietary crude protein level of the diet and supplementing it with limiting crystalline AA can reduce nitrogen excretion, which may prevent surface and ground water contamination (Lynch et al., 2007). The increasing availability of synthetic amino acids allows reduction of the crude protein level in piglet diets in association with adequate AA supplementation, which maintains sufficient essential AA supply with little or no decrease in growth performance (Figueroa et al., 2002). Urea excreted in urine is the main nitrogenous end-product from amino acids catabolism in pigs and plasma or serum urea concentrations may be indicative of excreted nitrogen in urine (Roth and Raczek, 2003). Serum or plasma urea nitrogen can be used in various animal species to quantity nitrogen utilization and excretion rates. Lower blood urea nitrogen indicated higher

availability of dietary nitrogen a better use for amino acids with the CP reduction (Toledo et al., 2014). No significant differences between groups in other serum parameters were found. The mean values of these parameters during the whole experimental period varied in control *vs.* experimental group in glucose (5.38 *vs*. 5.88 mmol/l), total lipids (1.39 *vs.* 1.76 g/l), and AST (0.30 *vs*. 0.33 μkat/l) respectively. The biochemical parameters in blood serum in weaning pigs oscillated within relatively wide ranges of physiological values for pigs, presented by the authors Kraft and Dürr (1992). Average concentrations of total protein in blood serum in weaning pigs were lower as presented by Doubek et al. (2010). These differences could be due to the very young category of the animals used in our experiment.

CONCLUSION

The present study demonstrated that feeding lower CP content in diet with addition of limiting amino acids (Lys, Met, Thr) for recommendation of ideal amino acids pattern for piglets after weaning reduces buffering capacity of diet (BUF4 48 mEq *vs.* 67mEq; difference - 19 mEq) and the blood urea concentration (average concentrations from four weekly collection 3.50 *vs.* 5.84 mmol/l), which means the increase of biological value of feed mixture.. The use of synthetic amino acids improves the use of dietary nitrogen, with lower nitrogen excretion into the environment.

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REFERENCES

AOAC Association of Official Analytical Chemists International, (2001). In Horwitz, W. (Ed.): Official Methods of Analysis, 17th edn., Arlington, USA. AOAC Inc.

Blank, R., Mosenthin, R., Sauer ,W.C., Huang, S. (1999) Effect of fumaric acid and dietary buffering capacity on ileal and fecal amino acid digestibilities in early-weaned pigs. J.Anim Sci 77, 11, 2974 – 2984.

Duan, Y., Duan, Y., Li, F., Li, Y., Guo, Q., Ji, Y., et al. (2016): Effects of supplementation with branched-chain amino acids to low-protein diets on expression of genes related to lipid metabolism in skeletal muscle of growing pigs. Amino Acids, 48, 9, 2131—2144.

Doubek, J., Šlosarková, S., Řeháková, K., Bouda, J., Scheer, P., Piperisová, I., et al. (2010): Interpretation of Basic Biochemistry and Haematology Findings in Animals (*In Czech*). Noviko a.s., Brno, 102 pp.

Figueroa, J.L., Lewis, P.S., Miller, R.L., Fishcher, R.S., Diedrichsen, R.M. (2002): Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid supplemented diets. Journal of Animal Science, 80, 11, 2911—2919.

Han, K. and Lee, H.J. (2000): The role of synthetic amino acids in monogastric animal production. Review. Asian-Aust. J. Anim. Sci., 1, 4, 543—560. DOI: 10.5713/ajas.2000.543

Kerr, B.J. (2006): Opportunities for utilizing crystalline amino acids in swine. Adv. Pork Prod., 17, 245—254.

Kraft, W. and Dürr, M. U. (2001): Reference values. *In* Hajko & Hajková: Clinical Laboratory Diagnosis in Vet. Medicine (Slovak/Czech edition), Bratislava, 365 pp.

Lawlor, Peadar, G., Lynch, P.B., Caffrey, P.J., O'Reilly, J.J, O'Connell. M.K. (2005): Measurements of the acid-binding capacity of ingredients used in pig diets. Irish Veterinary Journal 58 (8): 447-452.

Liao, S.F., Wang, T. and Regmi, N. (2015): Lysine nutrition in swine and the related monogastric animals: muscle protein biosynthesis and beyond. Springer Plus, 4, 1, 147.

Lynch, M.B., Sweeney, T., Callan, J.J., Flynn, B., O'Doherty, J.V. (2007): The effect of high and low dietary crude protein and inulin supplementation on nutrient digestibility, nitrogen excretion, intestinal microflora and manure ammonia emissions from finisher pigs. Animal, 1, 8, 1112—1121.

Roth, F.X. and Raczek, N.N. (2003): Nutritive effectiveness of sorbic acid, effects in piglet feeding. Kraftffutter, 86, 105—110.

Sajeev, E.P.M., Amon, B., Ammon, C., Zollitsch, W., Winiwarter, W. (2018): Evaluating the potential of dietary crude protein manipulation in reducing ammonia emissions from cattle and pig manure: A meta-analysis. Nutrient Cycling in Agroecosystems, 110, 1, 161—175.

Toledo, J.B., Furlan, A.C., Pozza, P.C., Carraro, J., Moresco, G., Ferreira, S.L., Gallego, A.G. (2014): Reduction of the crude protein content of diets supplemented with essential amino acids for piglets weighing 15 to 30 kilograms. Revista Brasileira de Zootecnia, 43, 6, 301-309.

EFFECTS OF SUPPLEMENTAL HUMIC ACIDS IN DIET ON SELECTED BLOOD INDICATORS IN FINISHING PIGS

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ABSTRACT

A twelve finishing pigs were used to determine the effects of humic acid (HA) supplement on blood characteristics - hematologic, mineral and biochemical profile. The finishing pigs were assigned randomly by weight to two treatments. The dietary treatments included: 1) Control (basal diet), 2) and Experimental (basal diet + 0.5% humic acid supplement). Results of the whole experimental period (42 days) showed that addition of 0.5% HA supplement at the expense of barley to the diet, significantly increased total protein level in blood serum ($P < 0.05$). Higher total protein levels observed in pigs receiving HA suggest that HA may modulate protein metabolism. At the end of the experiment, higher values of Ca ($P < 0.05$) and also Mg and Cu ($P < 0.01$) in the experimental group were observed. No significant differences were observed with regard to the haematological indicators of throughout experimental period. The results of this study suggest that HA supplement might be utilized as a feed additive in the diet. It could improve protein metabolism without negative effect on the level of blood minerals in finishing pigs.

Key words: additives; blood serum; humic acids; pigs; minerals

INTRODUCTION

As one of the alternative feed additives, humic substances (including humates, humic acids, and fulvic acid) have been used in animal husbandry to improve the economics and ecology of animal production by increasing the growth rate, improving feed efficiency and immunity, and reducing the risk of disease (Gropp 2005; Vucskits et al. 2010). However, dietary supplementation with humic substances in pig feed has not yet been fully investigated (Kim et al., 2004; Kunavue and Lien, 2012). In last two dacades, interest in the use of humic substances as a feed additive has increased, in particular because of its ability to prevent intestinal diseases and stimulate the growth of pigs (Trckova et al. 2005; Ponce et al., 2016).

Humic substances are defined as “a series of relatively high-molecular-weight, yellow to black colored substances formed by secondary synthesis reactions” (Stevenson, 1994). Although there is not enough evidence to hypothesize how humic substances promote growth, it is assumed that humates might increase the uptake of nitrogen and other nutrients due to their chelating properties (Kocabağli et al. 2002).

The objective of the present study was to investigate the effect of supplementation of natural humic acids supplement on selected biochemical, mineral and haematological indicators of finishing pigs.

MATERIAL AND METHODS

The animals in the experiment were fattening pigs in the weight category higher than 60 kg. The experiment ran for 42 days. Initial body weight of pigs at the start of experiment was 60.5 ± 3.99 kg in the control and 61.17 ± 3.37 in the experimental group.

The experimental group diet was supplemented with 0.5% humic acid (HA) supplement. The addition of the HA supplement into test diet was realized at the expense of barley. Composition of experimental and control diet, the calculated contribution of nutrients from the diets and analysis of both diets are shown in Table 1. The characteristics of the applied HA supplement were the following: size of particles up to 100µm, humidity max. 15%, content of humic acids min. 65% in dry matter (DM); minerals: Ca 42.28, Mg 5.10, Fe 19.05 g/kg and microelements Cu 15, Zn 37, Mn 142, Co 1.24, Se 1.67 as well as Mo 2.7mg/kg DM. Diets were analyzed for dry mater, crude protein, ether extract, crude fibre and ash by the AOAC (2001).

Blood samples were drawn from the cranial vena cava of the pigs. Serum biochemical parameters - total protein, albumin, urea, cholesterol, creatinine, aspartate aminotransferase (AST), alkaline phosphatase (AP) were measured using a fully automatic random access benchtop analyzer Ellipse (Italy). The concentration of minerals in serum were determined by means of flame atomic absorption spectrometry using a Unicam Solar 939 (Great Britain). Complete blood count was performed with an automated hematology analyzer (scil Vet ABC™Hematology Analyzer, scil animal care company GmbH, Germany). The variables evaluated in our study were hematocrit value (HCT), hemoglobin concentration (Hb), red blood cells count (RBC) and total white blood cell (WBC) count. Statistical analyses were

performed using Prism Free Trial software (GraphPad Software, USA). Microsoft Office Excel was used for calculation of mean values and standard deviation.

Table 1 Concentrations of ingredients and nutrient content after chemical analysis in control and experimental diets

Ingredients, %	**Experimental diet**	**Control diet**
Corn	25	25
Wheat	25	25
Barley	31.4	31.9
Soybean meal	15	15
Premix minerals-vitamins	3	3
Lysine	0.06	0.06
Methionine	0.03	0.03
Threonine	0.01	0.01
Humic acids supplement	0.5	
Calculated value		
ME (MJ/kg)	12.78	12.80
CP (%)	14.80	15.00
Lysine (%)	0.9	0.9
Methionine (%)	0.3	0.3
Analysis value, *% as fed*		
Dry matter	88.1	88.3
Crude protein	14.56	15.05
Crude fiber	3.89	3.84
Ash	5.46	5.41
Starch	46.6	47.2
Ca	0.69	0.6
P	0.44	0.45
Na	0.14	0.15

CP – Crude protein; ME – Metabolizable energy

RESULTS AND DISCUSSION

Blood collection for analysis was performed after experimental period (42 days, 6 weeks) in the control and experimental group. Average values of selected of biochemical, mineral and haematological parameters in both group are shown in table no. 2.

Not many studies on the effects of humic acid on blood parameters in finishing pigs have been observed. In our experiment, only total protein was found to be significantly different between the two groups, being higher in the experimental group. Higher total protein levels observed in pigs receiving HA suggest that HA may modulate protein metabolism. El-Zaiat et al. (2018) reported similar higher blood total protein (P =0.015) in goats administrated with HA showed. Almost all blood parameters were within their normal ranges and none of them had any significant differences. Only average values of total proteins and creatinine in the control group were slightly below normal range.

Table 2 Hematologic, mineral, biochemical profile

	Unit	Ref values	**Experimental group**	**Control group**	***P***
RBC	T/l	5.0–8.0	6.85 ± 0.25	6.80 ± 0.20	ns
WBC	G/l	11–18	12.34 ± 2.4	10.90 ± 0.60	ns
HCT	l/l	0.38–0.42	0.38 ± 0.02	0.38 ± 0.02	ns
Hb	g/dl	10–14	11.38 ± 0.48	11.34 ± 0.60	ns
Ca	mmol/l	2.2–3	2.91±0.2	2.64±0.11	*
P	mmol/l	1.6–3	2.06±0.12	2.08±0.13	ns
Mg	mmol/l	0.6–1.6	0.75±0.09	0.62±0.03	**
Cu	µmol/l	18–34	30.22±1.30	25.22±2.81	**
Zn	µmol/l	15–35	23.90±3.57	24.32±3.53	ns
Total protein	g/l	62–82	64.80 ± 2.24	61.60 ± 2.07	*
Urea	mmol/l	3.6–10.7	5.36 ±0.64	5.46 ± 0.53	ns
Albumin	g/l	19–39	38.32 ± 2.70	38.39 ± 2.40	ns
Glucose	mmol/l	4.7–8.3	5.62 ± 0.44	5.61 ± 0.48	ns

Cholesterol	mmol/l	2.6–3.9	2.60 ± 0.30	2.51 ± 0.50	ns
Creatinin	μmol/l	141–239	142.2 ± 10.80	134.80 ± 9.30	ns
AP	μkat/l	2.0–6.6	2.85 ± 0.50	3.20 ± 0.18	ns
AST	μkat/l	0.5–1.5	0.80 ± 0.06	0.82 ± 0.07	ns

RBC - Red blood cells; WBC - White blood cells; HCT - Hematocrit value; Hb – Hemoglobin; AP - alkaline phosphatase; AST - aspartate aminotransferase

$^{*}P \leq 0.05$; $^{**}P \leq 0.01$

This study found no significant effect in the number of blood and white cells as well as the hemoglobin level and the hematocrit value analyzed among the experimental and the control groups. None of the findings were significant, however WBC was higher in the experimental group after the experimental period.

The amount of Ca, Mg and Cu in blood serum were significantly higher in the experimental group after feeding of humic acid supplement in feed as compared to the control group. Higher serum levels of these minerals may result from their higher uptake in the experimental group due to their presence in the humic agent used. The other findings in minerals (phosphorus and zinc) were not deemed significant by statistical analysis.

Wang et al. (2008) used finishing pigs to determine the effects of humic substances (HS) on blood characteristics and meat quality. The results of this study suggest that HS might be utilized as a feed additive in the diet. As in our thesis no significant differences were observed with regard to the RBC and WBC throughout experimental period. Our results obtained concerning RBC, WBC and Hematocrit value parameters are in agreement with Rath et al. (2006). They reported that red blood cell, white blood cell and hematocrit values in broiler chicken were not affected after addition of HA to their diet. Similarly, Lala et al. (2016) reported that supplementation with humic acids had no significant effect on haematological indices of the broiler chickens. The results of Lala et al. (2016) also indicated that humic acid supplementation had a beneficial effect on mineral absorption in broiler chicken. Higher blood calcium and phosphorus concentrations were obtained in the treatments supplemented with humic acid than control. The results obtained by Zralý and Písaříková (2010) confirmed increased concentrations of blood serum calcium ($P < 0.05$) in experimental vs. control piglets. The objectives of the study Ipek et al. (2008) were to investigate the effects of supplementation of humic acids to the diet on some blood parameters. In contrast to our study

no significant differences were found in levels of plasma copper between the experimental and the control group.

CONCLUSION

Higher values of total proteins ($P < 0.05$) in the experimental group was observed in the collection at the end of experiment. No significant differences were observed with regard to the selected haematological indicators of throughout experimental period. The results indicated that humic acids supplementation had no unwanted effect on mineral absorption. After humic acids feeding, increased levels of some minerals and trace elements (such as Ca, Mg and Cu) in serum were recorded in our experiment.

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REFERENCES

AOAC Association of Official Analytical Chemists International 2001: Official Methods of Analysis. 17th ed. Horwitz W (Ed): AOAC Inc., Arlington, USA.

El-Zaiat, H. M., Morsy, A.S., El-Wakeel, E.A., Anwer, M.M., Sallam, S.M. (2018): Impact of humic acid as an organic additive on ruminal fermentation constituents, blood parameters and milk production in goats and their kids growth rate. J Anim. and Feed Sci. 27, 1230-1388.

Gropp, J. (2005): Humic acid substances in animal agriculture. Pakistan J Nutri. 4:126–34.

Ipek, H., Avci, M., Iriadam, M., Kaplan, O., Denek, N. (2008): Effects of humic acid on some hematological parameters, total antioxidant capacity and laying performance in Japanese quails. Archiv fur Geflugelkunde, 72(2), 56-60.

Kim S., Hulbert L., Rachuonyo H., McGlone J. (2004): Relative availability of iron in mined humic substances for weanling pigs. Asian Austr J Anim Sci. 17:1266–70.

Kocabağli N., Alp M., Acar N., Kahraman R. (2002): The effects of dietary humate supplementation on broiler growth and carcass yield. Poultry Science, 81: 227-230.

Kunavue N. and Lien T. (2012): Effects of fulvic acid and probiotic on growth performance, nutrient digestibility, blood parameters and immunity of pigs. J Anim Sci Adv. 2:711–21.

Lala, A. O., Okwelum, N., Irekhore, O. T., Ogunlade, B. A., et al. (2016): Haematological and Biochemical Parameters of Broiler Chicken Supplemented with Humic Acid in the Drinking Water. Applied Tropical Agriculture 21 (2) Special Issue, 74-78.

Ponce C., Arteaga C., Flores A. (2016): 1016 Effects of humic acid supplementation on pig growth performance, nitrogen digestibility, odor, and ammonia emission. J Anim Sci. 94:486–486.

Stevenson FJ. (1994): Humus Chemistry: Genesis, Composition, Reactions New York, NY: John Wiley and Sons.

Trckova M., Matlova L., Hudcova H., Faldyna M., Zraly Z., Dvorska L., et al. (2005): Peat as a feed supplement for animals: a review. Vet Med Praha. 50:361–77.

Vucskits A., Hullár I., Bersényi A., et al. (2010): Effect of fulvic and humic acids on performance, immune response and thyroid function in rats. J Anim Physiol Anim Nutri. 94:721–8.

Wang Q., Chen Y., Yoo J., Kim H., Cho J., Kim I. (2008): Effects of supplemental humic substances on growth performance, blood characteristics and meat quality in finishing pigs. Livestock Sci. 117:270–4.

Zralý, Z. and Písaříková, B. (2010): Effect of sodium humate on the content of trace elements in organs of weaned piglets. Acta Veterinaria Brno, 79 (1), 73-79.

MONITORING OF THE PRESENCE OF TREPONEMA SPP. IN ORTHOPEDIC PATIENTS OF THE CLINIC OF HORSES AND THE CLINIC OF RUMINANTS

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ABSTRACT

The aim of our work was to detect the presence of *Treponema* spp. in skin lesions of cattle with digital dermatitis and in horses with hoof canker, that were patients of the Clinic of Ruminants and the Clinic of Horses of the University of Veterinary Medicine and Pharmacy in Košice. Twenty-four samples of the affected skin of cattle and 20 hoof biopsies from horses were examined. Samples were collected on the farms in the Czech and Slovak Republic and were screened by PCR using primers for the detection of *Treponema pedis*, *Treponema brennaborense*, and the *Treponema denticola* group. In the cattle, *Treponema denticola* was detected in 10 samples, *Treponema pedis* was detected in 4 samples, *Treponema brennaborense* in only 1 sample. In horses, from 20 samples were 8 positive for *Treponema pedis*, 3 for *Treponema brenaborense* and 3 for *Treponema denticola*. Our results determined the presence of *Treponema* spp. in the digital dermatitis of the cattle and equine hoof canker, and the suitability of PCR as a method for their detection.

Keywords: equine hoof canker; digital dermatidis; DNA; *Treponema pedis*; *Treponema brennaborense*; *Treponema denticola*

INTRODUCTION

Equine hoof canker (*pododermatitis chronica verrucosa*) is defined as an infectious hoof disease that leads to chronic hypertrophy of horn-producing tissues. Digital dermatitis is an infectious inflammatory disease of bovine hooves. The clinical signs include painful ulcerative or proliferative changes that lead to lameness and thereby disturb the animal welfare but also negatively influence the economic efficiency of the breeding. The etiology of both diseases is unclear because attempts to identify the causative agents of bacteria, fungi or viruses have so far been unsuccessful. Nowadays, the effect of *Treponema* spp. and bovine papillomavirus is discussed as a potential etiological agent of these diseases. A multifactorial etiology with a significant influence of *Treponema* spp. is generally accepted. Some sources also mention the possibility of the interaction of *Fusobacterium necrophorum*.

Treponema spp. are anaerobic bacteria belonging to the phylum Spirochaetes. They are elongated, spirally twisted organisms composed of an outer membrane that exhibits on its surface a small amount of transmembrane proteins. This is likely to contribute to the ability of the bacteria to escape the host's immune system (Radolf, 1996). In horses, chronic hyperproliferative dermatitis of the hooves, also called equine hoof canker, seriously jeopardizes the use and welfare of the affected horses (Dietz, 2006). Some studies have provided the evidence of the presence of treponemal DNA in the affected hooves, suggesting that treponemal infection may contribute to the development and/or chronicity of the disease (Nagamine et al., 2005; Moe et al., 2010; Brandt et al., 2011). The aim of this study was to investigate the presence of treponemal DNA in hoof biopsies in horses affected with hoof canker and in the skin lesions of the cattle hooves affected by digital dermatitis using a PCR reaction.

MATERIAL AND METHODS

In this study were examined 24 samples of the cattle skin affected with digital dermatitis collected in Eastern Slovakia and 20 hoof biopsies collected from horses in the Czech and Slovak Republic. From the cattle with clinical signs of digital dermatitis was collected a part of the affected skin after the mechanical debris removal. Samples were stored at - 20 °C. We have also focused on the detection of Treponema spp. by PCR reactions to monitor the presence of treponemal DNA in hoof biopsies collected from horses during hoof trimming. A

previously standardized method of PCR with the DNA isolation using DNAzol® Direct (Molecular Research Center, iBiotech, USA) was used. Immediately after the collection, the biopsy samples were placed in a pre-prepared low binding 2 ml Eppendorf tubes (Eppendorf, Germany) containing 100 µl of DNAzol® Direct. The samples were then incubated in a dry shaking bath at 500 rpm for 15 min at 95 °C. The obtained 1 µl DNA sample was used as a template DNA for PCR reactions (Table 1). Mastermix (One Taq® 2X Master Mix with Standard Buffer (New England Biolabs) in a final volume of 50 µl was used in the reaction. PCR reaction conditions´ and specific primers (Table 1) were used, each with a volume of 1 µl and a concentration of 33 µM. PCR reactions for the identification of different Treponema spp. were designed based on a study by Brandt, Apprich et al., 2010. As positive DNA samples were used samples of *T. denticola* DSM 14222, *T. brennaborense* DSM 12168, and *T. pedis* DSM 18691 obtained from Deutsche Sammlung von Microorganismen und Zelikultren GmbH (DSMZ, Braunschweig, Germany).

Table 1

Detected species, gene	**Primers**	**PCR protocol**	**Expected product**
Treponema pedis, flaB2	TPed32f: 5´-CTTACTTACAGGAAACTACGGAC-3´; Tped-500r: 5´-GCAATGTTAATTCCTACAACCGTAAG-3´	94°C 5min 35x [94°C 30sec, 61°C 30sec,72°C 40sec] 72°C 5min	424bp
Treponema brennaborense, 16SrRNA	TBrenn-418f: 5´-GACAGCGTGGTGACAGTAGG-3´; TBrenn-1080r: 5´-CTTGCTGGTAACTGGCAGTAGG-3´	94°C 5min 35x [94°C 30sec, 61°C 30sec,72°C 40sec] 72°C 5min	663bp
Group *Treponema denticola, flaB2*	TMult-2f: 5´-ACGGYATTTCYTTTATTCAAGTTGC-3´; TMult-472r: 5´-CGAGTCTGTTYTGGTATGCACC-3´	94°C 5min, 45x [94 °C 30sec, 63°C 30sec, 72°C,40sec] 72 °C 40sec	470bp

RESULTS AND DISCUSSION

Using specific PCR were detected representatives of *Treponema* spp. in samples collected from skin lesions of cattle as well as in equine biopsy hoof samples. In cattle, *T. denticola* was detected in 10 samples from 24 samples. *T. pedis* was detected in 4 samples and *T. brennaborense* in only 1 sample. From 20 equine hoof samples was *T. pedis* detected in 8 samples, *T. brennaborense* in 3 samples and *T. denticola* in 3 samples. The sequencing of

PCR products revealed a particular region flanked by primers for specific *fla B2* genes and 16SrRNA gene of expected sizes. In the samples was by sequencing of amplification products determined the presence of *T. denticola* group, in particular, *T. medium* subsp. *bovis* T19 flagellin (flaB2) gene, partial cds (372/373) with a lower score for *T. vincentii* strain ATCC 35580 flagellin (flaB2) gene, partial cds (364/373). For *T. brennaborense* was determined a match with the *T. brennaborense* strain DD5/3 16S ribosomal RNA, partial sequence, *T. brennaborense* DSM 12168, for *T. pedis* was determined the most common match with *T. pedis* strain T3552B flagellin (flaB2) gene, partial cds.

Figure 1 *T. denticola* detected by PCR in cattle

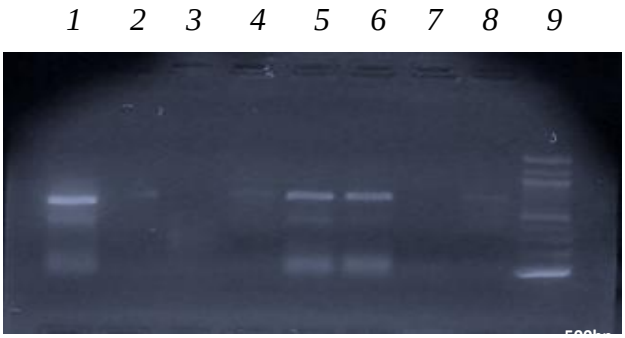
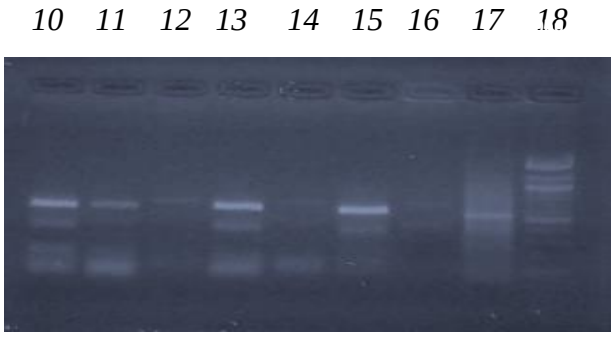


Figure 2 *T. denticola* detected by PCR in cattle



Legend: 1-8 and 10-17:tested samples, 9, 18: markers.

Figure 3 *T. pedis* detected by PCR in cattle

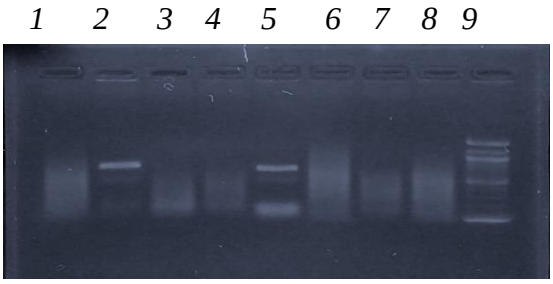
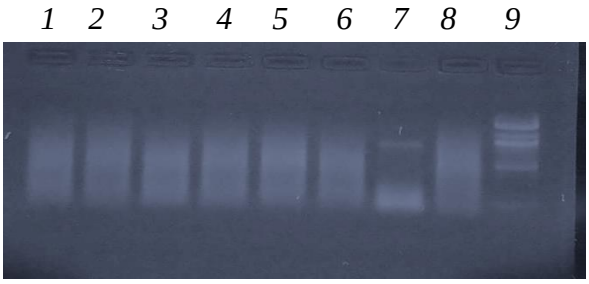


Figure 4 *T. Brennaborense* detected by PCR in cattle



Legend: columns 1-8: tested samples, 9: marker,

Treponema spp. has been accepted as a predominant bacterial species found in active dermatitis lesions (Demirkan et al., 2018). They are hardly cultivable and therefore their detection by PCR together with the histological examination is considered to be the best method for their diagnosis. According to Brandt et al. (2010) we amplified a specific fragment of the 16SrRNA gene for the detection of *T. brennaborense* and the gene encoding flagellin flaB2 for the detection of *T. denticola* and *T. pedis*. In their study were *Treponema* spp. found in 38 samples out of 45, and after using more sensitive qualitative PCR were determined in 42 samples out of 45, representing 93.3% Two of the three negative results were the samples from a chronic form of digital dermatitis. The most common treponema in this study was *T. pedis* determined in 51% of the samples, while the most common treponema in our study was *T. denticola* (41.7%/66.7%). Another difference in our findings was the discovery of one sample positive for *T. brennaborense*, which was not detected in this study. Wilson-Welder et al. (2015) also report its less frequent occurrence in digital dermatitis lesions. Rasmussen et al. (2012) reported in their study the possibility of *F. necrophorum* involvement in the development of digital dermatitis since this anaerobic bacterium was detected in biopsies from two lesions. Beninger et al. (2018) reported significantly higher amounts of *Treponema* spp. in active ulcerative lesions than in healing and chronic ones. In conclusion, with the use of the PCR method was determined the presence of *Treponema* spp. in skin lesions of the cattle affected by digital dermatitis.

Screening for the presence of Treponema DNA in biopsies collected from hooves affected by equine hoof canker and a healthy control hoof revealed the presence of treponemal DNA in both sample types which is consistent with findings of Sykora et al. (2015). However, this demonstrates that the presence of treponemal DNA is not limited to the hooves affected by canker, as reported by Moe et al. (2010). *Treponema* spp. are part of the horses´ intestinal microbiome, whether of healthy horses or horses suffering from laminitis, and the most commonly are determined *T. bryantii* and *T. succinifaciens* (Steelman, Chowdhary et al., 2012). In the future, we will monitor the presence of *Treponema* spp. in feces of horses and cattle.

CONCLUSION

The outcome of the study was the demonstration of the usefulness of PCR reactions for further study of the pathogenesis of equine hoof canker and digital dermatitis in cattle. The presence of several *Treponema* spp. was determined in the samples. In the cattle, the most

commonly present was *T. medium* subsp. *bovis* while in horses it was *T. pedis*. An interesting finding in this study was also that skin samples taken from digital dermatitis were still usable for PCR even after 6 months of storage at -20 °C. The DNA isolation method using DNAzol® Direct is not suitable for long-term storage of DNA samples at 4 °C and their later PCR processing, as some of the hoof and skin components will eventually deteriorate template DNA sample over the time. Therefore, the DNA sample thus obtained must be rapidly processed.

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REFERENCES

Apprich, V., Licka, T., Zipfl, N., Tichy, A, Gabriel, C. (2017): Equine Hoof Canker: Cell Proliferation and Morphology. Veterinary Pathology, 54, 661–668.

Beninger C, a kol. Associations between digital dermatitis lesion grades in dairy cattle and the quantities of four *Treponema* species. Veterinary Research. 2018;49:111.

Brandt S, a kol. Prevalence of bovine papillomavirus and Treponema DNA in bovine digital dermatitis lesions. Vet Microbiol. 2011;148(2-4):161-7

Brandt S. (2015): Occurrence of Treponema DNA in equine hoof canker and normal hoof tissue. Equine Veterinary Journal. 47, 627–63

Demirkan I, a kol. Isolation and identification of *Treponema pedis* and *Treponema phagedenis*-like organisms from bovine digital dermatitis lesions found in dairy cattle in Turkey. J dairy Sci.2018;101(11):10317-10326.

Rasmussen M, a kol. Bovine digital dermatitis: Possible pathogenic consortium consisting of *Dichelobacter nodosus* and multiple *Treponema* species. Vet Microbiology 2012; 160:151–161.

Steelman, S. M., B. P. Chowdhary, et al. (2012). Pyrosequencing of 16S rRNA genes in fecal samples reveals high diversity of hindgut microflora in horses and potential links to chronic laminitis. BMC Vet Res 8: 231.

Wilson-Welder J, a kol. Digital Dermatitis in Cattle: Current Bacterial and Immunological Findings. 2015;5(4):1114-1135.

THE INFLUENCE OF THE LUPIN BASED DIET ON THE FATTY ACID CONTENT IN EGG YOLK

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ABSTRACT

The aim of the experiment was to determine how the content of lupin meal in the diet for commercial laying hens would affect the quality of fat in the egg yolk. The results of experiment, using lupin seeds (unpeeled and peeled) in the feed mixture as a 50% and 100% replacement of extracted soybean meal, confirmed the positive effect of lupin based diets on egg yolk fat composition. Although the diets did not affect the fat content of the egg yolk, some other changes in the quality of the egg yolk were demonstrated during laying. These changes in egg yolk fat were characterized by decrease of saturated fatty acids (SFA), in some groups also by a slight decrease in monounsaturated fatty acids (MUFA) and, what is important, by a marked increase in polyunsaturated fatty acids (PUFA) from the omega 6 and omega 3 groups. From these results, it is evident that using lupin meal in the feed mixtures for commercial laying hens increases the quality of the produced eggs.

Keywords: commercial laying hens; lupin meal; quality of yolk fat, composition of yolk fat

INTRODUCTION

Current trends in the Czech Republic, but also throughout the European Union, are focused on the production of protein feeds in agricultural production. The reason behind this is to reduce imports of expensive soya products, which are an integral part of practically all feed mixtures intended for farm animal feed. One way, how to increase protein feed production is to increase the growth of legumes. Legume seeds are a rich source of protein. Some varieties,

such as lupin, have comparable or even higher protein content than soybeans (Straková et al., 2006; Saastamoinen et al., 2013; Straková and Suchý, 2016). From this point of view, the seeds of some varieties from the group of white and yellow cultivated lupins seem to be perspective. In local soil and climatic conditions, some white lupin varieties are especially promising, which, with proper agrotechnology, offer relatively high seed yields. Their advantage is that thanks to the tuberous root bacteria, which enrich the soil with nitrogen and their huge root system, leaves a large amount of organic matter in the soil. Also the fact that they are not genetically modified organisms is not negligible. Another advantage of the seeds of cultivated lupin varieties is that they contain the lowest amount of antinutritional substances compared to other types of legumes (peas, soybeans, beans) (Kurlovich et al., 2002). For these reasons, the seeds of white cultivated lupin varieties are of great interest as an alternative source of dietary protein in farm animal feeds, namely in pig fattening (Zralý et al., 2008; Kasprowicz-Potocka et al., 2016; Pieper et al., 2016) or in feed mixtures for rabbits (Volek et al., 2018; Zwoliński, 2016). Lupin seeds have been widely used in feed mixtures for poultry nutrition (Jeroch et al., 2016), especially in fattening broiler chickens (Geigerova et al., 2017; Chladek et al., 2017) or commercial laying hens. As a positive effect, commercial laying hens have an improved egg yolk colour and fatty acid profile in the yolk (Drażbo et al., 2014). Therefore, for several years, the authors have focused on the problem of using lupin seeds as a substitute for the extracted soybean meal. Based on the results of many experiments, it has been clearly shown that it is possible to replace 50-100% of soya meal with lupin seed meal in feed mixtures, especially for poultry, without adversely affecting production. On the contrary, in most experiments, an improvement in the health of the animals was noted, evaluated by a reduction in their mortality. Lupin seeds contain high-quality protein characterized by a high content of arginine (an essential amino acid for poultry) and white varieties, also, contain high-quality oil characterized by a high content of polyunsaturated fatty acids (Zapletal et al., 2015). These results have led the authors to find out whether the content of the lupin meal in diet would positively affects the quality of fat in the product - the fatty acid composition of egg yolk in this particular study.

MATERIAL AND METHODS

For the experiment with lupin containing feed mixtures was chosen hen breed Isa Brown. They are common commercial laying hens. At 18 weeks of age the laying hens were housed individually in three-level cage technology, with manual feeding (ad libitum) and automatic drinkers. In total, 5 groups of 70 laying hens were established, a control group (K 0%) and 4

experimental groups (N 50%, N 100%, L 50% and L 100%). During the laying period, three types of feed mixtures (N1 starter, N1 and N2) were given to the laying hens. The control group received a commercially produced feed mixture, while for the experimental groups, feed mixtures of similar component and nutrient composition were prepared, with the difference being that in the experimental mixtures, extracted soybean meal was replaced with lupin by 50% and 100% from unpeeled (N) or peeled (L) lupin seeds of the Zulika variety. During the laying period, 10 eggs were collected in 8-week intervals (5 times in total) from each group, where the fat content of the yolk was determined and analysed for fatty acids. The fat was determined by extraction with diethyl ether according to Soxhlet; for determining fatty acids, the Shimadzu GAS CHROMATOGRAF GC - 2010 analyser was used. The fat content of the egg yolk was expressed as a percentage and the fatty acid content in g per 100 g of fat.

Results were evaluated by mathematical-statistical methods using the statistical program Unistat 5.6; the evaluation of the mean values and their differences were performed by multiple comparisons using the Tukey-HSD test at the significance level $P \leq 0.05$. Each indicator is represented by the mean value (x) and the standard deviation (± SD).

RESULTS AND DISCUSSION

During the laying period, 10 eggs from each group were collected for 5 times in 8-week intervals, i.e. in the 8th, 16th, 24th, 32nd and 40th week of laying (in total 50 eggs per group). The yolk was separated from the egg, the fat content was determined, and its percentage calculated. For results see Figure 1. In general, regardless of the variety of diet, the percentage of fat in the yolk ranged from a relatively narrow range of 24.59% to 25.68%. The results show that diets based on lupin meal did not affect the percentage of fat in the yolk. Mean values of the control group was not significantly different from the mean values of the experimental groups.

Changes in the fatty acid composition in the egg yolk during the laying period

Saturated fatty acids (SFA)

From the results in Figure 2 is visible that laying hens fed with lupin based meal diets showed a significantly lower proportion of SFA in the egg yolk fat compared to the control group. Clearly, the lowest SFA content in the yolk fat was found in the group N 100%, which were

fed a 100% lupin meal from unpeeled lupin seeds. From these results, it is apparent that lupin meal can reduce the SFA content in the yolk fat.

Figure 1 Mean percentage of the fat content in the yolk in the control and experimental groups

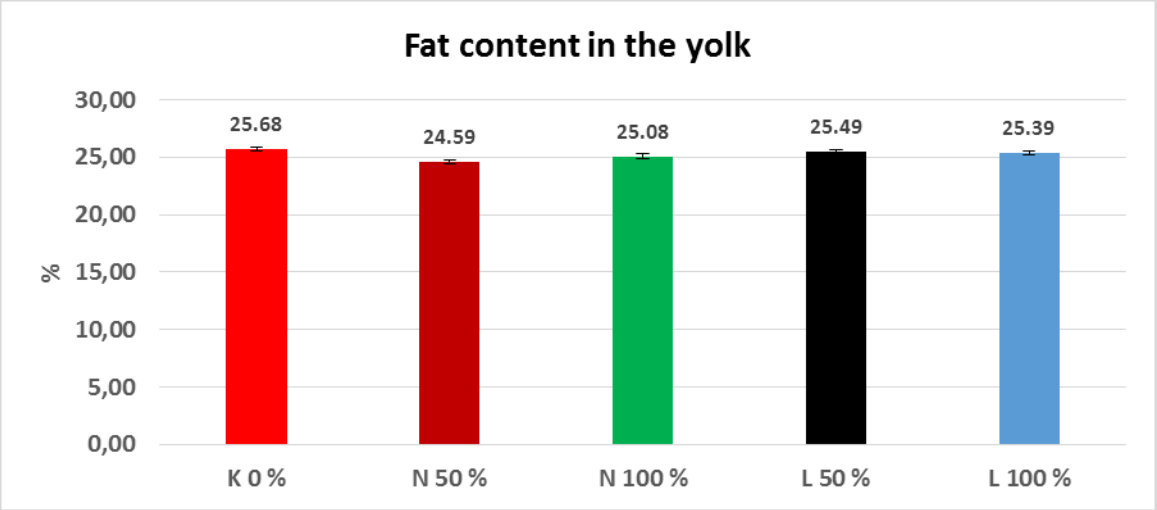
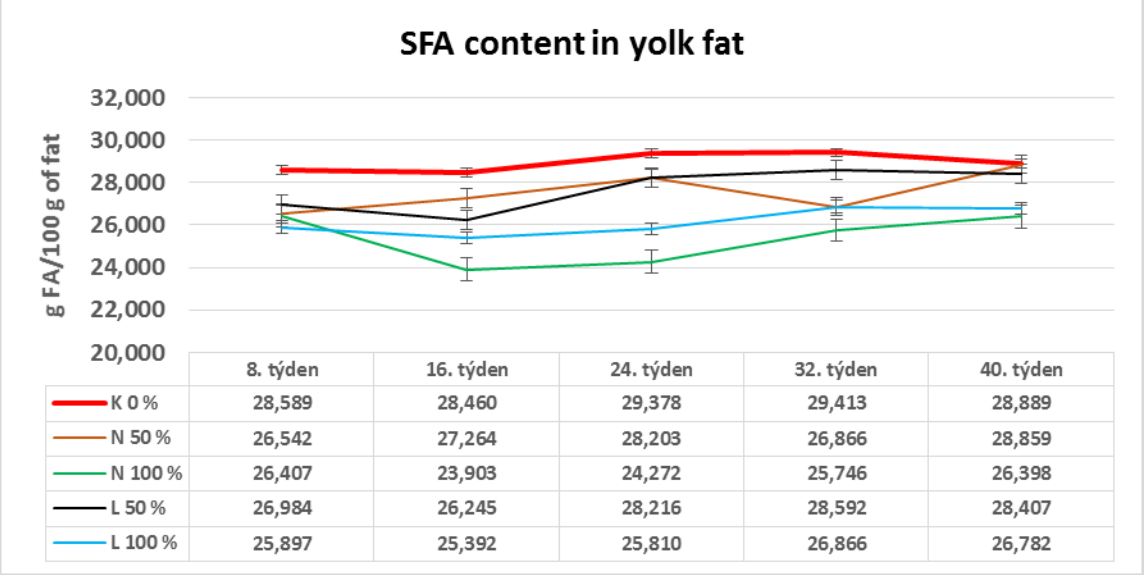


Figure 2 Mean SFA content in the yolk fat of the control and experimental groups

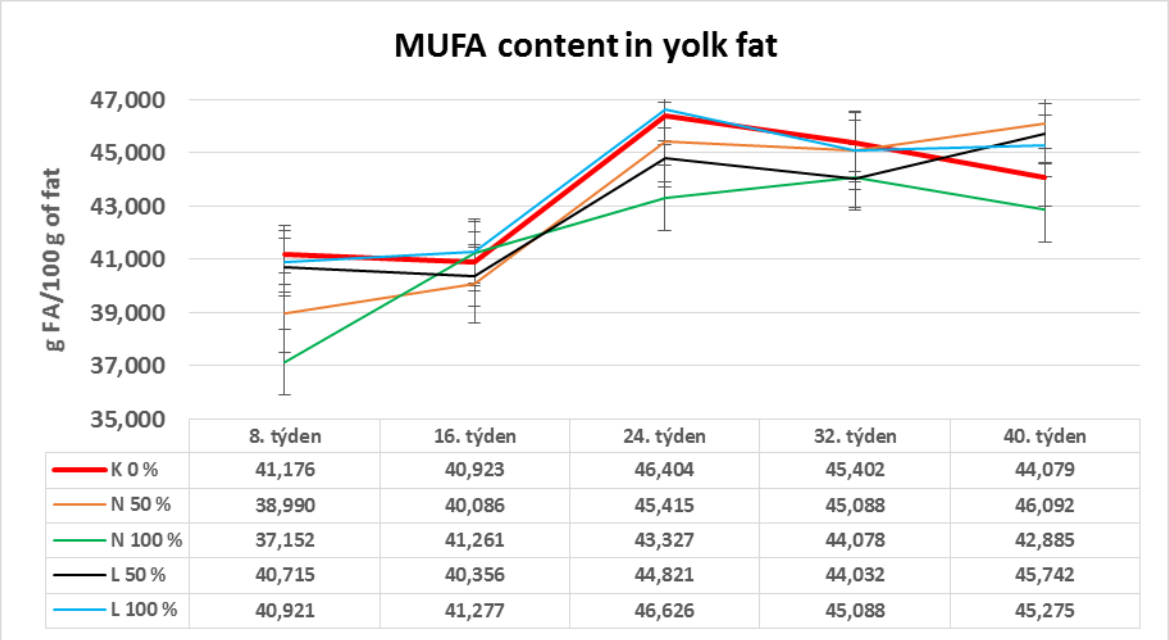


	8. týden	16. týden	24. týden	32. týden	40. týden
K 0 %	28,589	28,460	29,378	29,413	28,889
N 50 %	26,542	27,264	28,203	26,866	28,859
N 100 %	26,407	23,903	24,272	25,746	26,398
L 50 %	26,984	26,245	28,216	28,592	28,407
L 100 %	25,897	25,392	25,810	26,866	26,782

Monounsaturated fatty acids (MUFA)

The results of the MUFA analysis show different outcomes, see Figure 3. Compared to the control group, the mean MUFA fat content in the yolk fat during the laying period was comparable or slightly lower in the experimental groups, and even higher at the end. Clearly, the lowest MUFA fat content was detected in the experimental group N 100%.

Figure 3 Mean MUFA content in the yolk fat of the control and experimental groups



	8. týden	16. týden	24. týden	32. týden	40. týden
K 0 %	41,176	40,923	46,404	45,402	44,079
N 50 %	38,990	40,086	45,415	45,088	46,092
N 100 %	37,152	41,261	43,327	44,078	42,885
L 50 %	40,715	40,356	44,821	44,032	45,742
L 100 %	40,921	41,277	46,626	45,088	45,275

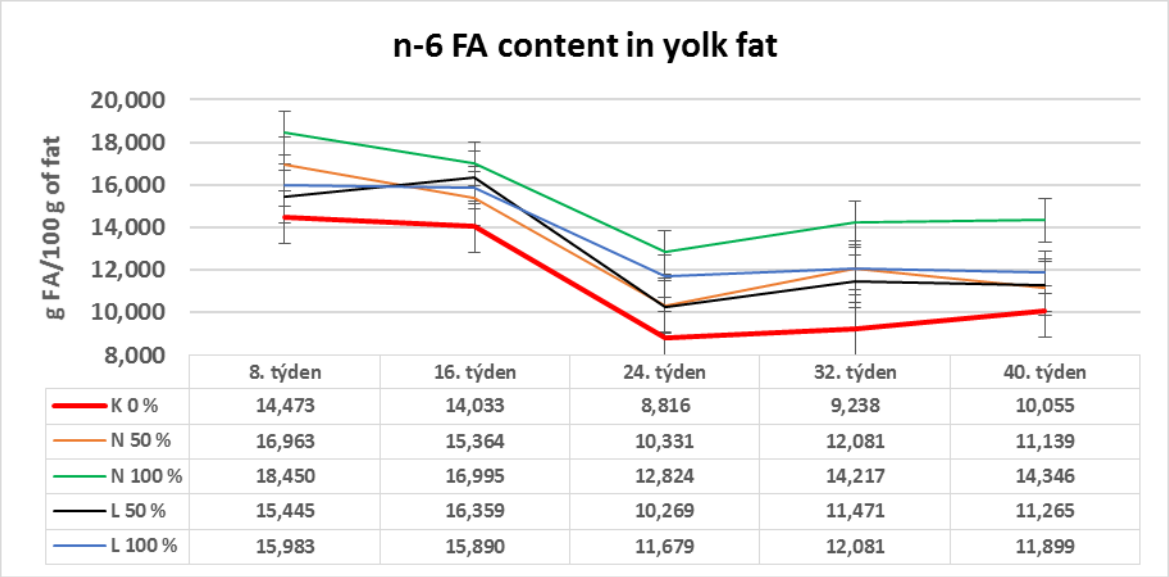
Polyunsaturated fatty acids (n-6 FA)

From the results shown in Figure 4, it is apparent that lupin based mixtures fed in experimental groups of laying hens had a significant effect on the increase of n-6 FA in the yolk compared to the control group. The highest content of n-6 FA was found in the egg yolk of N 100% experimental group (feed mixture with 100% replacement of soya meal with lupin meal). Changes of n-6 FA in the egg yolk were very dynamic. Gradual decrease of n-6 FA in the yolk fat was observed in all groups until the 24th week of laying. From the 24th week, the n-6 FA level stabilized and fluctuated within a relatively narrow range until the end of the experiment.

Polyunsaturated fatty acids (n-3 FA)

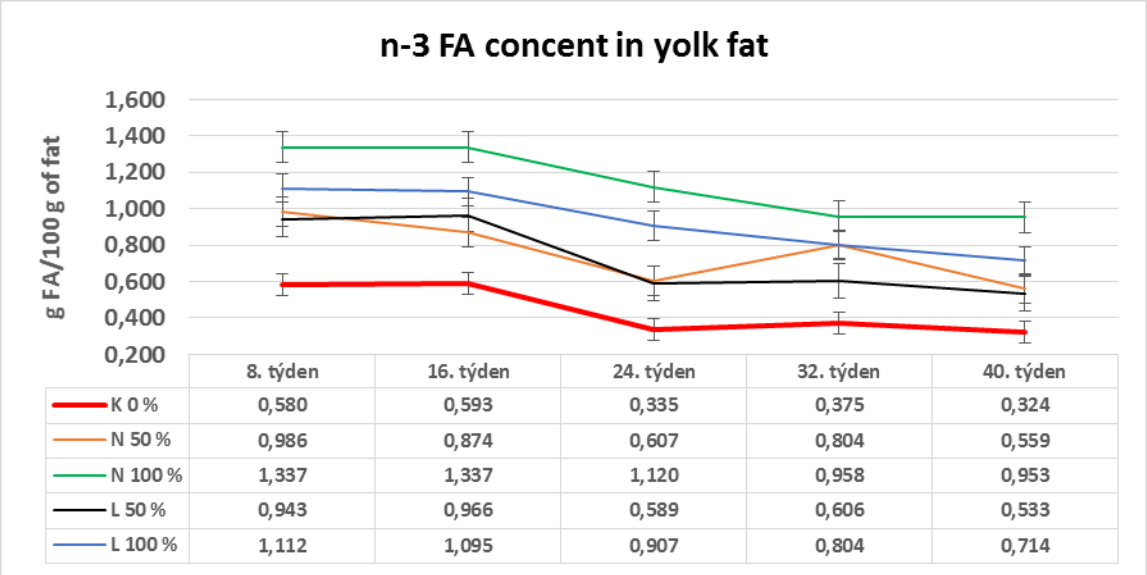
The effect of lupin meal in the feed mixture on the increase of n-3 FA in the egg yolk fat, was similar as at n-6 FA. Gradual decrease of n-3 FA in the yolk fat was observed in all groups until the 24th week of laying and then barely change (Figure 5).

Figure 4 Mean n-6 FA content in the yolk fat of the control and experimental groups



	8. týden	16. týden	24. týden	32. týden	40. týden
K 0 %	14,473	14,033	8,816	9,238	10,055
N 50 %	16,963	15,364	10,331	12,081	11,139
N 100 %	18,450	16,995	12,824	14,217	14,346
L 50 %	15,445	16,359	10,269	11,471	11,265
L 100 %	15,983	15,890	11,679	12,081	11,899

Figure 5 Mean n-3 FA content in the yolk fat of the control and experimental groups



	8. týden	16. týden	24. týden	32. týden	40. týden
K 0 %	0,580	0,593	0,335	0,375	0,324
N 50 %	0,986	0,874	0,607	0,804	0,559
N 100 %	1,337	1,337	1,120	0,958	0,953
L 50 %	0,943	0,966	0,589	0,606	0,533
L 100 %	1,112	1,095	0,907	0,804	0,714

CONCLUSION

In conclusion, the use of lupin based diets as a substitution for soybean diet had positive effect on the fatty acid composition in egg yolk fat. The main positive effects were

- reduction of the content of saturated fatty acids (SFA),
- increase of the content of polyunsaturated fatty acids omega 6 (n-6 FA)
- increase of the content of polyunsaturated fatty acids omega 3 (n-3 FA)

From the results, it is apparent that feeding lupin seed diet leads to the increase of the nutritional value of eggs - one of the most important products for human nutrition.

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REFERENCES

Drażbo A., Mikulski D., Zdunczyk Z., Szmatowicz B., Rutkowski A., Jankowski J. (2014): Fatty acid composition, physicochemical and sensory properties of eggs from laying hens fed diets containing blue Lupin seeds. Archiv fur Geflugelkunde, 78, 14.

Geigerová M., Švejstil R., Skřivanová E., Straková E., Suchý P. (2017): Effect of dietary lupin (*Lupinus albus*) on the gastrointestinal microbiota composition in broiler chickens and ducks. Czech Journal of Animal Science, 62, 369–376.

Chládek L., Plachý V., Vaculík P., Braný P. (2017): Evaluation of nutritional and physical values of pellets based on pea and Lupin with added yeast in chickens fattening. Agronomy Research, 15, 1550-1558.

Jeroch H., Kozłowski K., Schöne F., Zduńczyk Z. (2016):Lupins (*Lupinus spp*.) as a protein feedstuff for poultry. 1) varieties, composition and nutritional values for poultry. European Poultry Science, 80, 14.

Kasprowicz-Potocka M., Zaworska A., Kaczmarek S.A., Rutkowski A. (2016): The nutritional value of narrow-leafed Lupin (*Lupinus angustifolius*) for fattening pigs**.** Archives of Animal Nutrition, 70, 209-223.

Kurlovich B.S., Kartuzova L.T., Heinanen J., Benken I.I., Chmeleva Z.V., Bernatskaya M.L. (2002): Biochemical composition. Chapter 9. In: Kurlovich B.S. (ed.): Lupins (Geography,

Classification, Genetic Resources and Breeding). St. Petersburg, OY International Express, 241–268.

Pieper R., Taciak M., Pieper L., Święch E., Tuśnio A., Barszcz M., Vahjen W., Skomiał J., Zentek, J. (2016): Comparison of the nutritional value of diets containing differentially processed blue sweet lupin seeds or soybean meal for growing pigs. Animal Feed Science and Technology, 221, 79-86.

Saastamoinen M., Eurola M., Hietaniemi V. (2013): The chemical quality of some legumes, peas, fava beans, blue and white lupins and soybeans cultivated in Finland. Journal of Agricultural Science and Technology, 3, 92–100.

Straková E., Suchý P., Večerek V., Šerman V., Mas N., Jůzl M. (2006): Nutritional composition of seeds of the Genus Lupinus. Acta Veterinaria Brno, 75, 489–493.

Straková E., Suchý P. (2016): Lupin as a perspective inland protein source for animal nutrition (in Czech). In: Zborník prednášok Lazarove Dni Výživy a Veterinárnej Dietetiky XII, Košice, Slovak Republic. 223–225.

Volek Z., Ebeid T.A., Uhlířová L. (2018): The impact of substituting soybean meal and sunflower meal with a mixture of white Lupin seeds and rapeseed meal on rabbit doe milk yield and composition, and the growth performance and carcass traits of their litters. Animal Feed Science and Technology, 236, 187-195.

Zapletal D., Suchý P., Straková E., Karel K., Kubiska Z., Macháček M., Vopálenský J., Sedlákova K. (2015): Quality of lupin seeds of free varieties of white lupin grown in the Czech Republic. In: 14th International Lupin Conference, Book of Proceedings. Italy: International Lupin Association. 136 p.

Zralý Z., Písaříková B., Trčkova M., Doležal M., Thiemel J., Simeonovová J., Jůzl M. (2008): Replacement of soya in pig diets with white lupin cv. Butan. Czech Journal of Animal Science, 53, 418-430.

Zwoliński C., Gugołek A., Strychalski J., Kowalska D., Chwastowska-Siwiecka I., Konstantynowicz M. (2016): The effect of substitution of soybean meal with a mixture of rapeseed meal, white lupin grain, and pea grain on performance indicators, nutrient digestibility, and nitrogen retention in Popielno white rabbits. Journal of Applied Animal Research, 45, 570-576.

Vertebral Heart Score in two dog breeds – Cavalier King Charles Spaniel and Irish Wolfhound

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ABSTRACT

Presented X-ray study describes the numerical evaluation of vertebral heart score (VHS) measurements in clinically healthy Cavalier King Charles Spaniel (Cavalier) breed dogs n = 8 and Irish Wolfhound breed dogs n = 7 examined at the University of Veterinary Medicine and Pharmacy in Košice. Radiographs were performed in the laterolateral position on the right and left sides with alignment and collimation to patient's heart and taken at the maximal inspiration without sedation. We used indirect digitization of FireCR PLUS and FujiFilm Prima T2. VHS measurements were taken using short and long axis of heart compared with the vertebral bodies from 4 th thoracal vertebre. There was no statistical significance of VHS measurment in the laterolateral left and right position of the animals (P >0.05).

Keywords: VHS, heart siluet, Cavalier, Irish Wolfhound

INTRODUCTION

The popularity of the Cavalier breed dogs is currently increasing. This breed compared with the other breeds has specific reference values of VHS and also higher prevalence of the cardiovascular disease. Bavegems et al. 2005, Gugjoo et al. 2013, Lamb et al. 2001, Jepsen-Grant et al. 2013, Pinto et al. 2004, Kraetschmer et al. 2008, Cardoso et al. 2011 described these specific reference values of VHS especially in Wipet, Labrador Retriever, Doberman,

Pug, Pomerian, Boxer, Boston Terrier, Beagle, Bulldog, Poodle, Greyhound, American pit bull terrier breed dogs..

Radiological examination of the chest and heart is determined based on two perpendicular projections. These are laterolateral (LL) right and left and dorsovethral (DV) or ventrodorsal (VD) projection and it must be symmetrical position without rotation. Rotation of the patient may change the size and shape of the heart's siluet and be responsible for false positive evaluation of cardiomegaly. If an alveolar infiltrate or pleural effusion is present in adjacent parts of the heart silhouette, it is almost impossible to obtain an accurate results of measurement (Bradley, 2014, Azevedo et al., 2016).

Use of ventrodorsal position is contraindicated in dyspnoic patients. These patients accept well dorsoventral position or horizontal beam technique (Bradley, 2014). The standard width of a healthy heart silhouette in a dog's LL projection occupies 2.5-3.5 inter-rib spaces and occupies approximately 65-70% of the chest (Bradley, 2014).

MATERIAL AND METHOD

Our X-ray study describes a statistical evaluation of VHS measurements in clinically healthy Cavalier breed dogs and Irish Wolfhound breed dogs. The first group consisted of Cavalier n = 8, the second group included Irish Wolfhound n = 7. Radiographs were performed at the laterolateral projection with positionig of the animal on the right and left recumbency collimated onto heart under maximal inspiration. Dogs were not under sedation (Fig.1).

The study was conducted using a GIERTH 200A X-ray machine and indirect digitization of FireCR Plus and FujiFilm Prima T2.

Measured values of VHS were in the range of 9.8-11.2 in the Cavalier and in the Irish Wolfhound in the range 9.0-10.2 (Table 1, 2)

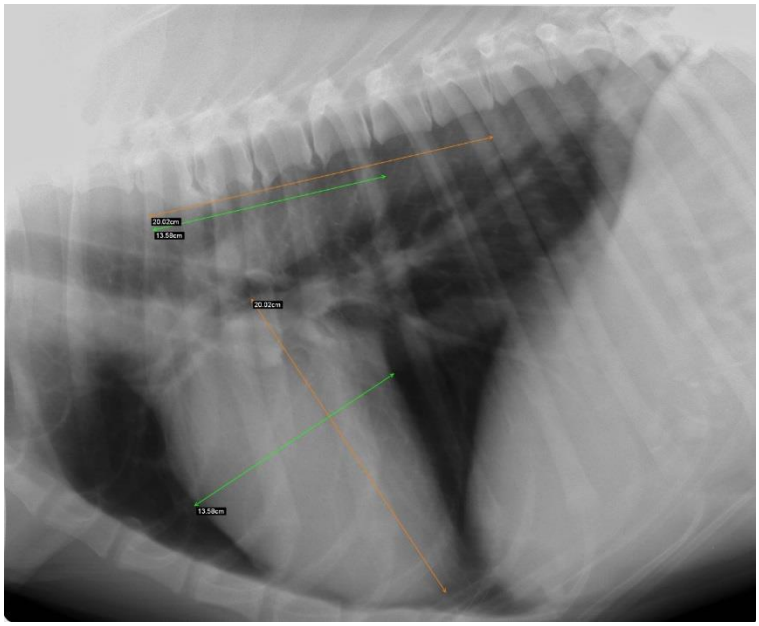
Table 1 Numerical parameters VHS – Cavalier, LL-laterolateral position

Dogs	LL right	LL left
1	10,6	10,4
2	11	10,8
3	11,2	10,8
4	10,8	11
5	9,8	10,8
6	11	11,2
7	10,5	10,1
8	10,8	10,3

Table 2 Numerical parameters VHS – Irish Wolfhound

Dogs	LL right	LL left
1	10	10,2
2	9,9	9,7
3	10,1	9,6
4	9,8	9,1
5	9,6	9,0
6	9,8	9,6
7	10,1	9,2

Fig.1 Laterolateral position – VHS – Irish Wolfhund



DISCUSSION

The VHS values measured based on laterolateral positions of the left and right side were statistically non significant (P >0.05). Bodh et al. (2016) described VHS in Labrador Retriever, Indian Spitz and Mongrel breed dogs. They have found statistical significance between the right and left LL position.

The Azavedo et al. (2016) described and compared this method with the CTR(Cardiothoracic Ratio) method in healthy poodle dog. They confirmed statistical significance in VHS

measurements between clinically healthy patients and with cardiovascular problem. Also, Lamb et al. (2000) confirm that a VHS value of greater than 10.7 indicates mild symptoms of heart disease with respect to their variability.

Buchana, Bucheler (1995) and Gugjoo et al. (2013) report that the chest-width-to-chest ratio does not have a significant effect on VHS, but thoracic vertebral malformations are directly related to higher VHS values for the Bulldog and Boston Terrier.

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REFERENCES

Azevedo, G.M., Pessoa G.T., Moura L.S. (2016): Comparative Study of the Vertebral Heart Scale (VHS) and the Cardiothoracic Ratio (CTR) in Healthy Poodle Breed dogs In Acta Scientiae Veterinariae 44, ISSN 1679-9216, 1-7.

Bavegems, V., Van Caelenberg A., Duchateau L., Sys S.U., Van Bree H., De Rick A. (2005): Vertebral heart size ranges specific for whippets In Veterinary Radiology & Ultrasound, 46(5), ISSN 1740-8261, 400-403.

Bodh, D., Hoque M., Saxena, A.Ch., Gugjoo, M.B., Bist, D., Chaudhary, J.K. (2016): Vertebral scale system to measure heart size in thoracic radiographs of Indian Spitz, Labrador retriever and Mongrel dogs. In Veterinary World, ISSN 2231-0910, 9 (4), 371-376.

Bradley, K. (2013): Radiology of the thorax. In Holloway, A., Mcconnell, F. BSAVA Manual of Canine and Feline Radiography and Radiology: A Foundation Manual, United Kingdom : Wiley, ISBN 1905319444, 109-122.

Buchanan, J.W., Bucheler, J. (1995): Vertebral scale system to measure canine heart size in radiographs. In Jurnal of the American Veterinary Medical Association, 206, (2), ISSN 1544-8412, 194-199.

Cardoso, M.J.L., Caludino, J.L., Melussi, A. (2011): Measurement of heart size by VHS method in healthy American Pitbull Terrier In Ciencia Rural, 41(1), ISSN 0103-8478, 127-131.

Gugjoo, M.B., Hoque, M., Saxena, A.Ch., Zama, M.M.S, Amarpal. (2013): Vertebral Heart Score in Dogs in thoracic radiographs. Advances in Animal and Veterinary Sciences, 1 (1), ISSN 2307 – 8316, 1 – 4.

Jepsen-Grant, K., Pollard, R.E., Johnson, L.R. (2013): Vertebral heart scores in eight dog breeds. Veterinary Radiology & Ultrasound, 54(1), ISSN 1740-8261, 3-8.

Kreatschmer, S., Ludwig, K., Meneses, F., Nolte, I. Simon, D. (2008): Vertebral heart scale in the Beagle dog. Journal of Small Animal Practice, 49(5), ISSN 1748-5827, 240-243.

Lamb, C.R., Wikeley, H., Boswood, A., Skelly, B.J., Cain, M. (2001): Use of breed-specific ranges for the vertebral heart scale as an aid to the diagnosis of cardiac disease in dogs In Veterinary Record 148(23), ISSN 1143-0680, 707-711.

Pinto, A.C.B., Iwasaki, M. (2004): Radiographic evaluation of the cardiac silhouette in clinically normal Poodles through the vertebral heat size (VHS) method In Brazilian Journal of Veterinary Research and Animal Science 41(4), ISSN 1678-4456, 261-267.

FEED ADDITIVES AFFECTS THE NON-RUMINATS PERFORMACE: A REVIEW

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ABSTRACT

The aim of this paper was to analyse the effect of selected feed additives in non-ruminants performance on the base of selected literature review. Feed additives are very important part in modern animal nutrition and feeding. There are many categories of feed additives, which should by apply in the diet of animals. The most analysed additives, mainly in poultry nutrition are probiotics, as active lactic acid bacteria, important microbes in gastrointestinal tract of animals. They can stimulate animal growth, increase feed intake, and have a positive effect on feed conversion ratio. Prebiotics are sources of nutrients and energy for probiotics, for host animal, they are undigestible. Active units of prebiotics are mainly fructooligosaccharides and mannan oligosaccharides. Combination of pre and probiotics are synbiotics as an additive with synergistic effect of both active units. Modern synbiotics are combining with another active units. Relatively young additives in animal nutrition are phytogenerics. Active units of these additives are aromatic herbs and spices. They stimulate feed intake and digestion processes in animal organism. Phytogenerics contains different chemical substances with fungicidal, virocidal and bactericidal effects. Additionally, they contains vitamins and enzymes important for nutrients digestibility.

Keywords: nutrition; supplements; livestock; nutrients; utilization

INTRODUCTION

Important part in modern animal nutrition and feeding are feed additives as a key factor to increase the animal performance and health. There are many functional groups of feed additives. However group of feed additives which can be applied by farmer are zootechnical

additives. This additives have a beneficial effect on feed nutrients utilization, as well as on gastrointestinal tract microbiota balance. From the practical point of view, in this group of additives are probiotics, prebiotics, synbiotics, phytogenerics, feed enzymes and acidifiers (Gálik et al, 2013). The term probiotics was first used by Lilly and Stillwell (1965), but the positive effect of probiotics on animal metabolism and health was probably suggested in 1906 by Mechnikov (Markowiak, Slizewska, 2018). Prebiotics are sources of nutrients for probiotic microbes, as naturally occur microbes in gastrointestinal tract of animals. Relatively new group of additives are phytogenics, with active aromatic plants and herbs. Feed enzymes are additives mainly for poultry nutrition as a solution of some cereals using in total feed mixtures (Gálik et al., 2018). The aim of this study was to analysed the effect of feed additives on non-ruminants performance on the base of references.

THE EFFECT OF FEED ADDITIVES ON NON-RUMINATS PERFORMANCE

Probiotics are the oldest feed additives in animal nutrition (except of antibiotic growth promoters). Those feed additives are typical with active lactic acid bacteria units with the beneficial effect on the intestine mucosa health and animal production stimulation (Gálik, 2012). The major effect of probiotics in animal organism is increasing the population of beneficial microorganisms including lactobacilli and bifidobacteria, and in this way inhibition of growth of harmful microorganisms by substances producing (FAO, 2016). Many experiments and reports with probiotics using were done with poultry. There are many published papers with the unclear results, means, that the effect of probiotics was insignificant and/or negative (f.e. Owings et al., 1990 or Quadros et al., 2001). However, probiotics can stimulate animal performance (Saleh et al., 2017). As table 1 shown, sometimes, after the probiotics application into the diet, the results should be not so fine. In experiment published Pelicano et al. (2003) different probiotics and different technologies of probiotics supplementation were used.

Table 1 The effect of probiotics supplementation on meat quality of Cobb broilers

	Yield (%)					
	Carcass	**Legs**	**Breast**	**Back**	**Wings**	**Fat**
	Probiotic in the diet					
Probiotic A	72.49	34.23	29.36	23.42	11.23	1.76
Probiotic B	70.77	34.16	29.01	23.82	11.35	1.66
Probiotic C	72.10	33.73	28.91	24.33	11.41	1.62
	Probiotic in drinking water					
No probiotic	71.65	34.29	29.06	23.70	11.27	1.68
Probiotic D	71.92	33.80	29.13	24.02	11.39	1.66

A: *Bacillus substilis*, 10^{10} CFU.g^{-1} of product, B: *Bacillus substilis*, $1.6x10^9$ CFU.g^{-1} and *Bacillus licheniformis*, $1.6x10^9$ CFU.g^{-1} of product, C: *Saccharomyces cerevisiae*, $8x10^9$ CFU.$g^{-1}$of product, D: *Lactobacillus reuteri*, $6.6x10^9$ CFU.g^{-1} and *Lactobacillus johnsoni*, $3.3x10^9$ CFU.g^{-1} of product into drinking water.

Pelicano et al. (2003).

As a Table 1 shown, authors find negative effect of different probiotics added into feed mixtures on carcass yield, as well as breast yield. However, there is a positive tendency on different probiotics on back yield and fat content. Pelicano et al. (2003) found a positive effect of probiotics on broiler meat production after the addition into drinking water. There are many published papers and guidelines, that addition of probiotics into drinking water is more effective from the results stability point of view. In another experiment, Saleh et al. (2017) focused the supplementation of probiotics into feed mixture as an effect on eggs production and quality. These authors found positive effect on feed conversion ratio, what means, that after probiotics supplementation, laying hens were able to better nutrients from the feed utilize. Also, these authors found a positive effect on egg weight (higher weight in average in group with probiotics) and cholesterol content in yolk (lower content in average in group with probiotics).

Table 2 The effect of probiotics supplementation on eggs production

	Group	
	Control	**Experimental**
Initial body weight (g)	1695	1797
Body weight gain (g/30 day)	103	73
Feed intake (g/kg)	152	141
Total egg weight (g)	6523	6547
Feed conversion ratio (kg of feed consumed/kg of egg produced)	2.95	2.79

Experimental: added *Lactobacillus paracase*i (KKP 824), *L. rhamnosus* (KKP 825) and. *L. rhamnosus* (KKP 826).

Saleh et al. (2017)

Another group of biological additives are prebiotics, undigestible ingredients for host animal, sources of nutrients and energy for selected microbes in gastrointestinal tract. These additives are also very effective growth promoters. As results of Bozkurt et al. (2009) in table 3 shown, after prebiotics supplementation, in broilers fattening, better feed intake and feed conversion ratio should be finding. The mode of the prebiotics action in animal organism is selective effect on beneficial microorganisms, like lactic acid bacteria, and in this way, stimulation of their activity. As Xu et al. (2003) reported, adequate intake of prebiotics by animals had a positive effect on the daily growth of animals, and on the growth of *Bididobacterium* and *Lactobacillus* bacteria, with a simultaneous inhibition of growth of *Escherichia coli* in chickens gastrointestinal tract (Markowiak and Śliżewska, 2018).

Table 3 The effect of prebiotics supplementation on male broilers production parameters

	Group	
	Control	**Experimental**
Body weight 22-42 d (g)	1776	1772
Feed intake 0-42 d (g)	4878	5074
Fede conversion ratio 0-42 d (g feed/g gain)	1.95	1.94

Experimental: added *mannan oligosaccharides*

Bozkurt et al. (2009)

However, some papers reported, that the effect of prebiotics can be insignificant, as well as any differences with and/or without prebiotics supplementation (Júskiewicz et al., 2006). Additionally, some study shows that excessively high prebiotic dose may have a negative impact on the gastrointestinal system and delay the process of growth of animals (Baurhoo et al., 2007). Another group of feed additives are synbiotics, historically as a combination of probiotics and prebiotics. Synbiotics means synergy of both active units (Cencic, Chingwaru, 2010). Relatively new group of feed additives in animal nutrition are phytogenerics (Gálik et al., 2018). Phytogenerics or phytogenic feed additives have a beneficial effect on nutrients utilization in animals (Gálik et al., 2015); additionally they are physiological and stimulate animal apetite. The mode of action of phytogenics is in active units, tannins, polyphenols and another different chemicals contains in different herbs and aromatic plants (Gálik et al., 2011). Interesting feed additives for animal nutrition are by-products from grape processing. Mainly grape pomace contains some specific and biological active substances with beneficial effect on animal metabolism (Gálik et al., 2019).

CONCLUSION

Feed additives are very important substances in non-ruminants nutrition. Except traditional additives like probiotics and prebiotics, synbiotics and phytogenerics are used for the non-ruminants performance and production quality improving. In adequate levels, effective combination and technology, these additives can help for growth stimulation, better nutrients utilization, higher content of some nutrients in meat or eggs products.

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REFERENCES

Baurhoo B., Letellier A., Zhao X., Ruiz-Feria C.A. (2007): Cecal populations of lactobacilli and bifidobacteria and Escherichia coli challenge in birds fed diets with purified lignin or mannanoligosacharides. Poultry Science, 86, 2509-2516.

Bozkurt M., Kucukyilmaz K., Cath A.U., Cmar M. (2009): The effect of single of combined dietary supplementation of prebiotics, organic acid and probiotics on performance and slaughter characteristics of broilers. South African Journal of Animal Science, 39, 197-205.

Cencic A., Chingwaru W. (2010): The role of functional foods, nutraceuticals, and food supplements in intestinal health. Nutrients, 2, 611-625.

FAO. 2016. Probiotics in Animal Nutrition: Production, impact and regulation by Yadav, S. et al. Rome: Food and Agriculture Organization of the United Nations, 89 p.

Gálik B., Bíro D., Halo M., Juráček M., Šimko M., Vaščáková V., Rolinec M. (2011): The effect of phytoadditives on macroelement digestibility of sport horses. Journal of Central European Agriculture, 12, 390-397.

Gálik B. (2012): The effect of phytogenic feed additives on nutrients utilization in non-ruminants: Assoc. prof. Thesis. Nitra, Slovak University of Agriculture, 109 p.

Gálik B., Arpášová H., Bíro D., Rolinec M., Šimko M., Juráček M., Novotná I. (2013): The effect of dietary Rhus coriaria L. supplementation on fatty acids composition in the table eggs. Acta fytotechnica et zootechnica, 16, 49-52.

Gálik B., Bíro D., Halo M., Rolinec M., Šimko M., Juráček M., Herkeľ R. (2015): The application impact of an origanum, anise and citrus oils blend in horses nutrition. Acta fytotechnica et zootechnica, 18, 103-105.

Gálik B., Bíro D., Šimko M., Juráček M., Rolinec M., Hanušovský O. (2018): Feeds in ruminants and non-ruminants nutrition. Slovak University of Agriculture, Nitra, Slovakia (in Slovak)

Gálik B., Kolláthová R., Rolinec M., Juráček M., Šimko M., Hanušovský O., Bíro D., Vašeková P., Kolesárová A., Barantal S. (2019): Grape by-products as bioactive substances in animal nutrition: a review. Agriculture and Food, 7, 167-172.

Júskiewicz J., Jankowski J., Zduńczyk K., Mikulski D. (2006): Performance and gastrointestinal tract metabolism of turkeys fed diets with different contents of fructoologisaccharides. Poultry Science, 85, 886-891.

Lilly, D.M., Stillwell, R.H. 1965. Probiotics: growth-promoting factors produced by micro-organisms. Science, 147, 747-748

Markowiak P., Śliżenska K. (2018): The role of probiotics, prebiotics and synbiotics in animal nutrition. Gut Pathogens, 10, 1-20.

Owings W.J., Reynolds D.L., Hasiak R.J., Ferket P.R. (1990): Influence of a dietary supplementation with Streptococcus faecium M-74 on broiler body weight, feed conversion, carcass characteristics and intestinal microbial colonization. Poultry Science, 69, 1257-1264.

Pelicano E.R.L., Souza P.A., Souza H.B.A., Oba A., Norkus E.A., Kondawara L.M., Lima T.M.A. (2003): Effect of different probiotics on broiler carcass and meat quality. Brazilian Journal of Poultry Science, 5, 207-214.

Saleh A.A., Gálik B., Arpášová H., Capcarová M., Kalafová A., Šimko M., Juráček M., Rolinec M., Bíro D., Abudabos A.M. (2017): Synergistic effect of feeding *Aspergillus awamori* and lactic acid bacteria on performance, egg traits, egg yolk cholesterol and fatty acid profile in laying hens. Italian Journal of Animal Science, 16, 132-139.

Quadros A.R.B., Kiefer C., Ribeiro N.L.C., Zink L.A. (2001): Caracteristicas qualitativas de carne de suinos alimentados com racoes contend ou nao probioticos. In: Anais da 38th Reuniao Annual da Sociedade Brasileira de Zootecnia, Piracicaba, Brasil, 794-795.

Xu Z.R., Hu Ch., Xia M.S. et al. (2003): Effect of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poultry Science, 82, 1030-1036.

SELECTION OF MAIZE HYBRIDS WITH THE USE OF HARVESTLAB ON JOHN DEERE FORAGE HARVESTER

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ABSTRACT

Selection of maize hybrids according nutritive value is very important factor for production of milk. High variability of maize hybrid according genetic potential is very wide and it is very high variability (40 to 70 %) of digestibility of NDF (DNDF) and that have very high effect for determination of NEL (Nett Energy of Lactation) of maize silage.

Keywords: DNDF, Nutritive value, potencial milk production, software

INTRODUCTION

Selection of maize hybrids according nutritive value is very important factor for production of milk. High variability of maize hybrid according genetic potential is very wide. According Oba and Allen (1999) is very high variability (40 to 70 %) of digestibility of NDF (DNDF) and that have very high effect for determination of NEL (Nett Energy of Lactation) of maize silage. Concentration of energy and level of DNDF have very high effect on intake of dry matter (DM) and potential production of milk. In the last time during harvesting of maize for silage was very popular to use NIR technology, which determines nutritive value of chopped maize. According content of nutritive value determinate by HarvestLab and determination of DNDF for different maize hybrid we evaluate special software for calculation NEL and production of milk per ha and kg of milk per 1 t of DM maize hybrids. New software is a good tool for the selection of maize hybrids for production of maize silage which is the main foodstuff for high-yield cows.

MATERIALS AND METHODS

On the spring 2017 we have planted 9 different maize hybrids (FAO 280 to FAO 290) at

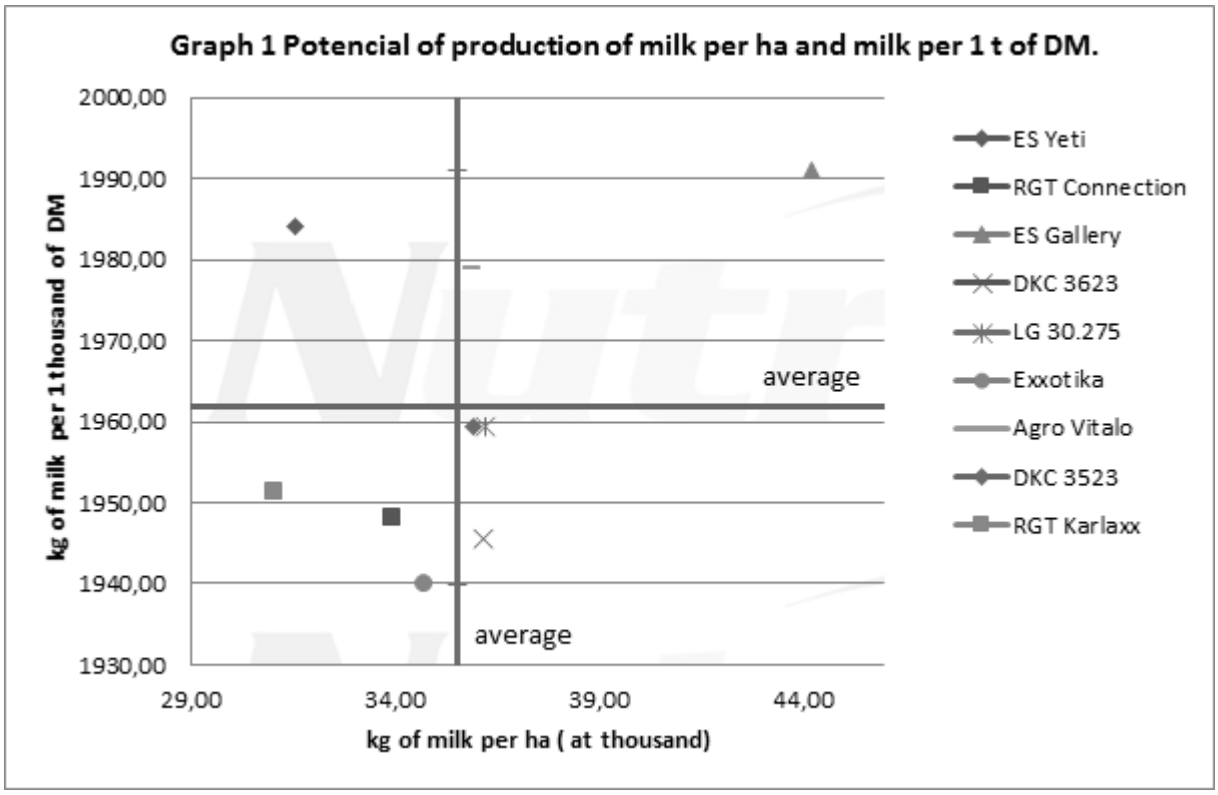
agriculture farm named Agrospol a.s. Knínice u Boskovic for plots. All hybrids we have harvested at the same day 15.9.2017 by harvestor John Deere with HarvestLab device. HarvestLab is NIR sensor developed by the John Deere Co. in conjunction with Carl Zeiss AG Co. already before more than 10 flights. John Deere developer verify the technology AutoLOC. AutoLOC system automatically adjusts longitude chopped forage depending on DM plus according to preset parameters attendance. John Deere its HarvestLab revamped about possibility scan of parameters like DM, ADF, NDF, Starch, Crude Protein (CP). HarvestLab with measurement frequency 17x per second will ensure so lump of dates that the designating high accurate average appreciate given to materials. The dates consumer from field chopper John Deere will relay either reinforcing USB flash disk or are automatically shipped on portal MyJohnDeere.com. For the calculation of concentration of NEL is important parameter of digestibility of fibre, resp. NDF. During harvesting is important to take 3 times of samples of each hybrid a determinate at laboratory for chemical analyses (AOAC, 2005) and DNDF by *in sacco* method (Ørskov and McDonald, 1979). For determination of nutritive value each maize hybrid was developed by new software which calculate all parameters which we need for the calculation of NEL. Software was prepared for HarvestLab as a new tool, which will to calculate ascertained nutritive funds chopped maize hybrids.

RESULTS

We added yield of green matter of chopped maize hybrids in tone per hectare, content of DM and nutritive value we determined by the new program and we calculated nutritive value for 9 maize hybrids and prepare table 1 and graph 1 where you can see order all of maize hybrids according their quality and yields. According results which you can see at graph 1 we can say that above - average values for milk yield per ha and milk per 1 t of DM was for 2 hybrids, e.g. ES Gallery and AgroVitalo. According the results which we take by HarvestLab we can advise both hybrids for next year to produce maize silage. For next year we advise two best hybrids and for next selection we advise to choose from offer next seeds companies next 4 or 6 hybrids.

Table1 The basic quality indicators of the tested hybrids

Hybrid	DM	DM yield	ADF	NDF	Starch	DNDF	NEL	Methane	Milk Production	
	%	t/ha	% DM	% DM	% DM	% DM	MJ/kg	l/kg DM	kg/ha in thousand	kg/t DM
ES Yeti	30.0	15.9	21.9	32.8	33.0	48.7	6.29	341	31.6	1984
RGT Connection	28.4	17.4	24.9	38.9	33.1	47.6	6.18	352	34.0	1948
ES Gallery	35.6	22.2	22.1	34.4	29.2	48.0	6.31	336	44.2	1991
DKC 3623	28.8	18.6	24.8	38.7	33.6	47.5	6.17	352	36.2	1945
LG 30.275	29.0	18.5	24.1	37.3	33.4	48.7	6.21	349	36.2	1959
Exxotika	29.9	17.9	24.3	38.0	32.5	45.8	6.15	348	34.8	1939
Agro Vitalo	35.3	18.2	22.0	33.9	30.7	46.8	6.27	336	35.9	1979
DKC 3523	30.9	18.3	23.6	36.7	33.3	47.0	6.21	346	35.9	1959
RGT Karlaxx	28.9	15.9	24.6	39.2	32.9	47.9	6.19	351	31.1	1951



CONCLUSIONS

According the yield of tested maize biomass, nutritive value determined by HarvestLab, and determinate DNDF, we can calculate at the new program other indicators of nutritive value quality and potential of milk production per ha and per 1 tone of DM. According results we can choose 2 of the best maize hybrids (Graph 1) for our conditions and production of maize silage and production of milk.

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References

AOAC (2005) Official Methods of Analysis. 18th Ed. AOAC International, USA.

Oba M. & Allen M.S. (1999) Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. Journal of Dairy Science, 82, 3, 589-596.

Ørskov E.R. & McDonald I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science, 92, 499-503.

THE CONTENT OF TESTOSTERONE IN DOGS BLOOD IN RELATION TO BODY WEIGHT

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ABSTRACT

The aim of this study was to evaluate testosterone levels in peripheral blood in dogs before and after stimulation of testosterone production. Another objective was to evaluate the relationship of the body weight on the testis morphometric parameters. Fifty male dogs were divided into 4 groups by weight. Testosterone levels were measured before and after a stimulation test using synthetic gonadorelin (Fertagyl ®, 10 μg / kg bw). The results revealed a positive correlation between the body weight and all observed morphometric parameters of the testes ($p < 0.001$) and a positive correlation between testosterone concentration before and after GnRH in peripheral blood in all groups ($p < 0.05$).

Keywords: testimetry; testosterone; dog; stimulation test

INTRODUCTION

Testosterone (TST) is a male sex hormone that is produced by Leydig testicle cells. It is regulated by inhibin via the hypothalamus, in which it inhibits gonadotropin releasing hormone (GnRH). Its production is stimulated through GnRH, a luteinizing hormone from the pituitary gland, which acts on the testicle Leydig cells, with subsequent production of testosterone. It plays an important role during spermatogenesis, maintains libido sexualis, it is essential for manifestations of sexual dimorphism, affects prostate growth and function. Testosterone is an essential hormone in the process of descent of the testes to the scrotum and

its lack of action leads to cryptorchidism. Due to the pulsative nature of TST secretion during the day, it is recommended to measure and evaluate its content after the GnRH stimulation test. In healthy male patients, TST release occurs in 60 minutes after the stimulation with GnRH. This test is also used to confirm the presence of the testicle in abdominal cryptorchids, where an increase in testosterone content in the second sample clearly confirms its presence (Hajurka, 2014). Basal serum TST is 1.82 ± 0.87 ng/ml (De Souza et al., 2015). The advantage of determining the TST concentration in peripheral blood is the differential diagnosis between intact, castrated and cryptorchid individuals.

MATERIAL AND METHODS

The study included 50 dogs of different body weight and age. The dogs were presented to the Small animal clinic for elective castration. All the dogs were privately owned, and the owners consent was obtained before the collection of samples. The study was conducted according to the regulations of the local Institutional Animal Care and Use Committee. The dogs were divided according to their body weight into 4 different groups (under 5 kg, >5,1-10 kg, >10,1-20 kg and over 20 kg). The dogs present in this study were all examined clinically and andrologically before the study.

Testimetry: Testicular size was evaluated after castration by testimetry. For each testicle, we measured its weight in grams, its height, width, and depth in millimeters. For the testimetric examination we used a digital precision caliper (PMS 150 with an accuracy of 0.01 mm) and the weight was determined on a digital laboratory precision scale (KERN 572-37 with an accuracy of 0.01 g).

Determination of testosterone: The testosterone concentration in the blood serum of dogs was observed before the stimulation and 60 minutes after stimulation with exogenous synthetic GnRH, which was administered intravenously via the cephalic vein. Gonadoreline was used for the stimulation (Fertagyl, Intervet/Schering-Plough Animal Health, Boxmeer, the Netherlands) in a dose of 10 μg/kg B.W. Blood samples from *v. cephalica antebrachii* were placed to the test tubes with aglutinative gel, the blood was then centrifuged and the blood serum was stored at -24ºC until the analysis. Testosterone value was quantitatively determined in a specialized RIA laboratory using a RIA Testosterone commercial kit (Immunotech, Beckman Coulter Ltd., Prague, Czech Republic).

Statistical Analysis: The values obtained were processed and evaluated using GraphPad Prism version 6.01 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. In the statistical analysis, basic data processing methods were used,

complemented with the Student's T-test at the significance level of P <0.05 (compared to individuals) and the non-parametric Mann Whitney test at the significance level of P <0.05.

RESULTS AND DISCUSSION

The measured values were expressed as mean ± SD (Table 1). The results revealed a positive correlation between weight and all observed testicular morphometric parameters (p <0.001).

Table 1 Mean ± standard deviation for testimetric values measured in both testes in dogs by weight category

	weight (g) mean ± SD	**CI 95%**	**width (mm) mean ± SD**	**CI 95%**	**height (mm) mean ± SD**	**CI 95%**	**depth (mm) mean ± SD**	**CI 95%**
up to 5 kg	2,8 ± 0,7	2,4 - 3,2	15,0 ± 1,5	11,2 - 15,8	21,1 ± 2,1	20,0 - 22,2	16,4 ± 1,7	15,5 - 17,3
up to 10 kg	5,3 ± 1,5	4,5 - 6,1	17,7 ± 1,8	16,8 - 18,7	27,2 ± 3,5	25,3 - 29,0	20,2 ± 2,2	19,0 - 21,3
up to 20 kg	8,1 ± 2,6	6,6 - 9,6	20,5 ± 2,2	19,3 - 21,8	31,4 ± 2,9	29,7 - 33,1	23,3 ± 2,2	22,0 - 24,6
over 20,01 kg	20,3 ± 6,8	16,6 - 23,9	28,8 ± 4,0	26,6 - 30,9	42,4 ± 5,7	39,3 - 45,4	33,3 ± 4,4	31,0 - 35,6

mean – average value, SD - standard deviation, CI - confidence interval

The increase of TST in peripheral blood after the stimulation test varried between the test groups. In all weight and age groups, we found a correlation between testosterone concentrations before and after GnRH administration in peripheral blood (p <0.05) (Table 2).

Table 2 Range of testosterone concentration in peripheral blood in different weight categories before and after the stimulation test

	Testosterone concentration (ng/ml) before ST mean ± SD	**CI 95%**	**Testosterone concentration (ng/ml) after ST mean ± SD**	**CI 95%**	***p***
up to 5 kg	2,1 ± 1,7	0,7 - 3,6	4,1 ± 2,3	2,2 - 6,0	*
up to 10 kg	2,3 ± 1,6	1,0 - 3,6	2,9 ± 1,6	1,6 - 4,2	*
up to 20 kg	1,6 ± 1,4	0,3 - 2,8	3,0 ± 2,0	1,2 - 4,9	*
over 20,01 kg	4,1 ± 3,6	1,1 - 7,1	5,7 ± 4,3	2,2 - 9,3	**

mean – average value, SD - standard deviation, CI - confidence interval, ST - stimulation test, p - statistical significance between average testosterone concentration in peripheral blood before and after stimulation test in each weight group, asterisk (*) indicates the level of statistics significance, $p < 0.05$ * $p < 0.01$ **

The TST concentration increased after the stimulation test from an average of 2.52 ± 2.07 ng/ml to an average of 3.93 ± 2.55 ng/ml. The peripheral blood TST values measured before the stimulation test are very similar to the reference values for dogs published by Feldman and Nelson (2004). According to these authors, the testosterone reference range is 0.4 – 10 ng/ml. In cryptorchid dogs, the concentration of TST in the peripheral blood is lower (0.09 - 1.99 ng/ml) and in castrated dogs it is less than 0.03 ng/ml (Feldman et al., 2004). Ortega - Pacheco et al. (2006) also confirm our results as their study showed an increase in testosterone blood concentrations one hour after intravenous GnRH administration from the original value of 1.21 ± 1.01 to 2.91 ± 1.1 ng/ml.

CONCLUSION

By evaluating individual testimetric parameters in healthy dogs divided into weight categories, we have obtained values that can serve as reference values for the assessment of testicular morphometric parameters in various pathological conditions present on dog testes. These results can also be used as a basis for a comprehensive assessment of the reproductive health of a dog. Monitoring of testimetric parameters is part of a complex andrological examination. Fully developed and formed testes are a prerequisite for properly functioning spermatogenesis and high male fertility. A direct correlation was found between testicular weight and sperm concentration in various animal species.

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REFERENCES

Caldeira B.C., De Paula T.A.R., Da Matta L.P., Balarini M.K., Campos P.K.A. (2010): Morphometry of testis and seminiferous tubules of the adult crab-eating fox (*Cerdocyon thous, Linnaeus*, 1766). Revista Ceres, 57, 569-575.

De Souza M.B., England G.C.W., Filho A.C.M., Ackermann C.L., Sousa C.V.S., DeCarvalho G.G., Silva H.V.R., Pinto J.N., Linhares J.C.S., Oba E., DaSilva L.D.M. (2015): Semen quality, testicular B-mode and Doppler ultrasound, and serum testosterone concentrations in dogs with established infertility. Theriogenology, 84, 805-810.

Feldman E.C., Nelson R.W. (2004): In Canine and feline endocrinology and reproduction. 3rd edition. St. Luis: WB Saunders, An Imprint of Elsevier., 943-963.

Hajurka J. (2014): Endokrinná kontrola reprodukcie. In: Hajurka J. a kol., Reprodukcia psov a mačiek. Košice: UVLF (in Slovak), 28-33.

Ortega- Pacheco A., Rodríguez-Buenfil J.C., Segura-Correa J.C., Jiménez-Coello M., Linde Forsberg C. (2006): Pathological Conditions of the Reproductive Organs of Male Stray Dogs in the Tropics: Prevalence, Risk Factors, Morphological Findings and Testosterone Concentrations. Reproduction in Domestic Animals, 41, 429-437.

MAIZE SILAGE MINERAL PROFILE CHARACTERISTICS AFTER UREA ADDITION

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ABSTRACT

In the experiment, maize (stay green hybrid FAO 480) was ensilaged in two variants. In control variant C (n = 3), silage matter without the addition of additive and in variant U (n = 3) with the addition of urea at a dose of 5 kg/t was ensilaged. The silage matter was stored in plastic silage units and after 3 months were samples taken for chemical analysis. The content of macroelements (Ca, P, Mg, Na, K) and microelements (Cu, Fe, Mn, Zn) was determined in maize silages. The results confirmed that urea addition influenced the macroelement profile of maize silages with statistically lower P, Mg and Na content. After application of urea statistically and significantly higher Cu, Fe and Zn content from microelement profile was found compared to control variant. The results confirmed, that the addition of urea influences the mineral profile of maize silages.

Keywords: macroelements; microelements; silage; maize; additive; urea

INTRODUCTION

Maize silage is an important component of feed rations, especially ruminants (Mlyneková, 2010; Rajčáková et al., 2012; Bíro et al., 2014). It is a typical feed with saccharide character that provides energy mainly in the form of starch and structural carbohydrates (Vršková and Bencová, 2011; Gálik et al., 2018). Maize silage is a feed with a low mineral content (Bíro et al., 2014). The addition of additives is one of the factors affecting the mineral content of maize silages (Juráček et al., 2018). The urea as a chemical additive affects the fermentation process, increases the quality and nutritional value of silage (a source of non-protein nitrogen) (Neumann et al., 2010; Rodriques et al., 2015; Doležal et al. 2017; Kang et al., 2018) and

reduces dry matter losses (Vieira et al., 2017). The aim of the work was to determine the effect of urea addition on the mineral profile of maize silage.

MATERIAL AND METHODS

In the experiment matter of maize in cooperation with University farm Kolíňany, dairy farm Oponice was ensilaged. Stay green hybrid maize (FAO 480) was harvested using a self-propelled chopper and cut to a theoretical chop length of 10 mm and then processed in two variants. In control variant C (n = 3), was ensilaged without the addition of additive and in variant U (n = 3) with the addition of urea at a dose of 5 kg/t. The silage matter was stored in plastic silage units and sealed. The silages were stored in the Laboratory of feed conservation and after 3 months, average samples were taken for analysis. In maize silages, the dry matter content (DM) and the mineral content were determined: macroelements (Ca, P, Mg, Na, K), whose content is given in g/kg of DM, microelement (Cu, Fe, Mn, Zn) content in mg/kg of DM. The dry matter content was determined by drying the sample at a temperature of 103 °C in a POL-EKO APARATURA oven. Silages of variant C had an average dry matter content of 401.32 g/kg and silages of variant U 393.15 g/kg. The mineral content was determined on a ContrAA 700 atomic adsorption spectrophotometer (ANALYTICS), only the P content was determined spectrophotometrically on a Spectrophotometer 6400 JENWAY at a wavelength of 666 nm. Statistical processing of results was performed using SPSS Statistics 20.0 (IBM) (ANOVA-Tukey test) and independent samples of T-test were used.

RESULTS AND DISCUSSION

The differences in Ca content between the variants were nonsignificant (Table 1). Statistically significant lower P content (by 11.1%) in maize silages with urea addition was found ($P<0.05$). The ratio of Ca to P was 1.15:1 for control silages and 1.32:1 for silages with urea. The values of Ca and P in the silages of both variants were lower than those reported by NRC (2001), Vyskočil et al. (2008), Loučka and Mikyska (2013). The maize silages without addive had higher content of Mg by 8.6% and Na by 36.4% ($P<0.05$). The differences in K content between the variants were nonsignificant. In experiment, the lower content of K in comparison to the results of Kung et al. (2008) was found. The ratio of K to Na was 26.1:1 for silages of C variant and 39.3:1 for silages of U variant. The content of Ca, P, Mg and K is in accordance with Feedipedia (2016) in comparable dry matter content. The Na content in both variants was higher compared to maximum value (0.1 g/kg of DM) in Kung et al. (2015) and

(0.2 g/kg of DM) Feedipedia (2016), but comparable to the results of Orosz et al. (2016). In silages with urea addition, a higher content of microelements in comparison with silages without an additive was found. The application of urea significantly ($P<0.05$) influenced the content of Cu, Fe and Zn in maize silages (higher values by 29.1%; 37.8% and by 29.5%). The content of microelements was different in contrary with Petrikovič et al. (2000) with the biggest differences in Fe content. The Fe content of the analyzed maize silages was lower by 54.7% (variant A) and 37.5% (variant B) than the average Fe content in the database of Petrikovič et al (2000) in maize silage with a dry matter content of 294 g/kg. The content of all analyzed microelements was in accordance with the Feedipedia (2016) database in maize silages in comparable dry matter content, but was not correspondent with the results in Skalická et al. (2013).

Table 1 Mineral profile of maize silages

n=3	Variant C				Variant U			
	$\bar{x}$	SD	Min	Max	$\bar{x}$	SD	Min	Max
Ca	2.17	0.096	2.05	2.31	2.22	0.131	2.05	2.34
P	1.89*	0.073	1.78	2.01	1.68*	0.118	1.58	2.34
Mg	1.63*	0.046	1.56	1.66	1.49*	0.070	1.40	1.82
Na	0.33*	0.128	0.22	0.50	0.21*	0.021	0.18	1.58
K	8.62	0.740	7.71	9.60	8.25	0.321	7.81	0.24
Cu	4.84*	0.391	4.33	5.28	6.25*	1.015	5.35	8.57
Fe	104.21*	4.746	98.99	110.09	143.61*	6.378	137.48	151.74
Mn	40.85	2.628	38.08	43.93	40.89	0.831	39.88	41.97
Zn	17.18*	2.286	13.97	19.54	22.25*	0.617	21.53	22.99

Ca, P, Mg, Na, K in g/kg of DM; Cu, Fe, Mn, Zn in mg/kg of DM

CONCLUSION

In general, the highest content from macroelements in the content of K and from microelements in the Fe content in maize silages was determined. Urea addition influenced the macroelement profile of maize silages with statistically lower P, Mg and Na content. The Ca and K content were not affected by treatment. The wider ratio of Ca to P and of K to Na in maize silages with urea addition was determined. After application of urea statistically and significantly higher Cu, Fe and Zn content from microelement profile was found. The Mn

content were not affected by treatment. The results confirmed, that the addition of urea influences the mineral profile of maize silages.

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REFERENCES

Bíro D., Juráček M., Šimko M., Gálik B., Rolinec M. (eds) (2014): Conservation and Adjustment of Feeds. Slovak University of Agriculture, Nitra, Slovak Republic. (in Slovak)

Doležal P., Dvořáček J., Szwedziak K., Tukiendorf M., Polanczyk E., Pavlata L. (2017): Urea as a conservative additive for grain silage. In: 12th Kabrt´s Dietetic Days, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic, 167–170.

Feedipedia (2016): Maize silage. [online], Available from www.feedipedia.org/node/13883 (accessed Oct 7, 2019).

Gálik B., Bíro D., Šimko M., Juráček M., Rolinec M., Hanušovský O. (eds) (2018): Feeds in Ruminant and Non-ruminant Nutrition. Slovak University of Agriculture, Nitra, Slovak Republic. (in Slovak)

Juráček M., Bíro D., Gálik B., Rolinec M. (eds) (2018): Conservation Additives and their Influence on Nutritional and Fermentation Quality of Silages. Slovak University of Agriculture, Nitra, Slovak Republic. (in Slovak)

Kang S., Wanapat M, Nunoi A. (2018): Effect of urea and molasses supplementation on quality of cassava top silage. Journal of Animal and Feed Sciences, 27, 74–80.

Kung L., Moulder B.M., Mulrooney C.M., Teller S.T., Schmidt R.J. (2008): The effect of silage cutting height on the nutritive value of a normal corn silage hybrid compared with brown midrib corn silage fed to lactating cows. Journal of Dairy Science, 91, 1451–1457.

Kung L., Lim J.M., Hudson D.J., Smith J.M., Joerger R.D. (2015): Chemical composition and nutritive value of corn silage harvested in the northeastern United States after tropical storm Irene. Journal of Dairy Science, 98, 2055–2062.

Loučka R., Mikyska F. (2013): Mineral contents of maize silages for ruminants. In: Xth Kabrt´s Dietetic Days, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic, 89–92.

Mlyneková Z. (2010): Comparison of nutritional quality of selected maize hybrids in relation to their use in ruminant nutrition. PhD. Diss. Nitra: Animal Production Research Centre, 70 p.

Neumann M., Oliboni R., Oliveira R.M., Faria M.V., Ueno R.K., Reinerh L.L., Durman T. (2010): Chemical additives used in silage. Pesquisa Aplicada & Agrotecnologia, 3, 197–207.

NRC /National Research Council/ (eds) (2001): Nutrient Requirements of Dairy Cattle. The National Academies Press, Washington, USA.

Orosz S., Simon Á., Szabó J., Podmaniczky T., Davies D.R., Szemethy D., Könyves L. (2016): Mineral content (Ca, P, K, Na, Mg, S, Mn, Zn, Cu) of the most important forages in Hungary (2013-2015). In: Forage Conservation, National Agricultural and Food Centre, Nitra, Slovak Republic, 153–154.

Petrikovič P., Sommer A., Čerešňáková Z., Svetlanská M., Chrenková M., Chrastinová Ľ., Poláčiková M., Bencová E., Dolešová P. (eds) (2000): Nutritive value of feeds 1st part. Research Institute for Animal Production Nitra, Nitra, Slovak Republic. (in Slovak)

Rajčáková L., Mlynár R., Loučka R., Jambor V. (2012): Effect of additives on fermentation process of maize silage with different dry matter content. In: Proceedings of the XVI International Silage Conference, MTT Agrifood Research Finland, University of Helsinki, Helsinki, Finland, 354–355.

Rodrigues P.R., Filho A.D.P., de Faria B.P., Freitas G.S., Freitas R.S., Deminicis B.B. (2015): Effect of additives on the physical and chemical characteristics of sugar cane silage. Ciências Agrárias, 36, 2753–2762.

Skalická M., Maskaľová I., Bujňák, L. (2013): Profile of selected mineral elements in feedingstuffs for dairy cows. In: Xth Kabrt´s Dietetic Days, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic, 45–48.

Vieira D.A., Cezário A.S., Valente T.N.P., Ribeiro J.C., dos Santos W.B.R., Ferreira P.R.N. (2017): Evaluation of the addition of urea or calcium oxide (CaO) on the recovery of dry matter of the by-product of sweet corn silage. Journal of Agricultural Science, 9, 141–148.

Vršková M., Bencová E. (2011): The quality of maize silage in Slovakia. In: IXth Kabrt´s Dietetic Days, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic, 148–153.

Vyskočil I., Zeman L., Kratochvílová P., Večerek M., Vašátková A. (eds) (2008): Pocket Feedstuffs. Mendel University of Agriculture and Forestry in Brno, Brno, Czech Republic. (in Czech)

MONITORING OF SELECTED CLIMA PARAMETERS AND TEMPERATURE OF DRINKING WATER DURING THE WINTER PERIOD IN DAIRY COWS HOUSED IN OLD TYPE OF FREE STALL STABLE

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ABSTRACT

Microclimate conditions and also drinking water influence significantly animals´ welfare, while environmental parameters affect also animal hygiene. The aim of the study was to evaluate the effect of the housing system and chosen bioclimatological indicators (air temperature, relative humidity of the air, THI) on the thermal comfort of dairy cows during the winter period. In addition, also temperature of drinking water was monitored. Our results show that selected microclimatic indicators during the winter period in the old type of stable were within the recommended values determined for dairy cows. Statistically significant differences between the indoor and outdoor environmental conditions were found in the air temperature, the humidity, and the temperature-humidity index ($P < 0.01$). Cubic capacity of the stable was assessed as inadequate. The average temperature of drinking water for dairy cows in winter months (December, January, and February) was 9.8°C.

Keywords: microclimatic conditions, temperature-humidity index, drinking water, cattle

INTRODUCTION

Factors influencing the thermal well-being of the organism play the most important role among the bioclimatic factors, because they can significantly influence thermoregulation and animal health, as well as milk and meat production (Roland et al. 2016, Summer et al. 2019). In addition, in dairy cows, alterations in the environmental conditions may cause changes in their behaviour (e.g. feed and water intake, duration of lying or place of lying, reluctance to

move). While these changes may vary depending on the microclimatic factors, especially in summer or winter period. Parameter of temperature-humidity index (THI) evaluate together influence of air temperature and air relative humidity and nowadays it can be classified as an main indicator for degree of stress on animals caused by weather conditions (Kudělková et al. 2018; Lallo et al. 2018). Many studies use THI in connection with heat stress of farm animals (Brouček et al. 2009; Weng et al. 2018). On the other hand, to monitor the cold stress of cows a modification formula of wind chill temperature (WCT) can be used (Tucker et al. 2007). The temperature of the stable environment in the winter is influenced by the outdoor air temperature, especially in the new types of stables (free stall barns with curtain walls) and low air temperature in the stables affect temperature of drinking water in the troughs.

The aim of our study was to monitor the main microclimatic indicators of the stable environment in the old type of stable for cattle, also in relation to the drinking water temperature.

MATERIALS AND METHODS

Study was performed in the old type of the stable. Stable was designed with lower concrete ceilings and the walls were built of bricks. A high of the longitudinal walls was 3 m and the ceiling was at the same high. A group of dairy cows were housed in the production pen of cubic capacity of 555.4 m^3. Natural and artificial methods of ventilation were used in relation to the climatic conditions. Artificial ventilation for air exchange was used mainly in winter and summer period. Watering equipment in the stable was presented by the water troughs. Size of the troughs was (132 x 22 x 20 cm). The temperature of drinking water in the trough was measured by hand-held MINI conduct meter in the morning (after milking cows). The measurement of selected microclimatic indicators (air temperature, relative humidity of the air) were recorded in six hour intervals by digital datalogger Comet (Comet System s.r.o.). First measurement point was located in the production pen of the free stall stable, while the sensor was placed in the zone occupied by the most productive animals. Second measurement point was located in outside environment on the farm. The following formula was used to calculate THI for dairy cows according Brouček et al. (2006):

$$THI = (0.8 \times Tmax) + (RV\ average / 100) \times (Tmax - 14.4) + 46.4$$

Dairy cows of the Czech Fleckvieh cattle and their crosses with the Red Holstein breed were housed in the pen with individual lying cubicles (n=17). The average milk production of cows

per the norm lactation was 8 700 l of milk. Bedding material was straw, which was padded once a day. Cows were milked 2 times per day.

The statistical analysis was performed with one-way ANOVA in the statistical program STATISTICA CZ. When ANOVA showed significant differences among groups, the HSD post-hoc test was used.

RESULTS AND DISCUSSION

Although ruminants are able to adapt to a relatively wide temperature range, the indoor stable air temperature should not fall too much, especially in the winter period. Temperatures around 0°C can cause low temperature of drinking water and the floor of the walking areas may get icy. This leads to the risk of slipping animals and/or workers. In these cases, the cows show reluctans to move on the slippery floors; standing and walking are difficult (Hulsen 2011). The results of the drinking waters´ temperature recorded in our study are presented in Table 1. Water in the troughs was clear without turbidity and other sensory changes (colour and smell). Although the average temperature was not low (9.8°C), it didn't fall within a recommended range of temperature for drinking water according to Doležal and Staněk (2015) published for the winter time.

Table 1 Drinking waters´ temperature

Temperature of drinking water (°C)	
December	10.7
January	10.6
February	8.2
Mean ± SD	**9.8** ± 1.42

If the temperature of drinking water in the troughs is about to 0°C, the dairy cows show a reduced intake of water of 15 to 20 l a day and during the arctic days the dairy cows prefer to drink water at temperature of 18 to 22°C. The optimal temperature of the drinking water in winter should be between 15 and 18°C, which can be achieved by using heating equipment (Doležal and Černá 2004; Doležal and Staněk 2015).

The average values of selected microclimatic indicators and the THI are presented in Table 2. Kožnarová and Klabzuba (2002) state that a minimum ambient temperature for dairy cows is 2°C, while temperatures between 4 to 10°C are considered to be optimal. Generally, for cattle is given a relatively wide range of the thermoneutral zone, which is dependent on the breed, age, performance or health status of cattle. According to Zejdová et al. (2012) the optimal ambient temperature for cows is between -5 to +20 °C, while Doležal and Staněk (2015) consider a thermoneutral zone for dairy cows between -6 to +16 °C.

Table 2 Microclimatic parameters

	Indoor air temperature (°C)	**Outside air temperature** (°C)	**Significance**
December	10.4	1.1	**
January	10.1	1.8	**
February	9.2	-2.2	**
Mean ± SD	**9.9** ± 0.62	**0.2** ± 2.14	
	Indoor relative humidity of the air (%)	**Outside relative humidity of the air** (%)	**Significance**
December	74.6	97.1	**
January	75.3	99.2	**
February	73.3	86.1	**
Mean ± SD	**74.4** ± 1.01	**94.1** ± 5.51	
	Indoor THI	**Outside THI**	**Significance**
December	50.4	37.4	**
January	50.0	38.1	**
February	48.7	32.7	**
Mean	**49.7**	**36.1**	

THI = temperature-humidity index; SD = standard deviation; ** = $P < 0.01$

Although dairy cows are able to tolerate low temperatures well compared to the other animals, the cold stress negatively affect milk yield due to reduction of nutrients´ availability (Novák and Rožnovský 2008). Although in our study we assessed an old type of stable, there was not high changes of climatic effects compared with a new type of stables constructed in nowadays.

Our results show (Table 2) that between indoor and outdoor microclimatic parameters (air temperature, relative humidity, THI) were found significant differences ($P < 0.01$) due to building construction of the stable. The average indoor air temperature during winter period was in recommended values for cows housed in a free stall stable. As for relative humidity, its outdoor environmental values in our study were high (mean 94.1 %). Contrary, values of RH (%) in indoor environment were in a recommended range. Values of THI have not been affected by an extreme limit, although scientific publications primarily concerned on the effect of heat stress (Zimbelman and Collier 2011). Many older types of cattle stables have low ceilings, when also the cubature of the stable in our study was inadequate with regard to the milk yield of current dairy cows. In high yielding dairy cows (producing above 8000 l of milk per lactation), there would be at least 6 m^3 of available air in housing per each 100 kg of cow´s live body weight (LW). According to Staněk (2019), the recommended cubature for dairy cows with yield over 8500 kg of milk per lactation should be 7.5 m^3/100 kg of LW, while in the future, there will be the expected stable cubic capacity of 8 m^3/100 kg of LW of dairy cow (Kozák 2007).

CONCLUSION

Based on the results of our study it can be stated that the microclimatic conditions in the winter period in the old type of free stall stable corresponded to generally recommended values for the air temperature, relative air humidity and THI. However, there was found slight imperfection in the cubature of housing system used for cows (m^3/100 kg LW of dairy cows) and also temperature of drinking water.

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REFERENCES

Brouček J., Mihina Š., Ryba Š., Tongeľ P., Kišac P., Uhrinčať M., Hanus A. (2006): Effects of high air temperatures on milk efficiency in dairy cows, Journal of Animal Science, 51, 93-101.

Brouček J., Novák P., Vokřálová J., Šoch M., Kišac P., Uhrinčať M. (2009): Effect of high temperature on milk production of cows frm free-stall housing with natural ventilation. Slovak Journal Animal Science, 42, 167-173.

Doležal O., Černá D. (2004): Napájení – napajedla, spotřeba a kvalita vody. Metodické listy. Praha – Uhříněves: Výzkumný ústav živočišné výroby. ISBN 80-86454-52-5.

Doležal O., Staněk S. (2015): Chov dojeného skotu – technologie, technika, management. Praha: Profi Press. ISBN 978-80-86726-70-0

Hulsen J. (2011): Cow signals. Praha: Profi Press. ISBN 978-80-86726-44-1.

Kozák L. (2007): Moderní ustájení dojnic. Náš chov, 67, 66-69.

Kožnarová V., Klabzuba J. (2002): Aplikovaná meteorologie a klimatologie. XI. Díl - Mikroklima stájí. ČZU Praha. ISBN 978-80-213-0870-1.

Kudělková L., Barešová N., Macháček M., Straková E., Suchý P., Šimek V. (2018): Effect of spring period on temperature humidity index in dwarf rabbits husbandry. In: Aktuální otázky bioklimatologie zvířat 2018, Praha – Uhříněves: Výzkumný ústav živočišné výroby, 26-28.

Lallo C.H.O., Cohen J., Rankine D., Taylor M., Cambell J., Stephenson T. (2018): Characterizing heat stress on livestock using the temperature humidity index (THI) – prospects for a warmer Caribbean. Regional Environmental Change, 18, 2329-2340.

Novák P., Rožnovský J. (2008): Vliv klimatických změn na organismus hospodářských zvířat. Náš chov, 68, 60-62.

Roland L., Drillich M., Klein-Jöbstl D., Iwersen M. (2016): Invited review: Influence of climatic conditions on the development, performance, and health of calves. Journal of Dairy Science, 99, 2438-2452.

Staněk S. (2019): Inovace v živočišné výrobě dojený skot – krávy. Praha – Uhříněves: Výzkumný ústav živočišné výroby.

Summer A., Lora I., Formaggioni P., Gottardo F. (2019): Impact of heat stress on milk and meat production. Animal Frontiers, 9, 39-46.

Tucker C.B., Rogers A.R., Verkerk G.A., Kendall P.E., Webster J.R., Matthews L.R. (2007): Effects of shelter and body condition on the behaviour and physiology of dairy cattle in winter. Applied Animal Behaviour Science, 105, 1–13.

Zimbelman R.B., Collier, R.J. (2011): Feeding strategies for high-producing dairy cows during periods of elevated heat and humidity. In: Tri-State Dairy Nutrition Conference, Indiana: Grand Wayne Center, 111-126.

Zejdová P., Chládek G., Falta D. (2012): Vliv stájového prostředí na chování a mléčnou užitkovost dojnic. Mendelova univerzita v Brně.

Weng X., Monteiro A.P.A., Guo J., Li C.,Orellana R.M., Marins T.N., Bernard J.K., Tomlinson D.J., DeFrain J.M., Wohlgemuth S.E., Tao S. (2018): Effects of heat stress and dietary zinc source on performance and mammary epithelial integrity of lactating dairy cows. Journal of Dairy Science, 101, 2617-2630.

IMPROVEMENT OF PRODUCTION AND HEALTH PARAMETERS OF WEANED PIGS AFTER PROBIOTICS ADMINISTRATION

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ABSTRACT

A total of 240 piglets were used in the trial, the pigs were divided into experimental group (n=120) and control group (n=120). Both groups of pigs were allocated to the trial within the same day. Control group was given normal standard diets for pigs in the range from weaning until 98th day of lives without additives. Experimental group was given control feed supplemented with 1000 g of probiotic preparation BioPlus 2B/t feed, which equals 3.2 x 10^9 CFU/kg feed. Preparation BioPlus 2B (Christian Hansen´s, Hørsholm, Denmark) consists of equal proportions of *Bacillus licheniformis* and *Bacillus subtilis* at a concentration of 3.2 x 10^9 in gram of powder.

After administration of BioPlus 2B to weaned pigs it can be concluded that the diarrhoea score in experimental group was lower than that in the control group. Incidence of diarrhoea was 13.3 % in experimental group and 20 % in control group. The piglets in experimental group reached higher average daily gains. The average daily gains in experimental group were approximately in 6 % higher than in control group in the first two weeks of the trial. Pigs in experimental group had lower mortality than those in control group. In experimental group, four weaned pigs died in the first two weeks of the trial, but six pigs died in control group in the same period.

Key words: weaned pigs, probiotics, diarrhoea, average daily gains

INTRODUCTION

The balance of microorganisms in the gut maintains a gentle interaction between its components, is called "eubiosis". On the contrary, "dysbiosis" means detrimental quantitative and qualitative changes in the gut microflora, its local distribution and metabolic activity (Gedek, 1993). Metabolic activity can be more important than numbers of selected bacterial species. Probiotics are able to maintain balance of microorganisms and improve metabolic activity of gastrointestinal microflora.

Salminen et al. (1999) defined probiotics as microbial cell preparations or components of microbial cell, which have positive effect on health and performance of the macroorganism. Chesson (1993) asserts that a probiotic strain should come from the intestinal tract (or tolerate conditions of the intestinal tract), adhere, maintain high vitality after freeze-drying, storing, produce inhibitors against pathogens and stimulate immunity of the macroorganism.

In the past decade the genus *Bacillus*, along with *Lactobacillus*, non-enterotoxic *E. coli*, *Bifidobacterium, Streptococcus* have been used as probiotics.

According to taxonomy studies, the genus *Bacillus* can be divided into 5 or 6 groups (Claus & Berkeley, 1986). In the *Bacillus subtilis* group usually five physiologically similar species are described – *Bacillus amyloliquefaciens, Bacillus atrophalus, Bacillus licheniformis, Bacillus pumilus, Bacillus subtilis.*

Barbosa et al. (2005) described the isolation of 237 presumptive gut-associated *Bacillus* spp, included *B. subtilis, B. pumilus, B. licheniformis, B. clausii* and species of the *B. cereus* group from the intestine. Isolates sporulated efficiently in the laboratory, and the resulting spores were tolerant to simulated gastrointestinal tract conditions. They exhibited also antimicrobial activity against a broad spectrum of bacteria, including food spoilage and pathogenic organisms. Results suggest that some of the sporeformers have the potential to persist in or transiently associate with the complex gut ecosystem and so influence the metabolism of guest.

MATERIAL AND METHOD

The feeding trial with weaned pigs was from weaning (mean age = 28 days; weight approx. 7.5 kg) and the following 10 weeks. All pens were assigned with pigs with the same distribution of genetic background. A total of 240 piglets were used in the trial, the pigs were divided into experimental group (n=120) and control group (n=120). Pigs in every group

were separated into five pens, each pen contained 24 weaned pigs. Both groups of pigs were allocated to the trial within the same day.

Control group was given normal standard diets for pigs without additives and without antibiotics (including omission of zinc and copper at a growth promotion level). Experimental group was given control feed with 1000 g of probiotic preparation Bioplus 2B/t feed which equals 3.2 x 10^9 CFU/kg feed. Preparation BioPlus 2B (Christian Hansen´s, Hørsholm, Denmark) consists of equal proportions of *Bacillus licheniformis* and *Bacillus subtilis* at a concentration of 3.2 x 10^9 in gram of powder. Control feeds were manufactured in different day than experimental diets. Diets were offered as dried mixed feed. Diets were stored in silos, clearly labelled with the diet code and date of manufacture. We observed incidence of diarrhoea, individual monitoring of pigs was done daily during the first two weeks of the trial.

$$\text{Diarrhoea score} = \frac{\text{all partial scores were added per pen for all days of observing}}{\text{days of monitoring}}$$

Scale for diarrhoea was 0 – 3 (0 = no diarrhoea, 1 = slight, 2 = some signs, 3 = acute).

Pigs were weighted on days 28, 42, 56 and 98 of their lives. Average daily gains were calculated by pigs´ weight. Health state of pigs was checked daily, we did evidence of treatment.

RESULTS AND DISCUSSION

The weaned pigs in experimental group reached higher average weight on 42nd day of life, even if they started with lower weight on 28th day of life. The average weight in experimental group was also higher compared with control group at the end of the trial. Although we did not find significant differences, the average daily gains in experimental group were approximately in 6 % higher than in control group in the first two weeks of the trial.

In experimental group, four weaned pigs died in the first two weeks of the trial, but six pigs died in control group in the same period. During the whole period of experiment, 14 pigs died in control group and 12 pigs died in experimental group (Table 1).

Diarrhoea occurred in both groups of pigs, but more often in control group. In experimental group, two weaned pigs suffered from diarrhoea markedly in the first pen, one pig in 2nd pen, four pigs in 3rd pen, four pigs in 4th pen and five in 5th pen.

In control group, five pigs suffered from diarrhoea markedly in 2nd control pen, four pigs in 3rd control pen, six pigs in 4th control pen and nine pigs in 5th control pen.

In general, 16 pigs suffered from diarrhoea in experimental group, from which 4 pigs died in the first two weeks of the trial. In control group, diarrhoea occurred in 24 pigs, from which 6 pigs died within the first two weeks of the trial. All pigs, which suffered from diarrhoea, were treated individually with antibiotics – enroxofloxacine.

Table 1 Weight of pigs, average daily gains (ADG) and mortality of pigs

	28 day of life	42 day of life	56 day of life	98 day of life
Weight – exp. group, kg	7.48±1.29	10.84±0.85	18.76±3.91	44.4±6.05
Weight – control group, kg	7.54±1.17	10.70±2.34	18.32±3.62	43.5±6.14
ADG – exp. group, kg/day		0.240	0.566	0.610
ADG – control, kg/day		0.226	0.544	0.599
% mortality, exp. group		3.33	5.83	0.83
% mortality, control group		5.0	6.66	0

In experimental group 13.3 % pigs suffered from diarrhoea, but 20 % of pigs suffered from diarrhoea in control group. Diarrhoea influenced average daily gains (ADG) of pigs.

Consequently, the ADG in experimental group were higher compared with control pigs (Table 2).

Table 2 Diarrhoea score and ADG of pig pens in the first two weeks of the trial

Pen	Group	Diarrhoea score	ADG between 28-42 days of life
1st pen	Experiment	0.34±0.076	0.249±0.101
2nd pen	Experiment	0.31±0.05	0.254±0.084
3rd pen	Experiment	0.54±0.14	0.241±0.109
4th pen	Experiment	0.49±0.15	0.246±0.125
5th pen	Experiment	0.69±0.29	0.210±0.102
Average	Experiment	0.48±0.16	0.240±0.104
1st pen	Control	0.29±0.13	0.277±0.090
2nd pen	Control	0.60±0.17	0.218±0.119
3rd pen	Control	0.42±0.08	0.255±0.132
4th pen	Control	0.78±0.23	0.191±0.080
5th pen	Control	0.84±0.24	0.189±0.130
Average	Control	0.59±0.23	0.226±0.110

ADG – average daily gains

It was described, that composition of the gut microflora in a healthy adult animal is almost constant (Tannock, 1990). On the other hand, scientific results show that selected probiotics at the concentration of 10^9 – 10^{11} decrease incidence of diarrhoea, and they can even influence metabolism of guest. Probiotic bacteria, even if they are given in large amounts, are rarely colonising permanently the intestinal tract and they are only seldom detected in faeces a few weeks after application (Sanders, 1999).

CONCLUSION

The aim of the experiment was to study the efficacy of probiotics based on *Bacillus spp*. in weaned and growing pigs.

After administration of BioPlus 2B to weaned pigs after weaning at the age 28 days to the age 98 days of life it can be concluded:

- The diarrhoea score in experimental group was lower than that in the control group.

Incidence of diarrhoea was 13.3 % in experimental group and 20 % in control group.

- The piglets in experimental group reached higher average daily gains in the first two weeks of experiment.
- Pigs in experimental group had lower mortality than those in control group.

REFERENCES

Barbosa, T. M., Serra, C. R., La Ragione, R. M., Woodward, M. J., Henriques, A. O., (2005). Screening for Bacillus isolates in the broiler gastrointestinal tract. Appl. Environ. Microbiol., 71, 968 – 978.

Claus, D. & Berkeley, C.W., (1986). Genus *Bacillus*, 1105 – 1139. In: Sneath P. H. A. (ed) Bergey´s manual of systematic bacteriology, vol. 2, Waverly Press, inc., Baltimore, USA.

Gedek, B., (1993). Darmflora – Physiologie und ökologie. Chemother. J. Suppl., 1, 2 – 6.

Chesson, A., (1993). Probiotics and other intestinal mediators. pp. 197 – 214. In: Cole, D. J. A., Wisiman, J., Varley, M. S. (eds) Principles of pig science. Loughbourough, UK, Nottingham University Press.

Salminen, S., Ouwehand, A., Benno, Y., Lee, Y., (1999). Probiotics: How should they be defined? Trends Food Sci. Technol., 10, 107 – 110.

Sanders, M., (1999). Probiotics. Foodtechnology, 53, 67 – 77.

Tannock, G. W., (1990). The microecology of Lactobacilli inhabiting the gastrointestinal tract. Adv. Microb. Ecol., 11, 147 – 171.

THE EFFECT OF PAIRED CALF HOUSING ON PERFORMANCE AND WELFARE

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ABSTRACT

The aim of this study was to evaluate the effect of a paired housing system on the daily activity of dairy calves, their performance and welfare. We analysed the 24-hour cycles of the basic daily activities of 20 calves housed in two different housing systems (individually and in pair) on one farm during the milk nutrition period. Daily behavioural activities of calves were evaluated by nonparametric test (Kruskal-Wallis ANOVA), quantitative parameters (live weight, average daily gain) using the F-test and the paired t-test in the Statistica 9 software package. Based on the results of selected behaviour indicators, it stands to reason that calves housed in pair were more active (by 3.6 %) compared to individually housed, which in contrast spent more time lying down (by 2.3 %). Calves housed in pair spent much more time in starter feed intake (by 1.0 %). Regarding the occurrence of negative signs of behaviours, calves housed in pair were found to have higher sucking rates of other calf (by 1.1 %), while in individually housed calves were the higher frequency of licking of surfaces of the hutches, runs and buckets (by 1.6 %). There was no statistically significant difference in body weight, average daily gain between the two various housing systems. In conclusion, it is possible to state that the pair housed calves reached a higher level of welfare due to the possibility of a wider range of natural behaviours.

Keywords: dairy calf; housing; daily time budget; average daily gain; welfare

INTRODUCTION

Presently there is a growing public interest in the welfare of farm animals (Vasseur et al., 2010). The public generally takes a negative attitude towards individual housing systems of calves. Points out that individual housing has a negative impact on the welfare of calves, because it usually significantly restricts physical activity; manifestations of natural behaviour, reducing space for resting, feeding and watering, protection against unfavourable climatic conditions and increasing susceptibility to infection (Hristov et al., 2011; Bolt et al., 2017).

In Europe, approximately 60% and in the Czech Republic around 75% of calves are housed individually during the milk feeding periods. (Doležal and Staněk, 2015; Bolt et al., 2017). The main reason for the expansion of individual housing systems of calves is a significant reduction of the pathogens transmission risk, the prerequisite for ensuring individual care of calves and at the same time the possibility of individual control of feed intake including easy identification of health problems (Svensson et al., 2003; Doležal and Staněk, 2015). Mutual contact between animals increases the risk of spreading pathogens, increases morbidity and, consequently, mortality in calves (Maatje et al., 1993; Gulliksen et al., 2009; Hulbert a Moisá, 2016).

Group housing of calves has recently become increasingly popular and desirable with regard to animal welfare because it improves cognitive performance, offers more movement and provides better opportunities for social behaviour (Chua et al., 2002; Wójcik et al., 2012; Gaillard et al., 2014). The advantage of paired and group housing is the possibility of social interactions and reduced stress levels when weaning calves are moving to the group (Babu et al., 2004). The disadvantages of group housing system are increasing the incidence of mutual sucking and the possibility of infection transmission by direct contact of calves (Gulliksen et al., 2009). As a result, there is an increase in the frequency of disease, especially diarrhoea and respiratory disease, with the consequent increase in treatment costs with a negative impact on the farm profitability (Svensson et al., 2003; Svensson a Liberg, 2006; Marcé et al., 2010).

The aim of this study was to evaluate the effect of a paired housing system on the daily activity of dairy calves, their performance and welfare.

MATERIAL AND METHODS

Observation was realized in 20 Holstein calves housed in the shelter during the milk feeding period. The shelter has a metal construction with roll-up anti-draft nets on both sides and front walls. The larger half of the roof is made of corrugated plastic covering and the other smaller half is fitted with a roll-up tarpaulin. On the concrete floor of the shelter, there are 40 plastic individual pens with metal net runs, separated by a gap, installed in two rows.

All calves were housed immediately after calving in individual pens with run. Two neighbouring calves were selected for pair housing on the basis of body weight and age at approximately 31 days of their age.

The calves remained in the original pens, which were separated by a central barrier. On the other hand, the runs of both pens were connected by removing the central barriers. The calves after connection could choose to spend resting, lying and sleeping time together in one pen or each separately in their own pen. The experimental group consisted of 12 calves i.e. 6 pairs, of it always one calf-bull and one calf-heifer. The control group was composed of 8 calves (4 calf-heifers and 4 calf-bulls). Nutrition (6 litres of milk per day, ad libitum amount of starter), water supply and straw were identical in calves housed individually and in pairs. Weaning of calves took place at 60 days of age.

The daily activities (lying, resting, standing and feeding, drinking and body parts and surface sucking and licking, etc.) of all calves was detected in the first week after the calves connection in pair, i.e. about 37 days of age, and in the last week before weaning (i.e. about 53 days of age), by a camera recording system of housing space evaluated in the 24-hour cycles. During the study was determined also birth weight and body weight measured by weekly interval during the milk feeding period. Starter consumption was measured daily.

Daily behavioural activities of calves were evaluated by non-parametrical Kruskal-Wallis ANOVA using statistical software Statistica 9. A statistically significant difference between groups was found by multiple comparison of the average ranking for all groups. The significant differences between groups of quantitative traits (birth weight, weaning live weight, mean daily gain, starter consumption) were verified by F-test and paired t-test at the corresponding significance levels (Statistica 9).

RESULTS AND DISCUSSION

The behavioural activities of calves often significantly vary and depend on the level of the breeding environment, housing system, management and health status. The mean values of the behavioural activities of calves, including the range of hours spent in the observed calves in two different housing systems, are summarized in Table 1.

Rest and sleep are the basic welfare indicators. Calves housed individually and in pairs, spent most of their daily time lying and resting (Table 1). Also, Chua et al. (2002), Hänninnen (2007) and Camiloti et al. (2012) found that calves spend of lying and resting approximately 17 to 19 hours per day, i.e. around 70 to 80% of the day.

Table 1 Behaviour of calves depending on housing system

Behaviour	Individual housing		Pair housing	
	Mean [hours a day]	Range of variation [hours a day]	Mean [hours a day]	Range of variation [hours a day]
Lying and resting	18.41	16.42 – 20.42	17.86	15.33 – 20.50
Standing	2.58	1.25 – 3.83	2.79	0.92 – 4.67
Starter intake	0.42	0.08 – 1.17	0.66	0.08 – 1.17
Starter intake	0.41	0.17 – 0.83	0.35	0.17 – 0.92
Hutch surfaces licking	1.28**	0.67 – 2.42	0.91**	0.25 – 2.92
Sucking of body parts	0.02	0.00 – 0.08	0.27	0.00 – 0.67
Comfort behaviour (grooming, scratching)	0.88	0.42 – 2.00	0.92	0.42 – 1.67
Agonistic behaviour (pushing)	0.00	0.00 – 0.00	0.23	0.00-0.67
Jump on other calves	0.00	0.00 – 0.00	0.01	0.00 – 0.08

Statistical significance:** ($p<0.01$)

The total length of calves lying depends not only on the depth of the litter but also on its moisture (Hänninnen, 2007). It is necessary to keep the dry bedding to secure the welfare of calves, as this reduces heat loss due to conduction and helps calves to cope with the cold environment (Camiloti et al., 2012). The lying and resting time of calves housed in pairs was shorter (by -2.3%) in compared to the individually housed of calves. The pair-housed calves searched for the close contact with the other calf during the lying; on an average both calves were in close proximity or touched directly 6.22 hours (1.17 to 16.67 hours) of the total rest period. While Chua et al. (2002) found that pair-housed calves spent a much shorter time in close contact (approximately 0.48 hours of total day-length).

Based on the results of selected behaviour indicators, it is evident that calves housed in pair were more active (by 3.6 %) compared to calves housed individually. Also Jensen et al. (1998) found that calves housed individually are less active than calves housed in the group. The pair-housed calves spent much more time in starter intake (by 1.0%) due to competition, which resulted in frequent overpressing of both calves in one bucket (Table 1). Similarly, De Paula Vieira et al. (2010) established that pair-housed calves more frequently visited the feeder with a starter and the total starter intake time was longer compared to individually housed calves.

The pair-housed calves also attended to much more time to comfort behaviour (i.e. grooming, scratching) by 0.2% than calves housed individually (Table 1).

Regarding the occurrence of negative signs of behaviours, calves housed in pairs devote to sucking the second calf by 1.1% more time per day. While individually housed calves spent by 1.6 % higher time of licking the surfaces of the pens, runs and buckets in compared to pair-housed calves. The frequency of calf mutual suckling is highest in group housing immediately after milk feeding. The need of calves to suck takes about 10 minutes after activation (i.e. the onset of milk feeding) and coincides with the average duration of the milk suction period under the cow (Graf et al., 1989). Mutual sucking is transmitted to a later age. Cows reared in group housing have twice higher the frequency of mutual sucking than cows reared individually (Debrecéni et al., 1999; 2000).

The values of the average daily gain of calves during milk feeding periods depending on the two different housing systems are shown in Table 2.

The birth weight of calves varied from 43.0 to 50.5 kg. Calves, destined for rearing in the pair, had a non-significantly higher birth weight (by +1.3 kg) compared to calves housed from birth to weaning in individually pens (Table 2).

Table 2 The average daily gain of calves depending on housing system

Housing system	N	Birth weight [kg]		Live weight at weaning [kg]		Average daily gain [kg]		Starter consumption [kg]	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Individual / pair	12	44.1	2.3	87.1	6.7	0.715	0.096	36.2	14.2
Individual	8	45.4	4.3	88.2	10.3	0.713	0.119	36.5	18.7
p		0.384		0.763		0.958		0.972	

The weaning weight of calf-bulls ranged from 78.0 to 104.5 kg. The calves housed in pairs from day 31 until weaning had a significantly higher body weight (+1.1 kg) compared to calves reared individually during the milk feeding period (Table 2).

The average daily increment of calves ranged from 0.588 kg to 0.934 kg during the milk feeding period. The average starter consumption varied during dairy nutrition from 17.9 kg to 66.7 kg. There was proved no statistically significant difference between the average daily gains or the average starter consumption by calves housed individually or in pairs (Table 2). These results are consistent with the study by Bolt et al. (2017), who also noted no effect of paired housing on the growth or consumption of the starter. De Paula Vieira et al. (2010) recorded significantly higher feed intake of pair-housed calves, but did not show any statistically significant increase in average daily gain. Also, Chua et al. (2002) found that pair-reared calves achieved higher growth and at the same time had higher starter consumption than individually reared calves. In contrast, Maatje et al. (1993) reported reduced feed intake and lower average daily increment in group housed calves due to competition for feed, respectively access to feed.

CONCLUSION

Based on the obtained data, it can be evident that there was proved no statistically significant difference in either live weight or average daily gains between the individual and pair calves housing systems during the milk feeding period. On the other hand, it is necessary to take into account the aspect of calf behaviour, which is an important indicator of welfare level. The calves housed in pair searched for close contact with the other calf during lying, when between 1.17 and 16.67 hours of total rest, both calves were in close proximity or were touching each other.

On the other hand competitive feeding behaviour (milk, starter) occurred in calves housed in pairs. Regarding the occurrence of negative signs of behaviours, calves housed in pair were found to have higher sucking rates of other calf (by 1.1 %), while in individually housed calves were the higher frequency of licking of surfaces hutches, runs and buckets (by 1.6 %). Paired housing creates the prerequisites for realized a wider range of natural behaviours, enough time to rest and feed, and provides the basis for maintaining a higher level of calf welfare as an important prerequisite for achieving optimal production indicators in adult rearing and breeding.

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REFERENCES

Babu L.K., Pandey H.N., Sahoo A. (2004): Effect of individual versus group rearing on ethological and physiological responses of crossbred calves. Appl. Anim. Behav, 87, 177-191.

Bolt S.L., Boyland N.K., Mlynski D.T., James R., Croft D.P. (2017): Pair Housing of Dairy Calves and Age at Pairing: Effects on Weaning Stress, Health, Production and Social Networks. PLOS ONE, 4: 1-18. Available from https://journals.plos.org/plosone/article?id=10.1371/

journal.pone.0166926 (Accessed 20.6.2018)

Camiloti T.V., Fregonesi J.A., Von Keyserlingk M.A.G., Weary D.M. (2012): Short communication: Effects of bedding quality on the lying behavior of dairy calves. J. Dary Sci., 95, 3380-3383.

De Paula Vieira A., Von Keyserlingk M., Weary D. (2010): Effects of pair versus single housing on performance and behavior of dairy calves before and after weaning from milk. J. Dairy Sci., 93, 3079-3085.

Debreceni O., Juhás P. (1999): Milk-sucking in dairy cattle in loose housing in Slovakia. Livestock Prod. Sci., 61, 1-6.

Debrecéni O., Juhás P., Strapák P. (2000): Výskyt a správanie dojníc-"cicaviek" v rôznych systémoch ustajnenia kráv a technike napájania teliat. Acta fytotechnica et zootechnica, 3, 2-5.

Doležal O., Staněk S. (2015): Chov dojeného skotu. Profi Pres s.r.o., Praha, 243.

Gaillard C., Meagher R. K., von Keyserlingk M. A. G., Weary D. M., McElligott A. (2014): Social housing improves dairy calves' performance in two cognitive tests. PLoS ONE, 9, e90205. Available from https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0090205

(Accessed 30.9.2019).

Graf B.,Verhangen N.,Sambraus H.H. (1989): Reduzierung des Ersatzsaugens bei künslich aufgezogenen Kälbern durch Fixierung nach dem Tränken oder Verlägerung der Saugzeit. Züchtungskunde, 61,: 384-400.

Gulliksen S.M., Lie K.I., Løken T., Østerås O. (2009): Calf mortality in Norwegian dairy herds. J Dairy Sci., 92, 2782-2795.

Hänninnen L. (2007): Sleep and rest in calves. Relationship to welfare, housing and hormonal aktivity. Academic dissertation. Finsko, Helsinki, Fakulty of Veterinary Medicine of the University of Helsinki, 76.

Hristov S., Stanković B., Todorović-Joksimović M., Mekić C., Zlatanović Z., Ostojić-Andrić D., Maksimović N. (2011): Welfare problems in dairy calves. Biotechnology in Animal Husbandry, 27, 1417-1424.

Hulbert L.E., Moisá S.J. (2016): Stress, immunity, and the management of calves. J. Dairy Sci., 99, 3199–3216.

Chua B., Coenen E., Van Delen J., Weary D.M. (2002): Effects of pair versus individual housing on the behavior and performance of dairy calves. J.Dairy Sci., 85, 360-364.

Jensen M.B., Vestergaard K.S., Krohn C.C. (1998): Play behaviour in dairy calves kept in pens: the effect of social Contact and space allowance. Appl. Anim. Behav. Sci., 56, 97-108.

Maatje K., Verhoeff J., Kremer W.D., Cruijsen A.L., van den Ingh T.S. (1993): Automated feeding of milk replacer and health control of group-housed veal calves. Vet. Rec., 133, 266-270.

Marcé C., Guatteo R., Bareille N., Fourichon C. (2010): Dairy cal housing systems across Europe and risk for calf infectious diseases. Animal, 4, 1588-1596.

Svensson C., Liberg P. (2006): The effect of group size on health and growth rate of Swedish dairy calves housed in pens with automatic milk-feeders. Prev. Vet. Med., 73,43–53.

Svensson C., Lundborg K., Emanuelson U., Olsson S.-O. (2003): Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Prev. Vet. Med., 58, 179-197.

Vasseur E., Borderas F., Cue R. I., Lefebvre D., Pellerin D., Rushen J., Wade K. M., de Passillé A. M. (2010): A survey of dairy calf management practices in Canada that affect animal welfare. J. Dairy Sci., 93,1307–1316.

Wójcik J., Pilarczyk R., Bilska A., Weiher O., Sanftleben P. (2012): Performance and health of group-housed calves kept in igloo calf hutches and calf barn. Pak. Vet. J., 33, 175–178.

SELECTED ENZYMATIC ACTIVITIES IN FAECES FROM BROILER CHICKENS AFTER PERORAL INTAKE OF HUMIC SUBSTANCES

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ABSTRACT

This study aimed to investigate the effects of the peroral intake of additives with humic substances on the amylolytic, cellulolytic and proteolytic activities in faeces of broiler chickens. One hundred and twenty, one-day-old broiler chickens of hybrid Ross 308, divided at random into 4 equal groups (A, B, C / control) were used in the experiment. The groups were fed with feed mixtures (BR1 – 197.85, BR2 – 194.03, BR3 – 183.70 g/kg CP) for 37 days. The humic substances were added into feeds as follows: A – 0.7% Humac natur AFM, B – 0.7% Humac natur AFM monogastric, C – 0.5% Humac natur AFM monogastric, control without additive). The applied additives Humac Natur AFM / Humac Natur AFM monogastric (Humac Ltd., Slovak Republic) contained humic acids min. 650/570, fulvic acids min. 50/50 g/kg, minerals Ca, Mg, Fe, Cu, Zn, Mn, Co, Se, Mo, V. The average weights of chickens in the groups were the following A: 2311.92, B: 2326.56, C: 2281.93 and control: 2291.79 g on day 37. The samples of faeces were used for the quantification of amylolytic, cellulolytic and proteolytic activities with the substrates starch, methyl hydroxyethylcellulose and azocasein. The addition of humic substances had a positive effect on the increase of cellulolytic activities in the gastrointestinal apparatus of chickens on day 3 ($P = 0.004$) and 24 ($P = 0.000$). The amylolytic and proteolytic activities were not statistically different.

Keywords: gut of poultry; humates; proteolytic; amylolytic; cellulolytic activities;

INTRODUCTION

The most used animal proteins worldwide are poultry meat and poultry eggs. The expectations are that the consumption will be enhanced because of the growth of population and the increasing needs of proteins for human nutrition. As for the increase of demand for the following raise of demand for poultry products is projected in South Asia by 271%, in Eastern Europe and Central Asia by 116%, in the Middle East and North Africa by 97% and in East Asia and the Pacific by 91% between 2000 and 2030 (FAO, 2015). The importance to improve the animal welfare and the potential development of antimicrobial resistance in humans influenced the changes in welfare legislation and subsequently the ban of the utilization of the antibiotic growth promoters in the European Union with the Regulation 1831/2003 (Castanon, 2007). The effect of this ban was the deterioration of animal performance and health as well as the increase of enteric disorders such as necrotic enteritis because of the loss of the lost prophylactic effects of the antibiotic substances (Pineda Quiroga et al., 2017). The rise in infections diseases caused the increase of the therapeutic use of antimicrobials for food animals in Europe (Casewell et al., 2003). The annual consumption of them was 148 mg/kg in 2010 and it is expected the increase by 67% untill 2030 (Van Boeckel et al., 2015). It appears that the significant reduction of the used of antibiotic growth promoters caused a higher incidence of animal welfare problems combine with health, leg and skin problems. The consequence is the rise of the public concern.

Humates are raw materials utilizable in the plant and animal husbandry as sources of organic and mineral substances with the positive effects on their biological characteristics. These natural products are characterized as geological deposits located in the earth superficial layer which originated from the process of decomposition of plant and animal matter via the activities of microorganisms (McMurphy *et al.*, 2011).

The scientific hypothesis was based on the positive effects of humic substances (HS) on the enzymatic activities in the gastrointestinal apparatus which have potential to improve digestion of proteins, starch and cellulose in the gut of poultry.

The study aimed to investigate the effects of the peroral intake of HS preparation on the amylolytic, cellulolytic and proteolytic activities in faeces of broiler chickens.

MATERIAL AND METHODS

One hundred and twenty, one-day-old broiler chickens of hybrid Ross 308, were delivered from a commercial hatchery. They were divided at random into 4 groups of 30 animals (A, B, C / control). The chickens were housed in four-floor pens located in one experimental hall of the University of Veterinary Medicine and Pharmacy (Košice, Slovak Republic) with constant access to feed and water. Both pens were identical concerning the same direction and the same area (0.12 m^2 per broiler chicken). The groups were fed with conventional commercial feed mixtures (BR1 – 197.85, BR2 – 194.03, BR3 – 183.70 g/kg CP) for 37 days.

The methionine was used as the first limiting amino acid. The diets were prepared and formulated without antibiotics and growth promoters. The anticoccidial agents were added into the starter and grower feed mixtures. The humic substances were added into feeds as follows: A – 0.7% Humac natur AFM, B – 0.7% Humac natur AFM monogastric, C – 0.5% Humac natur AFM monogastric, control without additive) for the whole experiment.

The characteristics of the applied preparations containing HS: Humac Natur AFM/Humac Natur AFM monogastric (Humac Ltd., Slovak Republic) were the following: the size of particles up to 100 □m, max. moisture 15%, the content of humic acids min. 650/570, fulvic acids min. 50/50 g/kg, macroelements Ca 42.28/51.1, Mg 5.11/4.86, Fe 19.05/18.09 g/kg and microelements Cu 15/14.25, Zn 37/35.15, Mn 142/135, Co 1.24/1.18, Se 1.67/1.59 as well as Mo 2.7/2.57, V 42.1/40 mg/kg dry matter (DM).

The body weights of chickens were assessed once a week. The average weights of chickens in the groups were the following A: 2311.92, B: 2326.56, C: 2281.93 and control: 2291.79 g on day 37. The feed was weighed to evaluate feed consumption.

The samples of faeces were placed into sterile tubes for digestive enzyme analyses. The preparation of samples for the quantification of digestion enzymes activities was performed as follows. One gram of fresh sample was diluted with 49 ml sterile TBS buffer (TRIS-hydroxymethyl aminomethane 10 mmol/l, HCl 0.5 mol/l, pH 7.0) and homogenised. The samples were subsequently taken for the measurement of nonspecific proteolytic activity (Broderick, 1987) with the substrate azocasein (Merck Ltd., Germany). The cellulolytic and the amylolytic activity (Lever, 1977) were analysed with the substrates methyl hydroxyethylcellulose (Merck Ltd., Germany) and starch (Fisher Slovakia Ltd.), respectively.

Table 1 Digestive enzyme activities in the faeces of broiler chickens (n = 6; mean ± SD)

Age (day)	Group	Amylolytic (glucose) (□ mol/l/min/g)	Cellulolytic activity (glucose) (□ mol/l/min/g)	Proteolytic activity (azocasein) (□ g/ml/min/g)
3	Control	3.85 ± 0.726	2.83 [a] ± 0.162	23.78 ± 2.779
	A	2.72 ± 0.604	3.98 ± 0.811	22.07 ± 7.220
	B	6.28 ± 3.440	5.325 [c] ± 0.614	26.57 ± 0.592
	C	4.26 ± 0.485	4.48 [b] ± 0.396	28.65 ± 2.588
10	Control	3.08 ± 0.520	2.63 ± 0.459	17.68 ± 1.206
	A	3.14 ± 0.706	3.08 ± 0.554	17.96 ± 1.850
	B	2.59 ± 0.213	3.10 ± 0.172	17.88 ± 1.162
	C	2.93 ± 0.117	2.52 ± 1.436	18.33 ± 1.063
17	Control	1.10 ± 0.481	3.14 [a] ± 0.769	26.13 ± 1.966
	A	0.87 ± 0.315	1.37 [b] ± 0.294	24.37 ± 2.188
	B	1.65 ± 0.409	1.15 [b] ± 0.267	26.67 ± 3.878
	C	1.10 ± 0.1202	2.05 ± 0.865	25.10 ± 3.194
24	Control	0.55 ± 0.418	0.36 [a] ± 0.085	17.53 ± 9.736
	A	0.37 ± 0.090	0.59 [b] ± 0.016	29.33 ± 1.404
	B	0.76 ± 0.263	1.38 [d] ± 0.198	26.028 ± 2.130
	C	0.46 ± 0.278	0.66 [b] ± 0.048	24.83 ± 3.739
31	Control	1.67 ± 0.390	2.26 ± 0.229	2.26 ± 0.229
	A	2.26 ± 0.229	3.01 ± 1.627	12.55 ± 2.442
	B	1.75 ± 0.533	2.99 ± 0.262	19.289 ± 3.090
	C	1.29 ± 0.169	2.16 ± 0.401	19.32 ± 5.012

Means with different superscript letters differed significantly: [a,b] $P < 0.05$; [a,c] $P < 0.01$; [a,d] $P < 0.001$

The data are expressed as means ± standard deviation (SD) of single values. Means of the results from the treatments were compared by one-way analysis of variance. Treatment means were statistically compared by Tukey-Kramer multiple comparison test. Significance was declared at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS AND DISCUSSION

The addition of HS had a positive effect on the increase of cellulolytic activities in faeces (Table 1) of chickens on day 3 (P = 0.004) and 24 (P = 0.000). In the case of the amylolytic and proteolytic activities, there were not observed significant differences in experimental groups compared to control. According to Rideau et al. (1983) it is proposed that the modification of the chyme and transit rates during the day may affect the pancreatic enzyme fate and distribution in the small intestine, as well as they observed that intestinal contents and enzyme activities were higher in egg-forming than in non-egg-forming laying hens.

Similarly, the addition of HS combined with urea had a positive effect on the amylolytic and cellulolytic activities in RF of sheep in EG (Marcin et al., 2018).

CONCLUSION

The addition of HS had a positive effect on the cellulolytic activities in the gut of chickens. The significant influence on the amylolytic and proteolytic activities was not observed.

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REFERENCES

Broderick, G. A. (1987): Determination of protein degradation rates using a rumen in vitro system containing inhibitors of microbial nitrogen metabolism. British Journal of Nutrition, 58, 463–475.

Casewell, M., Friis, C., Marco, E., McMullin, P., Phillips (2003): The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. Journal of Antimicrobial Chemotherapy, 52, 159-161.

Castanon, JIR. (2007): History of the use of antibiotic as growth promoters in European poultry feeds. Poultry Science, 86, 2466–2471.

Food and Agriculture Organization of the United Nations (2015): Global information and early warning system on food and agriculture. Available from http://www.fao.org/3/a-I5258e.pdf (accessed Oct 20, 2019).

Lever, M., 1977: Carbohydrate determination with 4-hydroxybenzoic acid hydrazide (PAHBAH): Effect of bismuth on the reaction. Analytical Biochemistry, 81, 21–27.

Marcin, A., Bujňák, L., Mihok, T., Naď, P. (2018): Effects of humic substances with urea on protozoal population and fermentation in the rumen of sheep. Bulgarian Journal of Veterinary Medicine (online first), DOI:10.15547/bjvm 2199.

McMurphy, C.P., G.C. Duff, S.R. Sanders, S.P. Cuneo & N.K. Chirase (2011): Effects of supplementing humates on rumen fermentation in Holstein steers. South African Journal of Animal Science, 41, 134-140.

Pineda Quiroga, C., Atxaerandio, R., Ruiz, R., Garcia, A. (2017): Effects of dry whey powder alone or combined with calcium butyrate on productive performance, duodenal morphometry, nutrient digestibility, and ceca bacteria counts of broiler chickens. Livestock Science, 206, 155–169.

Rideau, N., Nitzan, Z., Mongin, P. (1983): Activities of amylase, trypsin and lipase in the pancreas and small intestine of the laying hen during egg formation. British Poultry Science, 24, 1–9.

Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., Teillant, A., Laxminarayan, R. (2015): Global trends in antimicrobial use in food animals. PNAS, 112, 5649-5654.

CONTENT OF FATTY ACIDS IN INTRAMUSCULAR FAT OF HEAVY CARCASS LAMBS FROM PASTURE FATTENING

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ABSTRACT

The objective of this study was to assess the meat quality of the heavy slaughter lambs from pasture fattening, on the basis of selected fatty acids spectrum (FA) of intramuscular fat (IMF). Lambs fattening (10 pc.) was carried out in electrical fences after previous removal of young lambs at average weight 10 kg from their mothers. The lambs were slaughtered at an average weight of 28.68 ± 5.901 kg. The carcass yield was 46.03 ± 6.00 %. Based on the results obtained by analyzing the FA spectrum of IMF, it can be stated that pasture fattened lambs were characterized by extremely high meat quality. The lamb meat contained high levels of all health-beneficial fatty acids (e.g. trans-vaccenic FA - 3.73 g.100 g^{-1} fatty acid methyl esters - FAME, CLA - 1.16 g.100 g^{-1} FAME, EPA - 1.520 g.100 g^{-1} FAME, DHA - 0.438 g.100 g^{-1} FAME). The ratio of n-6/n-3 polyunsaturated FAs was also very favorable and the meat was relatively high in PUFA (21.99 ± 5.65 g.100 g^{-1} FAME) and low in SFA (43.31 ± 3.85 g.100 g^{-1} FAME). This is probably due to the fact that sheep grazing is generally regarded as a source of high ALA nutrient, which was also reflected in the examined lamb meat (3.245 g.100 g^{-1} FAME). The values of the atherogenic and thrombogenic index also indicate the high quality of the meat of the lambs being evaluated.

Keywords: lamb; pasture fattening; meat quality; intramuscular fat, fatty acid spectrum

INTRODUCTION

Worldwide, research is focused on revealing the potential benefits of consumption of lamb meat (Ponnampalam et al., 2014). There can be found many studies assessing the quality of lamb meat on the base of essential fatty acids (FAs) e.g. linoleic acid (LA), α-linolenic acid (ALA) and of health promoting polyunsaturated FAs (PUFA) in the intramuscular fat (Ponnampalam et al. 2014). Regarding PUFA, eicosapentaenoic acid (EPA) and

docosahexaenoic acid (DHA) are believed to be of anti-inflammatory effect, helping to protect human body against autoimmune diseases (McAfee et al., 2010). Regarding saturated FAs (SFA), high amounts of myristic acid and palmitic acid are assumed to increase the risk of cardiovascular diseases and the cholesterol amount (Howes et al., 2015). Research about essential and health beneficial FAs revealed that these depend on both production system and nutrition of lambs (Sinanoglou et al., 2013). In Slovakia, breeding of various meat and/or non-dairy dual-purpose breeds producing heavy lambs is increasing in Slovakia. Three basic production systems for fattening of heavy lamb are applied in Slovakia in larger commercial flock: the traditional intensive and semi-intensive system is usually applied (Margetin et al., 2018). However, heavy slaughter lambs are also produced exclusively by pasture fattening without their mothers. The lambs are bought most often after weaning from dairy sheep flock. After a short transitional period, the lambs are later fed to higher weights (25-40 kg) exclusively on pasture, using available grazing resources. The proportion of lambs fed in this way increases from year to year, but there is a lack of information on their nutritional quality. The aim of present paper, following the work of Margetin et al. (2018, 2019), was to determine the content of FAs in IMF of pasture fattened lambs.

MATERIAL AND METHODS

Description of biological material and method of lambs fattening

The biological material used in the study was a group of 10 lambs, which after being weaned from their mothers (average weight 10 kg) were transported to the pasture where the lambs were fattened. Lambs from commercial flock had different genetic proportions of the breed Improved Valachian, Tsigai, Lacaune and East Friesian breed. Lambs to the weaning (before transport) were raised under mothers using nurseries. After weaning (early May), the lambs were transported on pasture to the northern part of the cadastral area Kolárovice (foothill area of White Carpathians). After a short weekly transition period the lambs were since early May to late July pasture fattened and subsequently since early August were fattened in enclosed pasture area of 4.67 ha. Fattening lasted until the end of October. When the carcass weight was reached, the lambs were transported to the slaughterhouse. The slaughter of lambs was carried out according to STN 455220 "Slaughter sheep".

Analysis of fatty acids

Twenty-four hours after slaughter, meat samples were taken from *Musculus longissimus lumborum et thoracis* (MLLT) between the 9th and 13th vertebra. The analysis of the content of fatty acids (FAs) in the intramuscular fat (IMF) was undertaken in the laboratory of the Institute of Chemistry (Comenius University in Bratislava), following the procedure described in the study of Margetin et al. (2018). A total of 70 FA were identified. The atherogenic index (AI), thrombogenic index (TI) and hypocholesterolaemic FA/hypercholesterolaemic FA ratio (h/H ratio) were calculated according to Sinanoglou et al. (2013).

RESULTS AND DISCUSSION

The contents of FAs in IMF of MLLT samples from analysed group of lambs are shown in Table 1. Regarding individual saturated FAs (SFA), the values of saturated FAs had been extremely low (palmitic acid - 17.80 g.100 g^{-1} FAME; myristic acid – 1.28 g.100 g^{-1} FAME). Unlike study of Cividini et al. (2014), palmitic acid was only the second most common SFA. The content of palmitic acid (PA) was significantly lower like Margetin et al. (2018) found in pastured (21.80 g.100 g^{-1} FAME) and stabled (28.51 g.100 g^{-1} FAME) Ile de France (IF) lambs. The contents of remaining SFA i.e. lauric acid, myristic acid, margaric acid and stearic acid (SA) were found to be lower to the values in pastured IF lambs (Margetin et al., 2018). Unlike study of Cividini et al. (2014), stearic acid (SA) was in our study the first common SFA (20.34 g.100 g^{-1} FAME). Regarding individual monounsaturated FAs (MUFA), oleic acid (OA), was the most common MUFA. The value reported in this study was slightly lower than Aurousseau et al. (2007) reported for lambs on pasture those diets were enriched with concentrates. The content of trans-vaccenic acid (TVA) was 3.73 g.100 g^{-1} FAME. These value was higher than in pastured IF lambs (Margetin et al., 2018). Findings about higher contents of TVA (most important precursor of conjugated linoleic acid (CLA) in meat of grazed lambs agree with the study of Aurousseau et al. (2007), who found higher contents of TVA in grazed lambs as well.

Table 1 Basic statistical characteristics of chosen fatty acids in intramuscular fat (g.100g^{-1} fatty acid methyl esters)

Trait	n	Average	s	v%	Min.	Max.
C12:0 (lauric)	10	0.058	0.018	30.56	0.037	0.090
C14:0 (myristic)	10	1.28	0.288	22.54	0.844	1.75
C16:0 (palmitic)	10	**17.88**	**1.577**	**8.82**	**15.94**	**20.31**
C16:1 *cis9* (palmitooleic)	10	0.622	0.128	20.60	0.401	0.765
C17:0 (margarinic)	10	1.37	0.168	12.28	1.10	1.65
C18:0 (stearic)	10	**20.34**	**2.674**	**13.15**	**16.88**	**23.66**
C 18:1 *trans9* (elaidic)	10	0.235	0.037	15.75	0.193	0.270
C18:1 *cis9* (oleic, OA)	10	**26.21**	**15.938**	**60.80**	**23.08**	**29.73**
C18:1 *trans11* (*trans*-vaccenic)	10	3.73	0.844	22.63	3.46	4.15
C18:2 n-6 (LA)	10	**9.50**	**0.401**	**4.23**	**4.40**	**16.85**
C18:3 n-6 (GLA)	10	0.068	1.104	68.22	0.028	0.102
C18:3 n-3 (ALA)	10	3.25	16.876	20.06	2.22	4.17
C18:2 *cis9,trans11* (RA)	10	**1.16**	**0.193**	**16.64**	**0.68**	**1.57**
C20:4 n-6 (AA)	10	**2.44**	**1.006**	**41.19**	**1.02**	**3.64**
C20:5 n-3 (EPA)	10	1.52	0.532	34.98	0.79	2.21
C22:5 n-3 (DPA)	10	1.56	0.564	36.30	0.83	2.30
C22:6 n-3 (DHA)	10	0.438	0.203	46.39	0.200	0.804

Regarding individual polyunsaturated FAs (PUFA), γ-linolenic acid (GLA) was PUFA of the lowest content in analysed lamb groups (0.068 g.100 g^{-1} FAME). The essential linoleic acid

(LA) was of the highest content, i.e. 9.50 g.100 g^{-1} FAME. This value is extraordinarily high. Similarly the content of essential α-linolenic acid (ALA) was also extremely high (3.25 g.100 g^{-1} FAME). The contents of health beneficial PUFA i.e. arachidonic acid (AA), eicosapentaeonic acid (EPA), docosapentaeonic (DPA) and docosahexaeonic acid (DHA) were found as follows: 2.44, 1.52, 1.56 and 0.438 g.100 g^{-1} FAME. Values of these PUFA are extraordinarily high in comparison with papers published in Slovakia (Margetin et al., 2018, 2019).

Table 2 Basic statistical characteristics of chosen fatty acid groups, their ratios and indexes in the intramuscular fat (g.100g^{-1} fatty acid methyl esters)

Trait	n	Average	s	v%	Min.	Max.
SFA	10	**43.31**	**3.850**	**8.891**	**38.660**	**49.068**
MUFA	10	**34.70**	**3.229**	**9.306**	**30.292**	**38.946**
PUFA	10	**21.99**	**5.645**	**25.667**	**12.820**	**29.313**
Essential FA (LA+ALA)	10	12.74	3.943	30.947	6.624	19.644
n-6 PUFA	10	**12.27**	**4.604**	**37.515**	**5.723**	**20.579**
n-3 PUFA	10	6.81	1.926	28.291	4.180	9.518
LC n-6 PUFA	10	2.77	1.122	40.435	1.207	4.111
LC n-3 PUFA	10	**3.56**	**1.271**	**35.691**	**1.943**	**5.400**
CLA	10	**1.16**	**0.296**	**25.561**	**0.679**	**1.572**
PUFA / SFA	10	0.520	0.169	32.425	0.261	0.758
∑ n-6 PUFA/∑ n-3 PUFA	10	**1.82**	**0.654**	**35.872**	**1.369**	**3.326**
C18:2 n-6 / C18:3 n-3 (LA/ALA)	10	**2.98**	**1.340**	**44.916**	**1.941**	**6.027**
LC n-6 PUFA / LC n-3 PUFA	10	0.77	0.145	18.945	0.621	1.100
AI (atherogenic index)	10	0.43	0.078	18.033	0.340	0.570

TI (thrombogenic index)	10	0.91	0.210	23.058	0.654	1.299
h/H index	10	2.38	0.389	16.368	1.766	2.840
DFA (desirable FA)	10	77.03	1.793	2.328	74.478	79.053

Contents of FA groups (SFA, MUFA, PUFA, etc.), their ratios and indexes are shown in Table 2. The content of SFA group was 43.31 g.100 g^{-1} FAME, the content of MUFA was 34.70 g.100 g^{-1} FAME and the content of PUFA group was 21.99 g.100 g^{-1} FAME. The value of PUFA in the meat of our studied lambs is extremely high in comparison with published papers (Margetin et al., 2018, 2019). Health benefits of lamb meat were questioned due to its relatively high content of SFA and relatively low content of PUFA (McAfee et al., 2010, Howes et al., 2015). When comparing with study of Margetin et al. (2018), both contents of *n-6* PUFA and *n-3* PUFA (12.27 and 6.81 g.100 g^{-1} FAME) were much higher than respective contents found for pastured IF animals (8.50 and 4.55 g.100 g^{-1} FAME). The content of essential FAs (summed LA and ALA) was 12.74 g.100 g^{-1} FAME what is much higher content like in the study of Margetin et al. (2019). The content of CLA tend to accord with respective values for stabled and pastured IF lambs (Margetin et al., 2018). The ratio of *n-6*/*n-3* PUFA were 1.82; this value agree with the recommendation to be below 4 as proposed by Wood et al. (2003). The ratio of LC *n-6*/LC *n-3* PUFA was 0.77 and this value was lower than Aurosseau et al. (2007) reported (0.8 and 1.2). The ratio of PUFA/SFA was 0.520 and is only slightly lower from the recommendations to be above 0.7 (proposed by Raes et al., 2004). The AI was found to be much lower like number 1 (0.43) and agreed with the recommendation of Sinanoglu et al. (2013), who proposed this index to be 1 at a maximum. The same recommendation was proposed for thrombogenic index (TI = 0.91). Taking into account AI, TI ratios, meat of studied lamb group should be considered as healthy food consisting of beneficial FAs that may help in prevention of cardiovascular diseases.

CONCLUSION

Based on the results obtained by analysing the fatty acid spectrum of IMF of heavy slaughter lambs from pasture fattening, it can be concluded that the lambs fattened in this way are characterized by extremely high meat quality. The meat of the pasture fattened lambs

contained a high content of all health-beneficial fatty acids and the omega 6 and omega 3 FA ratios was also very favourable.

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REFERENCES

Aurousseau, B. et al (2007): Indoor fattening of lambs raised on pasture: (1) Influence of stall finishing duration on lipid classes and fatty acids in the *longissimus thoracis* muscle. Meat Science, 76, 241–252.

Cividini, A. et al. (2014). Fatty acid composition of lamb meat from autochthonous Jezersko-Solčava breed reared in different production systems. Meat Science, 97, 480–485.

Howes, N.L. (2015): Opportunities and implications of pasture-based lamb fattening to enhance the long-chain fatty acid composition in meat. Comprehensive Reviews in Food Science and Food Safety, 14, 22–36.

Margetín, M. et al (2018): Fatty acids in intramuscular fat of Ile de France lambs in two different production systems. Archives Animal Breeding, 61, 395–403.

Margetín, M. et al (2019): Fatty acids in intramuscular fat of Berrichon du Cher and Suffolk heavy lambs kept in semi-intensive production systems in Slovakia. Slovak J. Anim. Sci., 52, 81-89.

McAfee, A.J. et al (2010): Red meat consumption: An overview of the risks and benefits. Meat Science, 84, 1–13.

Ponnampalam, E.N. et al (2014): Sources of variation of health claimable long chain omega-3 fatty acids in meat from Australian lamb slaughtered at similar weights. Meat Science, 96, 1095–1110.

Raes, K. Et al. (2004): Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. Animal Feed Science and Technology, 113, 199–221.

Sinanoglou, V.J. et al (2013): Lipid quality indices: Differentiation of suckling lamb and kid breeds reared by traditional sheep farming. Small Ruminant Research, 113, 1–10.

Wood, J.D. et al (2003): Effects of fatty acids on meat quality: a review. Meat Science, 66, 21–32.

USAGE OF UREA ANALYSIS IN MILK FOR EVALUATION EFFICIENCY OF PROTEIN TRANSFORMATION IN DAIRY COWS

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ABSTRACT

The objectives of the study are to evaluate the relationship between nutrition, content of milk urea, transformation and excretion of N in the urine in the farm conditions. The content of crude protein in daily feed ration has a significant effect on milk production and constituents, urea content in blood and milk and the evaluation of nitrogen balance (N intake, digestibility and utilization of nitrogen, excretion of nitrogen in faeces and urine, nitrogen production in milk). Milk urea serves as an important marker for monitoring and evaluating protein nutrition and metabolism of nitrogen in lactating dairy cows, specifically for assessing the quantity and quality of protein and the proportion of carbohydrates in TMR. Monitoring and evaluation of milk urea on farms provides an opportunity to formulate rations and adjust levels of protein to optimize effective utilisation of nitrogen in order to increase milk and milk protein production and to avoid the negative effects of urea excretion in urine. In summary, milk is a useful indicator of the animal nutritional (protein and energy specific) status.

Keywords: *crude protein, milk protein, milk urea, milk urea nitrogen, urea nitrogen in urine*

INTRODUCTION

Increasing of milk production on farms in today's places great emphasis on the nutritional requirements and mainly on the level of protein nutrition in high-yielding dairy cows through the supply of protein and amino acids without environmental burden. For nutritional and ecological reasons in recent years has dominated the question of increasing the effective

utilization of proteins and reducing the excretion of nitrogen into the environment. Effective utilization of nitrogen (EUN) is the ratio between the content of nitrogen in milk and the amount of nitrogen received from TMR. The effectiveness of nitrogen transformation for production of milk protein rarely exceeds 30%, with more than 70% of received crude protein representing 30% loss in faeces and 40% loss in urine, mostly in the form of urea (Yan et al., 2006). Efficiency of protein utilization is often in the range of 25 to 35% (Sinclair et al., 2014), but a study (Higgs et al., 2012) shows an increase in utilization efficiency of nitrogen of herds in the range of 30-31% with proper formulation of feed ration. The efficiency utilization of nitrogen from TMR is most limited by the proportion and degradability of proteins and the amino acid composition of digestible proteins at the level of small intestine (Misciatelli et al., 2003). An important factor affecting EUN is the concentration of crude protein in TMR itself (Huhtanen and Hristova 2009). In general, increasing the amount of crude protein in TMR and increasing its intake results in higher excretion of urea nitrogen in urine and decreased EUN without affecting milk protein production. Effective utilization of nitrogen (EUN) is the ratio between the content of nitrogen in milk and the amount of nitrogen received from TMR. The effectiveness of nitrogen transformation for production of milk protein rarely exceeds 30%, with more than 70% of received crude protein representing 30% loss in faeces and 40% loss in urine, mostly in the form of urea (Yan et al., 2006).

Analysis of levels of protein and urea in milk are important indicators for evaluation of the intake and efficiency transformation of crude protein. Level of milk urea is a suitable indicator for assessing phase nutrition in terms of monitoring the protein-energy ratio of nutrients in feed rations. Given the highly significant relationship between levels of rumen ammonia, levels of blood urea and levels of milk urea, as well as the unassuming method of determining and obtaining samples, such a method for evaluation of the level of phase nutrition in a herd is very advantageous.

The aim of study was in our farms conditions by analysis of milk urea in relation to intake and concentration of nutrients to evaluate nitrogen metabolism through efficiency of nitrogen utilization, excretion of nitrogen in urine and emission of ammonia.

MATERIAL AND METHODS

The evaluations were carried out on feed trials within 30 herds with a controlled nutritional level system and with an average annual production of between 8,500 and 9,500 kg per cow.

In dairy cows (n = 3,150) at the peak of lactation evaluated the level of protein intake, the efficiency of utilizing N for milk protein and excretion N as a marker of environmental burden. Samples of prepared TMR in the monitored farms were taken from the feed manger on the control day and were analyzed for dry matter (DM), crude protein (CP), acid and neutral detergent fibre (ADF, NDF), starch and ether extract (EE) contents according to conventional methods according to the Commission Regulation (EC) no. 691/2013.

Analysis of production parameters on the control day on individually collected milk samples was evaluated for milk production levels in dairy cows, milk components and milk urea. Milk samples were analysed the total protein content, fat, lactose and urea concentration by near infrared spectrophotometric assay using MilkoScan FT+ and BENTLEY FTS at the Central Analytical Laboratory of Milk with accreditation under registration number 096/5878/2015/2. The analysed urea in milk (MU) was converted to urea nitrogen in milk (MUN) using the equation by Oudah 2009.

RESULT AND DISCUSSION

In feed trials on a total of 30 farms and 3,150 individual dairy cows in the first lactation phase, daily feed intake in the group and the nutritional composition of TMR were intensively monitored. The average annual production on evaluated farms varied in the range of 8,500 – 9,500 kg per cow with a total number of lactating cows from 200 - 600 pieces. The number of evaluated dairy cows in the 1st phase of lactation was 105 ± 53 on average with a minimum number of 43 and a maximum of 168 per group in the holding.

Composition of daily feed rations. Daily feed rations on evaluated farms were predominantly based on corn and alfalfa silage, supplemented with a different species of carbohydrates (cereal grains and cereal grain by-products) and protein supplements (soybean meal and rapeseed meal) fed as TMR. Average concentrations of CP of evaluated rations were 16.2 ± 0.8 % of dry matter (range 14.4 -17.8) and NEL 6.76 ± 0.20 MJ/kg of dry matter (range 6.38-7.13). The formulated rations contained on average 24.3 ±3.5 % starch (range 16.9-29.5) and 34.5± 2.9 % of NDF in dry matter (range 28.0-38.7), respectively.

The overall dry matter intake of the monitored groups of dairy cows in the first lactation phase was on average 22.4 ± 1.3 kg and ranged from 20.8 to 24.9 kg/day. The average CP intake was 3.62 ± 0.2 kg/day (range 3.2 to 4.0), starch intake and NDF intake were 5.60 ± 0.9 kg or 7.68 ± 0.9 kg (range from 16.9 to 29.8, or 28.0 to 38.7)

The average milk production 35.4 ± 6.4 kg/day at the content of milk protein 3.15 ± 0.2%, milk fat 3.62 ± 0.4% and content of milk urea 27.4 ± 3.6 mg/dl, reflects different composition of TMR, the different content of nutrients, genetic production potential and the order of lactation. The parameters of milk production and composition of TMR showed significant differences in chemical composition of nutrients against actual production and composition of milk on evaluated farms.

1.) ***Evaluation of the effect of crude protein content in feed rations on transformation of nitrogen*** is summarized in Table 1.

At an average content of crude protein of 16.3 ± 0.9% was confirmed the content of milk urea on average 27.4 ± 3.6 mg/dl. Dividing farms according to content of crude protein (CP) in TMR confirmed the dependence of increasing CP with concurrently decrease content of starch on increase content of milk urea, on excretion of nitrogen in urine and emission of ammonia into the environment. The effect of crude protein in direct dependence influenced the increase of content of milk urea and significantly increased the level of nitrogen excreted in the urine and decreased the efficiency of N utilization.

Table 1 ***Effects of CP content (%) dry matter of TMR on transformation of nitrogen***

Range of CP %	***CP % DM of TMR***	***Milk urea mg/dl***	***EUN % by MUN***	***UN g/d by MUN***	***Emission of NH_3 g/d***
15 – 16	15.4 ± 0.4	25.2 ± 3.9	30.4 ± 0.9	191.1 ± 14.9	82.3 ± 4.7
>16 - 17	16.5 ± 0.3	27.6 ± 1.7	29.0 ± 0.8	215.6 ± 12.6	90.0 ± 3.9
>17 - 18	17.3 ± 0.3	31.7 ± 2.8	27.2 ± 0.9	246.4 ± 21.8	99.7 ± 6.7
X	**16.2 ± 0.8**	**27.4 ± 3.6**	**29.2** ± 1.4	**211.8** ± 24.3	**81.7 ± 7.6**

An increase of 1% in crude protein content ranging from 14 to 18% in TMR corresponds to – increase in content of milk urea of 3.4 mg/dl, - an increase in urinary N excreted by 25.61 g / day, and - a reduced efficiency utilization of nitrogen of 1.48%. The decrease of EUN of 1.48% when increased the content of CP in TMR about 1%, what is lower value of decrease as compared to the value of 1.86% determined (Olmos Colmenero and Broderick 2006), resp. 1.76% (Broderick 2003). Values in the study (Wattiaux and Ranathunga 2016) assume that each decrease of CP in feed ration by one percentage point in the range of the content of CP 19 and 13% (in DM) reduces urea nitrogen in urine by 28.1 g/d.

2.) The effect of starch content in TMR and its different content on nitrogen transformation is summarized in Table 2. Content of starch in dry matter of TMR shows an indirect dependence of correlation and with increasing content of starch there is reduced content of milk urea, excretion of nitrogen in urine and partial efficiency utilization of nitrogen.

Table 2 ***Effects of starch content (%) dry matter of TMR on transformation of nitrogen***

Range Starch %	***Starch % DM of TMR***	***Milk urea mg/dl***	***EUN % by MUN***	***UN g/d by MUN***	***Emission of NH_3 g/d***
19 – 23	19.9 ± 1,6	29.9 ± 3.4	27.6 ± 1.1	233.3 ± 26.3	95.6 ± 8.3
>23 - 27	25.4 ± 1,0	26.9 ± 2.6	29.5 ± 1.0	211.2 ± 11.6	88.6 ± 6.2
>27 - 30	28.3 ± 1,0	26.0 ± 4.3	30.0 ± 0.9	199.0 ± 40.4	83.8 ± 5.5
X	**25.0 ± 3.3**	**27.4 ± 3.6**	**29.2** ± 1.4	**211.8** ± 24.3	**81.7 ± 7.6**

The most significant effect of CP content in TMR on the concentration of MUN (R^2 = 0.93) with a less significant impact of energy concentration and protein degradability in TMR was confirmed in the study (Broderick and Huhtanen 2013).

3.) The effect of the ratio of starch content to CP (Table 3) shows a negative dependence of correlation, and as the ratio starch CP increases, was confirmed a decrease in the content of urea in milk, a decrease in excretion of nitrogen in urine and increased efficiency utilization from feed to milk.

Table 3 ***Effects of ratio Starch/CP in dry matter of TMR on transformation of nitrogen***

Range Starch/CP	***Starch/CP***	***Milk urea mg/dl***	***EUN % by MUN***	***UN g/d by MUN***	***Emission of NH_3 g/d***
1.0 – 1.4	1.21 ± 0.1	25.2 ± 3.9	27.6 ± 1.0	233.5 ± 24.4	95.6 ± 7.6
>1.4 – 1.7	1.59 ± 0.1	26.9 ± 2.4	29.5 ± 0.9	210.7 ± 17.6	88.5 ± 5.5
>1.7 – 2.1	1.83 ± 0.1	25.7 ± 4.6	30.3 ± 0.9	191.9 ± 16.8	82.6 ± 5.3
X	**1.55 ± 0.3**	**27.4 ± 3.6**	**29.2** ± 1.4	**211.8** ± 24.3	**81.7 ± 7.6**

The low efficiency utilization of nitrogen is directly related to the high content of crude protein in feed rations (Huhtanen and Hristov 2009; Aguilar et al., 2012). High intake of crude protein conditions the accumulation of ammonia in the rumen and increases its

absorption with higher production of urea in the liver (Marini and Van Amburgh 2003; Reynolds and Kristensen 2008), thereby increasing the content of urea in blood and milk and directly correlating with increased excretion of nitrogen and reduces the utilization of N for milk protein (Recktenwald et al., 2014). This process is directly influenced by the content of crude protein and the ration starch to crude protein and less the content of starch in the TMR. The increase of nitrogen intake has the effect of an increase of concentrations of MUN and UN and thereby contributed to the reduction in the efficiency of N utilisation (Nousianen et al.2004; Spek et al., 2013). Regression analysis confirmed (Castilo et al., 2000) a positive linear correlation between intakes of nitrogen and proportionate representations of excreted nitrogen in faeces, urine and milk up to intake of 400 g N/d. However, it is necessary to ensure that improving the efficiency of N utilization does not affect milk production (Pereira 2012). Increasing the nitrogen intake over 400 g N/d exponentially increased the excretion of nitrogen in the urine and the excretion of nitrogen in the faeces and milk decreased linearly.

CONCLUSION

Based on the obtained results of farm trials we can conclude that concentrations of milk urea are simple and non-invasive approaches to examine the metabolism of nitrogen fed to dairy cattle. Milk urea is economically advantageous, and provide information on nutrition status, metabolic load and ecological burden and production health of cows, also allowing the evaluation of the efficiency of nitrogen utilization from feed to milk protein and prediction of excretion of nitrogen in urine.

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REFERENCES

Aguilar, M., Hanigan M. D., Tucker H. A., Jones B. L., Garbade S. K., and McGilliard M. L. (2012). Cow and herd variation in milk urea nitrogen concentrations in lactating dairy cattle. J. Dairy Sci. 95:7261-7268.

Broderick G., Huhtanen P. (2013) Application of milk urea nitrogen values [Internet], (asccessed 19.10.2013), Available from www.researchgate.net/publication/43267630

Broderick, G.A. (2003) Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci,86:1370-1381

Castillo A.R., Kebreab, E., Beever, D.E., France. J.A. (2000). A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. J. Anim. Feed Sci.; 9:1-32.

Huhtanen P., Hristov A.N., (2009). A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. J. Dairy Sci. 92:3222-3232

Marini, J.C., and M.E. Van Amburgh (2005). Partition of nitrogen excretion in urine and the feces of Holstein replacement heifers. J. Dairy Sci. 88:1778–1784

Misciattelli, L., Kristensen, V.F., Vestergaard, M., Weisbjerg, M.R., Sejrsen, K., Hvelplund, T., (2003). Milk production, nutrient utilization, and endocrine responses to increased postruminal lysine and methionine supply in dairy cows. J. Dairy Sci. 86, 275–286.

Nousiainen J., Shingfield K.J., Huhtanen P. (2004). Evaluation of milk urea nitrogen as a diagnostic of protein feeding. Journal of Dairy Science, 2004;87:386–398

Oudah, E.Z.M. (2009) Non genetic factors affecting somatic cell count, milk urea content, test day milk yield and milk protein percent in dairy cattle of Czech Republic using individual test day records. Livestock Research for Rural Development, 2009; 21(5):1-25

Olmos Colmenero J.J., Broderick G.A. (2006) Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:1704–1712

Pereira, Andre de Barros Duarte, (2012) "Reduced protein for late-lactation dairy cows“, Louisiana State University, Master's Theses 224, 2012, available from http://digitalcommons.lsu.edu/gradschool_theses/224

Recktenwald, E. B., Ross, D. A., Fessenden, S. W., Wall, C. J., and Van Amburgh, M. E (2014). Urea-N recycling in lactating dairy cows fed diets with 2 different levels of dietary crude protein and starch with or without monensin. J. Dairy Sci. 97, 1611-1622

Reynolds, C.K. and Kristensen, N.B. (2008). Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis. J. Anim. Sci.86 (E. Suppl.): E293–E305

Spek J.W., Dijkstra J., van Duinkerken G., Hendriks W.H., Bannink A. (2013.) Prediction of urinary nitrogen and urinary urea nitrogen excretion by lactating dairy cattle in northwestern Europe and North America: A meta-analysis. J. Dairy Sci., 96:4310–4322

Wattiaux M.A., Ranathunga S. (2016) Milk urea Nitrogen as a tool to assess efficiency of Nitrogen utilization in dairy cows, In Proceedings of Four-State Dairy Nutrition and Management Conference 2016, (Dubuque Iowa, June 15-16, 2016), pp 79-88

THE EFFECT OF HUMIC ACIDS ON THE PRODUCTION PARAMETERS AND PRODUCT QUALITY OF CHICKENS

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ABSTRACT

The effects of humic acids on health, production parameters, and quality of products were monitored in the experiment with the broiler chickens. The average live weight of broilers achieved in the control group was 2319 g and in the experimental group 2377 g at the end of the experiment. The use of humic substances did not affect the overall feed conversion in the concentration of 0.5% in the feed mixture. It was 1.51 kg in the control group and 1.53 kg in the group with the addition of humic acids. Significant differences were found in the average weight of the chickens after swallowing (control group 1.711 kg; experimental group 1.793 kg) and in the recovery rate of 73.8 resp. 75.3%. The fat content in the femoral muscle of the experimental group was also significantly higher, 3.72% more than in the control group. The significant differences were ascertained in the average weight of chickens after autopsy (control group 1.711 kg; experimental group 1.793 kg) and in the case of yields 73.8% and 75.3%, respectively. The fat content in the femoral muscle of the experimental group was also significantly higher, 3.72% more than in the control group.

Key words: humic substances; health; production; quality of products; poultry

INTRODUCTION

Humic substances are characterized as soil components, which have been created by the decomposition of biological matter containing plant and animal residues and by the activity of microorganisms found in the soil. They occur in natural materials such as sediment, turf, brown coal, lignite and other materials. Due to their chemical structure formed by the aromatic nucleus and the functional group, they are efficient to bind the polar and non-polar

compounds. Therefore, they are having the ability to influence the availability of various nutrients in the animal organism (Tichá et al., 2009). Humic substances are used to influence soil fertility as well as in the animal husbandry to reduce environmental load and the odour in housing objects (Greene et al, 2000; Rashid et al., 2018). Their application is in human and veterinary medicine for prevention, stimulation of the immune system and in the supportive treatment of some diseases too. The substances are characteristic with the detoxifying effect when they are binding various toxic substances and ensure their removal through excrements. They can activate metabolism, promote health, reduce mortality, improve the growing intensity and the feed conversion as well as the fattening efficiency index (Veselá et al., 2005; Vaško et al., 2012; Mudroňová et al., 2018). The effect of humic acids on livestock production parameters, feed conversion and product quality in poultry was studied (Herzig et al., 2009; Lala et al., 2016; Arpášová et al., 2016). Also in broiler chickens alone or in combination with probiotics or plant extracts (Demeterova et al., 2009; Pistová et al., 2016) and laying lay (Hakan et al., 2012; Arafat et al., 2015).

We observed the effect of humic substances on the health and production parameters in the experiment with the broiler chickens.

MATERIAL AND METHODS

There were used 60 broiler chickens in the experiment, divided into two groups (n = 30). The animals were housed in the experimental conditions of the breeding establishment, providing the conditions for the fattening of broiler chickens. The control and experimental groups were fed with a conventional commercial feed mixture (FM). In the experimental group, 0.5% concentration of humic substances was added to the compound feed. The preparation contains 40% of natural humic acids (including 60% humic acid, 5% fulvic acid). Chickens were given feed and water ad libitum during fattening. The mortality was monitored in the course of the experiment. The chicks were weighted at weekly intervals, their weight and the feed consumption were observed. The average daily weight gains and the feed conversion was calculated.

Broiler chickens were weighed, stunned on day 37 and then sacrificed by cervical dislocation. In addition to total recovery, carcass yields of the thigh (whole, skin and bones), pectoral muscle, trunk and wings were determined. Water and dry matter content was determined in the samples of pectoral and femoral muscle. Proteins were determined by the Kjeldahl method. Fat content by the Soxhlet method.

The comparison of the observed production parameters between groups was done using the t-test using Prism Free Trial software (GraphPad Software, USA).

RESULTS AND DISCUSSION

The effects of humic acids on production parameters, health and quality of products were monitored in the experiment with the broiler chickens. The mortality of 1 chicken was registered in the control group in the second week. In the third week, one chicken was eliminated from both groups because of more pronounced lag in growth. The starting average weight was 50g in both groups. At day 37 chicks reached the average weight of 2319.3 g in the control group and 2377.8 g in the experimental group. Demeterová and Šamudovská (2011) reported no significant differences in weight between groups using sodium humate in their broiler experiment as well. The weight of chicks in the control group was 2476.6 g and in the experimental group 2481.5 g on day 37. The weight of the feed mixture in the control and experimental groups is in Table 1. There were fed 114.21 kg FM in total in the experimental group and 107.33 kg in the control group. The average consumption of FM of one chicken per one day increased in the control group from 27.9 g in the first week, to 161.2 g in the fifth week. This parameter was in the experimental group from 29.1 g in the first week to 177.8 g in the finish trial. The feed conversion reached 1.51 kg in the control and 1.53 kg in the experimental groups. It can be stated that the use of humic substances in concentrations of 0.5% in feed mixture, did not affect the overall feed conversion in the case of comparison of the control and experimental groups. There was not observed the statistically significant difference.

Table 1 Quantity of the utilized feed mixture during fattening

Week	Utilized FM (kg)	
	Control group	**Experimental group**
1	5,45	5,68
2	12,92	12,68
3	20,80	21,20
4	27,60	28,40
5	31,60	36,10
6 (2 days)	8,96	10,15
Total	**107,33**	**114,21**

The humic substances at the concentration of 0.5% in FM did not affect the average feed consumption. The feed consumption was 3.58 kg/chicken in the experimental group and the

control group, it was by 0.07 kg/chicken lower. Marcinčáková et al. (2018) found no positive effect of the addition of humic acids on the feed consumption (control group 3.89 kg, experimental group 3.68 kg) and the feed conversion (control group 1.64, experimental group 1.63) in the experiment in the case of concentration of the feed additive 0.8%.

Positive effects of the addition of humic substances were observed in the reduction of mortality in the experimental group, where no individual chickens died compared to the control group with one case of mortality. Vaško et al. (2010) reported the beneficial effects of the addition of humic acids on the reduction of livestock mortality, too.

The difference in mean weight in the control and experimental groups, before gutting, was statistically insignificant (Table 2). Statistically significant differences (P <0.05) were observed in the post-evisceration weight, which was up to 81.7 g higher in the broiler chickens with the addition of humic substances than in the control group. A statistically significant difference (P <0.05) was also found at slaughter yield. In the experimental group, recovery was 1.5% higher compared to the control group. A significant difference was also found with the percentage of wings (P <0.05). In the control group 1% higher compared to the experimental group. At percentages of pectoral muscle and thighs, the values were statistically insignificant.

Table 2 Carcass weight comparison

	Control group	**Experimental group**
carcass weight (g)	2319,3±92,6	2377,8±133,2
weight after gutting (g)	1711,9±81,8b	1793,7±158,5a
slaughter yield (%)	73,8±1,8b	75,3±3,2a
breast meat (g)	522,1±50,4	512,1±67,1
breast meat (%)	30,5±2,8	28,5±2,7
thigh meat (g)	484,6±43,7	522,9±62,1
thigh meat (%)	28,3±2,6	29,2±2,8
wings (g)	175,3±14,3	164,4±16,8
wings (%)	10,2±0,7a	9,2±0,5b
hull of a chicken (g)	454,3±51,8	496,2±72,4
hull of a chicken (g)	26,5±2,7	27,6±2,7

$^{a, b}$ – statistically significant difference P < 0,05

The chemical composition of pectoral muscle is shown in Table 3. The higher dry matter content (by 0.63%) was determined in the pectoral muscle samples from the experimental

group. In the control group, a higher fat content of 0.53% and a protein content of 0.23% were found. However, the measured values were not statistically significant differences.

Table 3 Chemical composition of pectoral muscles

	pectoral muscles (in %)			
	dry matter	**fat**	**water**	**protein**
Control group	25,46±0,11	2,94±0,1	74,2±0,38	21,48±0,13
Experimental group	26,09±0,34	2,41±0,86	74,04±0,25	21,25±0,60

In the femoral muscle, the dry matter content was 0.3% and the protein content 1.03% higher in the control group compared to the experimental group (Table 4). These differences were not statistically significant. A statistically significant difference in fat content ($P < 0.05$) was found in the femoral muscle samples, from the group of broilers fed with 0.5% humic substances. In the control group, the mean value was 3.72% lower.

Table 4 Chemical composition of thigh muscles

	thigh muscles (in %)			
	dry matter	**fat**	**water**	**protein**
Control group	28,28±0,2	9,28±0,20b	71,71±0,19	19,93±1,55
Experimental group	27,98±0,4	13,0±0,35a	72,02±0,4	18,90±0,53

$^{a, b}$ – *statistically significant difference $P < 0,05$.*

CONCLUSION

Feed additives are used in livestock nutrition for the improvement of the production health, the nutrient utilization and the quality of products. The achievement of positive results at their application is influenced by several factors. We did not determine any statistically significant differences of the observed production parameters in the experiment with broiler chickens after the application of humic substances added into the feed mixture at the concentration of 0.5%. Statistically significantly higher values were found in the experimental group only at broiler weight after dissection and in the fat content of the thigh muscle.

REFERENCES

Arafat R.Y., Khan S.H., Abbas G., Igbal J. 2015 Effect of dietary humic acid via drinking water on the performance and egg quality of commercial layers, American Journal of Biology and Life Science, 3, (2), 26-30

Arpašová H., Pospišilová D., Pistová V. (2016) Využitie huminových kyselín vo výkrme brojlerových kurčiat, Slovenský Chov, 8: 32-33

Demeterová M., Mariščaková R., Pistl J., Naď P., Šamudovská A. (2009) The effect of the probiotic strain Enterococcus faecium DSM 7134 in combination with natural humic substances on performance and health of broiler chickens, Berliner und Munchener Tierarztliche Wochenschrift 122, 9-10: 364-369

Demeterová M., Šamudovská A. (2011) Lepší rast brojlerov a konverzia krmív: Prírodné humínové látky vplývajú priaznivo na zdravotný stav a produkciu. Slovenský chov. 16, 8: 37-38.

Greene L. W., Cole A. (2000) Efficient waste and odor management for feedlots. The Agriculture Program, Texas A&M University System, AGCOM 5-1-00. http://agprogram.tamu.edu; press release

Hakan K.B., Gulketin Y., Ozge S. (2012) Effects of boric acid and humate supplementation on performance and egg quality parameters of laying hens, 14, 4: 233-304

Herzig I., Navrátilová M., Totušek J., Suchý P., Večerek V., Bláhová J., Zralý Z. (2009) The effect of humic acid on zinc accumulation in chicken broiler tissues, Czech Journal of Animal Science, 54, 3: 121-127

Lala A.O., Okwelum N., Bello K.O., Famakinde N.A., Alamu M.O. (2016) Comparative study between ISA Brown and Fulari ecotype chickens supplemented with humic acid, Slovak Journal of Animal Science, 49, 2: 68–75

Marcinčáková D., Mačanga J., Bartkovský M.,Semjon B., Hudák M., Jaďuttová I., Marcinčák S. (2018) Vplyv skrmovania humínových látok na produkčné ukazovatele brojlerových kurčiat, profil mastných kyselín a kvalitu produkovaného mäsa. Lazarové dni výživy a dietetiky XIII. zborník UVLF Košice, 120-123

Mudroňová D., Pešulová T., Karaffová V., Koščová J., Marcinčáková D., Marcinčák S. (2018) Vplyv humínových kyselín na črevnú mikrobiotu a imunitnú odpoveď brojlerov. Lazarové dni výživy a veterinárnej dietetiky XIII.: zborník UVLF Košice, 186-189

Pistová V., Arpášová H., Hrnčar C., Kačaniová M., Haščík P. (2016) The effect of the humic acid and herbal additive supplement on production parameters of broiler chicken, Animal Science and Biotechnologies, 49, 2: 166-169

Rashid I., Murtaza G, Zahir A. Z., Farooq M. (2018) Effect of humic and fulvic acid transformation on cadmium availability to wheat cultivars in sewage sludge amended soil, Environmental Science and Pollution Research, 25:1671–1679

Tichá A., Hyšpler R., Kriesfalusová L., Ježková D., Zadák Z. (2009) Vliv huminových látek na absorpci cholesterolu. Klinická biochemie a metabolismus, 17, 1: 37-41

Vaško L., Vašková J., Černeková D. (2010) Skúsenosti s aplikáciou humínových kyselín u hydiny. Chovateľ, 46, 3: 20-21 ISSN 0862-5573

Vaško L., Vajda V., Szanyi G. (2012) Humínové kyseliny vo výžive a ich vplyv na metabolizmus a produkčné zdravie. Slovenský chov. 5: 40-41

Veselá L., Kubal M., Kozler J., Innemanová P. (2005) Struktura a vlastnosti přírodních humínových látek typu oxihumolitu. Chem. Listy, 99: 711-717

EFFECT OF PARITY ON DYNAMICS OF MILK YIELD, MILK COMPONENTS AND MILK UREA CONCENTRATION IN DAIRY COWS

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ABSTRACT

The aim of the study was to evaluate the effect of lactation order on dynamics of milk yield, milk components and milk urea concentration in Holstein dairy cows with yearly monitoring of feed ration composition and nutrient intake. By evaluating of lactation order in relation to dynamics of milk yield, milk components and milk urea for standard lactation in primiparous cows compared to multiparous cows was confirmed: – significantly ($P < 0.001$) lower milk yield (32.5 ± 6.4 and 37.5 ± 9.9 kg/d, respectively), – significantly ($P < 0.001$) lower milk urea content (27.42 ± 6.8 and 28.06 ± 7.2 mg/dl) with lower individual numbers of dairy cows (34.6% of primiparous cows and 40.0% of multiparous cows) with over-the-limit milk urea content (34.53 ± 3.9 and 35.07 ± 4.2 mg/dl), – significantly higher ($P < 0.001$) milk fat content (3.73 ± 0.7 and 3.59 ± 0.8%), – significantly higher ($P < 0.001$) milk protein content (3.25 ± 0.3 and 3.21 ± 0.4%), – significantly lower ($P < 0.001$) milk lactose content (4.82 ± 0.2 and 4.95 ± 0.2%), – significantly lower ($P < 0.001$) daily protein yield (1.05 ± 0.2 and 1.18 ± 0.3 kg/d), fat yield (1.20 ± 0.3 and 1.31 ± 0.4 kg/d) and lactose yield (1.61 ± 0.3 and 1.82 ± 0.5 kg/d). The results of the study confirm the influence of non-nutritional factors on the milk yield, milk components and milk urea content in dairy cows.

Keywords: lactation order; urea in milk; milk quantity; composition of milk; lactation stage

INTRODUCTION

Lactation order is generally correlated with age of the cows. Primiparous cows in the 1st lactation use ingested nutrients from the feed ration to complete the growth and development of the body frame and resulting is limited milk synthesis with changes in its composition. With advancing age, milk production increase to reach the maximum at the 3rd to 4th

lactations, then starts again to decrease (Baul et al., 2013). Urea as a final product of protein metabolism in dairy cows is synthesized in the liver from excess ammonia released from dietary protein degradation in the rumen or deamination of amino acids in excess of requirements. Urea is removed from the blood by recycling into the rumen, excreted through urine into the environment and secreted into the milk. Milk urea (MU) is a good indicator of appropriate balancing of the feed ration for dairy cows in terms of protein and energy (Miglior et al., 2006). In addition to the nutritional factors that largely affect MU content, milk yield and milk components, these parameters are according to Arunvipas et al. (2003) to 13.3% affected by non-nutritional factors (parity, season) and according to Hojman et al. (2004) to 37% production factors (milk yield and milk components). The aim of this study was to evaluate the effect of parity on dynamics of milk yield, milk components and milk urea concentration in dairy cows.

MATERIAL AND METHODS

Data collection and management of dairy herd

The study was performed on a dairy farm with Holstein dairy cows during the year 2017. Dairy cows were fed and selected into groups according to the lactation order to dairy cows in the 1st lactation (primiparous cows) with weight 600 kg (n = 2239) and mature cows (multiparous cows) in the 2nd to 8th lactations with weight 650 kg (n = 3558). Furthermore, according to days in milk (DIM) were divided into the following groups: until 30 DIM (*postpartum*), 31-120 DIM (early lactation), 121-210 DIM (mid lactation), 211-305 DIM (late lactation).

Groups of dairy cows were fed a total mixed ration (TMR) formed monthly depending on the nutrient requirements according to milk yield and capacity of dry matter intake alike for primiparous and multiparous cows in *postpartum* period, mid and late lactation, where cows were kept in a common group. In a separate group were kept primiparous and multiparous cows in early lactation. Samples of TMR were analysed by conventional methods (Commission Regulation EC No. 152/2009 of 27.1.2009) to examine the nutrients such as a dry matter (DM), crude protein (CP), fat, neutral detergent fibre (NDF), acid detergent fibre (ADF), starch and ash. Non-fibre carbohydrates (NFC) and net energy of lactation (NEL) were calculated by the equations based on the analysed nutrients content of the feeds (NRC, 2001).

The cows were milked twice a day and milk samples were analysed once per month to evaluate the milk yield, milk protein, fat, lactose and MU in the Central Milk Laboratory in

collaboration with The Breeding Services of Slovakia, using the Breeding Information System. Milk components and MU content were determined by the Fourier transformed infrared analyser method MilkoScan FT+ and Bentley FTS and daily yields of protein, fat and lactose were calculated by multiplying the percentage of milk components with milk yield per day.

Statistical analysis

Obtained data were processed by the IBM SPSS Statistics version 24.0 and expressed as mean ($\bar{x}$) and standard deviation (SD). The unpaired Student's *t*-test was used to compare the results of milk yield, milk components and MU content in primiparous and multiparous cows on average for standard lactation as well as in individual lactation stage.

RESULTS AND DISCUSSION

The analysed nutrients content of TMR

The composition and analysed nutrients content of TMR in primiparous and multiparous cows according to lactation stage are presented in Table 1.

Table 1 The composition and analysed nutrients content of TMR in primiparous and multiparous cows according to lactation stage

Variable	***Postpartum*** Primiparous cows Multiparous cows	**Early lactation** Primiparous cows	**Early lactation** Multiparous cows	**Mid lactation** Primiparous cows Multiparous cows	**Late lactation** Primiparous cows Multiparous cows
Composition of TMR					
Corn silage kg	7.0 - 20.0	19.0 - 33.0	20.0 - 36.0	19.0 - 39.0	8.0 - 36.0
Clover silage kg	7.0 - 12.0	6.0 - 14.0	6.0 - 16.0	4.0 - 10.0	8.0 - 14.0
Grass silage kg	3.0 - 4.0	2.0 - 3.0	2.0 - 5.0	4.0	4.0 - 6.0
Alfalfa silage kg	-	-	-	13.0	22.0
Rye silage kg	7.0	8.0	8.0	12.0	17.0
Oat silage kg	2.0 - 3.0	-	-	4.0 - 8.0	4.0 - 17.0
Alfalfa hay kg	0.8 - 2.0	0.7 - 1.0	0.8 - 1.0	0.8 - 2.0	1.0 - 3.0
Alfalfa hay kg	-	0.8	1.0	-	-
Wheat straw	0.5	0.5 - 0.8	0.5 - 0.8	0.5	1.0

kg					
Cereal grain mixture kg	1.5 - 3.7	0.8 - 3.6	0.5 - 4.5	0.8 - 3.5	1.0 - 1.3
Rapeseed meal kg	0.4 - 2.9	1.5 - 3.8	2.0 - 4.0	3.2 - 5.3	2.5 - 6.0
Wet distillers grains kg	4.0	4.0	4.0	4.0	-
Concentrate mixture kg	3.5	4.2	4.5	1.5	0.3
Nutrients content of TMR					
DM g/kg	466.9 ± 25.8	444.8 ± 18.2	446.8 ± 16.8	422.2 ± 25.1	410.9 ± 32.4
CP g/kg DM	169.7 ± 2.5	157.8 ± 2.0	157.6 ± 2.2	154.2 ± 3.6	157.0 ± 2.9
Starch g/kg DM	230.1 ± 10.1	251.2 ± 6.4	251.9 ± 6.4	232.6 ± 16.5	162.3 ± 33.5
Fat g/kg DM	50.1 ± 4.6	43.6 ± 1.0	43.3 ± 0.8	37.6 ± 0.7	35.7 ± 0.9
NDF g/kg DM	336.6 ± 8.8	342.7 ± 11.5	341.9 ± 10.8	377.5 ± 7.9	417.8 ± 12.9
ADF g/kg DM	209.3 ± 5.1	212.3 ± 5.6	211.8 ± 5.5	229.8 ± 5.1	267.3 ± 13.8
NFC g/kg DM	360.6 ± 10.8	381.3 ± 9.1	382.4 ± 9.2	357.0 ± 10.5	308.9 ± 21.2
NEL MJ/kg DM	6.6 ± 0.1	6.6 ± 0.1	6.6 ± 0.1	6.4 ± 0.1	6.1 ± 0.2
Ash g/kg DM	83.2 ± 4.2	74.7 ± 3.0	74.5 ± 3.2	73.8 ± 4.9	80.6 ± 9.4

The analysed average nutritional composition of TMR for the year fluctuated in the range, which was recommended by the nutrient requirement (NRC, 2001) for the *postpartum* period, mid and late lactation. The analysed nutrients content of TMR for all groups of dairy cows, independent of the lactation stage and milk yield showed a balanced CP content (150-160 g/kg DM) but low starch content (250 g/kg DM) to reach the production potential for early lactation in dairy cows. Separate formed TMR for primiparous and multiparous cows in early lactation showed no significant differences in the analysed nutrients content.

Milk production, milk components and MU

The production data for the primiparous and multiparous cows during lactation are presented in Table 2 and 3.

Table 2 Milk yield, milk components and MU during lactation in primiparous cows

Primiparous cows					
Lactation stage	$\bar{x}$ ± **SD**	***Postpartum***	**Early lactation**	**Mid lactation**	**Late lactation**
Number of measurements	2239	66	717	723	733
DIM	162 ± 80	25 ± 2	76 ± 26	165 ± 26	256 ± 26
Dry matter intake kg/d	21.2 ± 1.8	19.0 ± 0.6	23.1 ± 0.9	22.2 ± 0.6	20.4 ± 0.7
Milk yield kg/d	32.5 ± 6.4[a]	29.0 ± 7.4[a]	34.1 ± 6.2[a]	33.4 ± 5.9[a]	30.2 ± 6.4
MU mg/dl	27.42 ± 6.8[b]	24.42 ± 7.6	26.54 ± 6.2[b]	27.84 ± 6.8	28.13 ± 7.0[a]
MU > 30 mg/dl % of group	34.53 ± 3.9 34.6%	34.70 ± 3.5 22.7%	34.44 ± 4.1 25.0%	34.71 ± 4.0 37.1%	34.42 ± 3.7 42.7%
Fat %	3.73 ± 0.7[c]	4.23 ± 0.7	3.52 ± 0.6[c]	3.66 ± 0.6[b]	3.97 ± 0.6[b]
Fat yield kg/d	1.20 ± 0.3[d]	1.22 ± 0.3[b]	1.19 ± 0.3[d]	1.21 ± 0.2[c]	1.19 ± 0.3
Protein %	3.25 ± 0.3[e]	3.46 ± 0.3	3.07 ± 0.3[e]	3.25 ± 0.3[d]	3.40 ± 0.3
Protein yield kg/d	1.05 ± 0.2[f]	1.00 ± 0.2[c]	1.04 ± 0.2[f]	1.08 ± 0.2[e]	1.02 ± 0.2
Lactose %	4.82 ± 0.2[g]	4.81 ± 0.2	4.90 ± 0.2[g]	4.83 ± 0.2[f]	4.71 ± 0.2[c]
Lactose yield kg/d	1.61 ± 0.3[h]	1.40 ± 0.4[d]	1.71 ± 0.3[h]	1.66 ± 0.3[g]	1.43 ± 0.4[d]

Table 3 Milk yield, milk components and MU during lactation in multiparous cows

Multiparous cows					
Lactation stage	$\bar{x}$ ± **SD**	***Postpartum***	**Early lactation**	**Mid lactation**	**Late lactation**
Number of measurements	3558	110	1225	1172	1051
DIM	154 ± 80	26 ± 3	75 ± 26	164 ± 25	255 ± 27
Dry matter intake kg/d	21.7 ± 2.6	19.0 ± 0.6	25.0 ± 0.7	22.2 ± 0.6	20.4 ± 0.7
Milk yield kg/d	37.5 ± 9.9[a]	37.2 ± 7.6[a]	43.1 ± 8.3[a]	38.0 ± 8.6[a]	30.4 ± 8.4
MU mg/dl	28.06 ± 7.2[b]	25.23 ± 9.1	27.36 ± 7.1[b]	28.31 ± 7.2	28.98 ± 6.9[a]
MU > 30 mg/dl % of group	35.07 ± 4.2 40.0%	36.03 ± 4.8 34.5%	35.10 ± 4.1 34.4%	35.10 ± 4.2 40.5%	35.00 ± 4.3 44.1%
Fat %	3.59 ± 0.8[c]	4.19 ± 0.8	3.36 ± 0.8[c]	3.54 ± 0.8[b]	3.81 ± 0.7[b]
Fat yield kg/d	1.31 ± 0.4[d]	1.57 ± 0.4[b]	1.43 ± 0.4[d]	1.32 ± 0.3[c]	1.13 ± 0.3

Protein %	3.21 ± 0.4[e]	3.41 ± 0.3	3.02 ± 0.3[e]	3.20 ± 0.3[d]	3.39 ± 0.2
Protein yield kg/d	1.18 ± 0.3[f]	1.26 ± 0.3[c]	1.30 ± 0.2[f]	1.21 ± 0.3[e]	1.02 ± 0.3
Lactose %	4.95 ± 0.2[g]	4.82 ± 0.2	5.01 ± 0.2[g]	4.96 ± 0.1[f]	4.90 ± 0.2[c]
Lactose yield kg/d	1.82 ± 0.5[h]	1.79 ± 0.4[d]	2.12 ± 0.4[h]	1.84 ± 0.4[g]	1.49 ± 0.3[d]

$\bar{x}$ ± **SD**: P < 0.001: a,b,c,d,e,f,g,h; ***Postpartum***: P < 0.001: a,b,c,d; **Early lactation:** P < 0.001: a,c,d,e,f,g,h; P < 0.01: b; **Mid lactation:** P < 0.001: a,b,c,d,e,f,g; **Late lactation:** P < 0.001: b,c; P < 0,05:a; P < 0.01:d;

Daily milk yield for standard lactation was significantly (P < 0.001) lower (32.5 ± 6.4 kg/d) in primiparous cows compared with daily milk yield in multiparous cows (37.5 ± 9.9 kg/d). Daily milk yield in primiparous cows accounted 86% of the milk production in multiparous cows. According to Bailey and Currin (2009) primiparous cows produce 75 to 80% from the milk production of multiparous cows. In all stages of lactation, a lower daily milk yield in primiparous cows compared with multiparous cows was confirmed, which was significant (P < 0.001) in *postpartum,* early and mid-lactation. The lactation order is associated with age of the cows, when the first-lactation cows use nutrients from TMR to complete development of udder and body frame, but milk synthesis is limited (Nyamushamba et al., 2014).

The mean value of MU for standard lactation in primiparous cows was 27.42 ± 6.8 mg/dl with over-the-limit MU (above 30 mg/dl) at 34.53 ± 3.9 mg/dl individually in 34.6% of cows. In multiparous cows, the MU content for standard lactation was 28.06 ± 7.2 mg/dl with over-the-limit MU at 35.07 ± 4.2 mg/dl individually in 40.0% of cows. By evaluating the MU content according to lactation stage, a lower content in primiparous cows compared with multiparous cows was found, which was significant for standard lactation (P < 0.001), in early (P < 0.01) and late (P < 0.05) lactation. Confirmed lower MU content in primiparous cows compared with multiparous cows is conditioned by continued growth and a correspondingly higher efficiency of amino acid utilization. Thus, the deamination of amino acid and synthesis of urea in the liver are reduced (Cao et al., 2010).

The milk fat content for standard lactation was significantly (P < 0.001) higher (3.73 ± 0.7%) in primiparous cows than in multiparous cows (3.59 ± 0.8%). According to lactation stage a significantly (P < 0.001) higher milk fat content in primiparous cows compared with multiparous cows in early, mid and late lactation was confirmed. Daily fat yield for standard lactation was significantly (P < 0.001) lower (1.20 ± 0.3 kg/d) in primiparous cows compared with multiparous cows (1.31 ± 0.4 kg/d). According to lactation stage a significantly (P <

0.001) lower daily fat yield in primiparous cows compared with multiparous cows in *postpartum* period, early and mid-lactation was confirmed.

The milk protein content was significantly ($P < 0.001$) higher (3.25 ± 0.3%) in primiparous cows than in multiparous cows (3.21 ± 0.4%) for standard lactation. According to lactation stage a higher milk protein content in primiparous cows in all lactation stages, which was significant ($P < 0.001$) in early and mid-lactation compared with multiparous cows was confirmed. Daily protein yield for standard lactation was significantly ($P < 0.001$) lower (1.05 ± 0.2 kg/d) in primiparous cows compared with the average daily protein yield (1.18 ± 0, 3 kg/d) in multiparous cows. According to lactation stage, a significantly lower ($P < 0.001$) daily protein yield in *postpartum* period, early and mid-lactation in primiparous cows than in multiparous cows was confirmed. Higher milk fat and protein content in primiparous cows was connected with the lower milk yield in primiparous cows. The limiting milk yield associated with the continued development of the mammary gland and body frame in primiparous cows promotes a higher fat and protein contents in milk, whereas with increasing lactation order, milk yield increases and the contents of milk protein and fat decrease (Nyamushamba et al., 2014).

The milk lactose content was significantly lower ($P < 0.001$) in primiparous cows compared with multiparous cows for standard lactation, in early, mid and late lactation. This dependence is due to increase of milk yield with increase of lactation order, when the synthesis of lactose in the mammary gland regulates the milk secretion (Miglior et al., 2006). Daily lactose yield was significantly lower for standard lactation ($P < 0.001$) as well as in *postpartum* period ($P < 0.001$), in early ($P < 0.001$), mid ($P < 0.001$) and late ($P < 0.01$) lactation in primiparous cows compared with multiparous cows. Increased need of nutrients for continued growth of primiparous cows, limits milk yield with a decrease in protein, fat and lactose yield compared with multiparous cows (Nyamushamba et al., 2014).

CONCLUSION

Confirmed effects of lactation order on milk yield, milk components and MU content indicate a significant impact of non-nutritional factors with the possibility of their use as part of a comprehensive assessment of dairy protein nutrition that influence milk quantity, milk components and utilisation of N into milk with negative impact on the environment as well as on reproduction in dairy cows.

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REFERENCES

Arunvipas, P., Dohoo, I. R., VanLeeuwen, J. A., Keefe, G. P. (2003): The effect of non-nutritional factors on milk urea nitrogen levels in dairy cows in Prince Edward Island, Canada. Preventive Veterinary Medicine, 59, 83–93.

Bailey, T., Currin, J. (2009): Milk production evaluation in first lactation heifers. Virginia Cooperative Extension [serial online], 404–285, 1–4. Available from https://pubs.ext.vt.edu/404/404-285/404-285.html (accessed January 31, 2019).

Baul, S., Cziszter, L. T., Acatincai, S., Cismas, T., Gavojdian, D., Tripon, I., Erina, S., Buzamat, G. (2013): Effect of parity on dynamics of milk yield and composition during normal lactation in dairy cows. Animal Science and Biotechnologies, 46, 285–289.

Cao, Z., Huang, W., Wang, T., Wang, Y., Wen, W., Ma, M., Li, S. (2010): Effects of parity, days in milk, milk production and milk components on milk urea nitrogen in Chinese Holstein. Journal of Animal and Veterinary Advances, 9, 688–695.

Hojman, D., Kroll, O., Adin, G., Gips, M., Hanochi, B., Ezra, E. (2004): Relationships between milk urea and production, nutrition and fertility traits in Israeli dairy herd. Journal of Dairy Science, 87, 1001–1011.

Miglior, F., Sewalem, A., Jamrozik, J., Lefebvre, D. M., Moore, R. K. (2006): Analysis of milk urea nitrogen and lactose and their effect on longevity in Canadian dairy cattle. Journal of Dairy Science, 89, 4886–4894.

NRC (2012): Nutrient Requirements of Dairy Cattle: 7th ed. National Academy Press, Washington DC, 3–204.

Nyamushamba, G. B., Halimani, T. E., Imbayarwo-Chikosi, V. E., Tavirimirwa, B. (2014): Comparative evaluation of non-genetic factors affecting milk yield and composition of Red Dane and Jersey cattle in Zimbabwe. Springer Plus, 3, 88.

THE EFFECT OF TECHNOLOGICAL PROCESSING OF SOYBEAN ON *IN VITRO* ORGANIC MATTER DIGESTIBILITY

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ABSTRACT

The aim of this study was to compare the ruminal organic matter digestibility of ground soybean (GS), solvent-extracted soybean meal (SSBM) and extruded defatted soybean (EDSB) determined *in vitro* using a rumen fluid buffer system. The rumen fluid was taken from a lactating cow fed a diet based on maize (22 kg) and lucerne (12 kg) silages, hay (3 kg) and a supplemental mixture (6.7 kg, all on as fed basis). Samples of studied feedstuffs (0.5 g/tube) were incubated in five replications with buffered rumen fluid (40 ml) at 39°C for 24.0 h. Content of crude protein in GS, SSBM and EDSB was 377.0, 499.1 and 495.1 g/kg dry matter, respectively. Generally, nutritive value of GS was comparable to table values while nutritive value of SSBM and EDSB agreed with the available feed composition data in crude protein and fat contents but differed in the content of crude fibre, NDF and ADF. Ruminal *in vitro* organic matter digestibility was the lowest in GS (47.6 %) and was lower than in SSBM (51.4 %, $P<0.05$) and tended to be lower than in EDSB (52.4 %, $P=0,057$).

Keywords: ground soybean; solvent-extracted soybean meal; extruded defatted soybean; rumen digestibility; *in vitro*

INTRODUCTION

Soybean and soybean products, mainly solvent extracted soybean meals, are commonly used supplemental plant proteins in the dairy nutrition. They are characterized by high palatability and well-balanced and available essential amino acids (EAA) contents (Awawdeh et al., 2007). Indeed, the EAA index of soybean bypass protein was the second best after microbial protein among 11 evaluated protein sources (Chandler, 1989). On the other hand, extensive

ruminal degradability being 74% for whole soybeans (NRC, 2001) limits their utilization as sources of RUP in dairy nutrition. That is why various feed processing techniques have been used to treat soybean and soybean products to alter their ruminal degradability. In recent years the extrusion technique is extensively used, because it has numerous advantages, such as improvement in digestibility of starch, protein and amino acids, destruction of antinutritional factors and making starch easily accessible to digestive enzymes (Dust et al., 2004; Liu et al., 2013; Al-Marzooqi and Wiseman, 2009) as well as the possibility of wide application, high productivity, energy efficiency and high quality of the resulting product (Moritz et al., 2005).

The aim of the study was to compare the nutritive value and the ruminal organic matter digestibility of ground soybean (GS), solvent-extracted soybean meal (SSBM) and extruded defatted soybean (EDSB) using *in vitro* methods.

MATERIAL AND METHODS

Samples of ground soybean (GS), solvent-extracted soybean meal (SSBM) and extruded defatted soybean (EDSB) were analysed for the content of dry matter (ČSN 46 7092-3), crude protein (ČSN EN ISO 5983), crude fibre, NDF, ADF (ČSN EN ISO 6865, ČSN EN ISO 13906 and ČSN EN ISO 16472, respectively), crude fat (ČSN 46 7092-7) and ash (ČSN 46 7092-9) prior the experiment.

The ruminal digestibility was performed *in vitro* using a rumen fluid buffer system. Rumen fluid was collected from a lactating dairy cow fed a diet consisted of 22 kg maize silage, 12 kg lucerne haylage, 3 kg meadow hay, 1.5 kg straw, 6.7 kg supplemental mixture and 0.1 kg mineral supplement (on as fed basis). Rumen fluid was collected 1 h before morning feeding via a flexible, stainless-steel stomach tube, placed in bottles with N_2, and transferred to the laboratory in a heat-stable box. Immediately, pH was measured and the rumen fluid was mixed and filtered through 4 layers of cheesecloth. The fluid was then mixed with a prewarmed buffer (39°C) in a ratio of 1:1. The buffer (pH 6.9) was composed of solutions A and B (A:B = 5:1) with following compositions: solution A = 10.0 g/L KH2PO4, 0.5 g/L MgSO4·7H2O, 0.5 g/L NaCl, 0.1 g/L CaCl2·2H2O, and 0.5 g/L urea; solution B = 15.0 g/L Na2CO3 and 1.0 g/L Na2S·9H2O. The incubation process was conducted in 90-mL (thick-walled) glass tubes containing 40 mL of incubation solution, 0.5 g of the feed sample ground through a 1-mm screen. Each sample was incubated in five replications. Tubes were purged with N_2 before being sealed with rubber stoppers with a Bunsen gas release valve. Then, samples were incubated at 39°C for 24.0 h with gentle manual shaking 3 times per day. After

incubation samples were cooled in ice water to stop the fermentation and filtered. Residues of feed samples were analysed for content of dry matter and ash as mentioned above.

Digestibility of organic matter of each feedstuff was calculated and obtained results were analysed using one-way analysis of variance (Statistica 13).

RESULTS AND DISCUSSION

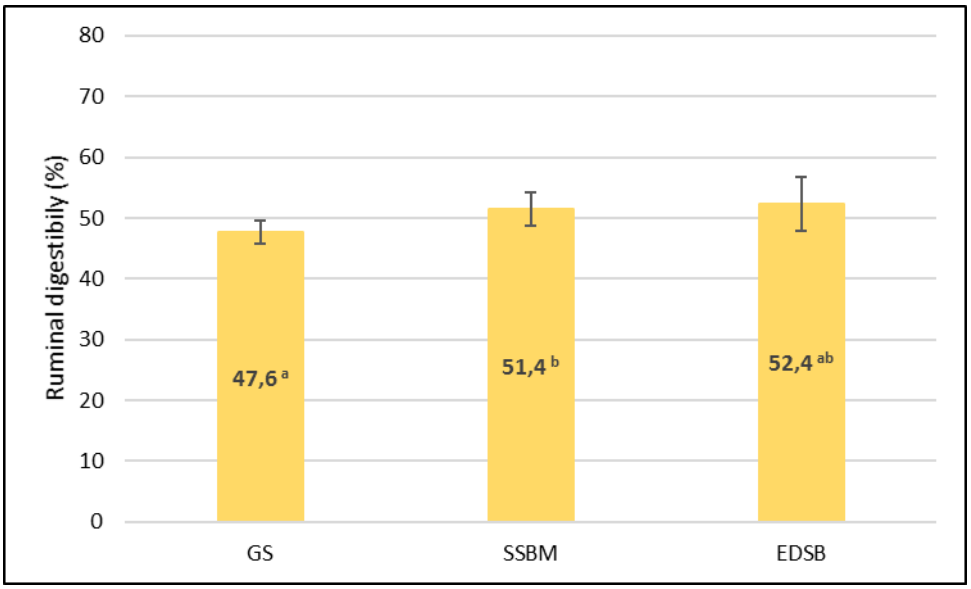
The chemical composition of studied soybean components is presented in Table 1. Crude protein content of GS was lower than in the other two feedstuffs and also slightly lower than average value reported in Feedipedia or in NRC (2001). Crude protein content of SSBM was comparable to published values for SSBM 46% (Sauvant et al., 2004, INRA-CIRAD-AFZ Feed tables, NRC 2001) and was similar to protein content in EDSB. Content of fat in GS corresponded to table values for full-fat raw soybean (Feedipedia, NRC, 2001). Content of fat in SSBM and EDSB was identical and was comparable to soybean meal and extruded soybean meal, respectively (Sauvant et al., 2004, INRA-CIRAD-AFZ Feed tables). The crude fibre, NDF and ADF contents of SSBM and EDSB differed from the values reported by INRA-CIRAD-AFZ Feed tables or by Sauvant et al. (2004). In case of GS, the content of crude fibre was comparable to values in Feedipedia while contents of NDF and ADF were higher. Similarly, differences in the content of NDF and ADF between several raw full-fat soybean samples were observed by Ravindran et al. (2014).

Table 1 Composition of ground soybean (GS) solvent-extracted soybean meal (SSBM) and extruded defatted soybean (EDSB)

Item	Units	GS	SSBM	EDSB
Dry matter	g/kg	906.1	922.6	900.9
Crude protein	g/kg DM	377.0	499.1	495.9
Fat	g/kg DM	205.4	13.4	13.4
Crude fibre	g/kg DM	64.7	42.2	34.6
NDF	g/kg DM	189.1	295.5	227.0
ADF	g/kg DM	83.3	59.6	59.1
Ash	g/kg DM	51.7	71.6	70.8

In vitro organic matter digestibility of studied feedstuffs is shown in Figure 1. Ruminal *in vitro* organic matter digestibility was the lowest in GS (47.6 %) and was lower than in SSBM (51.4 %, P<0.05) and tended to be lower than in EDSB (52.4 %, P=0,057). On the other hand, Doreau et al. (2009) did not find any significant differences in *in vitro* organic matter digestibility of untreated and extruded linseed.

Figure 1 *In vitro* ruminal organic matter digestibility (%) of ground soybean (GS), solvent-extracted soybean meal (SSBM) and extruded defatted soybean (EDSB)



CONCLUSION

Extraction process as well as extrusion of defatted soybean seeds increased *in vitro* ruminal digestibility of organic matter compared to untreated raw soybeans.

ACKNOWLEDGEMENT

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REFERENCES

Al-Marzooqi W., Wiseman J. (2009): Effect of extrusion under controlled temperature and moisture conditions on ileal apparent amino acid and starch digestibility in peas determined with young broilers. Animal Feed Science and Technology, 153, 113–130.

Awawdeh M.S., Titgemeyer E.C., Drouillard J.S., Beyer R.S., Shirley J.E. (2007): Ruminal degradability and lysine bioavailability of soybean meals and effects on performance of dairy cows. Journal of Dairy Science, 90, 4740–4753.

ČSN 46 7092-3 (1999): Metody zkoušení krmiv: Stanovení obsahu vlhkosti. Český normalizační institut. Praha (in Czech).

ČSN 46 7092-7 (1999): Metody zkoušení krmiv: Stanovení obsahu tuku. Český normalizační institut. Praha (in Czech).

ČSN 46 7092-9 (1998): Metody zkoušení krmiv. Stanovení popela. Český normalizační institut. Praha (in Czech).

ČSN EN ISO 13906 (2011): Krmiva - Stanovení obsahu acidodetergentní vlákniny (ADF) a acidodetergentního ligninu (ADL). Český normalizační institut. Praha (in Czech).

ČSN EN ISO 16472 (2012) Krmiva - Stanovení obsahu neutrálně detergentní vlákniny po úpravě vzorku amylázou (aNDF). Český normalizační institut. Praha (in Czech).

ČSN EN ISO 5983 (2012): Krmiva - Stanovení obsahu dusíku a výpočet obsahu hrubého proteinu. Český normalizační institut. Praha (in Czech).

ČSN EN ISO 6865 (2001): Metody zkoušení krmiv - Stanovení obsahu vlákniny - Metoda s mezifiltrací. Český normalizační institut. Praha (in Czech).

Doreau M., Aurousseau E., Martin C. (2009): Effects of linseed lipids fed as rolled seeds, extruded seeds or oil on organic matter and crude protein digestion in cows Animal Feed Science and Technology, 150, 187–196.

Dust J.M., Gajda A.M., Flickinger E.A., Burkhalter T.M., Merchen N.R., Fahey G.C. (2004): Extrusion conditions affect chemical composition and in vitro digestion of select food ingredients. Journal of Agricultural and Food Chemistry., 52: 2989–2996.

Feedipedia. Animal feed resources information system. Available from https://www.feedipedia.org/ (accessed Oct 15, 2019).

Chandler P.T. (1989). Achievement of optimum amino acid balance possible. Feedstuffs, 61, 14–25.

INRA-CIRAD-AFZ Feed tables. Composition and nutritive values of feeds for cattle, sheep, goats, pigs, poultry, rabbits, horses and salmonids. Available from https://www.feedtables.com/ (accessed Oct 15, 2019).

Moritz J.S., Parsons A.S., Buchanan N.P., Calvalcanti W.B., Cramer K.R., Beyer R.S. (2005): Effect of gelatinizing dietary starch through feed processing on zero- to three-week broiler performance and metabolism. Journal of Applied Poultry Research.,14, 47–54.

NRC 2001. Nutrient Requirements of Dairy Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

Ravindran V., Abdollahi M., Bootwalla S. (2014): Nutrient analysis, apparent metabolisable energy and ileal amino acid digestibility of full fat soybean for broilers. Animal Feed Science and Technology, 197, 233–240.

Sauvant D. et al. (eds) (2004): Tables of composition and nutritional value of feed materials. Wageningen Academic Publishers, The Netherlands and INRA, Paris, France.

IDENTIFICATION OF THE CULTIVABLE AUTOCHTHONOUS MICROBIOTA OF THE CANINE DENTAL PLAQUE

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ABSTRACT

The oral microbiota has an important function of protecting against colonization of exogenous bacteria which could cause a systemic disease. On the other hand, the most common oral diseases, such as periodontitis or dental caries, are caused by microorganisms. A deeper microbiological study of this environment is needed to understand the relationships between the members of the microbiota and to identify microorganisms involved in the development of oral diseases. In this study, we focused on identifying autochthonous cultivable bacteria of canine dental plaque. For this purpose the samples were gathered twice with a three month interval between the collections. Dental plaque samples were taken from five dogs of small breeds living in one household. Samples were cultured on blood agar under aerobic and anaerobic conditions. Selected bacterial colonies were identified with help of PCR with universal primers (27F and 1492R) and comparing the sequence of the 16S rRNA gene to the sequences available in the GenBank database using BLASTn analysis. Species *Actinomyces hordeovulneris*, *Corynebacterium canis*, *Neisseria animaloris*, *Neisseria shayeganii*, *Pasteurella canis*, *Porphyromonas gulae*, *Porphyromonas macacae*, and *Schaalia canis* were identified as members of autochthonous microbiota. In dental plaque samples, representatives of genera *Arcobacter*, *Bacteroides*, *Campylobacter*, *Frederiksenia*, *Fusobacterium*, *Globicatella*, *Haemophilus*, *Kingella*, *Lampropedia*, *Leucobacter*, *Ottowia*, *Staphylococcus*, *Stenotrophomonas* and *Streptococcus* were also detected.

Keywords: dog; dental biofilm; oral microbiota; oral bacteria

INTRODUCTION

The oral cavity represents a very suitable environment (temperature, pH, water and nutrients) for the growth of microorganisms (Deo and Deshmukh 2019). It hosts a diverse group of microorganisms, including bacteria, viruses, fungi, and protozoa that colonize teeth, tongue, oral mucosa, hard palate, caries lesions, periodontal pocket, and similarly. The distribution of microorganisms in the oral cavity is not random; most species prefer certain places over others due to the specific local conditions these sites provide, such as the anaerobic environment of the gingival sulcus (Wade 2010; Huang et al. 2011). However, the environment in the oral cavity is also hostile to microbial life, therefore only a certain group of microbes entering it is able to colonize and survive in this environment. Microorganisms must attach to the surface and form biofilms in order to remain there (Marsh 2005). Dental plaque, also called dental biofilm, consists of a large number of bacteria involved in the extracellular matrix of organic polymers produced by bacteria or derived from saliva (Yu et al. 2018). The formation of dental biofilm in the oral cavity is a multi-stage process (Dhir 2013). It can be divided into four main stages: pellicle formation, initial bacterial adhesion, plaque maturation, and, finally, bacterial dispersion (Huang et al. 2011). The representatives of the genera *Neisseria*, *Corynebacterium*, and *Stenotrophomonas* are involved in the formation of canine dental biofilm as primary colonizers. The most common species of the genus *Neisseria* are *N. zoodegmatis*, *N. animaloris*, and *N. weaveri*. The role of secondary colonizers is probably played by the representatives of the genera *Actinomyces*, *Porphyromonas*, *Moraxella*, *Leucobacter*, and the families *Peptostreptococcaceae*, and *Pasteurellaceae*. Species *Actinomyces canis*, and *Porphyromonas gingivicanis* show high levels of biofilm incorporation. The species which featured most frequently in the role of third community member are *Peptostreptococcaceae* sp., *Porphyromonas gingivicanis*, and *Leucobacter* sp. (Holcombe et al. 2014).

MATERIAL AND METHODS

Dental plaque samples were taken from five dogs of small breeds living in one household. There were 2 males (9 and 13 years old) and 3 females (1, 5 and 7 years old). The samples were gathered twice with a three month interval between the collections. The samples were taken from the buccal surfaces of dentes canini and upper premolars with a sterile syringe needle. They were immediately placed in a sterile Eppendorf tube containing 500 µl sterile filtrated phosphate-buffered saline and processed within 2-3 hours after collection. The

Eppendorf tubes were vortexed (20 seconds) and shaked (20 minutes, 400 RPM) for content homogenization. At the first sampling, a volume of 25 µl was inoculated to trypticase soy agar (TSA; Carl Roth GmbH, Germany) containing 5% ram's blood. At the second sampling, plaque samples were serially diluted in phosphate-buffered saline and subsequently volume of 25 µl was inoculated to TSA agar (Carl Roth GmbH, Germany) containing 5% ram's blood. Samples were cultured under aerobic and anaerobic conditions at 37°C. BBL GasPak™ Plus (Becton, Dickinson and Company [BD], Maryland, USA) were used for anaerobic cultivation. After 2 days of aerobic cultivation, and after 3 and 7 days of anaerobic cultivation, individual solitary colonies were subcultured to obtain pure bacterial cultures.

DNA was isolated from solitary colonies using DNAzol direct (Molecular Research Center Inc., Cincinnati, USA) according to the manufacturer's instructions. The 16S ribosomal RNA (rRNA) genes from the isolates were amplified by PCR using primers 27F (5-AGAGTTTGATCMTGGCTCAG-3) and 1492R (5-CGGYTACCTTGTTACGACTT-3). Reaction conditions were: 94°C for 5 min; 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 3 min; and 72°C for 10 min. PCR was performed on thermocycler (TProfesional Basic, Biometra GmbH, Göttingen, Germany), aliquot PCR products were separated by horizontal 3% agarose gel electrophoresis in Tris-acetate-EDTA buffer (pH 7.8) and visualized with GelRed (Biotium, Inc., Hayward, CA, USA) under UV light. The amplification products were sent for purification and sequencing with using primer 1492R (Microsynth, Austria). The obtained chromatograms of sequences of approximately 1,100 bases were validated using Geneious 8.0.5 (Biomatters, Auckland, New Zealand). The 16S rRNA genes sequences were compared with other 16S rRNA gene sequences in GenBank using the NCBI Basic Local Alignment Search Tools, nucleotide (BLASTn) program (http://www.ncbi.nlm.nih.gov/BLAST/). After identification of all isolates, the phylogenetic tree was prepared from alignment of the sequences of individual isolates with a length of 900 bases using the Geneious 8.0.5 program (Biomatters, Auckland, New Zealand).

RESULTS AND DISCUSSION

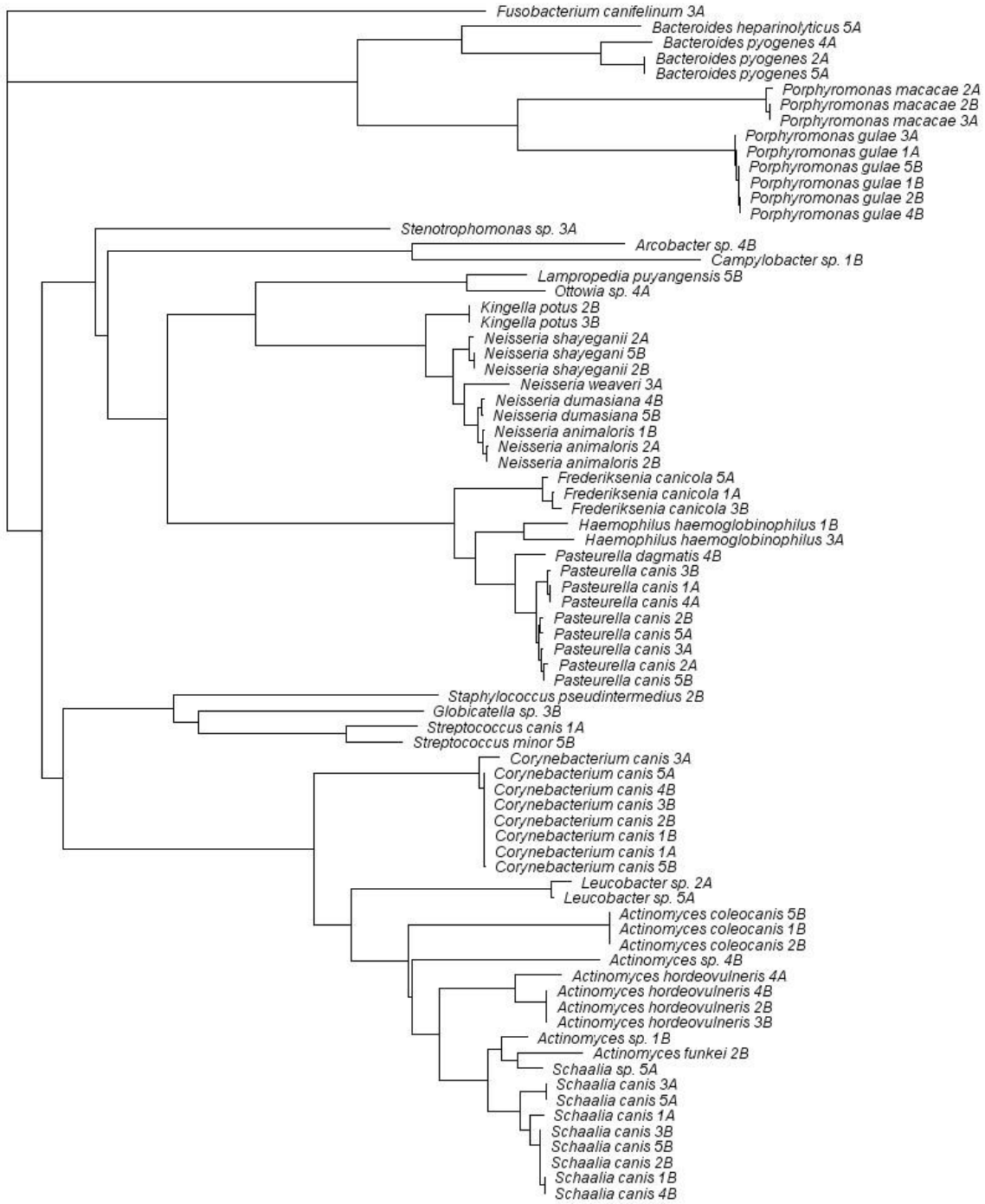
Various species of microorganisms were identified in the samples (table 1). Species *Actinomyces hordeovulneris*, *Corynebacterium canis*, *Neisseria animaloris*, *Neisseria shayeganii*, *Pasteurella canis*, *Porphyromonas gulae*, *Porphyromonas macacae* and *Schaalia canis* were identified in both samplings. Identified species with high quality of chromatogram

are showing by phylogenetic tree (figure 1), that arrange bacteria into the group based on similarity of 16S rRNA gene.

Table 1 Comparison of bacteria identified at the first and second sampling

Dog	First sampling	Second sampling	Both samplings
No. 1	*Corynebacterium canis* *Frederiksenia canicola* *Fusobacterium canifelinum* *Pasteurella canis* *Porphyromonas gulae* *Schaalia canis* *Streptococcus canis*	*Actinomyces* sp. *Actinomyces coleocanis* *Campylobacter* sp. *Corynebacterium canis* *Corynebacterium freiburgense* *Haemophilus haemoglobinophilus* *Neisseria animaloris* *Porphyromonas gulae* *Schaalia canis* *Schaalia cardiffensis*	*Corynebacterium canis* *Porphyromonas gulae* *Schaalia canis*
No. 2	*Bacteroides pyogenes* *Leucobacter* sp. *Neisseria animaloris* *Neisseria shayeganii* *Pasteurella canis* *Porphyromonas macacae*	*Actinomyces coleocanis* *Actinomyces funkei* *Actinomyces hordeovulneris* *Corynebacterium canis* *Kingella potus* *Neisseria animaloris* *Neisseria shayeganii* *Pasteurella canis* *Porphyromonas gulae* *Porphyromonas macacae* *Schaalia canis* *Staphylococcus pseudintermedius*	*Neisseria animaloris* *Neisseria shayeganii* *Pasteurella canis* *Porphyromonas macacae*
No. 3	*Corynebacterium canis* *Fusobacterium canifelinum* *Haemophilus haemoglobinophilus* *Neisseria weaveri* *Pasteurella canis* *Porphyromonas gulae* *Porphyromonas macacae* *Schaalia canis* *Stenotrophomonas* sp.	*Actinomyces hordeovulneris* *Corynebacterium canis* *Frederiksenia canicola* *Globicatella* sp. *Kingella potus* *Pasteurella canis* *Schaalia canis* *Streptococcus minor*	*Corynebacterium canis* *Pasteurella canis* *Schaalia canis*
No. 4	*Actinomyces hordeovulneris* *Bacteroides pyogenes* *Ottowia* sp. *Pasteurella canis*	*Actinomyces* sp. *Actinomyces hordeovulneris* *Arcobacter* sp. *Corynebacterium canis* *Neisseria dumasiana* *Pasteurella dagmatis* *Porphyromonas gulae* *Schaalia canis*	*Actinomyces hordeovulneris*
No. 5	*Bacteroides heparinolyticus* *Bacteroides pyogenes* *Corynebacterium canis* *Frederiksenia canicola* *Leucobacter* sp. *Pasteurella canis* *Schaalia canis* *Schaalia* sp.	*Actinomyces* sp. *Actinomyces coleocanis* *Corynebacterium canis* *Lampropedia puyangensis* *Neisseria animaloris* *Neisseria dumasiana* *Neisseria shayegani* *Pasteurella canis* *Porphyromonas gulae* *Schaalia canis* *Streptococcus minor*	*Corynebacterium canis* *Pasteurella canis* *Schaalia canis*

Figure 1 The phylogenetic tree showing isolates of both samplings



Legend: The phylogenetic tree is based on an alignment of 900 bases. Sequences are identified by their originating species or genus identification. The numbers 1 to 5 indicate individual dogs, A indicates the first sampling and B indicates the second sampling.

The genera most frequently isolated from our samples were *Actinomyces*, *Corynebacterium*, *Neisseria*, *Pasteurella*, *Porphyromonas* and *Schaalia*. In a study Oh et al. (2015), representatives of genera A*ctinomyces*, *Porphyromonas*, *Fusobacterium*, *Neisseria*,

Pasteurella, *Lampropedia*, *Capnocytophaga*, *Frigovirgula*, *Conchiformibius*, *Filifactor*, *Eubacterium*, *Streptococcus*, *Corynebacterium*, and *Derxia* were identified in the canine oral samples. Given that different methods of inoculation were used, whereas the second method proved to be better, it could be that some bacteria were not captured at the first sampling. Nevertheless, the results show that we were able to detect the presence of same bacterial species in the same dogs over time. They can be considered as autochthonous microbiota of canine dental plaque.

CONCLUSION

Based on this study, we can assume that species *Actinomyces hordeovulneris*, *Corynebacterium canis*, *Neisseria animaloris*, *Neisseria shayeganii*, *Pasteurella canis*, *Porphyromonas gulae*, *Porphyromonas macacae* and *Schaalia canis* are members of autochthonous microbiota. Since different microorganisms have been identified in the samples and only a part of the microorganisms of the dental biofilm are cultivable, it is necessary to study biofilm using culture-independent molecular methods such as 16S rRNA gene amplicon sequencing.

ACKNOWLEDGEMENT

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REFERENCES

Deo, P. N. and Deshmukh R. (2019): Oral microbiome: Unveiling the fundamentals. J Oral Maxillofac Pathol 23(1): 122-128.

Dhir, S. (2013): Biofilm and dental implant: The microbial link. J Indian Soc Periodontol 17(1): 5-11.

Holcombe, L. J. et al. (2014): Early canine plaque biofilms: characterization of key bacterial interactions involved in initial colonization of enamel. PLoS One 9(12): e113744.

Huang, R. et al. (2011): Bacterial interactions in dental biofilm. Virulence 2(5): 435-44.

Marsh, P. D. (2005): Dental plaque: biological significance of a biofilm and community life-style. J Clin Periodontol 32 Suppl 6: 7-15.

Oh, C et al. (2015): Comparison of the Oral Microbiomes of Canines and Their Owners Using Next-Generation Sequencing. PLoS One 10(7): e0131468.

Wade, W. G. (2010): New aspects and new concepts of maintaining "microbiological" health. J Dent 38 Suppl 1: S21-5.

Yu, O. Y. et al. (2018): Dental Biofilm and Laboratory Microbial Culture Models for Cariology Research. Dent J (Basel) 5(2).

COMPARISON OF AMINO ACID CONTENT IN GREEN MATTER BY SELECTED WHITE LUPIN VARIETIES GROWN IN THE CZECH REPUBLIC

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ABSTRACT

The purpose of the study was to compare the nutritional value of white lupin green matter (Amiga, Dieta, Zulika) cultivated on the land owned by ŠZP Nový Jičín VFU Brno and on land belonging to ZZN Pelhřimov a.s. The study is focused on variability of white lupin varieties grown in the same localities. Next part of the study is dealing with nutritional value of protein of white lupin green matter compared to other commonly grown green matter. For the samples (age was 15 weeks of growth), assessment of nutritional quality based on amino acid spectrum was accomplished: essential amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine), non-essential amino acids (aspartic acid, serine, glutamic acid, proline, glycine, alanine, tyrosine). By nutritional point of view the variety Dieta has higher content of crude protein, than Amiga and Zulika. The content of crude protein is related to total ($\sum$ AA) amino acids, at Dieta variety was detected statistically higher ($P \leq 0.01$) content of essential amino acids (Thre, Val, Met, Ile, Leu, Lys), than at the Zulika variety. By the results of nutritional analysis is evident that between varieties (Dieta, Amiga, Zulika), and even at the same variety of white lupin, are differences. Considering the content of crude protein, the lupine white matter is very valuable source of protein. The lupine growth is characterized by high production ability and green matter nutritional value is also high, that is comparable with other commonly grown green matter.

Keywords: Amiga; Dieta; Zulika; nutritional quality; amino acid spectrum

INTRODUCTION

White lupin (*Lupinus albus* L.) is one of the 200 species of lupins, a genus of multipurpose annual legumes grown throughout the world both for their seeds used in feed and food, and

for forage. Lupin seeds can be an alternative to soybean in all livestock species due to their high content in good quality protein (in the 30-40 % range) (Jansen, 2006). In the chemical composition of individual varieties of the *Lupinus* genus is a large difference. However, it is important that lupin are characteristic for its high content of proteins. Compared to soy protein, the amino acid composition is characterized with a low content of methionine, cysteine, lysine, threonine, and tryptophane, while the arginine content is significantly higher (Suchý et al., 2006, Stanek et al., 2006). High arginine content is characteristic for lupin protein. White lupin originates from South-Eastern Europe and Western Asia. It was cultivated in Greece, Italy and Egypt and Cyprus 2000 years BCE (Terres Univia, 2017; Clark, 2014). It is mainly cultivated in Northern Europe, Russia, Germany, France and Poland (Clark, 2014; Cowling et al., 1998). The largest producer and exporter of lupin is Western Australia, where mainly lupine is cultivated. In the Czech Republic, lupin is grown on an area of approximately 7,000 ha, but the tendency of sowing areas is relatively variable (Suchý et al., 2006).

White lupin seeds are used to feed livestock and aquaculture species (Jansen, 2006). In addition to seeds of high protein content (Costa and Rezio, 2000), increased attention is currently paid also to the use of the whole plant. The plant may also be grazed during late winter and early spring or cut for forage or silage. As a legume, white lupin plant is used for green manuring (in Southern Europe, it is traditionnally used in vineyards and olive plantations), and for soil improvement (Jansen, 2006). Though white lupin is mainly sown as a sole crop, it can be also grown in association (Arvalis, 2014; Likawent Yeheyis et al., 2010). In in organic systems in Europe, white lupin can be cultivated with oats, barley and triticale (Milleville, 2014). *Lupinus albus* whole plant is a forage known in Europe. Used as only feed, its nutritive value was estimated similar to that of green alfalfa. Effectively dried lupin forage can be used as the main source of fibre similarly or even better than alfalfa meal (Harries et al., 1999).

MATERIAL AND METHODS

The aim of the study was to compare the nutritional value of green matter of selected varieties from the group of white lupins grown in 2016 at the ŠZP Nový Jičín VFU Brno and on land belonging to ZZN Pelhřimov a.s. The Dieta, Amiga and Zulika varieties were used for field cultivation at the locality of the ŠZP Nový Jičín. Each of the selected varieties was grown on an area of 10 ha. The individual varieties were sown in the amount of 2 q/ha for Dieta and

Amiga, in the case of the Zulika variety in the amount of 1.7 q/ha. The growing of the variety Dieta was carried out on an area of 63 ha, on the AGS AGRO České Budějovice plot under ZZN Pelhřimov a.s. in the amount of 1.8 q/ha. The Amiga variety was grown on an area of 36 ha in the locality DZV Nova, a.s. belonging to ZZN Pelhřimov a.s., the sowing rate was 2 q/ha. Sampling of green matter was realized by random selection of 8 samples from an area of 1 m^2 from each variety. Green matter for laboratory analysis was collected in the 15^{th} week of the age of the stand, when the stands were in the stage of fully developed green pods. In the following period, the volume of vegetation did not increase significantly, followed by ripening of green pods and drying of green matter.

The content of individual amino acids was determined using the AAA400 instrument (*INGOS a.s. Praha*) using liquid ion exchange chromatography by photometric detection after post-column derivatization with ninhydrin. The sample was first hydrolysed with HCl to cleave the peptide bonds in the protein chain, followed by pH adjustment of the hydrolysate. The acid hydrolysis was carried out in a thermostat at 110 °C for 24 hours. The sample so treated was dosed into an automatic amino acid analyser. The amino acids in the hydrolysate were separated by ion exchange chromatography and the colour formation reaction of AA with the oxidizing agent ninhydrin was detected. Among the partial amino acids, our attention was focused on: Asp (aspartic acid), Thre (threonine), Ser (serine), Glu (glutamic acid), Pro (proline), Gly (glycine), Ala (alanine), Val (valine), Met (methionine), Ile (isoleucine), Leu (leucine), Tyr (tyrosine), Phe (phenylalanine), His (histidine), Lys (lysine) and Arg (arginine). The statistical significance of the results of our analyses of lupine stands was evaluated using the statistical program Unistat CZ version 5.6 for Excel. The evaluation of the mean values and their differences by subsequent multiple comparisons was performed using the Tukey HSD (Honest Significant Difference) test at a significance level of $P \leq 0.01$ and $P \leq 0.05$.

RESULTS AND DISCUSSION

The assessment of the nutritional value of the green matter protein of selected lupine varieties was performed through the representation of individual amino acids from the ranks of essential and non-essential and subsequent evaluation of inter-variety differences of the amino acid spectrum. The results presented in Tables 1 and 2 show that there is a significant inter-variety variability in lupine stands within the group of white-flowering lupins.

The content of the individual amino acids in the dry matter green matter of the three varieties of white lupine tested from ŠZP Nový Jičín is shown in Table 1. Very interesting is arginine,

which represents the highest proportion of EAA lupine protein. The arginine content of the Zulika variety 6.54 g/kg, compared to the Amiga 15.63 g/kg and Dieta 15.27 g/kg varieties, was the lowest ($P \leq 0.01$). In contrast, the least abundant amino acid in the lupine protein is methionine in all varieties. The variety Dieta showed a statistically significantly highest ($P \leq 0.01$) methionine content of 0.97 g/kg, compared to the variety Amiga 0.83 g/kg and Zulika 0.84 g/kg. The results show that aspartic acid is the most abundant amino acid in the lupine protein in all studied varieties. From the results it is evident that the high Asp content was found in the Dieta variety 44.18 g/kg, which was highly significantly ($P \leq 0.05$) higher than in the Amiga variety 36.93 g/kg. The highest content Asp ($P \leq 0.01$) was in the variety Zulika 48.22 g/kg compared to Amiga. The analysis also included a comparison of the proportion of total amino acids (Σ AA). The results show that the highest content of total amino acids was found in the Dieta variety 191.61 g/kg, which was highly significantly higher ($P \leq 0.01$) compared to the variety Zulika 142.98 g/kg. Highly significant ($P \leq 0.01$) was the Σ AA even in the Amiga variety 175.98 g/kg compared to the variety Zulika.

Santamaría-Fernandez et al. (2017) studied suitable raw materials (green matter) for the production of feeds rich in organic proteins. In this context, for example, alfalfa, the concentration of EAA in dry matter in fresh alfalfa reached Arg 10.5 g/kg, His 5.1 g/kg, Ile 10.0 g/kg, Leu 16.2 g/kg, Lys 13.5 g/kg, Met 3.4 g/kg, Phe 10.8 g/kg, Thr 9.7 g/kg, Val 12.4 g/kg, NeAA represented Ala 11.6 g/kg, Asp 31.1 g/kg, Cys 2.5 g/kg, Glu 20.7 g/kg, Gly 11.0 g/kg, Pro 9.7 g/kg, Ser 11.1 g/kg, Σ AA as essential and non-essential were 189.1 g/kg.

Table 1 Content of essential and non-essential amino acids in green matter by the three white lupine varieties tested (g/kg)

EAA	DIETA	AMIGA	ZULIKA	NeAA	DIETA	AMIGA	ZULIKA
Thre	6.31^{B}	6.05^{B}	4.31^{A}	**Asp**	44.18^{b}	36.93^{A}	48.22^{B}
Val	7.64	7.19	6.84	**Ser**	8.73^{B}	8.44^{B}	6.10^{A}
Met	0.97^{A}	0.83^{B}	0.84^{B}	**Glu**	34.81^{B}	31.47^{B}	15.57^{A}
Ile	7.76^{A}	7.19	6.20^{B}	**Pro**	8.96^{B}	8.44^{b}	5.78^{A}
Leu	13.21^{B}	12.51^{B}	8.62^{A}	**Gly**	7.76^{B}	6.78^{B}	4.84^{A}
Phe	9.08	8.55	9.15	**Ala**	6.90	7.31	7.14

His	4.73^{B}	4.90^{B}	3.27^{A}	**Tyr**	6.30^{B}	6.47^{B}	3.89^{A}
Lys	8.25^{B}	7.30^{B}	5.15^{A}	**Σ AA**	191.61^{B}	175.98^{B}	142.98^{A}
Arg	15.27^{B}	15.63^{B}	6.54^{A}				

Thre threonine, Val valine, Met methionine, Ile isoleucine, Leu leucine, Phe phenylalanine, His histidine, Lys lysine, Arg arginine, Asp aspartic acid, Ser serine, Glu glutamic acid, Pro proline, Gly glycine, Ala alanine, Tyr tyrosine, Σ average content of total amino acids, AB highly significant difference $P \leq 0.01$, Ab significant difference betwwen average values $P \leq 0.05$

Table 2 shows the content of individual amino acids in the dry matter green matter of two studied varieties of white lupine from ZZN Pelhřimov a.s. Highly statistically significant differences were confirmed between varieties and in arginine content. A significantly lower ($P \leq 0.01$) Arg content was found in the Amiga variety 6.64 g/kg compared to the 10.44 g/kg Dieta variety. The Amiga variety showed a statistically significantly higher ($P \leq 0.05$) methionine content of 0.97 g/kg compared to the Dieta variety of 0.90 g/kg. The results of the non-essential amino acid content show that a higher aspartic acid content was found in the variety Dieta 37.88 g/kg, which was highly significantly ($P \leq 0.01$) higher than the variety Amiga 21.73 g/kg. The results show that a higher content of total amino acids was found in the variety Dieta 140.93 g/kg, which was highly significantly higher ($P \leq 0.01$), compared to the variety Amiga 112.56 g/kg.

The green matter of alfalfa was also monitored, when the Σ AA analysed was 138 g/kg in dry matter, the EAA content in fresh matter in g/100g was Arg 5,6, Cys 1,5, His 2,5, Ile 4, 9, Leu 8.7, Lys 6.2, Met 1.9, Phe 5.9, Thr 5.3, Val 6.1 and the NeAA content in fresh matter in g/100g Ala 6.7, Asp 12, 7, Glu 11.6, Gly 5.7, Pro 6.2, Ser 5.1, Tyr 3.6 (Edmunds et al., 2013).

Table 2 Content of essential and non-essential amino acids in green matter in two white lupine varieties tested (g/kg)

EAA	DIETA	AMIGA	NeAA	DIETA	AMIGA
Thre	5.17	4.83	**Asp**	37.88^{A}	21.73^{B}
Val	6.38^{A}	5.31^{B}	**Ser**	6.63^{A}	5.67^{b}
Met	0.90^{b}	0.97^{A}	**Glu**	18.09	16.63

Ile	5.74	5.07	**Pro**	6.66	6.99
Leu	9.23	7.96	**Gly**	5.74	5.18
Phe	7.28^{A}	5.78^{B}	**Ala**	4.37^{B}	6.27^{A}
His	4.27^{A}	3.61^{b}	**Tyr**	4.51^{A}	3.74^{b}
Lys	7.65^{A}	6.17^{b}	**Σ AA**	140.93^{A}	112.56^{B}
Arg	10.44^{A}	6.64^{B}			

Thre threonine, Val valine, Met methionine, Ile isoleucine, Leu leucine, Phe phenylalanine, His histidine, Lys lysine, Arg arginine, Asp aspartic acid, Ser serine, Glu glutamic acid, Pro proline, Gly glycine, Ala alanine, Tyr tyrosine, Σ average content of total amino acids, AB highly significant difference $P \leq 0.01$, Ab significant difference between average values $P \leq 0.05$

CONCLUSION

From the nutritional point of view, the variety Dieta can be evaluated positively, as it contained the most CP in green matter, i.e. Σ AA and especially EAA (Thre, Val, Met, Ile, Leu, Lys). The Amiga variety can be evaluated positively as well. Based on the analysis of nutritional indicators, it can be concluded that between varieties (Amiga, Dieta, Zulika), in the same group of lupines (white lupins), there is considerable variation between varieties even under the same growing conditions. Due to its CP content, lupine green matter is a very valuable bulky protein feed. Lupine stands have a high production potential and represent a nutritionally high quality product comparable to a number of commonly grown fodder plants.

ACKNOWLEDGEMENT

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REFERENCES

The literature sources used are available with the author.

FOCAL INFECTION OF DENTAL ORIGIN. ALTERNATIVE OPTIONS IN REGENERATIVE PROCESSES IN OROFACIAL AREA

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ABSTRACT

Microbiological analysis of oral microbiota is still a challenge the science has to face. Up to this day, we have knowledge of only a portion of microorganisms living in the oral cavity. Their research is very important from the point of view of prevention, diagnostic and treatment of oral and general diseases. Dental caries is the most common chronic disease in the world affecting people regardless of sex, age and ethnic origin, although it affects more the individuals with low social-economic status. Streptococcus mutans was identified as the causative agent of this disease. Presented were also results indicating participation of acidogenic bacteria in the process of its development. These bacteria are generally called cariogenic bacteria. However, no pathogen is the direct and only cause of the development of dental caries or periodontitis. More profound knowledge of microbial composition of the oral biofilm of humans on the surface of teeth or in the subgingival space can help to understand better the complexity of pathogenesis of the development of dental diseases, and find new ways how to affect positively the oral health. The oral cavity is a constantly changing habitat. Traditional methods intended for the studies of diversity of mirobiocenoses are basedon conventional isolation of bacteria by cultivation, their morphology and identification by means of their biochemical properties. These methods do not suffice to ensure concise characterisation and quantification of microbiota, are time demanding, provide results not earlier than after 48 hours and involve only cultivable bacteria. High percentage of bacteria is cultivated only with difficulties due to unknown requirements on their growth. Currently, a number of genetic techniques intended for quantification, identification and characterisation of bacterial communities are available. The study of the external influence on oral cavity microbiocenosis is inevitable due to high incidence and prevalence of dental caries or periodontopathies, despite the current widespread use of oral hygiene preparations. Today's

market offers a multitude of such preparations, and also, alternative approaches for the improvement of oral health are available. Scientific studies presented interesting knowledge about beneficial bacteria capable of inhibiting the growth of pathogenic bacteria by their bioactive products.

The cells and tissues interaction of various embryonal origin, genetic code used in periodontal regeneration with adequate amount of periodontal tissues are significant factors of embryogenesis. Dental tissue renewal caused by focal infection, trauma, disease or non-created tooth germs presents for dentists the basic everyday routine. In praxis it means better tissue regeneration process in more numerous mesenchymal cells amount especially fibroblasts, cementoblasts and osteoblasts. There are successfully used very important bioactive growth factors in periodontal regeneration, which has parallel functions of periodontal tissues during regeneration process. The aim of the study is to analyse periodontium the group of the patients in focus on periodontal processes, to evaluate regeneration process after pathological process in periodontium, to evaluate regeneration process on experimental animals in orofacial region, to evaluate possibilities of tissue engineering in follow-up experiment to optimize standards of using therapeutical methods.

Keywords: regenerative medicine, focal infection of dental origin, periodontal tissues, experimental animals, biomaterials, tissue engineering

INTRODUCTION

Knowledge of the embryonic development of periodontal structures is now successfully used in different therapeutic regenerative methods (Ebrahimi and Botelho, 2017). The basic principle of regeneration is the imitation of the individual conditions during which individual periodontal tissues arise in the process of embryogenesis. The basic principles of embryogenesis are genetic code, sufficient number of cells, the interaction of cells, tissues and extracellular matrix, the presence of paracrine and humoral biologically active substances. Regenerative medicine is a multidisciplinary, rapidly developing area of medicine scientific research and clinical applications, where the goal is correction, replacement or regeneration of cells, tissues, organs with a view to the recovery of their functions, which were damaged due to any cause and cannot be treated with the methods of conventional medicine (Suma et al., 2015). Usage of multiple technological procedures, combines aspects of medicine, cell and molecular biology, biotechnology and tissue engineering. Biomaterials are natural or synthetic

materials which are possible to implant for the purpose of tissue regeneration. Properties of biomaterials that allow to incorporate into the body without adverse response is known as biocompatibility. Cells represent a vital part of tissue engineering. In vivo incorporation of cells into matrices of biomaterials includes the selection of appropriate cells, the methodology of their obtaining, processing and introduction into the final product. It is recommended to use even immature cells in sufficient quantity in the process of growth. In order to periodontium to heal without the inflammation and resorption, it must be on the surface of the root of the tooth to create a sufficient number of young viable cells. The cells are exposed to both mechanical damage to the surrounding structure in the untreated extraction, biomechanical damage to the various pH or the osmotic pressure of the surrounding environment, dehydration (Rahmati et al., 2018). Autologous cell therapy is characterized by the fact that autologous cells are obtained from the patient, cultured and expanded in vitro, then inserted into the damaged places. Self-repair therapy is attractive, it does not require the treatment with immunosuppressants. It requires a surgical intervention, applicable in the tissues of the tolerated intervention and ex vivo cultivation, they are immunological ideal. Allogenic tissues sometimes require treatment with immunosuppressants, however, deproteinized and demineralized tissues, do not require immunosuppressive treatment. Allogenic stem cells have osteoinductive and osteoconductive properties, membrane techniques associated with the controlled tissue regeneration. Mesenchymal stem cells are known as autologin source for tissue regeneration. The discovery of the therapeutic regeneration potential ordinary cells isolated from the tissues of the adult individual there was a large progression (Wu et al., 2014). Biosynthetic materials in the regeneration of periodontium are divided into inorganic materials (hydroxyapatite, beta-TCP), natural biodegradable polymers–collagen-Nio-Gide and Bio-Oss, starch, hyaluronic acid, propolis, fibrinogen, chitosan, synthetic biodegradable polymers–acid polyglycol, polyactolic acid and their copolymers.

MATERIAL AND METHODS

We performed identification of cultivable microbiota representatives from the extracted tooth surface in patients with hard and soft dental tissues. DNA was isolated from solitary colonies using DNAzol direct (Molecular Research Center Inc., Cincinnati, USA) according to the manufacturer's instructions. 16S rRNA genes from isolates were amplified by PCR primers 27F (5-AGAGTTTGATCMTGGCTCAG-3and1492R (5-CGGYTACCTTGTTACGACTT-3) (synthesized by Sigma Aldrich). Cycle conditions were 5 minutes at 94 °C, 1 minute at 94 °C,

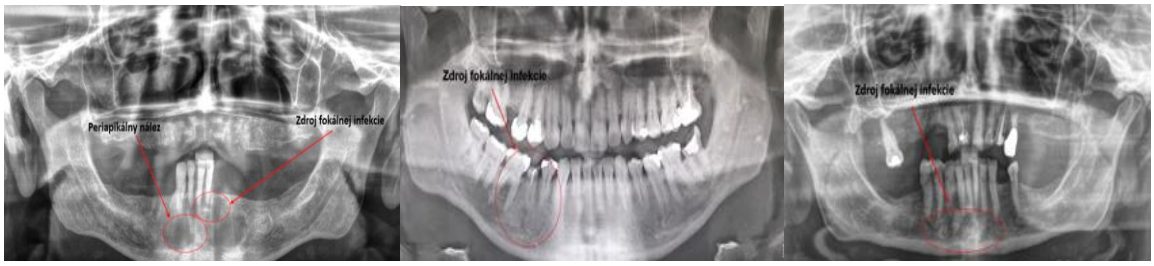
1 minute at 55 °C and 3minutes at 72 °C and 10 minutes at 72°C. PCR was performed on thermocyclers (TProfessional Basic, Biometra GmbH, Göttingen, Germany) PCR products were visualized by gel red biotia UV on 3% TAE. agarose gel electrophoresis The 1400 bp amplification products were purified and sequenced in both directions (Microsynth AG Postfach 58 6961 Wolfurt-Bahnhof Austria), and the cultured bacterial sequences obtained were compared to those in the GenBank database by BLASTn

RESULTS AND DISCUSSION

Inflammations in the periodontium region affect several types of tissues such as parts of the suspension apparatus of teeth, compacta, spongiosis of alveolar bone and root surface cementum. Such changes are collectively referred to as periodontitis. The causes of periapical inflammation may include infections, chemical irritation and acute or chronic trauma. The most frequent cause of the development of periapical focus is necrotic, passively infected tooth pulp in the root canal. This way altered dental pulp contains compound microbiota with predominance of Gram-positive streptococci, but also enterococci, lactobacilli, Candida and Neisseria species and anaerobic bacteria such as Fusobacteria and Bacteroides. Infection causes softening of the dentin wall of the root canal and the metabolic products of microorganisms induce inflammatory conditions in the periodontium region. The most frequent site of the development is the apex of the tooth root, but the inflammation process is observed also in the areas of lateral ramifications or sub-pulpal tooth canal. The inflammation is acute or primarily chronic, or chronic with acute exacerbation.

Genotyping results and x-ray treatment of focal infection

Case No.1 **Case No.2** **Case No.3**

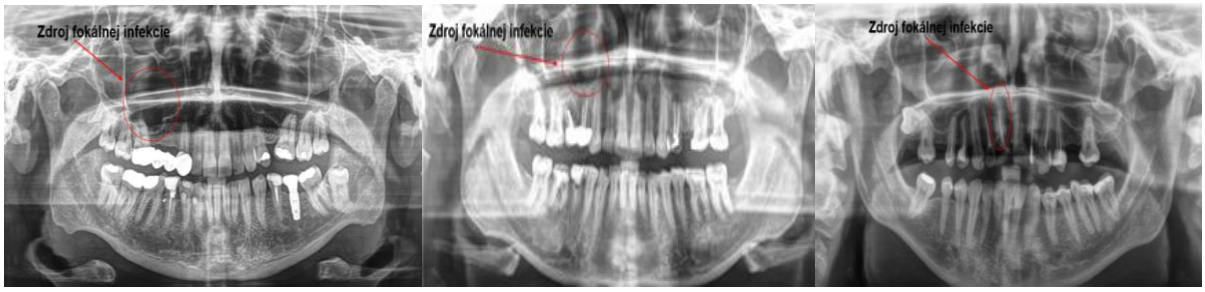


Case No. 1 P1/ 43/3 *Streptococcus anginosus*, P1/43/8/2 *Staphylococcus epidermidis*, P1/43/1B *Streptococcus anginosus*, P1/43/4 *Streptococcus anginosus*, P1/43/5 *Streptococcus anginosus*, P1/43/6 *Staphylococcus epidermidis*, P1/43/1D *Streptococcus anginosus*; P1/43/2 *Staphylococcus epidermidis*; P1/41/1 *Staphylococcus epidermidis*; P1/41/4 *Staphylococcus epidermidis*; P1/41/5 *Staphylococcus epidermidis*; P1/41/6 *Staphylococcus hominis*, P1/41/7 *Staphylococcus epidermidis*

Case No. 2 ALV1/1 *Streptococcus salivarius*, ALV1/1V *Staphylococcus hominis*, ALV1/2/MK *Staphylococcus hominis*, ALV1/2/M *Lactobacillus fermentum*, ALV1/2/Mhem *Lactobacillus fermentum*, ALV1/1 *Streptococcus salivarius*

Case No. 3 MM3 *Streptococcus salivarius*, MM3V *Enterococcus faecalis*, MM5 *Streptococcus parasanguinis*

Case No.4 **Case No.5** **Case No.6**



Case No. 4 H2 *Lactobacillus rhamnosus*, H4 *Staphylococcus hominis*, H3 *Streptococcus* sp., H1D *Lactobacillus rhamnosus*, H2M *Staphylococcus epidermidis*

Case No. 5 G3M *Streptococcus* sp. (*Enterococcus faecium*), G4VM *Staphylococcus epidermidis*

Case No. 6 P1 *Staphylococcus epidermidis*, P3 *Streptococcus anginosus*, P6/1 *Staphylococcus epidermidis*, P4 *Staphylococcus epidermidis*, P6 *Staphylococcus epidermidis*

In our study, we analyzed oral microbiota of various disease states of soft and hard dental tissues that are the source of focal infection. Clinical findings are shown on individual X-ray images. During X-ray examination we examined the condition of apical periodontal tissues, periapical lesions, their size and shape. In our study, most of the isolates identified belong to the genera Streptococcus and Stafylococcus. To a lesser extent, the genera Lactobacillus and Enterococcus are represented.

CONCLUSION

The future of molecular dental medicine should include the collection of patient's stem cells, or the preparation of their population under laboratory conditions. This is a way to overcome the problem of current therapeutic interventions in conflict resolution. Replacement of bone loss as a result of diseases, trauma or congenital anomalies is a fundamental problem in maxillofacial surgery, orthopedics and traumatology. The limited amount of autologous graft tissue and the problems associated with the use of allografts and xenografts call scientists and clinicians to use tissue engineering techniques to regenerate bone tissue.

ACKNOWLEDGEMENT

Vega 1/0788/19 Study of changes in microflora of dental biofilms in humans and dogs to harmonize oral microbiocoenoses using selected oral probiotics

REFERENCES

Ebrahimi M., Botelho M. (2017): Adult Stem Cells of Orofacial Origin: Current Knowledge and Limitation and Future Trend in Regenerative Medicine. Journal of Tissue Engineering and Regenerative Medicine, 14,6, 719-733.

Suma G. N., Arora M. P., Lakhanpal M. (2015): Stem cell therapy: A novel treatment approach for oral mucosal lesions. Journal of Pharmacy and Bioallied Sciences, 7, 1, 2-8.

Rahmati M., Pennisi C. P., Budd E., Mobasheri A., Mozafari M. (2018): Biomaterials for

Regenerative Medicine: Historical Perspectives and Current Trends. Cell Biology and Translational Medicine, 4, 1-19.

Wu S. M., Chiu H. C., Chin Y. T., Lin H. Y., Chiang C. Y., Tu H. P. (2014): Effects of enamel matrix derivative on the proliferation and osteogenic differentiation of human gingival

mesenchymal stem cells. Stem Cell Research & Therapy, 5, 52.

PROXIMODISTAL ALIGNMENT OF THE CANINE PATELLA IN SMALL BREEDS OF DOGS

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ABSTRACT

Proximo-distal change in position of patella is one of the supposed factors contributing to the pathogenesis and postoperative recurrence of patellar luxation. Among the radiographic techniques described to evaluate the proximodistal alignment of the patella is Insall-Salvati index and modified Insall-Salvati index. The purpose of the present study was to determine whether patellar luxation grade in small dog breeds has influence on the IS and mIS indexes and if the length of PLL is increasing with higher grade of patellar luxation as it is increasing in large breed dogs. The proximo-distal position of the patella was not different in our sample of small dog breeds, measured by IS index and neither was PLL. Values measured by mIS index are significantly lower in 3th grade of patellar luxation compared to the control and 2th grade of patellar luxation group, with statistical significant difference values of patellar ligament length in 3th grade of patellar luxation compared with control group. Based on the results of this study the length of patellar ligament probably does not play a role in the pathophysiology of medial patellar luxation in small breed dogs.

Keywords: dog, patellar luxation, proximo-distal position, *patella alta, patella baja*

INTRODUCTION

Proximo-distal change in position of patella is one of the supposed factors contributing to the pathogenesis and postoperative recurrence of patellar luxation, especially in large breed dogs. Pathological changes in the vertical patellar position can be proximal- *patela alta* or distal- *patella baja*.

Patella alta is defined as the proximal displacement of the patella within the femoral trochlear groove (Johnson et al.,2002). In large breed dogs *patella alta* is associated with medial

patellar luxation (MPL) (Mostafa et al.,2008). If the patellofemoral articulation moves completely proximal to the femoral trochlear groove during stifle extension, the buttressing effects of the trochlear ridges would be lost, resulting in an increased risk of luxation as the patella begins to move distally within the femoral trochlear groove during stifle flexion (Johnson et al., 2006).

Patella baja, also known as *patella infera*, is an abnormal distal position of the patella within the femoral trochlear groove (Noyes et al., 1991). Patella baja is much less common in dogs and has rarely been documented in veterinary literature. It develops as a developmental defect or is most often the result of premature closure of the tuberositas tibiae growth plate, secondary to the therapy of the Salter-Harris avulsion fracture of tuberositas tibiae using tension cerclage (Griffon, 2011).

Among the radiographic techniques described to evaluate the proximodistal alignment of the patella in humans, Insall- Salvatti index (IS) provides the most applicable and convenient method of diagnosing *patella alta* in humans. Insall's index has been modified (mIS) to overcome the artefact created by long distal patellar facets that artificially increase the ratio and may mask the presence of *patella alta*. In spite of this modification, the clinical utility of Insall's index and Insall's modified index remains limited by the difficulty in precisely determining the point of insertion of the patellar ligament on the tibial tuberosity (David-Chausse´ J, Vignes L, 1982).

Mostafa and colleagues evaluated the L: P ratio in medium to large breed dogs using a mIS. An L: P ratio greater than 2.06 was indicative of *patella alta*. These authors concluded that medial patellar luxation is associated with a relatively long patellar ligament in medium to giant breed dogs (Mostafa et al., 2008). Another study defined vertical patellar position, by IS index, in large-breed dogs with clinically normal stifles and compare the L: P of large-breed dogs with medial patellar luxation (MPL). They conclude that large-breed dogs with MPL had a significantly more proximal vertical patellar position compared with large-breed dogs with clinically normal stifles. Large-breed dogs with L: P values >1.97 were considered to have *patella alta* (Johnston et al., 2006). The findings of these studies suggest that proximal displacement of the patella in medium, large, and giant breed dogs with *patella alta* may create a patella-femoral articulation that extends proximal to the femoral trochlear groove during extension of the stifle, resulting in a loss of the buttressing effects of the proximal end of the trochlear ridges (Johnston et al., 2006; Mostafa et al., 2008). Three studies have evaluated the L: P ratio in small-breed dogs with medial patellar luxation. Neither study found

a significant difference in L: P ratio (Towle et al., 2005; Mortari et al., 2009, Wangdee et al. 2015).

The purpose of the present study was to determine whether the IS and mIS indexes are influenced by patellar luxation grade in small dog breeds and if the length of PLL is increasing with higher grade of patellar luxation as it is increasing in large breed dogs.

MATERIALS AND METHODS

Medical and radiographic records of small breed dogs admitted between January 2017 and September 2019 were retrieved. Information retrieved was breed, age, weight, type of luxation and preoperative radiographs. Inclusion criteria for the patellar luxation group were: small breed dog (<10kg), skeletally mature (≥1 year of age), documented non-traumatic patellar luxation in at least 1 stifle, no detectable evidence or knowledge of previous stifle surgery and presence of a good quality straight lateral radiograph of that stifle. The case definition for a diagnosis of patellar luxation did not require a radiograph and was based on a history of intermittent pelvic limb lameness and the presence of a grade I–IV patellar luxation on orthopaedic examination of the affected pelvic limb. The control group consisted of dogs with no history of stifle disease and good quality straight lateral radiograph of that stifle radiographs. Based on orthopaedic examination dogs were divided into the following groups: dogs without patellar luxation- control group, dogs with grade two patellar luxation and dogs with grade three patellar luxation. Dogs with grade one patellar luxation were not included because of their small number. Dogs with grade fourth patellar luxation were not included either, because of superimposition of patella with femur and with difficulties of its edges detection. All dogs were sedated (medetomidine 0,015-0,02mg/kg i.v and butorphanol 0.2 mg/kg i.v.) for radiographic examination. Positioning was judged as satisfactory if both femoral condyles were superimposed on lateral projections.

IS index was obtained by dividing the greatest diagonal length of the patella by the length of the patellar ligament to calculate a ratio (Fig. 1) (Insall and Salvati, 1971). MIS index was obtained by dividing the distance between the inferior aspect of the articular surface of the patella and the insertion of the patellar ligament by the length of the patellar articular surface (Fig.2) (David- Chausse´ J, Vignes L, 1982).

All data were included into a spreadsheet program (Microsoft Office Excel 2016) and imported into statistical software (GraphPad Prism 8) for analysis. Student's t-test was used to compare values between each group of dogs obtained by IS and mIS, separately. ANOVA

were used to compare PLL values between each group of patellar luxation obtained by IS and mIS. Significance was set at $P < 0.05$.

Fig 1. Mediolateral radiograph of canine stifle illustrating the measurements to determine PL- red line and PLL- green line for IS index.

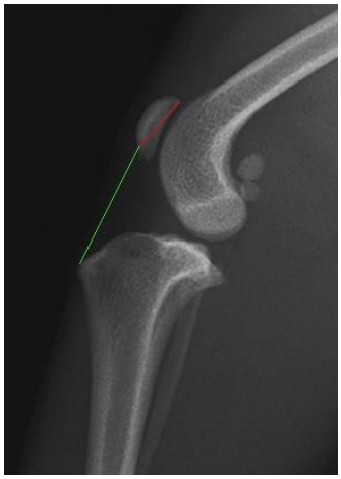
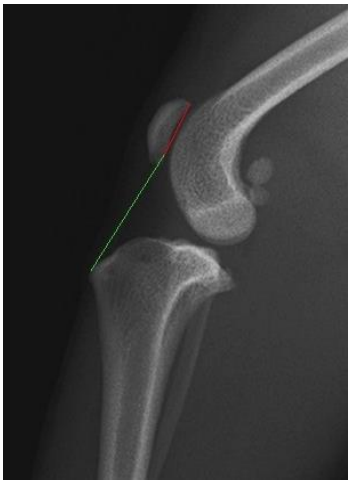


Fig 2 Mediolateral radiograph of canine stifle illustrating the measurements to determine PL- red line and PLL- green line for mIS.



RESULTS

Eighty three limbs and radiographs met the criteria for inclusion. Twenty limbs were identified that fit the inclusion criteria for the control group. Fifty two limbs were in-group of second grade of patellar luxation and eleven limbs were in group of third grade of patellar luxation. Breeds were: Yorkshire terrier (34), Maltese (7), Chihuahua (6), and Jack Russel Terrier (4). Body weight and age did not differ significantly between 3 groups of dogs studied (control, 2th grade of luxation, 3th grade of luxation). Mean body weight was 4,5 kg (2,4-6,6kg) and age was between 10 to 96 months.

IS index

Results are reported in Table 1. There was no statistically significant (P>0,05) difference between the values of individual groups of dogs obtained by IS index. The average value of

IS index in the control group was 2,02 mm (1,76- 2,53mm); the average value of the IS index in the 2th grade of patellar luxation group was 2,01mm (1,61- 2,66mm) and the average value of the IS index in the 3th grade of patellar luxation group was 1,95mm (1,55-2,34mm). The average value of PLL in control group was 17,23mm (9,9- 21,6mm); in group of 2th grade patellar luxation average value was 16,66 (11- 24,6mm); in group of 3th grade patellar luxation average value was 15,65mm (12,1-20,1mm). There was no statistical significance (P> 0,05) between the values of PLL between groups of dogs.

mIS

Results are reported in Table 1. There was statistical significance (P < 0,05) between the values of control group and 3th grade of patellar luxation (P =0,002) and between 2th and 3th grade of patellar luxation (P=0,0004) obtained by mIS. The average value of mIS index in the control group was 2,57mm (2,2- 3,06mm); the average value of mIS index in the 2th grade of patellar luxation group was 2,56mm (2,09- 3,24mm); the average value of mIS index in the 3th grade of patellar luxation group was 2,21mm (1,68- 2,52mm). The average value of PLL in control group was 18,63mm (14,3-23mm); in group of 2th grade patellar luxation average value was 18,05mm (14,1- 27,8mm); in group of 3th grade patellar luxation average value was 16,36mm (12,8- 21,28mm). Between the control group and 3th stage of luxation was significant difference in PLL (P=0,03), but there was no difference between the control group and 2th grade of patellar luxation and either was between 2th and 3th grade of patellar luxation.

Table 1

Mean values of IS measurements in mm.				**Mean values of mIS measurements in mm.**			
	PL	PLL	IS		PL	PLL	mIS
Control group	8,53	17,23	2,02	Control group	7,2	18,66	2,57
2th grade patellar luxation	8,3	16,66	2,01	2th grade patellar luxation	7,1	18,05	2,56
3th grade patellar luxation	8,09	15,65	1,95	3th grade patellar luxation	7,4	16,36	2,21

DISCUSSION

Insall's index has been modified to overcome the artefact created by long distal patellar facets that artificially increase the ratio and may mask the presence of *patella alta*. The purpose of the present study was to determine whether patellar luxation grade in small dog breeds has influence on the IS and mIS indexes.

In our study, IS index in control group was not significantly different from patellar luxation groups. This finding was consistent with earlier findings for small breed dogs with medial patellar luxation (Towle et al., 2005; Mortari et al., 2009). In contrary, Johnson and colleagues reported that large-breed dogs with MPL had a significantly more proximal vertical patellar position (1,87 ±0,025) compared with large-breed dogs with clinically normal stifles (1,71±0,020) (Johnston et al., 2006).

The values obtained by mIS in control and 2th grade patellar luxation group were significantly higher than 3th grade of patellar luxation group, but there was no significant difference between the control and 2th grade patellar luxation group. Wangdee et al., conclude that a proximo-distal patellar position is not associated with medial patellar luxation in Pomeranians, Chihuahuas, and Toy or Standard Poodles (Wangdee et al., 2015). In contrary, previously described results from medium to large breed dogs reported that the mIS of clinically normal stifle joints was lower than in dogs with medial patellar luxation (Mostafa et al.,2008).

Our results of PLL obtained by IS and mIS did not show significant statistical difference, but there is trend of decreasing values depending on the increasing degree of patellar luxation. In contrast, another study in medium to large breed dogs reported that MPL is associated with a relatively long patellar ligament compared to the control groups (Mostafa et al., 2008). On the basis of these findings, unlike large-breed dogs, medial patellar luxation does not appear to be associated with longer PLL and more proximal patellar position in small breed dogs, what corresponds with the results of another study (Wangdee et al. 2015).

The major limitation of our study is that the measurements were made by only one observer and were not repeated. Another limitation was the low number of dogs and the uneven number of dogs between groups.

CONCLUSION

The proximo-distal position of the patella was not different in our sample of small dog breeds, measured by IS index and neither was PLL. Values measured by mIS index are significantly lower in 3th grade of patellar luxation compared to the control and 2th grade of patellar luxation group. The length of patellar ligament probably does not play a role in the pathophysiology of medial patellar luxation in small breed dogs. In fact, the pathophysiology of patellar luxation may be influenced not only by breed and size, but also by age and musculoskeletal abnormalities, including coxofemoral and tibiotarsal joints, and the presence of muscle and soft tissue adjacent to the stifle joint. These factors explain various

abnormalities seen in dogs with patellar luxation. Study with larger number of dogs and a more balanced number of dogs in groups is essential.

ACKNOWLEDGEMENT

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REFFERENCES

David- Chausse´ J, Vignes L: Critical study of patella alta. II. Patella alta and femoropatellar pathology. Rev Rhum Mal Osteoartic 49:507–513, 1982

Griffon DJ. Assessment and correction of Patellar Alta/Baja. In ESVOT Seminar on patellar luxation: proceedings. Lyon, 2011, p: 19-22.

Insall J, Salvati E. Patellar position in the normal knee joint. In Diagnostic Radiol, 1971, 101, p: 101-104.

Johnson AL, Broaddus KD, Hauptman JG, et al: Vertical patellar position in large-breed dogs with clinically normal stifles and large-breed dogs with medial patellar luxation. Vet Surg 35:78–81, 2006

Johnson AL, Probst CW, DeCamp CE, et al: Vertical position of the patella in the stifle joint of clinically normal large-breed dogs. Am J Vet Res 2002, 63:42–46.

Mortari AC, Rahal SC, Vulcano LC, et al. Use of radiographic measurements in the evaluation of dogs with medial patellar luxation. Can Vet J 2009; 50: 1064–1068

Mostafa AA, Griffon DJ, Thomas MW, et al. Proximodistal alignment of the canine patella: radiographic evaluation and association with medial and lateral patellar luxation. Vet Surg 2008; 7: 201–211.

Noyes FR, Wojtys EM, Marshall MT. The early diagnosis and treatment of developmental patella infera syndrome. In Clin Orthop Relat Res, 1991, 265, p: 241-252

Simmons E, Cameron JC. Patella alta and recurrent dislocation of the patella. In Clin Orthop Rel Res, 1992, 274, p: 265-269

Towle HA, Griffon DJ, Thomas MW, et al. Pre- and postoperative radiographic and computed tomographic evaluation of dogs with medial patellar luxation. Vet Surg 2005; 4: 265–272.

Wangdee C., Theyse L.F.H., Hazewinkel H.A.W., Proximo- distal patellar position in three small dog breeds with medial patellar luxation. Vet Comp Orthop Traumatol 2015; 4; 270-273.

PARTICLE SIZE EVALUATION IN HENS DIET AND FEED RESIDUES

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ABSTRACT

A total of 16 Bovans Brown hens were included into the experiment. The trial was performed from the age of 75 weeks to 79 weeks of hens age. The diets were fed in non-pelleted form. The experimental feed mixture (Coarse mixture) contain a coarser particles. The control feed mixture (Fine mixture) contain a finer particles. The weight of the hens and the feed consumption were not significant different during the experiment. A statistically significant difference was found when comparing the fine feed residues with the fine feed mixture. The comparison of the coarse feed mixture and the coarse feed residues showed a uniform loss. Reducing the proportion of fine particles of compound feeds significantly affects dustiness, loss of feed, as well as animal health, as numerous studies document the negative impact of high proportions of fine particles on the digestive tract.

Keywords: sieve analysis; hammer mill; roller mill; poultry nutrition

INTRODUCTION

Sieve analysis is used in feed analysis and studies physiology of digestive system with various approaches to describe an mean value of particle size which can serve to compare different samples (Fritz et al., 2011). Feeds or digesta are not composed of particles of a uniform size but represent conglomerates of differently sized particles. The analysis of the particle size distribution of such substrates is usually performed by sieve analysis, in which the sample is fractionated by a cascade of sieves of different mesh sizes – using typically dry samples when analysing feeds (Ehle, 1984; Giger-Reverdin, 2000) and a wet sieving technique when analysing digesta or faeces (Udén and Van Soest, 1982). The particle size distribution is then determined by the weight of the residues on the individual sieves (Fritz et al., 2011).

A compound feed with a higher proportion of smaller particle fractions (< 0.5 mm) led to a reduction in feed intake, increased feed selection, resulting in greater feed waste and increased dust pollution; the result may also be malnutrition of animals (Safaa et al., 2009). Svihus (2011) recommends the inclusion of 20–30% of cereal particles larger than 1 mm in compound feed for poultry. Many literatures suggest that in the manufacture of compound feed for animals, a certain uniformity of the components should be maintained to ensure a homogeneous compound feed. Most importantly, in the manufacture of such a compound feed, it has the highest proportion of intermediate particle fractions (from about > 0.5 mm to > 1.4 mm). The optimal distribution of the individual fractions of the particles in the compound feed will ensure an optimal course of peristalsis, digestibility and nutrient utilization throughout the animal digestion process, and will have a positive effect on the health and performance of the whole organism. In addition, a compound feed with a low proportion of fine (i.e. dusty) particles will not jeopardize production and human and animal health.

The aim of the study was to determine whether different particle size of the feed mixture affect the feed consumption of hens.

MATERIAL AND METHODS

A total of 16 Bovans Brown hens were included into the experiment. The trial was performed from the age of 75 weeks to 79 weeks of hens age. Hens were divided into two groups per 8 hens with 4 repetitions. The diets were fed in non-pelleted form. The experimental feed mixture (Coarse mixture) contain a coarser particles (wheat and corn were crushed by a roller mill). The control feed mixture (Fine mixture) contain a finer particles (wheat and corn was crushed with a hammer mill with 3 mm screen). The experiment was divided into preparatory and experimental periods. The preparation period lasted 14 days and the experiment period lasted 21 days. The nutrient composition of diets corresponds to the recommended nutrient requirements of the relevant category according to the recommended nutrient content in feed mixtures for poultry (Zelenka *et al*., 2007). Table 1 shows composition and nutrient content of used diets. The chemical composition of nutrient content of diets were determined for dry matter, crude protein, ether extract, crude fibre, and ash according to Commission Regulation (EC; Commission Regulation, 152/2009). The room temperature, relative humidity and light mode were controlled. The light regime was set to 18 hours of light and 6 hours of darkness with gradual dimming and flashing. The experimental animals were regularly monitored for

health and deaths were recorded. Hens had an *ad libitum* access to feed and water. The feed was weighted daily. The feed residue was collected and weighted daily as well to calculate total feed consumption. The animals were weighed every week in the same time.

Table 1 Composition and nutrients content of the experimental diets for laying hens.

Components (g/kg)	Fine mixture (control)	Coarse mixture (experiment)
Maize	333	333
Wheat	330	330
Soybean meal	193.8	193.8
Limestone	74.1	74.1
Rapeseed oil	31.7	31.7
Vitamin-mineral premix[1]	30	30
Monocalcium phosphate	5	5
L-Lysine	1.4	1.4
DL-Methionine	1	1
Analysed composition (per kg)		
AME_N (MJ)[2]	11.45	11.45
Dry matter (g)	880	880
Crude protein (g)	161.2	164.1
Ether extract (g)	44.6	44.7
Crude fiber (g)	18.0	18.4
Ash (g)	117.2	124.8
Calcium (g)	35.6	33.6
Total phosphorus (g)	62.2	65.4

[1]Vitamin-mineral premix per kg diet: 0.39 g lysine; 1.35 g methionine; 8.85 g Ca; 2.01 g P; 1.38 g Na; 9.00 mg Cu; 54.00 mg Zn; 60 mg Fe; 72.00 mg Mn; 0.9 mg I; 0.24 mg Se; 9,900 IU vitamin A; 3,000 IU vitamin D_3; 15.00 mg vitamin E; 1.2 mg B_1; 3.6 mg B_2; 1.62 mg B_6; 12.00 mg B_{12}; 0.09 mg biotin; 0.9 mg folic acid; 12.6 mg niacinamide; 7.5 mg calcium

pantothenate; 180 mg choline chloride; 0.3 mg butylhydroxyanisole; 1.5 mg butylhydroxytoluene; 3 mg etoxyquin.

[2]AME_N – apparent metabolizable energy (calculated value).

Sieve analysis

The feed mixtures (n=7) and feed residues (n=24) were analyzed with a Retsch AS 200 Control sieve set for 10 minutes with amplitude 1.8 mm/g. All samples were sieved over a cascade of 5 sieves with square holes (sieve 1 = 3.0 mm, sieve 2 = 2.0 mm, sieve 3 = 1.5 mm, 4 = 1.0 mm, 5 = 0.3 mm) and particles passing the finest sieve (0.3 mm) were weighted as well (pan = 0 mm).

Statistical analysis

Data were processed by Microsoft Excel (USA) and StatSoft Statistica version 12.0 (USA). The basic statistical characteristics of the set of values (means and standard errors) were calculated from the results of the individual groups. One-way analysis of variance (ANOVA) was used. To ensure evidential differences Scheffe's test was applied and $P < 0.05$ was regarded as statistically significant difference.

RESULTS AND DISCUSSION

The weight of the hens was not significant different during the experiment. Hens receiving fine mixture achieved an average feed consumption of 115 g per hen and day, and hens receiving coarse mixture reached 122 g per hen and day. These differences were not statistically significant. Table 2 shows the particle size distribution of the feed mixtures and the respective feed residues.

The sum of proportion of fine particles in the fine feed mixture was 65% compared to 32% in coarse feed mixture. The sum of proportion of intermediate particles in the fine feed mixture was 31% compared to 23% in coarse feed mixture. The sum of proportion of coarse particles in the fine feed mixture was 5% compared to 45% in coarse feed mixture. The fine feed mixture contains more fine particles (about 33%) than coarse feed mixture. Coarse feed mixture contained more coarse particles (about 40%) than fine feed mixture.

A statistically significant difference was found when comparing the fine feed residues with the fine feed mixture. On the 0.3 mm sieve was found significantly more feed residues than

contained fine feed mixture on sieve 0.3 mm (Table 2). This means that the animals ate more coarse particles of the feed and more dust particles remained in the feeder with fine feed mixture feeding.

Table 2 Particle Size Distribution of Fine and Coarse Feed Mixture and Fine and Coarse Feed Residues (mean±standard error)

	Sieve Size (mm)	Sieve Size Interval (mm)	Part of Interval (%)			
			Fine feed mixture	Coarse feed mixture	Fine feed residues	Coarse feed residues
Fine particles	0	0–0.3	13±1.05	9±0.49	10±0.54	9±1.31
	0.3	0.31–1.0	52±0.64[a]	23±0.51	60±0.94[b]	22±0.68
Intermediate particles	1.0	1.01–1.5	23±0.47	12±0.13	21±0.55	12±0.30
	1.5	1.51–2.0	8±0.16	11±0.05	6±0.38	11±0.39
Coarse particles	2.0	2.01–3.0	4±0.15	27±0.38	2±0.26	27±0.91
	3.0	> 3	1±0.09	18±0.47	0±0.03	18±0.37

[a, b] – different letters means statistically significant different ($P < 0.05$)

The comparison of the coarse feed mixture and the coarse feed residues showed a uniform loss. This means that the hens received the coarse feed mixture uniformly, rather than picking different sized particles.

In ration (containing in dominant proportions corn, wheat and soybean meal) for fattened chickens, a spectrum of six different particle sizes from < 0.6 mm to > 2.36 mm was analysed for crude protein, calcium and phosphorus content. The authors found that a diet with a higher proportion of larger particles retained more nitrogen and less calcium and phosphorus than a diet with a higher proportion of finer particles (Portella et al., 1988).

CONCLUSION

The size of the particles and their shape fundamentally influence the evaluated physical quality parameters of feed, which are essential for transport, packaging, storage and feeding technique. Reducing the proportion of fine particles of compound feeds significantly affects

dustiness, loss of feed, as well as animal health, as numerous studies document the negative impact of high proportions of fine particles on the digestive tract.

REFERENCES

Ehle F.R. (1984): Influence of particle size on determination of fibrous feed components. Journal of Dairy Science, 67, 1482–1488.

EUROPEAN COMMISSION. 2009. Laying down the methods of sampling and analysis for the official control of feed. Commission regulation (EC) 152/2009. Brussels: The commission of the European Communities.

Giger-Reverdin S. (2000): Characterisation of feedstuffs for ruminants using some physical parameters. Animal Feed Science and Technology, 86, 53–69.

Portella F.J., Caston L.J., Leeson S. (1988): Apparent feed particle size preference by broilers. Canadian Journal of Animal Science, 68, 923–930.

Safaa H.M., Jimenez-Moreno E., Valencia D.G., Frikha M., Serrano M.P., Matheos G.G. (2009): Effect of main cereal of the diet and particle size of the cereal on productive performance and egg quality of brown egg-laying hens in early phase of production. Poultry Science, 88, 608–614.

Svihus B. (2011): The gizzard: function, influence of diet structure and effects on nutrient availability. World's Poultry Science Journal, 67, 207–224.

Udén P., Van Soest P.J. (1982): The determination of digesta particle size in some herbivores. Animal Feed Science and Technology, 7, 35–44.

Zelenka J., Heger J., Zeman L. (2007): Recommended nutrient content in feed mixtures and nutritional value of feed for poultry [in Czech: Doporučený obsah živin v krmných směsích a výživná hodnota krmiv pro drůbež]. Brno, MZLU.

THE APPLYING QUANTUM THERAPY ON WOUND HEALING IN ONCO PATIENTS IN THE POST - OPERATIVE PERIOD

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ABSTRACT

Quantum Therapy (RIKTA) is a non-invasive method that is based on the photophysical and photochemical response of tissues after light absorption. In our study, we used quantum therapy in 10 bitches with a mammary gland tumor in the post-operative period of 4096, 512 and 64 Hz for 5 minutes. The general effect of quantum therapy is to activate non-specific immunity.

Keywords: dogs; mammary tumours; wound healing; quantum therapy; RIKTA

INTRODUCTION

A wound is an open injury in the skin. Wound heal within a few weeks in general, however, the healing process may delay due to circulation problems, diabetes, collagen, or autoimmune diseases. Wound healing is a highly complex process involves a set of overlapping coordinated processes. The wound healing process can be divided into four major phases:

(1) Coagulation or hemostasis: immediately after the injury the initial response is hemostasis, which results in clot formation due to the action of platelets, endothelial cells, fibrin, and fibronectin which act to block or, at least, decrease the bleeding, and secretion of kinins and other factors.

(2) Inflammation: the inflammation phase begins shortly after the injury, as the kinins and other factors attract neutrophils, lymphocytes, and macrophages. This phase is responsible for clear cellular debris, and control wound contamination and infection.

(3) Migration, proliferation, and angiogenesis: cells needed for wound closure multiply or move into the wound site for several days. This phase is characterized by formation of granulation tissue, capillary and blood vessels proliferate.

(4) Remodeling: scar formation may last for week or months.

Wound healing may be accelerated by low-level laser therapy, however, the therapeutic effects may vary between individuals due to types, sizes, and causes of wounds (Hamblin et al. 2016).

Quantum therapy is defined as a therapy in which light energy is absorbed by the intended part of the body and the temperature is not increased more than 1°C (Bodnár et al. 2011). Low-energy quantum laser therapy is known for its beneficial wound care to promote tissue healing and pain relief. Research on low-level laser therapy assess the estimated effects of this kind of therapy on healing by using experimental wound model in humans; the results have been measured by wound contraction. The research shows that treated wounds contracted more than the untreated wounds and also healed at a faster rate. The exact mechanism by which low-level laser therapy promote wound healing is not fully known, however, several theoretic may help to explain the succeeding of wound contraction achieved by this kind of therapy and include increased fibroblast proliferation which may facilitate fibroplasia during the repair phase of tissue healing; transformation of fibroblasts into myofibroblasts which are directly involved in granulation tissue contraction; undirected healing effect on the surrounding tissues by enhanced releasing of growth factors; other suggestions are facilitation of collagen synthesis, and keratinocyte cell motility (Hopkins et al. 2004).

For this work has been used the RIKTA device, which is a magnet-infrared-laser (quantum) irradiation therapeutic device, that is designed for painless, non-invasive, and non-medicament treatment, with a multiple therapeutic effects for a wide range of diseases (Bodnár et al. 2011). Magnet-infrared-laser irradiation therapy consists of pulsating wide-band infrared irradiation, pulsating red light, and static magnetic field upon the patient (Bodnár et al. 2011). It has been reported that animals respond well to treatment, compared to human, thanks to better perceiving of laser and infrared light (“Quantum Therapy Devices. RIKTA.”). Low-level laser therapy exploits electromagnetic light energy with photons that transfer energy (Stubblefield 2019). Due to the strong electric field at the surface, the absorption and scattering of the impulses of infrared laser irradiation is strongly enhanced and

penetrates tissues deeply up to 10-13 cm, thus improve blood circulation, stimulate cell growth and metabolism, promote tissue regeneration, pain-relieving effect, and an anti-inflammatory effect (Bodnár et al. 2011, Huang et al. 2006).

MATERIAL AND METHODS

Animals

The group of mammary glands tumour included 10 bitches (aged 5 to 15 years). Specific patient data included signalment, medical history data and description of the current health state.

Clinical examination

All animals were subjected to an initial clinical examination, i.e. visual inspection and palpation of all sets of mammary glands, regional lymph nodes and possible formations. If mammary gland pathology was diagnosed as a malignant tumor, cancer stage was determined using the TNM system (Owen 1980). Subsequently, three thoracic X-ray projections were performed: dorsoventral or ventrodorsal; and right and left laterolateral projections. Before surgery, animals were subjected to biochemical (BCH) and hematological (H) examinations as well as preoperative ECG. Venous blood was taken from *v. cephalica antebrachii* or *v. saphena medialis (lateralis)* of all bitches. Blood samples were placed to the test tubes with agglutinative gel using sterile single-use needles (21G x 38 mm, 0.8 x 38 mm). The blood was then centrifuged at 3500 rpm for 10 min (Eppendorf centrifuge 5702), where the blood serum was separated. Obtained blood serum was stored at -20 ºC until the determination of tumor markers was performed.

Surgical removal

A variety of procedures for removing tumors on mammary gland, and choice of procedure were determined by size, fixation to surrounding tissue and number of lesions. Surgical removal was performed as per surgical oncological protocol designed by Gilson and Stone (1990).

Post-operative therapy

Post-operative care was provided with antibiotics (amoxicillin potentiated by clavulanic acid 15mg/kg) and non-steroidal anti-inflammatory drugs (meloxicam 0.2 mg/kg). Quantum therapy - RIKTA for 10 days was used to speed up the healing of surgical wounds.

1st - 3rd day frequency 4096 Hz, 5 minutes, blue light, Day 4: frequency 512 Hz, 5 minutes, blue light

Day 5 - 7: frequency 512 Hz, 5 minutes, red light, Day 8-10: frequency 64 Hz, 5 minutes, red light

RESULTS AND DISCUSSION


Figure 1a

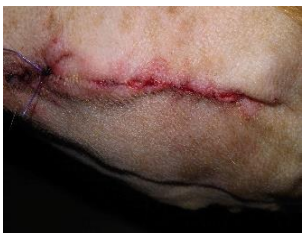
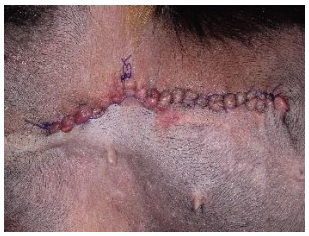


Figure 2a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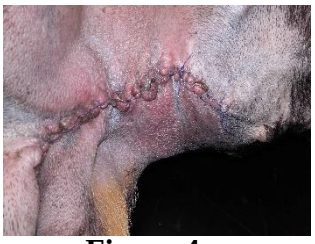
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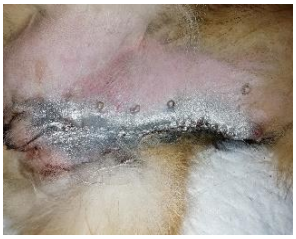
Figure 5a

Figure 1a – 5a Bitches after surgical removal tumour of mammary gland (day 1)

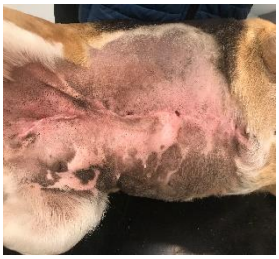
Figure 1b

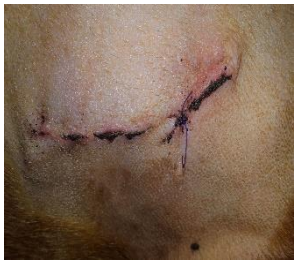
Figure 2b


Figure 3b

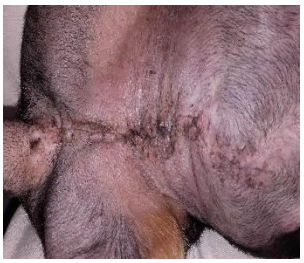
Figure 4b

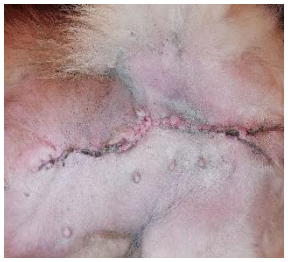
Figure 5b

Figure 1b – 5b Bitches after surgical removal tumour of mammary gland (day 10)


Figure 6a


Figure 7a


Figure 8a


Figure 9a


Figure 10a

Figure 6a – 10a Bitches after surgical removal tumour of mammary gland (day1)


Figure 6b

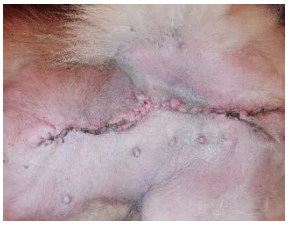
Figure 7b


Figure 8b

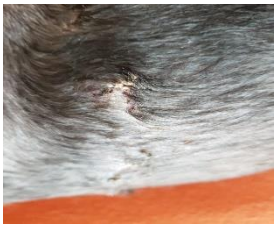
Figure 9b

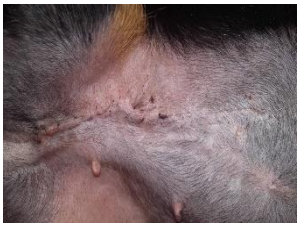
Figure 10b

Figure 6b – 10b Bitches after surgical removal tumour of mammary gland (day 10)

Portable semiconductor laser based instrumentation has found widespread use in modern medical practice (Isaev 2001). Quantum therapy, as a new direction of therapy, began to develop in veterinary medicine in the 1990s, along with the production of portable laser devices - RIKTA (Kataranov et al. 2003). Quantum therapy provides ecologically pure, non-invasive, non-medicamentous, highly effective therapy and prevention options for a wide range of diseases. The main goal of quantum therapy is the combined potentiated impact of the infrared laser beam pulse, the pulsating broadband infrared band, the pulsating red light and the constant magnetic field acting on the biological structures of living organisms. Pulsed infrared laser radiation penetrates 10 - 13 cm in tissue depth, has a powerful stimulating effect on blood circulation and membrane cell metabolism, activates neurohumoral factors and immunocompetent systems while harmonizing hormonal factors of metabolism (Ingr 1995).

CONCLUSION

Based on available literature and practical experience, quantum therapy can be used in various areas of veterinary medicine (surgery, orthopedics, internal medicine, dentistry, pulmonology, gastroenterology, gynecology, urology, neurology and dermatology). Our results confirm that quantum therapy - RIKTA, used mainly in cancer patients in the post-operative period, improves wound healing, since these wounds heal worse than in clinically healthy individuals.

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REFERENCES

Bodnár M., Nagy J., Popelka P., Koréneková B., Mačanga J., Nagyová A. (2011): “Effect of Quantum Therapy on Pork Quality.” Journal of Zhejiang University: Science B. https://doi.org/10.1631/jzus.B1000319.

Gilson S.D., Stone E. A. (1990): Principles of oncologic surgery. *Continuing Education of Small Animal*; 12(6): 827-835.

Hamblin M.R., Agrawal T., De Sousa M. (2016): Handbook of Low-Level Laser Therapy. Handbook of Low-Level Laser Therapy. https://doi.org/10.1201/9781315364827.

Hopkins J., Ty T., McLoda A., Seegmiller J.G,, Baxter G. D. (2004): “Low-Level Laser Therapy Facilitates Superficial Wound Healing in Humans: A Triple-Blind, Sham-Controlled Study.” Journal of Athletic Training.

Huang X., El-Sayed I.H., Qian W., El-Sayed M.A. (2006): “Cancer Cell Imaging and Photothermal Therapy in the Near-Infrared Region by Using Gold Nanorods.” Journal of the American Chemical Society.

Ingr I., (1995): Ageing Processes in Meat. *In*: Steinhauser, L., Beneš, J., Budig, J., Gola, J., Hofmann, I., Ingr, I., Kameník, J., Klíma, D., Kozák, A., Kužniar, J., *et al*. (Eds.), Meat Hygiene and Technology. LAST, Brno, 387-404.

Isaev A.K. (2001): Milta-f magnetic infrared laser therapeutic apparatus with a photographic recorder for diagnosis, prognosis, and treatment of inflammatory diseases of uterine appendages. *Biomed. Eng.*, 35(6):334-337. [doi:10.1023/A:1014642525004]

Kataranov A.A., Avdeenko V.S., Stravskij A.S. (2003): Correction of Immunologic Deficiency in Newborn Calfs by Laser Radiation. Proceedings “Quantum Therapy in Veterinary Medicine”. Moscow, Milta, 48-49.

Owen L.N. (1980): Classification of tumors in domestic animals, Geneva, World Health Organization.

Stubblefield M. D. (2019): Cancer Rehabilitation: Principles and Practice. Second Edi. https://books.google.sk/books?id=DiFbDwAAQBAJ&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false.

PRODUCTION AND VEAL QUALITY FROM DAIRY FARMS: A REVIEW

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ABSTRACT

Recently, meat has been ranked among the frequently discussed nutritive because of possible health risks, particularly in connection with the development of humans´ vascular and coronary diseases. On the other hand, there is no doubt about the importance of animal proteins, minerals and essential fatty acids in human nutrition. Demand for quantity and quality of the final product is constantly increasing. Nowadays, level of knowledge in the field of genetics, nutrition, technological systems of housing, feeding, fulfillment of animal health aspects, welfare, etc. allows a wide range of production systems for the veal and beef industry. In the European Union, the rearing and fattening of calves is regulated by legislative requirements, with the basic framework since 1991 given by Council Directive 91/629/EEC and its subsequent amendments. Since 2008, this Directive has been replaced by Council Directive 2008/119/EC, which sets out 15 animal welfare rules in Annex I. A key aspect in choosing a suitable breeding system is the target age and weight at slaughter, which inevitably determines the feeding system needed to achieve the required performance.

Keywords: colour; feeding system; slaughter weight; veal production; welfare

INTRODUCTION

According to OECD (2018), global meat consumption in 2016 was 317 million tonnes. Beef and veal consumption in EU 28 increased up to 7.2 million tonnes. In terms of beef and veal production, the number of slaughtered animals increased in 2018 by 1.3% year on year (Josrová, 2018). Despite the potential health risks of red meat intake, it is important to note that red meat is full of nutrients. Meat, especially red meat as a nutritive is important source of high biological value protein and essential micronutrients (Wyness et al., 2011). In the

bovine meat industry, the main objective is to increase the quality and safety of products sold to consumers, which are mostly determined by the total amount of fat and the proportion of SFA, MUFA and PUFA (Warren et al., 2008). Meat is a source of range of fatty acids (omega-6, omega-3, PUFA) (Wyness et al., 2011). Veal is rich in content of Vitamin B3 (6.0 mg), which has a beneficial effect on human metabolism. Red meat also has a high amount of heme-iron - which is better absorbed than plant-derived iron, vitamin B-6, selenium and other vitamins and minerals (URL 1). Moreover, calf meat is soft, tasty, easily digestible and thus also suitable for people with digestive disorders or carbohydrate diets.

THE AIM, MATERIAL AND METHODS

The aim of this work was to process veal production systems in the European Union, mainly of the Holstein breed and to describe the achieved quality of the slaughter product as well. The paper was elaborated on the basis of data from national and foreign scientific literature as well as on the own achieved results.

POSSIBILITIES OF VEAL PRODUCTION

According to Breitenstein et al. (1972, cited in Krása et al., 1995) is possible to produce meat from all healthy calves regardless of breed or utility type with respect of individual establishment, breeding and sex. In the European Union (EU), beef is produced on two categories of farms: specialized farms with suckler cows or young cattle; and dairy farms for which beef production is a by-product of dairy production (Hocquette - Chatellier, 2011). The first mention about veal in Europe is dated to ancient Romans (URL 2). Prior to the establishment of the veal industry after 1900s, all male calves in the United States were sold to the butcher a few days after birth as 'bob veal', as they were considered an undesirable product of the dairy industry. After Second World War, several changes occurred, especially in the dairy industry, and so veal has become more readily available to the general consumer. Nowadays, due to consumers demand for high quality product, most male and some female calves are used to produce “special-fed veal calves” (URL 3). The modern veal production originates in the Netherlands; calves are fed with a specific nutrient – balanced feed mixture including liquid whey, known as special-fed calves, resulting in a product with the unique properties of light pink color and smooth taste (Ngapo-Gariépy, 2006). In many countries for calves raised for meat production are limited age of 7 months and a weight of 250 kg

(Domaradzki et al., 2017). However, in accordance with current EU legislation, veal is since 2008 defined as a meat from bovine animals aged between 0 and 12 months in accordance with the requirements of the country-specific rules (URL 4; UNECE, 2011). There are two categories: for animals less than 8 months at slaughter (category V) and category Z for bovines aged between 8 and 12 months at slaughter (Domaradzki et al., 2017). Market of this category (Table 1) is subject to the appropriate trade names given for each Member State, eg. 'Jungrindfleisch' (Austria, Germany), 'rosé veal' (Ireland) or 'carne de ternera' (Spain) (AND International, 2014).

Table 1 Commercial label for cattle under 12 months of age in selected EU Member States

Member State	Category V	Category Z
Belgium	veau, viande veau Kalfsvlees / Kalbfleisch	jeune bovin, viande de jeune bovin Jongrundvlees / Jungrindfleisch
Netherlands	Kalfsvlees	rosé Kalfsvlees
France	veau, viande de veau	jeune bovin, viande de jeune bovin
Germany	Kalbfleisch	Jungrindfleisch
Italy	vitello, carne di vitello	vitellone, carne di vitellone
Portugal	vitela	vitelão
Poland	cielęcina	mloda wolowina
Slovakia	teľacie mäso	mladé hovädzie mäso

Source: AND International (2014)

The sector of veal and young beef produces around 6 million calves with main production (in thousands of tonnes of carcass weight) in Spain (237.6), the Netherlands (217.2), France (209.2) and Italy (101.7), which together account for 78% of the total production in this category (EUROSTAT, 2015; Sans - de Fontguyon, 2009). The highest average per capita consumption of beef, including veal, is reported in Denmark, France and Sweden (> 24 kg). Contrariwise, the least red meat (<10 kg / person/year) is consumed in Poland, Hungary, the Czech Republic, Slovakia and Romania (Roubalová - Vodička, 2015; Haley, 2017). Three different definitions or category of veal apply in Europe in accordance with European Union legislation. *White veal*, *milk-fed veal* or *special-fed veal* is a traditional form of veal production with a central position in the European industry. It is characterized by the pale color of the meat (1-10 points) and must come from bovine animals less than 8 months old (EC 566/2008). To obtain pale meat (due to the low myoglobin content in the muscles), calves are kept under specific housing conditions (without access to bedding or plant feed) and are fed specific milk diets to ensure low iron intake (Pardon, 2012, Pardon et al., 2014). However, the EU accepted calf welfare regulations and, since 31 December 2003, Council of

Europe Directive 97/2/EC (EU Council, 1997) mandates group housing and the provision of solid feed to calves for meat production (Cozzi-Ragno, 2003). The pale creamy-pink veal with a firm, soft and velvety texture is produced by a specific fattening known as *special-fed veal*. Such meat is obtained by a complete milk nutritional supplement for fattening up to the age of 18-22 weeks and a weight of 204-227 kg (URL 3). Red veal 'rosé veal' (grain-fed veal or non-specifically-fed 'non-formula-fed') with a slightly reddish coloration (11-14 points) is obtained from calves under 8 months of age depending on nutrition; the protein supplement must not be of animal origin (EBLEX, 2011). Ngapo-Gariépy (2006) describes 'red veal' as calves fed with milk replacers, along with hay and grains. In general for veal calves is used following feeding systems: mother cow milk (*mother-fed*), dairy feed mixture (*formula-fed*) or compound feed, which may include milk, milk replacers, silage, cereals, grains or other plant products (*non-formula-fed*). Under the legislation of specific EU Member States, feed should not contain fishmeal, additives of animal origin, growth promoters or products derived from genetically modified organisms (UNECE, 2011).

QUALITY OF SLAUGHTER PRODUCT

Meat production is based on the animal growth rate, which depends on several environmental factors as well as management practices. There is currently no consensus about precise explanation of the concept of meat quality, because is generally considered to be a combination of two main elements. On the one hand, the overall quality of meat and meat products includes measurable properties - microbiological state, tenderness, colour, juiciness, shelf life, pH value. On the other hand, meat quality includes personal consumers´ perception of the value of meat and meat products (Feiner, 2006). Intramuscular fat content and also composition is influenced by the feeding method, sex of animals, slaughter weight and slaughter age, as well as the duration of suckling (Moreno, 2006). Table 2 presents an overview of average daily gains, live weight and most important carcass characteristics of Holstein calves.

Table 2 Slaughter characteristics of veal from Holstein breed

Feeding	ADG (kg)	Slaughter weight (kg)	Carcass weight (kg)	Dressing percenta-ge (%)	Source
Milk	1.36	247.3	145	58.6	Xiccato et al., 2002
Maize	1.43	256.7	152	59.1	
Roughage, straw		270	141	59.1	Yim et al., 2015
FM, hay	0.72	145.60	75.70	51.09	Vavrišínová et al., 2013
FM, hay, maize silage	0.75	149.08	67.75	45.47	
Feed mixture	-	144.6	67.89	46.88	Dias et al., 2018
Feed mixture	-	179.8	87.24	48.26	
50% barley, 50% corn	1.55	257.6	158.2	61.4	Noon et al., 1998
TMR	-	143.83	73.69	51.22	Vavrišínová et al., 2018
TMR	-	194.25	100.68	51.81	

ADG – average daily gain, FM – feed mixture, TMR – total mixed ration

Meat from milking breeds shows the good quality in lower slaughter weights and therefore it is possible to cover the lack of veal mainly with meat from calves of dairy breeds. Sensory attributes of the meat quality – colour, texture and taste are influenced by the growth rate and management, age and weight at slaughter, gender, nutrition as well as by the length of fattening (Çatikkaş – Koç, 2017). The results of the vast majority of proximate composition analyses concern mainly sections of *M. longissimus dorsi*. The reported values for the content of moisture, protein and intramuscular fat are given in Table 3.

Table 3 Chemical composition of veal longissimus muscle from Holstein breed

Slaughter weight (kg)	Feeding	Protein content (%)	Moisture content (%)	IMF content (%)	Source
247.3	Milk	21.16	75.98	-	Xiccato et al., 2002
256.7	Maize	21.07	75.71	-	
270	Roughage, straw	24.21	74.90	0.65	Yim et al., 2015
130.4	TMR	22.45	75.02	1.31	Vavrišínová et al., 2019
210	TMR	23.00	74.65	1.57	

TMR – total mixed ration, IMF – intramuscular fat

With increasing slaughter weight of animal, the individual proportions of tissues in carcass change; while proportion of bones decline and proportion of meat increase. Table 4 describes proportion of muscles, bones and separable fat in carcass halves of Holstein calves.

Table 4 Proportion of individual tissues of the Holstein veal carcasses

Slaughter weight (kg)	Feeding	Meat (%)	Bones (%)	Separable fat (%)	Source
143.83	TMR	62.05	28.11	7.55	Vavrišínová et al., 2018
194.25	TMR	62.61	27.98	8.36	
144.6	FM	69.07	20.88	8.95	Santos et al., 2013
227.5	FM	67.09	19.43	12.71	
210	TMR	63.16	27.98	8.86	Vavrišínová et al., 2019

TMR – total mixed ration; FM – feed mixture

The most widely assessed non-nutritional qualitative parameters are physicochemical characteristics, including color, pH, cooking loss and Warner-Bratzler shear force. The intrinsic properties are given in Table 5.

Table 5 Parameters associated with meat quality from Holstein veal

Feeding	pH_{24}	CL (%)	WBSF (kg/cm^2)	CIE *L**	CIE *a**	CIE *b**	Source
Milk	5.53	24.95	2.15	53.26	11.50	6.67	Xiccato et al., 2002
Maize	5.53	22.41	2.11	53.52	11.32	6.86	
Roughage, straw	4.72	16.83	4.39	45.54	9.06	2.41	Yim et al., 2015
Milk replacer, feed mixture	5.59	-	-	46.1	7.9	11.9	Skřivanová et al., 2007
Milk replacer, feed mixture, Se supplement	5.65	-	-	46.7	7.0	12.0	
Concentrate, hay	5.84	-	-	44.12	5.99	10.23	Vavrišínová et al., 2013
Concentrate, hay, maize silage	6.12	-	-	47.53	4.73	11.16	
Feed mixture	5.58	31.50	9.00	35.88	7.99	2.26	Cho et al., 2014
TMR	5.61	37.4	5.60	32.7	5.0	17.1	Titi et al., 2008
TMR, yeast culture	5.66	37.5	5.95	35.5	5.1	11.5	
Milk replacer	5.50	29.0	4.91	44.1	12.3	7.5	Scheeder et al., 1999
Maize silage	5.58	28.9	4.17	40.6	14.6	8.2	

CL – cooking losses, TMR – total mixed ration, WBSF – Warner-Bratzler shear force

CONCLUSION

Within the European Union countries, the most preferred production system for veal calves is traditional fattening for pale coloured veal, known as “special-fed calves”, which combine milk replacers, grains and forages, such as hay, straw or silage. Calves are mostly slaughtered at weight of 140 kg or 250 kg. The average dressing percentage ranged between 45.47% and

66.2%. In term of veal colour, achieved results of the lightness (L^*) varied between 35.5 and 53.52; redness (a^*) was determined in range of 4.73 and 14.6.

REFERENCES

The literature used is available from the authors.

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DIFFERENCES IN COMPOSITION BETWEEN CARP MUSCLE AND REPRODUCTIVE ORGANS. PILOT STUDY.

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ABSTRACT

The aim of this pilot study was to find out the composition of carp soft and hard roe and compare it with the composition of carp muscle. Pilot study was interested in protein and fat content, amino acid composition, trace elements quantity and fatty acid composition. Results shown that carp gonads in many parameters skipped the meat. These results will help us to decide which direction we should continue in future research.

Keywords: *Cyprinus carpio*, hard roe, soft roe, meat composition, fatty acids

INTRODUCTION

The pond farming is an aquaculture technology tightly linked with its surrounding environment. The extensive and semi-intensive management systems, typically for Czech pond aquaculture include complex production methods with many important links to the surrounding ecosystem. Carp (*Cyprinus carpio*, Linnaeus 1758) farming has a long tradition in the Czech Republic, with many ponds originating from early medieval times. Over such a long period, the ponds have become an important part of the countryside. In summary, ponds function as landscape components, they retain water, and they are irreplaceable for fish production, which means high nutritional quality food production.

High nutritional quality is the most important health benefit of fish meat due to its high content of n–3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), (Calder and Yagoob, 2009; Adamkova et al., 2011). PUFA, especially n-3 long-chain HUFA (highly unsaturated fatty acids – where exactly EPA

and DHA belong) found mainly in fish have an indisputable effect on human health, especially, prevention of human coronary disease, a healthy lifestyle and weight reduction (Adamkova et al., 2011; Lund, 2013). Furthermore, not in every country, people consume fish and their products frequently. For example, in the Czech Republic, the annual fish consumption in 2017 was 3.8 kg per capita (Fish Annual Report, 2017).

Lund (2013) states that fish meat is healthy not only thanks to the essential fatty acid content, but also due to the favourable composition of proteins, minerals and vitamins. Many factors influence the fish meat composition, these factors include e.g. fish species, size and age of fish, season, water temperature, geographic location. The major factors influencing fatty acid profiles of the freshwater fish flesh are the diet (Ehsani et al., 2013) and the rearing conditions (Ozogul et al., 2007).

Fish gonads (hard and soft roe) are on the menu more or less seasonal. They are, usually consumed in special fish Christmas soup in majority of the families. Data about their nutritive value do not exist. The aim of this pilot study was to compare the composition of carp muscle and gonads and find out potential differences between them. The results of this pilot study should clear potential differences up and show the direction where we should focus our attention.

MATERIAL AND METHODS

Because this study was done as pilot study, there was used only two market size carps from the south Moravian semi-intensive pond. Carps were caught in the beginning of the June in spawning period. Fish, were stunned by a blow to the head and killed by bleeding. Fillets (meat without bones) from them were skinned and only left fillet was homogenized for the further analyses. Also carp gonads (male and female) were analysed. Analyses started immediately after processing in the laboratory at the University of Veterinary and Pharmaceutical Sciences in Brno.

The homogenized fillet was dried at 105 °C under prescribed conditions, and the dry matter was determined. The content of proteins was determined using the Kjeldahl method on a Buchi Analyzer (Centec automatika, Czech Republic), and the content of nitrogenous matter (crude protein) was calculated by multiplying the value of nitrogen by the coefficient of 6.25. Fat was determined by the ANKOMXT10 Fat Analyzer (O.K. SERVIS BioPro, Czech Republic). Determination of mineral substances was done by the Agilent ^{240} AA device using flame atomic absorption spectrometry (FAAS). Amino acids were analysed by gas chromatography. The extraction of fat from the muscle to determine fatty acids was

performed according to Hara and Radin (1978). In addition, the standard ČSN EN ISO 12966-1, 12966-2 were used. Fatty acid esters, were detected by gas chromatography using a Gas Chromatograph GC-2010 Analyzer (Shimadzu, Japan) with a flame ionisation detector and evaluated in the GC PostRun program. Some fatty acids in the samples reached very low values, therefore only fatty acids occurring more than 1g/100g of fat are in Table 4, but despite this, they are included in total sums. Samples were converted to the content of the given fatty acid in the sample (g/100 g of fat). To ensure more accurate reproducibility, all the results are presented in the muscle dry matter (g/kg of dry matter).

RESULTS AND DISCUSSION

Values of proteins ranging between 633.8 and 683.9 g/kg (Table 1) and were similar regardless of source (gonads and/or muscles). Different situation was in fat content where in soft roe and muscles were values of fat oscillating 351.2 g/kg (mean value) while hard roe contained 512.6 g/kg of fat (increase of 46 %). A high impact on the fat composition of fish has exactly reproduction period. Lipids that are mobilized potentially for the formation and growth of the gonads are stored in muscle, liver, and abdominal regions before the reproduction period (Kiessling et al., 1989). Our samples were from the spawning period, so lipids in the hard roe were used as a source of energy for reproduction by the organism.

Table 1 Basic parameters of carp muscles and gonads (g/kg of dry matter).

	Dry Matter	**Protein**	**Fat**	**Ash**
Carp Soft Roe	306.0	680.0	365.2	128.0
Carp Hard Roe	314.4	683.9	512.6	75.4
Carp Muscle 1	208.5	665.1	317.5	45.7
Carp Muscle 2	232.6	633.8	370.9	38.5

Amino acid composition did not vary between muscles and gonads, but differences were evident between the two muscles (Table 2). Feed is the main factor influencing the amino acid composition and fish were from the semi-intensive pond that means they could consume nature diet as well as grains (often used for supplemental feeding) and this would be probably the reason for different values.

Results of trace elements in carp gonads shown interesting variation (see Table 3) mainly in copper levels (4 times higher in soft roe), iron levels (1.6 times higher in soft roe) and zinc levels (3.6 times higher in hard roe). Trace element composition in both muscle samples was similar with only moderate variations. Generally, we can say that carp gonads are rich in

phosphorus, and iron and hard roe additionally in zinc in comparison with muscles. Bastić et al. (2002) found 25.96% of zinc, 11.52% of iron and 0.98% of copper in crucian carp (*Carrassius carrassius*) muscles (native matter) from the pond. Recounting our values on native matter, we found our results lower in zinc and copper but higher in iron, which is certainly positive.

Table 2 Amino acids composition in carp muscles and gonads (g/kg of dry matter).

	Carp Soft Roe	Carp Hard Roe	Carp Muscle 1	Carp Muscle 2
Asp	35.0	31.9	62.5	69.2
Thr	25.3	28.3	22.8	24.8
Ser	22.3	40.8	20.4	22.0
Glu	62.0	96.7	98.7	105.8
Pro	24.4	38.1	19.5	19.5
Gly	34.5	45.0	29.2	28.9
Ala	38.9	49.4	37.6	40.1
Val	27.9	45.0	30.9	33.1
Met	5.1	14.6	7.8	8.9
Ile	22.1	38.6	27.4	30.1
Leu	41.6	62.8	42.7	47.7
Tyr	17.9	24.2	18.3	20.7
Phe	18.8	27.6	37.1	40.4
His	11.8	18.7	17.1	19.2
Lys	70.0	45.6	68.5	72.4
Arg	47.1	38.6	27.4	28.6

Table 3 Trace elements in carp muscle and gonads (g/kg of dry matter (K, Na, Ca, P, Mg) and mg/kg (Cu, Fe, Mn, Zn)).

	Carp Soft Roe	Carp Hard Roe	Carp Muscle 1	Carp Muscle 2
K	9.28	6.49	12.42	9.93
Na	2.32	1.97	3.12	2.49
Ca	0.33	1.18	2.11	1.72
P	22.7	22.7	7.2	4.4
Mg	0.59	0.48	0.86	0.73
Cu	4.9	1.2	3.9	2.1
Fe	313.8	198.4	29.9	22.9
Mn	20.6	16.1	24.6	26.7
Zn	26.2	94.2	43.3	38.1

Most often fatty acids (FA) were monounsaturated FA (MUFA) in the muscles just as in the gonads (Table 4). Quantity of n-3 FA was low in comparison with n-6 FA but the ratio

of n-3 to n-6 FA was very favourable in the gonads. In muscles was the ratio of n-3 to n-6 FA very low. With respect to FA composition, gonads are (hard roe more than the soft roe) foodstuff very beneficial for human health. Nevertheless, we have to take into consideration that FA composition can influence many factors, e.g. climatic conditions (Varga et al., 2013), feedstuffs (Acar and Türker, 2018), sex (Arslan, 1993) and last but not least the part of the fish´s body (Dong et al., 2017).

Table 4 Most often fatty acids in carp muscles and gonads (g/100 g of fat).
Note: Sums contain even values lower than 1 g/100 g of fat.

	Carp Soft Roe	**Carp Hard Roe**	**Carp Muscle 1**	**Carp Muscle 2**
C14:0	0.78	0.84	1.09	1.08
C16:0	15.77	16.35	17.31	17.13
C18:0	5.28	5.66	4.71	4.66
ΣSFA	**22.66**	**23.56**	**23.85**	**23.56**
C16:1	6.67	6.19	8.59	8.50
C18:1n9	42.41	41.96	34.95	35.2
C20:1n9	1.61	1.37	1.49	1.39
C22:1n9	0.66	0.18	1.28	1.26
ΣMUFA	**51.61**	**49.89**	**46.69**	**46.72**
C18:3n3	0.77	0.45	2.32	2.30
Σn-3 FA	**1.21**	**0.74**	**2.95**	**2.91**
C18:2n6	4.66	4.37	5.67	5.61
C20:4n6	1.54	1.09	0.75	0.74
Σn-6 FA	**7.23**	**6.58**	**7.05**	**6.98**
Σn-3:Σn-6	**1:6.0**	**1:8.9**	**1:2.4**	**1:2.4**
ΣSFA:ΣUFA	**1:2.7**	**1:2.4**	**1:2.4**	**1:2:4**

CONCLUSION

In this pilot study were proved differences between the muscle and gonads composition from the point of view of protein and fat content and amino acids, trace elements and fatty acids composition. These results will help us to decide which direction we should continue in future research.

REFERENCES

Acar Ü., Türker A. (2018). Response of Rainbow trout (*Oncorhynchus mykiss*) to unrefined peanut oil diets: Effect on growth performance, fish health and fillet fatty acid composition. Aquaculture Nutrition, 24, 292-299.

Adamkova V., Kacer P., Mraz J., Suchanek P., Pickova J., Kralova Lesna I., Skibova J., Kozak P., Maratka V. (2011). The consumption of the carp meat and plasma lipids in secondary prevention in the heart ischemic disease patients. Neuroendocrinological Letters, 32, 17-20.

Arslan A. (1993). Microbiological and chemical quality of carp (*Cyprinus carpio* L.) in Keban Lake, Doga. Turkish Journal of Veterinary and Animal Sciences, 17, 251–259.

Bastić L., Kočovski T., Antonović D., Vidarić D. (2002). The meat quality of some freshwater fish – nutritive and technological aspects. Acta Veterinaria (Beograd), 52, 259-266.

Calder P. C., Yaqoob P. (2009). Omega-3 polyunsaturated fatty acids and human health outcomes. Biofactors, 35, 266–272.

ČSN EN ISO 12966-1. Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters – Part 1: Guideline for advanced gas chromatography of fatty acid methyl esters

ČSN EN ISO 12966-2. Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters – Part 2: Preparation of fatty acid methyl esters

Dong X. P., Wu Q., Li D. Y., Wang T., Pan J. F., Zheng J. J., Chen G. B. (2017). Physicochemical, micro-structural, and textural properties of different parts from farmed common carp (*Cyprinus carpio*). International Journal of Food Properties, 20, 946-955.

Ehsani A., Jasour M. S., Khodayari M. (2013). Differentiation of common marketable-size rainbow trouts (*Oncorhynchus mykiss*) based on nutritional and dietetic traits: a comparative study. Journal of Applied Animal Research, 41, 387-391.

Fish Annual Report, Czech Ministry of Agriculture, Prague, 2017, 28 pages (2017).

Hara A., Radin N. S. (1978). Lipid extraction of tissues with a low-toxicity solvent. Analytical Biochemistry 90, 420-426

Kiessling A., Johansson L., Storebakken T. (1989). Effects of reduced feed ration levels on fat content and fatty acid composition in white and red muscle from rainbow trout. Aquaculture, 79: 169–175.

Lund E. K. (2013). Health benefits of seafood; is it just the fatty acids? Food Chemistry, 140, 413–420.

Ozogul Y., Ozogul F., Alagoz S. (2007). Fatty acid profiles and fat contents of commercially important seawater and freshwater fish species of Turkey: a comparative study. Food Chemistry, 103, 217–223.

Varga D., Hancz C., Horn P., Molnár T., Szabó A. (2013). Environmental factors influencing the slaughter value and flesh quality of the common carp in four typical fish farms in Hungary. Acta Alimentaria Hungarica, 4**2**: 495–503.

INFLUENCE OF NATURAL FEEDING SUPPLEMENTS ON THE GROWTH AND HEALTH IN CALVES

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ABSTRACT

The aim of this study was to prove the hypotheses that the growth and health in calves are dependent on feed supplements with antidiarrhoeic effect. Total 60 calves were included in the experiment. After birth the calves were divided into three treatment groups: *Ascophyllum nodosum* (brown seaweed hydrolyzate, prebiotics), *Lactobacillus sporogenes (*probiotics), and control group. All calves were weighed within two hours after birth. The growth and health were investigated from the birth to the fourth week of age. Compared to the control, the significant influence of applied feed supplements was found in the *Lactobacillus sporogenes* group in body weight at 28th day of life ($P<0.01$) and average daily gains ($P<0.001$). We concluded from the analysis, that the use of *Lactobacillus sporogenes* had a positive influence on the growth increasing only. Neither of the two supplements had a positive impact on the health of calves.

Keywords: calf; probiotics; growth; health; diarrhea

INTRODUCTION

In an intensive management system of farm animals, especially in calf rearing without mother, the natural acquisition of autochthonous microflora is drastically reduced by changing the intestinal environment and allowing pathogens to colonize the intestinal microflora (Rosmini et al. 2004). The incidence of metabolic disorders in dairy calves in the Czech Republic represents a highly actual problem and that one of the significant factors that influence this condition is the insufficient care for and the related insufficient colostral nutrition of the calves (Podhorský et al. 2007; Šlosárková et al. 2014).

The importance of probiotics and prebiotics lies in their ability to stabilize the inner intestinal microflora and to influence the calf health and the calf welfare. Positive effects of Ascophyllum nodosum on the reduction of pathogen E. coli O157:H7 were proved in the case of cattle and sheep (Bach et al. 2008). The effect of Lactobacillus sporogenes on Salmonella dublin was verified by Frizzo et al. (2011), the effect of Lactobacillus on the started feed intake and on the weight gain by Higginbotham and Bath (1993) and the effect of Lactobacillus acidophilus on the occurrence of calf diarrhoeas by Tarboush et al. (1996).

MATERIAL AND METHODS

60 Holstein calves (20 in *Lactobacillus sporogenes* group, 20 in *Ascophyllum nodosum* group and 20 in control group) from one dairy cows herd were included in the experiment. After birth the calves were randomly divided into three treatment groups: group 1 *Ascophyllum nodosum*, group 2 *Lactobacillus sporogenes* and control group 3. They were separated and weaned from mothers on the first day after birth. Calves were reared in individual littered hutches from the second day of life to weaning. They received colostrum and mothers milk ad libitum three times a day from a bucket with nipple from the second to fourth day. From the fifth day they received 4.5 kg of milk replacer per day divided into 3 portions and could eat starter mixture and alfalfa hay ad libitum until weaning. Colostrum and subsequently milk replacer were administered to calves in plastic buckets with nipples that were fitted in the hutches at a height of 40 cm above the ground. The calves had a free access to drinking water for the entire experimental period.

The *Ascophyllum nodosum* experimental group received orally 5 ml of hydrolyzate from brown seaweeds in addition to colostrum and milk replacer. The *Lactobacillus sporogenes* experimental group received orally 1 tablet of probiotics added to colostrum at first and then to milk replacer and thoroughly mixed. The formulation of 1 tablet of probiotics was as follows $4x10^7$ *Lactobacillus sporogenes*. Experimental groups were administered these feed supplements one time a day (at the second feeding). Both supplements were applied to experimental groups within the first fortnight after birth. The control group received an unsupplemented diet, consisted 1.5 kg of milk replacer per feeding (totally 4.5 kg), starter mixture and alfalfa hay ad libitum. All calves were observed until the 28th day of life.

All calves were weighed within two hours after birth. They were weighed regularly every week. The classical method of for the evaluation and expression of diarrhea according to Larson et al. (1977) was used. Observations of feces and health condition was evaluated twice

a day together with rectal temperature measurements at the time of feeding. Respiratory condition was assessed by the types of symptom (normal, runny nose, heavy breathing, and cough – moist or dry). Other frequency of cough (possible respiratory disorder) as occasional, intermittent, or persistent. Operators observed the condition of hair and eyes (dullness and brightness) and signs of dehydration (sunken eyes, inelastic skin, and prostration).

During long lasting diarrheal diseases calves from all treatment groups were treated using the preparation Argivo Se (Deltavit, France) at 40 g per day.

The data were analyzed using a General Linear Model ANOVA (four ways with the interactions) of the statistical package STATISTIX 12 (Analytical Software, Tallahasee, FL, USA).

RESULTS AND DISCUSSION

The calves from the 2nd treatment group (probiotics) reached the highest live body weight at the 28th day. Differences were significant in comparison to 1st group and control group (53.77±6.18 kg vs 51.27±4.71 kg, $P<0.05$; 53.77±6.18 kg vs 50.15±5.61 kg, $P<0.01$). Similarly, the of average daily gains for all observed period were also the highest in the probiotics (2nd) group (0.39±0.09 kg vs 0.33±0.10 kg, $P<0.05$; 0.39±0.09 kg vs 0.30±0.10 kg, $P<0.01$) (Table 1).

At the present work we studied the impacts of two feed supplements. However, the significant effect was showed only 2nd treatment group, which received probiotics. These calves had the most intensive growth of livebody weight.

A positive influence of the use of *Lactobacillus sporogenes* on weight gains of calves was also reported by Soto et al. (2014), Frizzo et al. (2010), Fuller (1989), Tarboush et al. (1996), Schneider et al. (2004) and Timmerman et al. (2005). A low or no influence on an increase in weight gains of animals in the group with *Ascophyllum nodosum* may be a result of the availability of a sufficient amount of prebiotics in ordinary feed like oats, barley and wheat while the prebiotic availability is not a limiting factor (Gaggía et al. 2010).

Table 1 The influence of applied supplements on the growth of calves

Variables	N	Treatment groups			*P*	Significance
		Ascophyllum nodosum	*Lactobacillus sporogenes*	Control		
		$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD		
BW at birth (kg)	60	41.49±5.11	42.11±5.28	40.99± 4.70	0.4642	
BW in 28th day (kg)	60	51.27±4.71	53.77±6.18	50.15±5.61	0.0012**	2:3**, 1:2*
ADG from birth to 28th day (kg)	60	0.33±0.10	0.39±0.09	0.30± 0.10	0.0000	2:3**, 1:2*

*P *< 0.05;* **P*<0.01;* ***P*<0.001; *SD = standard deviation; ADG = average daily gains; BW = body weight;* P *= significance; N = number (1 – Ascophyllum nodosum, N=20, 2 - Lactobacillus sporogenes, N=20; and 3 – control, N=20)*

CONCLUSION

The using of *Ascophyllum nodosum* has no meaning for improving of growth and health of calves. The results did not show a positive effect of both observed supplements (*Ascophyllum* nodosum nor *Lactobacillus sporog*enes) on health and specially scour incidences. We concluded from the analysis, that the effect of the probiotics (*Lactobacillus sporogenes*) was manifested only in the increased growth of calves.

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REFERENCES

Bach S. J., Wang Y., McAllister T. A. (2008): Effect of feeding sun-dried seaweed (*Ascophyllum nodosum*) on fecal shedding of *Escherichia coli* O157:H7 by feedlot cattle and on growth performance of lambs. Animal Feed Science and Technology, 142, 17-32.

Frizzo L. S., Soto L. P., Bertozzi E., Zbrun M. V., Signorini M. L., Sequeira G., Armesto R. R., Rosmini M. R. (2011): Intestinal populations of lactobacilli and coliforms after in vivo salmonella dublin challenge and their relationship with microbial translocation in calves supplemented with lactic acid bacteria and lactose. Animal Feed Science Technology, 170, 12-20.

Frizzo L. S., Soto L. P., Zbrun M. V., Bertozzi E., Sequeira G., Rodriguez M., Armesto R. R., Rosmini M. R. (2010): Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. Animal Feed Science Technology, 157, 159–167.

Fuller R. (1989): Probiotics in man and animals. Journal of Applied Bacteriology, 66, 365–378.

Gaggía F., Mattarelli P., Biavati B. (2010): Probiotics and prebiotics in animal feeding for safe food production. International Journal Food Microbiology, 141, 15–28.

Higginbotham G. E., Bath D. L. (1993): Evaluation of lactobacillus fermentation cultures in calf feeding systems. Journal Dairy Science, 76, 615-620.

Larson L. L, Owen F. G., Albright J. L. (1977): Guidelines toward more uniformity in measuring and reporting calf experimental data. Journa Dairy Science 60, 989-991.

Podhorský A., Pechová A., Dvořák R., Pavlata L. (2007): Metabolic disorders in dairy calves in postpartum period. Acta Veterinaria Brno, 76, 45-53.

Rosmini M. R., Sequeira G. J., Guererro-Legarreta I., Martí L. E., Dalla-Santina R., Frizzo L., Bonazza J. C. (2004): Production of probiotics for animals: importance of using internal intestinal microflora (in Spanish). mexican journal chemical engineering, 3, 181–191.

Schneieder R., Rosmini M.R., Eehrmann M., Vogel R. (2004): Identification of lactic acid bacteria form the typical microbiota found in artificial reared calves. FAVE - Ciencias Veterinaria, 3, 7–15.

Soto L. P., Zbruna M. V., Frizzo L. S., Signorinia M. L., Sequeira G. J., Rosmini M. R. (2014): Effects of bacterial inoculants in milk on the performance of intensively reared calves. Animal Feed Science and Technology, 189, 117–122.

Šlosárková S., Fleischer P., Pěnkava O., Skřivánek M. (2014): The assessment of colostral immunity in dairy calves based on serum biochemical indicators and their relationships. Acta Veterinaria Brno, 83, 151-156.

Tarboush H. M. A., Saiady M. Y. A., Din A. H. K. E. (1996): Evaluation of diet containing lactobacilli on performance, fecal coliform, and lactobacilli of young dairy calves. Animal Feed Science Technology, 57, 39-49.

Timmerman H. M., Mulder L., Everts H., Van Espen D. C., Van Der Wal E., Klaassen G., Rouwers S. M. G., Hartemink R., Rombouts F. M., Beynen A. C. (2005): Health and growth of veal calves fed milk replacers with or without probiotics. Journal Dairy Science, 88, 2154–2165.

IMPACT OF FLIGHT LOAD ON THE HEALTH OF RACING PIGEONS

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ABSTRACT

Increased stress and short time for regeneration during the race are an important factors that significantly affects the health of racing pigeons and their flight performance. The aim of this study were to observe the health status of 80 racing pigeons (*Columba livia f. dom.*) in age 1.5 - 4 years originated from holding on west of Slovakia, which for three months completed eighteen races at a distance of 120 - 1150 km. At the beginning and at the end of the race season were taken the samples of swabs from cloaca and oropharynx. Comparing the most common diseases of pigeons, were found an increased incidence of coccidiosis (52.2%), trichomoniasis (31.9%), respiratory syndrome (7.2%) and ectoparasitosis (5.8%) after the race season. Results are showing that also despite increased prevalence of monitored diseases, the pigeons are able to provide flight performance and cope with changes in their organism during this difficult race period. The development of prophylactic programmes is an indispensable part of the proper functioning of breeding and disease prevention, adherence to which contributes to optimum performance of individuals and to reducing losses during the races themselves.

Keywords: pigeons; flight performance; coccidiosis; trichomoniasis; respiratory syndrome

INTRODUCTION

The most vulnerable to infections are racing and highfliers pigeons, as they perform a large number of flights in the so-called racing season. This leads to substantial exhaustion of birds and, consequently, increases their susceptibility to various diseases. The diseases that affect pigeons are divided in to contagious diseases and non-contagious diseases. Viruses, bacteria, fungi and parasites are the main causes for contagious diseases while non-contagious diseases are resulted from the absence of proper nutrients as well as poisonous elements in the food (Rupipper, 1998a,b).

One of the most common parasitic diseases is coccidiosis. This parasitic protozoon organism affects the bird's intestines. Two types of coccidia affect pigeons: *Eimeria columbanum* and *Eimeria labbeanna*. The clinical disease due to these organisms is identical. Coccidia in the intestines produce 'oocysts', which are passed out in the faeces. These mature in the environment and will then affect other birds if ingested. Most adult birds carry low levels of these parasites. Only when large numbers of parasites are present is treatment necessary. Usually raised levels of coccidial oocysts are associated with sub-optimal performance but in young birds and adults under stress an acute clinical form of the disease may be seen. The parasite affects the lining of the intestine causing diarrhea and blood may be present (**Fig. 1**). Affected birds are depressed, rapidly become emaciated and may die (Struhár, 2015).

Figure 1 Coccidiosis

Legend: Coccidiosis x 400. Section of small intestine infected with *Eimeria* spp. The lumen is full of frank blood with flecks of clotted blood. Note the necrotic, hemorrhagic, debris within the lumen (green arrow).

The second most common cause of reduced pigeon performance is the canker (**Fig. 2**). Canker is caused by a parasitic organism called *Trichomonas colombi* and three forms are recognized that affect the pharynx (throat), navel and internal organs respectively (Scullion et Scullion,

1997). The majority of adult pigeons are symptomless carriers of the organism but clinical cases may occur if the bird is under stress and in young pigeons the disease may be severe and even fatal. The disease is spread from adults to squabs in the crop milk and between pigeons by the oral route (Struhar, 2015).

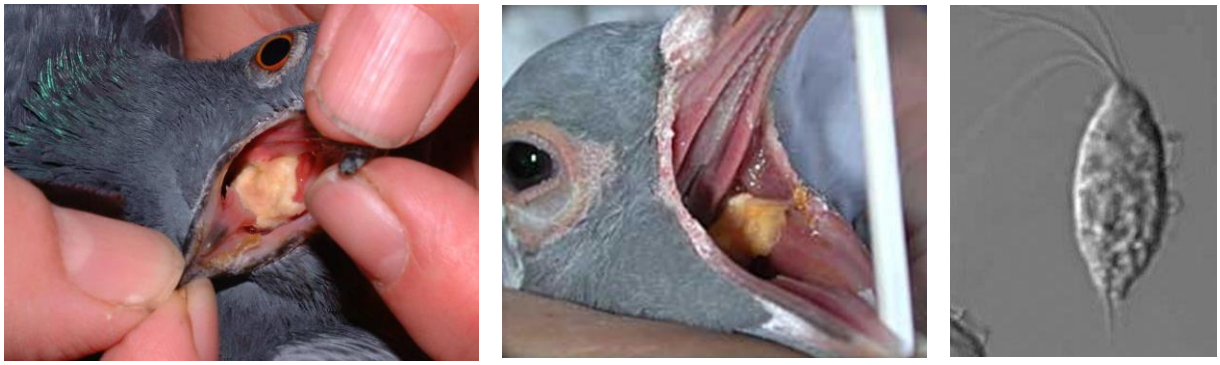

Figure 2 Canker caused by a*Trichomonas colombi* - pharynx form

Additionally, birds may contract combined infections, known as “ornithosis complex”, "ornithose," "ornithosis complex," "coryza," and "one-eye colds." This group disease can be caused by infection with *Chlamydia psittaci*, Pasteurella, or mycoplasma organisms. Other gram-negative bacteria *E. coli, Yersinia spp., Enterobacter* spp., and viral agents such as herpesvirus may also play a role (Zigo et al., 2017).

The current physical overload of racing pigeons not only weakens their organism, but also increases their susceptibility to various diseases. Therefore, the aim of this study was to monitoring and characterize the most common diseases of racing pigeons before and after the race season.

MATERIAL AND METHODS

Birds and samples

The study included 80 pigeons in age 1.5 - 4 years originated from holding on west of Slovakia, which for three months completed eighteen races at a distance of 120 - 1150 km. Clinical examination of health status at the beginning (may) and on the end (july) of race season was performed according to Scullion et Scullion (1997). Faecal samples, swabs of the cloaca and oropharynx were collected from all 80 pigeons at the beginning of race season. On the end of race season were taken samples of faeces and swabs from 69 pigeons because 11 pieces were lost during the races.

Diagnostic and laboratory analysis

Diagnostic of ornithosis complex was performed according to Rupipper (1998) and Smith (1996) based on the evaluation of clinical signs such as: rhinitis, conjunctivitis, eye wiping, nasal discharge, feather loss around the eye, epiphora, sinusitis, coughing, sneezing, fluffing and poor race performance.

The floatation technique was used for detecting coccidiosis and endoparasitosis from faecal samples according to Dranzoa et al. (1999) and Stenzel et Koncicki (2007). Microscopic determination of swabs from the oropharynx and crop to demonstrate the presence of trichomonads was performed according to Rupipper et al. (1998b). The presence of ectoparasitosis caused by *Columbicola columbae, Campanulotes compar, Hohorstiella lata* and *Menacanthus stramineus* on the skin, flight and tail feathers was performed according to Struhár (2015).

Statistical analysis

Statistical analysis was performed using software Chi quadrate test for comparison of the most common diseases of pigeons and isolated strains from swabs of the cloaca and oropharynx before and after race season. Differences were considered as significant at the level of 0.05 or less.

RESULTS AND DISCUSSION

According to Balicka et Pilarczyk (2014) the most common diseases pigeons include coccidiosis, trichomoniasis and respiratory infections, which is also confirmed in our study. After race season were increased ($P \leq 0.05$) incidence of coccidiosis (52.2%), trichomoniasis (31.9%), respiratory syndrome (7.2%) and ectoparasitosis (5.8%) (Tab. 1).

Scullion (2007) focused on clinical manifestations of various pigeon diseases in his work. He describes that the incidence of coccidia (*Eimeria spp*.) Confirmed in faecal samples does not always have a clinical course, rather the subclinical course observed in these individuals. On the contrary, the clinical manifestations of trichomoniasis increase with increasing occurrence of *Trichomonas* spp. isolated from oropharynx swabs.

Except for trichomoniasis and coccidiosis was respiratory syndrome very common diseases in monitored flock. Respiratory syndrome is the major cause of poor performance and pigeon loss during the race season. Young birds under stress are most at risk of contracting

respiratory diseases, although healthy old birds can fall ill when exposed to respiratory diseases in the race basket. Race birds with respiratory infection can be difficult to detect and yet, like a human athlete with flu, cannot compete (Rupipper, 1998b).

Table 1 The most common diseases of pigeons in the monitored flock during race season

Diseases	**Before race season** (80)		**After race season** (69)	
	n	%	n	%
Coccidiosis	9	**11.3[a]**	36	**52.2[b]**
Trichomoniasis	6	**7.5[a]**	22	**31.9[b]**
Respiratory syn.[1]	0	**0[a]**	5	**7.2[b]**
Ectoparasitosis[2]	0	**0[a]**	4	**5.8[b]**
Endoparasitosis[3]	0	**0**	2	**2.9**

Note: n – number of isolated strains from total 80 investigated pigeons before race and from 69 pigeons after race, Respiratory syn.[1] - respiratory syndrome determined according to clinical manifestations. Ectoparasitosis[2] - caused by *Columbicola columbae*, *Ceratophylus columbae*, Endoparasitosis[3] - caused by *Ascaridia* spp., *Capillaria* spp.

Clinical respiratory infection in pigeons is the end result of the interplay of a number of factors but, in particular, the type of infective organism and the vulnerability of the birds to infection are important. The classic clinical symptoms of respiratory infections include mucous in the throat, open beak and heavy breathing, rasping or gurgling while breathing, watery discharge from eyes, sometimes associated with swelling in the eye area (Struhár, 2015).

Other symptoms include discharge from the nasal area and occasionally air sac swelling or crop swelling as torn air sacs trap air under the skin. As is usually the case with pigeons, other diseases can quickly manifest themselves when birds are in distress, so other symptoms can occur, such as loose, greenish droppings and loss of weight (Hand, 2004).

Common problems during the transport and feeding of pigeon are mixed endoparasitosis infections. Mixed infections with intestinal nematodes and coccidia were found in 42% of domestic pigeons and in 14.3% of the wild. As can be seen from the research, parasitic infection was greater in domestic pigeons than in the wild (Rupipper, 1998b). In our study before race season we recorded low level of coccidiosis without endoparasitosis and

ectoparasitosis. After the race season were increased ($P \leq 0.05$) incidence of coccidiosis and endoparasitosis.

CONCLUSION

Many factors will affect the performance of racing pigeons. Results are showing that also despite increased prevalence of coccidiosis, trichomoniasis and respiratory syndrome, the pigeons are able to provide flight performance and cope with changes in their organism also during this difficult race period. Maintaining sound husbandry practices and preventive medicine principles is vital to flock health. Many of these diseases can be prevented with proper management. In those flocks that require veterinary assistance, intervention can provide a rapid diagnosis, effective treatment, and enhanced performance.

REFERENCES

Balicka-Ramisz A., Pilarczyk B. (2014): Occurrence of coccidia infection in pigeons in amateur husbandry. Diagnosis and prevention. An. of Par., 60: 93-97.

Dranzoa C., Ocaido M., Katete P. (1999): The ecto, gastro-intestinal and haemo parasites of live pigeons (*Columba livia*) in Kampala, Uganda. Av. Pat., 28: 119.

Hand O. (2004): The Effects of Stress on Racing Pigeons. [Online] Available: URL: http://www.pigeonbasics.com/articles/article79.html

Rupiper D.J. (1998): Diseases that affect race performance of homing pigeons, Part I: husbandry, diagnostic strategies, and viral diseases. J. Avian Med Surg. 12: 70-77.

Rupipper D.J (1998): Diseases that affect race performance of homing pigeons. Part II: Bacterial, fungal, and parasitic diseases. Journal of Avian Medicine and Surgery. 12 (3): 138-148.

Scullion F.T, Scullion M.G. (1997): Investigation of diseases in young racing pigeons. Available:http://www.wmenews.com/Information/Other/Section4Scullionetalpp73-75pdf.pdf

Scullion FT. (2007): Phatologicfindings in racing pigeons (*Columba livia domestica*) with „young bird sickness“. [Online] Available: URL: http://www.ncbi.nlm.nih.gov/pubmed/18069166

Smith S.A. (1996): Parasites of birds of prey: their diagnosis and treatment. Semin Avian Exotic Pet. Med. 5(2): 97-105.

Stenzel T, Koncicki A. (2007): Occurrence of parasitic invasions in domestitc pigeons (*Columba livia domestica*) in the Northern Poland. Pol. J. of Vet. Sci., 10: 275 – 278.

Struhár M. (2015): Holuby a ich zdravie, Kníhtlač Gerthofer, Zohor, 74 s. ISBN 978-80-89783-02-1.

Zigo F., Takáč L., Chripková M. et al. (2016): Infectious diseases of carrier pigeons and antimicrobial resistance in isolated bacteria. Int. J. of Sci. Res., 5: 2277 – 8179.

ANALYSIS OF WELFARE, HEALTH AND BIOSECURITY IN CALVES FARMS

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ABSTRACT

The study is focused on the analysis of basic critical points of the calf's life, which influence the level of their health, welfare and biosecurity, including the determination of simple selected indicators of calf health directly applicable in farm practice. The critical points of calf rearing begin during the period of insemination, continue of pregnancy, transition and calving, and are completed by the rearing of calves after birth to weaning. A vital role in calf life plays colostrum.

Keywords: calf, insemination, pregnancy, dry and transition period, calving, weaning

INTRODUCTION

At present more and more emphasis is placed on creating a comfortable breeding environment for calves as one of the prerequisites for maintaining good health, adequate levels of welfare and biosecurity. Without the above mentioned it is not possible to achieve genetically given performance and consequently, of course, the economic efficiency of the farmers. At the same time, it creates conditions for reducing the negative impact of extreme climatic conditions (Mandel et al., 2016). The success to keep calves healthy and growing starts well before the calf is born – even prior to conception. In fact, the cow's health can be a strong predictor of the calf's potential for growth and production (Ryan, 2017).

Calves are the most sensitive category of cattle and are highly susceptible to disease because their immune system is not yet fully developed (Fotheringham, 1995; Cortese, 2009). For calves, everything starts, but of course everything can end there prematurely. A poorly rearing calf will never grow into a top dairy cow.

The most common causes of calf loss (70-80%) are diarrhoea and respiratory disease (Virtala et al., 1996; Illek, 2007). Infectious pressure on farms and stables increases in proportion to the concentration of animals and the duration of their stay in the different calf housing systems (Novák, Malá, 2012). This can cause growth depression and increased incidence of

calf health problems. Therefore, preventive measures, including sanitation, should be included in all housing systems in order to maintain good hygiene standards in the various technological systems of farming.

MATERIAL AND METHODS

The work is focused on the analysis of selected critical points of the calf's life, which affect the level of their health, well-being and biosecurity. The paper is based on the analysis of both Czech and foreign professional and scientific literary sources and above all on the practical experiences of the authors obtained in the framework of the solution of scientific research projects and field studies in livestock breeding.

RESULTS AND DISCUSSION

The prevention and protection of calves' health begins as early as during the period of insemination of the mothers, continues during the pregnancy period ending in calving and the following period of calves rearing until weaning. Appropriate breeding conditions and rearing management determine the level of welfare and calf health. Early identification of sick calves and provision of supportive therapy, enhances their survival, improves their welfare levels and minimizes negative effects on their long-term productivity.

First of all, it is necessary pay attention to health control of calves after birth. The simplest system of health indicators evaluation include calf standing position, eye and nostrils, faeces consistency, colour and odour, respiratory and pulse rate and rectal temperature. These simple indicators can be used to quickly assess the health status of a large number of calves, which indicates what steps need to be taken to improve the health and welfare levels of the calves. The advantage of most of the above-mentioned calf health indicators, in addition to rectal temperature, is the ability to evaluate using our senses (sight, hearing, touch, smell).

There are two major diseases of calves - scours and pneumonia, which account for 80% of all calf deaths. The cause of scours in calves under 21 days of age is difficult to determine. There is usually not one single cause, but an interaction between calf management, diet, the environment, poor immunity, and the presence of pathogenic viruses and bacteria. Most of the scours can be controlled through good management and appropriate preventative measures.

Pneumonia is a problem with housed calves, particularly when stocking density is high and ventilation is poor. Control is mainly through improved housing (Moran, 2012).

Insemination period

Lower body condition score, hot weather (≥29°C) at the time of first artificial insemination and the pre-existence of peri- or postpartum disorders are important risk factors that limit first service conception in dairy herds, and that a failure of FSC is associated with an economic loss for farmers. Thus, nutritional, environmental, and management strategies to maintain BCS ≥3.0, prevent heat stress during the insemination period, and reduce the incidence of or effectively treat peri- and postpartum disorders might be required to improve FSC rate in dairy herds with a high yield under intensive production systems, thereby reducing the cost of reproductive management (Kim and Jeong, 2019).

Dry and transition period

The dry period is the most important phase of a dairy cow's lactation cycle. During this phase, the cow and her udder are prepared for the next lactation; hence any abnormalities during the dry period will have a negative effect on the cow's health and milk production after calving.

During dry period, the following significant changes take place: acceleration of foetal growth, regeneration and growth of new milk tissues in the udder, hormonal changes and increased the intensity of metabolism.

The goal of providing the calf with good-quality colostrum to maximize passive immunity can best be achieved by providing pregnant and dry cows with adequate protein, energy, vitamins and minerals including the dry matter intakes. Low dry matter intakes may result in lower volumes of colostrum. Approximately 60 % of fetal growth occurs during the last three months of gestation. Adequate dry cow mineral supplementation is a must for calf health and the development of a healthy immune system. The key period in dairy cattle breeding is the care of high-pregnant cows approximately 3-4 weeks before and 3 to 4 weeks after delivery (transition period), because the profitability of the whole breeding is decided especially at this time (Mudřík, 2013).

Calving

The main principle of trouble-free calving is that a cow needs for calving quiet place and time. Calving should be intervened only when absolutely necessary (e.g. if calving lasts too long, calf is too large, etc.). Cows near to calving are more ideally housed and calved ideally in individual pens, which are well-bedded with fresh, clean, dry bedding to reduce the amount of mud and faecal contamination. It is also necessary to ensure adequate air exchange in the delivery area, sufficient light. Sanitation (cleaning, washing and disinfection) between every calving cow prevent the disease transmission. Cows must have permanent access to water and feed in the

farrowing pen. High level of farrowing pen hygiene and the birth itself, as well as appropriate care of the newborn calves contributes to its successful rearing.

The right routine of calf treatment immediately after birth is to remove mucus from the nostrils, clean the oral cavity, navel disinfection and dry the body surface by licking the mother or manual drying the calf surface using straw, colostrum drinking and calf removing to the hutches.

Housing environment

To ensure calf welfare, it is necessary to provide dry litter bedding; is very important for calves and their thermoregulation as it significantly reduces heat loss from the body by conduction and thus helps animals to overcome low ambient temperatures (Webster, 1984). Wet litter reduces the ability of calves to plunge into the litter and increases the amount of heat loss from the surface of lying animals (Hill et al., 2011). Inadequate ventilation (resulting in inappropriate airflow, low or high temperatures, high humidity and poor air quality), poor floor conditions (wet floor, without bedding) can cause exposition to pathogens causing respiratory and gastrointestinal disorders (Maunsell et al., 1999).

Colostrum management

The calf is born without protective antibodies and depends on the uptake of colostral antibodies (immunoglobulins) to protect it against common diseases (passive immunity). Immunoglobulins are absorbed across the small intestine wall from colostrum during the first 24 h after birth, 48 hours after calving the total closing the small intestine wall occurs. Achieving adequate passive transfer of immunoglobulins is a function of quality and quantity of colostrum as well as the timing of colostrum feeding. The best colostrum calf feeding system is based on 1-2-3 rule: 1st feed within 2 hours after birth with 3 litres of colostrum (about 10-12% of the calf's live birth weight) of high quality (≥ 50 mg / heated to temperature of about 40 ° C. To ensure adequate colostral immunity – it is necessary regularly check the colostrum quality, quantity and frequency of calf feeding during the first 24 hours after calving.

The immunoglobulins contained in colostrum provide passive immunity. While active immunity of the calf organism is created gradually from the third week after birth (Hulbert and Moisá, 2016). First-lactation heifers produce lower yields of colostrum, lower total mass of Ig, and lower IgG concentration in colostrum than cows in their second or greater lactation. The quality of colostrum continues to increase with parity after the second calving, and older cows generally have the best quality colostrum (Morrill et al., 2012). Volume of colostrum produced may be less in cows with a dry period <40 days (Rastani et al., 2005). However, short dry periods are associated with reduced colostral IgG concentrations in first lactation animals (Annen et al., 2004).

Calves weaning

Weaning can be a stressful time for calves as they change from a liquid diet from predominantly animal protein sources to a solid diet from vegetable protein sources. To reduce potential stress, concentrates (starter) should be introduced early, from a few days after calving, to encourage intake. Concentrates should be highly palatable and of a high nutritional quality. Forage should also be offered, usually straw or hay. Fresh water must be available during the 24 hour of a day. Weaning should be done gradually by reducing milk fed over a period of 7 to 14 days. This will lead to increased concentrate intake, avoid a growth check after weaning and minimise weaning distress. Calves should only be weaned after they have been eating at least 1kg of starter per day for three consecutive days. The aim of the breeder is to maintain the average daily gain increase in calves during weaning while minimizing the risk of their disease. Calves weaned before five weeks of age tend to be more susceptible to respiratory diseases.

CONCLUSION

All technological systems, components and equipment on livestock farms should comply with welfare requirements in terms of ecology, ethology and ethics. Adherence to the appropriate hygienic standard of breeding is a prerequisite for maintaining good health and achieving a high level of production and reproductive performance. The basic hygienic principles of rearing calves in the dairy period can be summarized in ten basic points:

1) High level of care from insemination through the pregnancy to the dry period cows - technological systems of breeding and nutrition management;

2) Transition period and calving - hygiene of farrowing pens, including control of the delivery and, where appropriate, assistance during calving;

3) Care of calf after birth - treatment of nasal and oral cavity and navel, drying of body surface, possible application of vitamins and supporting products;

4) Ensuring adequate colostral immunity - checking colostrum quality, quantity and frequency of calf feeding during the first 24 hours after calving;

5) Adequate level of nutrition - regular watering with milk or milk replacer, from third day after birth - access to water, from one week starter, hay or TMR;

6) Quality of breeding environment – ensuring welfare and comfort, spatial isolation between calves, protection against climatic extremes (wind, rain, snow, ...), dry bed (sufficient amount of quality bedding);

7) All in all out system of rearing - optimally 7 days between following batches;

8) Hygiene and sanitation - cleaning, washing, disinfection and insect and rodent control;

9) High level of nursing care;

10) Health herd management.

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REFERENCES

Annen, E.L. (2004): Effect of modified dry period lengths and bovine somatotropin on yield and composition of milk from dairy cows. J. Dairy Sci. 87: 3746-3761.

Cortese, V.S. (2009): Neonatal Immunology. In: Smith GW. Bovine Neonatology. Vet. Clin. North Am. Food Anim. Pract. 25, 221-227.

Fotheringham, V.J.C. (1995): Disinfection of livestock production premises. Rev. sci. tech. Off. Int. Epiz. 14 (1): 191-205.

Hill, T. M., Bateman, G., Aldrich, J.M., Schlotterbeck, R.L. (2011): Comparison of housing, bedding, and cooling options ford dairy calves. J.Dairy Sci. 94:2138-2146.

Hulbert, L.E., Moisá, S.J. (2016): Stress, immunity, and the management of calves. J.Dairy Sci. 99 (4): 3199-3216.

Illek, J. (2007): Závažná průjmová onemocnění telat. Zemědělec, 19, 7: 2.

Mandel, R., Whay, H. R., Klement,E., Nicol, C. J.(2016): Environmental enrichment of dairy cows and calves in indoor housing. Invited review. J. Dairy Sci. 99 (3): 60-69.

Maunsell, F.P., D.E. Morin, P.D. Constable et al. (1999): Use of mammary gland and colostral characteristics for prediction of colostral IgG1 concentration and intramammary infection in Holstein cows. JAVMA. 214:1817-1823.

Moran,J.(2012): Rearing Young Stock on Tropical Dairy Farms in Asia.CSIRO Publishing. 296p.

Morrill, K.M., E. Conrad, A. Lago et al. (2012): Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. J. Dairy Sci. 95:3997-4005.

Mudřík, Z.: Tranzitní období a následná produkce. Zemědělec 2.8. 2013

Novák, P., Malá, G. (2012): Obecné zásady biosecurity v chovech hospodářských zvířat. Metodika, Praha Uhříněves: Výzkumný ústav živočišné výroby, v.v.i., 55 s.

Rastani, R., Brummer, S.J. Bertics et al. (2005): Reducing dry period length to simplify feeding transition cows: milk production, energy balance and metabolic profiles.J.Dairy Sci. 88:1004-1014.

Ryan, J. (2017): Calf Health Starts Before Birth. Available from https://blog.vet-advantage.com › livestock › calf-health-starts-before-birth (accessed Feb 17, 2019).

Virtala, A. M. K., Mechor, G. D. Gröhn, Y. T., Erb, H. N. (1996): The effect of calfhood diseases on growth of female dairy calves during the first 3 months of life in New York State. J. Dairy Sci. 79:1040-1049.

Webster ,J. (1984): Calf husbandry, health and welfare. London, UK: Collins, 202p.

PREFERENCE OF EARTHDOG´S WORK – TERRIERS AND DACHSHUNDS IN DEN TRIALS FROM THE WELFARE POINT OF VIEW

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ABSTRACT

The aim of the work was to compare the preference of earthdogs´ work (Terriers and Dachshunds) in earthdogs hunting examinations within the contact mode of action from the welfare point of view. Examination rules are valid at the examination tests in Slovakia, where the dog and the fox come into the direct contact. Tests are carried out in an artificial den**.** We evaluated international tests in den trials in Slovakia within the period of five years (2014 – 2018), where three ways of work were preferred: hard dog 54.32 %, bayer 23.20 % and bolter 22.48 %. There are fights between the dog and the fox in the earthdogs´ work, and we can see various bites as well as various fox and dog injuries. Welfare principles are not observed in such trials. From the practical use point of view, the work of the bolter is the most suitable, however, the work of the hard dog (54.32 %) was the most frequent. We evaluated the hard dog work according to the place, where the fox has been nipped. The most common way of working preference in Terriers was „muzzle in the muzzle“ (30.93 %). The working preference in Terrier breeds and Dachshunds, too, was the nip on the back half of the fox's body (32.41 %). This way of working prepares the earthdogs for competitions in the den trials but not for practical use, therefore, we propose a change in the examination order for tests in den trials in Slovakia, so that the dog and the fox are not in the direct physical contact.

Keywords: terrier; dachshund; fox; welfare; earthdog; bolter; bayer; hard dog

INTRODUCTION

Earthdog work, as a mean of red fox (*Vulpes vulpes*) population control, has a long hunting tradition. The red fox is the most widespread carnivore in the world and occurs almost all over Europe. The narrow and slender body of the fox is adapted to live in lair, which either digs itself or uses another lair. The fox often stays in the lair especially in winter when the weather is bad, at the time of hunting, and in the spring when fox cubs are raised. Fox is a game with year-round hunting in Slovakia. One of the forms of regulation of fox numbers is underground hunting with the help of earthdogs. The group of earthdogs consists of Terriers and Dachshunds that are bold, courageous and passionate. They have a strong prey drive and hunting instinct, which allows them to work fearlessly underground, but also on the surface, especially when hunting wild boar.

MATERIAL AND METHODS

We compared the working methods of earthdogs (Terriers and Dachshunds) at International Den Trials in the period 2014 – 2018 in Slovakia according to the current requirements related to animal protection and welfare. Information were received from the Slovak Club of Jagdterriers, the Slovak Club of Terriers and Fox Terriers and the Slovak Dachshund Club, which organized these events.

RESULTS AND DISCUSSION

We focused on rules that observe the protection and welfare of foxes and dogs used in den trials in this work. We compared the working preference of earthdogs according to dogs of Terriers and Dachshunds breeds at the international earthdog trials within the period 2014 – 2018 in Slovakia. In these trials, the dog and the fox come into direct contact. Hunting in Slovakia is governed by hunting legislation, which includes hunting cynology. In Slovakia, hunting is regulated by the Act no. 274/2009 Coll. on Hunting and on Amendments to Certain Acts and by Decree no. 344/2009 Coll. International earthdog trials were organized according to the valid examination rules (Slovak Hunting Chamber, 2015). The trials shall be carried out in an artificial den, the scheme of which is a part of the examination rules. One dog testing lasts 10 minutes (5 minutes in international den trials), if the dog does not end the trial sooner by grabbing or expelling the fox from the burrow. Dogs usually work in three different ways: as a bayer, bolter and a hard dog. The work of the bolter is characterized by the expulsion of the fox from the artificial lair, if the dog persistently barks throughout the trial period, this dog is evaluated as being a bayer. The third way of working is called hard dog work, it means that

the dog grabs on any part of the fox´s body. Depending on where the fox is grasped by the dog, the hard dog´s technique is divided into 6 areas: head, hip, throat, muzzle and the front or back part of fox´ body (SHCh, 2015). The arranging organisation is obliged to provide for a sufficient number of healthy, mature and veterinary checked foxes. For International den trials, 1 fox must be present for 3 dogs. In addition, 50 % of the additional foxes must be prepared as substitutes. If the dog has grabbed the fox, it must be replaced and another fox will be used for the next dog. The injured fox is replaced by a reserve fox in order, and must not be used in the exams anymore (SHCh, 2015). We found that in the international earthdog trials in the screening industry within the period 2014 – 2018, the most prevalent work was the work of the hard dog 830 times (54.32 %), the second was the work of the bayer 355 times (23.20 %) and the third way was the work of the bolter 343 times (22.48 %) (Table 1). We separately evaluated the work of terrier breeds, where the hard dog occurred 540 times (62.94 %) and the work of the bolter only 157 times (18.30 %). The hard dog in dogs of dachshunds breed occurred 290 times (43.28 %) and the bolter 186 times (27.76 %) (Table 1). It can be stated that the work of the bolter occurred several times (27.76 %) in dachshunds compared to the terriers (18.30 %). The best work of the earthdogs from the welfare point of view is the work of the bolter, where the dog drives the fox out of the den. That way of dog´s work is the best for practical use.

Table 1 The overall evaluation of the number of ways of earthdogs in the International Den Trials in Slovakia in 2014 – 2018

Earthdogs	**The way of work**					
	BOLTER		**BAYER**		**HARD DOG**	
	number	**%**	**number**	**%**	**number**	**%**
Terriers	157	18,30%	161	18,76%	**540**	**62,94%**
Dachshunds	186	27,76%	194	28,96%	**290**	**43,28%**
TOTAL	343	22,48%	355	23,20%	**830**	**54,32%**

The dog gains the hunting ability to control foxes in the nature after passing the den trials. These trials do not prepare earthdogs for ideal work in nature, but for competitions in den trials.

Table 2 Distribution of the hard dog work by grasping the fox site at the International Den Trials in 2014 – 2018 in Slovakia

Earthdogs	**Hard dog (grasping the fox)**					
	Neck	**Hip**	**Head**	**Muzzle in muzzle**	**Front body part**	**Back body part**
Terriers	114 (21,11%)	44 (8,15%)	58 (10,74%)	**167 (30,93%)**	74 (13,70%)	83 (15,37%)
Dachshunds	56 (19,31%)	11 (3,80%)	31 (10,69%)	62 (21,38%)	36 (12,41%)	**94 (32,41%)**
TOTAL	170 (20,48%)	55 (6,63%)	89 (10,72%)	**229 (27,59%)**	110 (13,25%)	177 (21,33%)

We evaluated as well the work of the hard dog according to the site where the fox was grasped by the dog and we found that the Terrier breeds preferred mostly muzzle in muzzle way of grasping 167-times (30.93 %) compared to the Dachshunds, they gripped the fox by the back body part 94 times (32.41 %) (Table 2).

CONCLUSION

We focused on the earthdogs´ ways of working from the perspective of welfare in Slovakia. In Slovakia, den trials are contact, that means the dog comes into direct physical contact with the fox. There is fighting in the artificial den, where bites and injuries happen mainly in foxes, but in dogs, too. Ethical principles and welfare are not followed in such den trials. Contact den trials are not important for practical use, but they can prepare dogs for competitions in artificial den. Therefore, we propose a change in the den trials in Slovakia, from the welfare point of view. We recommend those trials contactless, with the possibility to assess the talents of earthdogs for working underground. Den trials are also contactless in neighboring countries: the Czech Republic, Poland and Hungary.

REFERENCES

Ciberej J., Karolová R., Kiš O., Oberhauserová K. (2013): Hunting Cynology. Rev. Jozef Jursa. 2. Publishing. Zvolen, Technical University, (v slovenčine), 104 pp., ISBN 978-80-228-2561-0.

Červený J., Hell, P., Slamečka, J. et al. (2004): Encyclopedia of Hunting, Ed. Prague Otto publishing house (v slovenčine), 591 pp., ISBN 80-7181-902-6.

Slovak Hunting Chamber (2015): Test rules and breeding of hunting dogs in the pocket. Publisher Epos, 192 pp. ISBN 978-80-562-0083-4.

Slovak Club of Jagdterriers' breeders (2014): International Club Tests in Den Trials in Jagdterriers 2014. Yearbook of Club News 2015, pp. 90.

Slovak Club of Jagdterriers' breeders (2015): International Club Tests in Den Trials in Jagdterriers 2014. Yearbook of Club News 2016. pp. 70.

Slovak Club of Dachshunds´breeders (2014): International Den Trials of Dachshunds. Club Newsletter Dachshund 2014, pp. 83.

Slovak Club of Dachshunds´breeders (2015): International Den Trials of Dachshunds [online]. [cit. 2019-03-04]. Available at the Internet: http://www.jazvecik.sk/medzinarodne-klubove-skusky-jazvecikov-v-brlohareni-msblj/

Slovak Club of Dachshunds´breeders (2016): International Den Trials of Dachshunds. Club Newsletter Dachshund 2016, pp. 80.

Slovak Club of Dachshunds´breeders (2017): International Den Trials of Dachshunds. Newsletter SKCHJ Dachshund 2017, pp. 138.

Slovak Club of Dachshunds´breeders (2018): International Den Trials of Dachshunds. Newsletter SKCHJ Dachshund 2018, pp. 165.

Slovak Club of Terriers and Fox terriers breeders (2014): International Den Trials of Terriers. Club Newsletter, XXIV, 2/2014, pp. 82, 33 – 39.

Slovak Club of Terriers and Fox terriers breeders (2015): International Den Trials of Terriers. Club Newsletter, XXV, 2/2015, pp. 102.

Slovak Club of Terriers and Fox terriers breeders (2016): International Den Trials of Terriers [online], [cit. 2019-03-04]. Available at the Internet: http://terriers.sk/dokumenty/1195_MSBLT_Tura_Luka_2016.pdf

Slovak Club of Terriers and Fox terriers breeders (2017): International Den Trials of Terriers. Club Newsletter, XXVII, 2/2017,pp. 66.

Slovak Club of Terriers and Fox terriers breeders (2018): International Den Trials of Terriers. Club Newsletter, XXVIII, 2/2018, pp. 58.

USE OF SOFTWARE TO MONITOR THE HEALTH OF DAIRY COWS

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ABSTRACT

Good dairy health is a prerequisite for achieving the desired production and reproductive performance. The occurrence of serious health problems results in large economic losses due to decreased milk yield, deterioration of milk quality, shortening of lactation and premature braking. Computer-based accurate health records and the use of appropriate technological equipment lead to the prevention and optimization of herd health.

Key words: health, evidence, technological equipment

INTRODUCTION

The continuously increasing requirements for safe medicines manipulation forces the zootechnics to closely monitor all the medical interventions that are carried out on animals. Each farm has to keep records of all used medicines. To facilitate the keeping of recordings, our company has developed the treatment module in the Farmsoft program. This module is a part of program offers, and the most majority of zootechnics use it.
One of the first promoters of our program is MVDr. Libor Borkovec from the company Zoetis. He uses our treatment module in his breedings, especially the mastitis evaluation report (see Figure 2). Thanks to this report, he can observe the development of the health condition of the udder of dairy cows throughout the whole year (and according to the given

stable). We can find there the total number of treated animals, the number of new mastitides, recurrences and incidences. Health is an important part of animal welfare (Smutný, 2015). All diseases worsen the level of animal welfare, but vice versa, a worse welfare level increases the susceptibility of animals to disease.

LITERARY OVERVIEW

To obtain information about animals it is necessary to collect, process and evaluate a large amount of input data based on measurements of selected physiological parameters such as milk size, conductivity and milk temperature, feed use, physical activity or body weight. These accurate and up-to-date data must be continuously processed, sorted, analyzed and presented to the breeder through a quality software system (Smutný et al., 2002, Smutný 2000, Bouška et al., 2006, Stupka 2013, Doležal 2015).

Mastitis is generally considered to be the most expensive disease in dairy cattle, which manifests by reduced milk yield, increased production costs and decreased milk quality (White 1994, Wilson 2004, Borkovec 2014).

Health is an important part of animal welfare. All diseases worsen the welfare level of the animals, but vice versa, a worse welfare level increases the susceptibility of the animals to the disease (Broom 2002).

METHODOLOGY

Farmsoft is a Czech zootechnical software for everyday zootechnics work. The Farmsoft program is used by 171 businesses at 285 stations. The Farmsoft program includes the Health module, which is used to optimize health, well-being and production in production herds by systematically analyzing relevant data.

The database in this treatment module contains all necessary data such as various diagnoses, drugs (their quantities and kinds), date of intervention, withdrawal periods for meat and milk. All this information can be viewed, printed or introduced to the inspection authorities at any time. For this purpose, the treatment module generates a report called the Record of drug administration (see Figure 2).

The role of zootechnics is to enter the input data on the healing and status of drugs in stock. The task of the program is to follow the withdrawal periods of milk and meat of treated animals, the quantities of used drugs and their stock flows. The possibility of entering a

treatment plan, where we can determine the healing procedure or insemination interventions (preparation for ovsynch, presynch) several days in advance, represents a significant help for zootechnics and veterinary services as well.

The frequency of the disease per 100 cows per year was monitored for individual diseases. The observation also corresponded to data from literature literatury Hodges (2001) and Eddy (1980). Data from 45 enterprises were used where it was assumed that data on disease and treatment were accurately recorded.

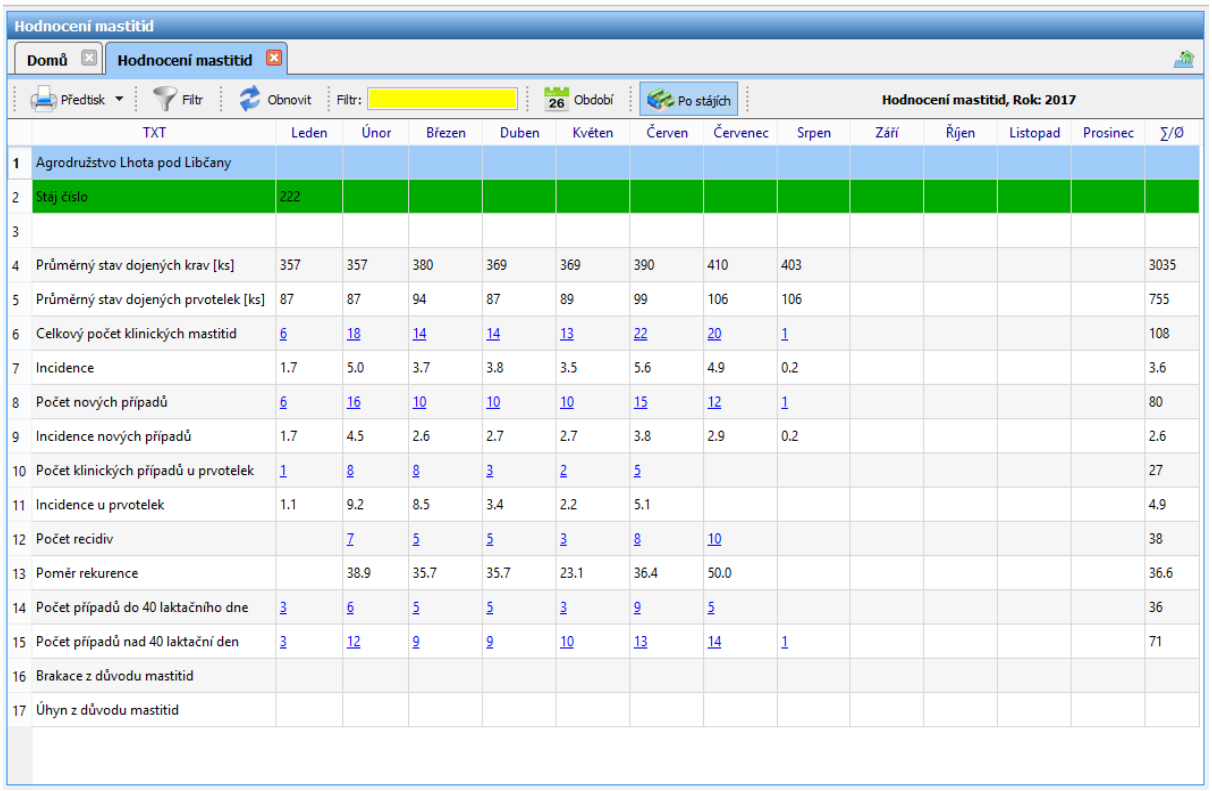
RESULTS

Table 1 Health evaluation is based on the interval distribution of points in relation to the frequency of disease per 100 cows

	5	**4**	**3**	**2**	**1**
Mastitis	under 20 0 farms	20 - 40 12 farms	40 - 70 16 farms	70 - 100 12 farms	over 100 5 farms
Extremities	under 5 5 farms	5 - 15 7 farms	15 - 30 11 farms	30 - 50 22 farms	over 50 0 farms
Reproduction	under 10 4 farms	10 - 35 28 farms	35 - 60 6 farms	60 - 90 7 farms	over 90 0 farms
Metabolism	under 5 19 farms	5 - 10 14 farms	10 - 15 10 farms	15 - 20 2 farms	over 20 0 farms
Other	0 18 farms	1 - 5 18 farms	5 - 10 7 farms	10 - 15 2 farms	over 15 0 farms

Based on the evaluation of Sad and Hodges, we find that in the mentioned breeds the lowest number of metabolic and reproductive problems is observed, on the contrary, the highest occurrence is observed in the occurrence of mastitis

Picture 1 Evaluation of mastitis in the herd



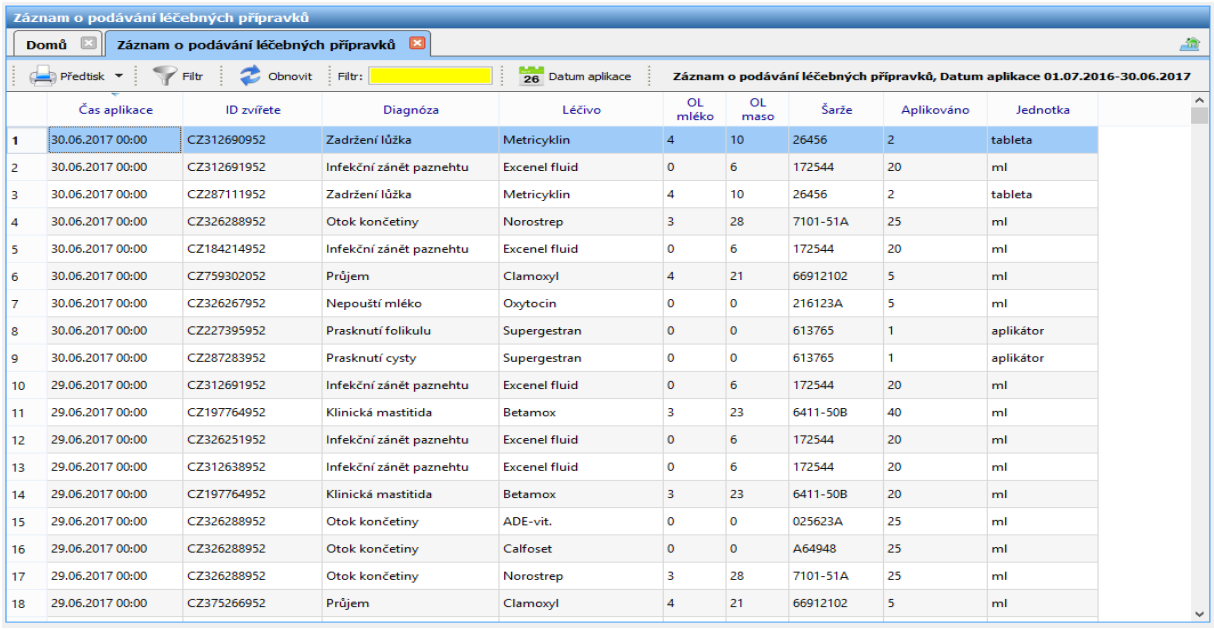
Hodnocení mastitid

Domů | Hodnocení mastitid

Předtisk | Filtr | Obnovit | Filtr: | Období | Po stájích | Hodnocení mastitid, Rok: 2017

	TXT	Leden	Únor	Březen	Duben	Květen	Červen	Červenec	Srpen	Září	Říjen	Listopad	Prosinec	∑/Ø
1	Agrodružstvo Lhota pod Libčany													
2	Stáj číslo	222												
3														
4	Průměrný stav dojených krav [ks]	357	357	380	369	369	390	410	403					3035
5	Průměrný stav dojených prvotelek [ks]	87	87	94	87	89	99	106	106					755
6	Celkový počet klinických mastitid	6	18	14	14	13	22	20	1					108
7	Incidence	1.7	5.0	3.7	3.8	3.5	5.6	4.9	0.2					3.6
8	Počet nových případů	6	16	10	10	10	15	12	1					80
9	Incidence nových případů	1.7	4.5	2.6	2.7	2.7	3.8	2.9	0.2					2.6
10	Počet klinických případů u prvotelek	1	8	8	3	2	5							27
11	Incidence u prvotelek	1.1	9.2	8.5	3.4	2.2	5.1							4.9
12	Počet recidiv		7	5	5	3	8	10						38
13	Poměr rekurence		38.9	35.7	35.7	23.1	36.4	50.0						36.6
14	Počet případů do 40 laktačního dne	3	6	5	5	3	9	5						36
15	Počet případů nad 40 laktační den	3	12	9	9	10	13	14	1					71
16	Brakace z důvodu mastitid													
17	Úhyn z důvodu mastitid													

Picture 2 Record of administration of medicinal products



Záznam o podávání léčebných přípravků

Domů | Záznam o podávání léčebných přípravků

Předtisk | Filtr | Obnovit | Filtr: | Datum aplikace | Záznam o podávání léčebných přípravků, Datum aplikace 01.07.2016-30.06.2017

	Čas aplikace	ID zvířete	Diagnóza	Léčivo	OL mléko	OL maso	Šarže	Aplikováno	Jednotka
1	30.06.2017 00:00	CZ312690952	Zadržení lůžka	Metricyklin	4	10	26456	2	tableta
2	30.06.2017 00:00	CZ312691952	Infekční zánět paznehtu	Excenel fluid	0	6	172544	20	ml
3	30.06.2017 00:00	CZ287111952	Zadržení lůžka	Metricyklin	4	10	26456	2	tableta
4	30.06.2017 00:00	CZ326288952	Otok končetiny	Norostrep	3	28	7101-51A	25	ml
5	30.06.2017 00:00	CZ184214952	Infekční zánět paznehtu	Excenel fluid	0	6	172544	20	ml
6	30.06.2017 00:00	CZ759302052	Průjem	Clamoxyl	4	21	66912102	5	ml
7	30.06.2017 00:00	CZ326267952	Nepouští mléko	Oxytocin	0	0	216123A	5	ml
8	30.06.2017 00:00	CZ227395952	Prasknutí folikulu	Supergestran	0	0	613765	1	aplikátor
9	30.06.2017 00:00	CZ287283952	Prasknutí cysty	Supergestran	0	0	613765	1	aplikátor
10	29.06.2017 00:00	CZ312691952	Infekční zánět paznehtu	Excenel fluid	0	6	172544	20	ml
11	29.06.2017 00:00	CZ197764952	Klinická mastitida	Betamox	3	23	6411-50B	40	ml
12	29.06.2017 00:00	CZ326251952	Infekční zánět paznehtu	Excenel fluid	0	6	172544	20	ml
13	29.06.2017 00:00	CZ312638952	Infekční zánět paznehtu	Excenel fluid	0	6	172544	20	ml
14	29.06.2017 00:00	CZ197764952	Klinická mastitida	Betamox	3	23	6411-50B	20	ml
15	29.06.2017 00:00	CZ326288952	Otok končetiny	ADE-vit.	0	0	025623A	25	ml
16	29.06.2017 00:00	CZ326288952	Otok končetiny	Calfoset	0	0	A64948	25	ml
17	29.06.2017 00:00	CZ326288952	Otok končetiny	Norostrep	3	28	7101-51A	25	ml
18	29.06.2017 00:00	CZ375266952	Průjem	Clamoxyl	4	21	66912102	5	ml

CONCLUSION

The introduction of software records of the health status of individual cattle categories on farms facilitates the work of the zootechnician and veterinarian. Despite the time-consuming data acquisition, unlike manual records, the use of Farmsoft will result in a better overview of herd morbidity, preventive action planning, drug consumption overview, and better health.
Last but not least, the Farmsoft treatment module is also a great helper for the staff involved in applying for and processing subsidies for livestock production. The program can prepare detailed documents necessary for grant applications.

ACKNOWLEDGEMENT

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REFERENCES

Bouška, J., Doležal, O., Jílek, F., a Kudrna, V. *et al.*: *Chov dojeného skotu.* Profi Press, Praha, 2006, s. 186, ISBN 80-86726-16-9.

Borkovec, L. (2014).: *Jakým směrem ke zdravé mléčné žláze?* Náš chov 5/2012.

Broom, D. M.: Indicators of poor welfare, pure and applied zoology, 2002, vol. 142, p. 524–526.

Doležal, O., Staněk, S. (2015): Chov dojeného skotu, PP Praha 2015, ISBN 978-80-86726-70-0.

Eddy, R. G., Scott, CP. (1980): Some observations on the incidence of lameness in dairy cattle in Somerset. Vet. Rec, 106, 140-144.

Hedges, V. J., Blowey, R. W., Packington, A. J., O'Callaghan, C. J., Green, L. E. (2001): Alongitudinal field trial of the effect of biotin on lameness in dairy cows. J. Dairy Sci., 84, 1969-1975.

Smutný, L.: *Počítač pomocníkem při chovu hospodářských zvířat*. Náš chov. 2000, č. 11.

Smutný, L., Šoch, M., a Novák, P.: Individuální péče o dojnici formou elektrického sledování a vyhodnocování zootechnických údajů. In: Sborník z konference „Ochrana zvířat a welfare 2002“ Brno: Veterinární a farmaceutická univerzita Brno. 2002, s. 217-218.

Smutný, L.: Funkčnost dojících robotů a jejich vliv na welfare dojnic. DiP, ZF, JU 2015.

Stupka, R. (2013): Chov zvířat, Powerprint Praha, 2013, ISBN 978-80-87415-66-5.

White, T. C., Madsen, K. S., Hintz, R. L., Sorbet, R. H., Collier, R. J., Hard, D. L., Hartnell, G. F., Samuels, W. A., Kerchove, F., Adriaens, F., Craven, N., Bauman, D. E., Bertrand, G., Bruneau, P., Graven, G. O., Head, H. H., Huber, J. T., Lamb, R. C., Palmer, C, Pell, A. A., Hipps, R., Weller, R., Piva, G., Irijpkema, Y., Skarda, J., Vedeau, F., Wollny, C. (1994):

Wilson, D. J., Gonzales, R. N., Hertl, J., Schulte, H. F., Bennett, G. J., Schukken, Y.H.,

ETHICAL ASPECT OF SELECTED HUNTING DOGS TRIALS IN SLOVAKIA AND THE CZECH REPUBLIC

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ABSTRACT

The aim of the work was to compare selected disciplines of hunting dogs involved in the field trial competition and to evaluate compliance with ethical principles in Slovakia and the Czech Republic. We focused on selected trials of hounds, den trials, special trials in water and the individual trials of scent hounds, too. During the den trials in Slovakia, the dog comes into a direct physical contact with a fox, whereas trials take place without a direct contact between the dog and the fox in the Czech Republic. We compared the discipline of courage in a wild boar enclosure, where the dog and the wild boar are in a direct contact in Slovakia and there is not a direct contact between animals in the Czech Republic. Ethical principles are not respected during these disciplines in Slovakia. Moreover, in Slovakia, a part of special trials in water discipline and trials of bringing a small game by retrievers is a discipline called a duck´s scent on water surface. In this discipline, a duck cannot fly, because it is released with one flight feather modified. In the Czech Republic, this discipline is not a part of the trials as ethical principles are not respected. We also compared the individual trials of scent hounds, which take place on deliberately shot even-toed ungulates. In order to comply with ethical principles and welfare, we propose changes in examination rules of above mentioned disciplines in Slovakia.

Keywords: hunting dogs trials; hounds; ethical aspects; welfare; den trial

INTRODUCTION

Hunting in Slovakia is regulated by the Act No. 274/2009 Coll. on Hunting and on Amendments to Certain Acts, and implementing Decree no. 344/2009 Coll. The Decree requires hunting ground users to use hunting dogs in the exercise of their hunting rights. Hunting in the Czech Republic is regulated by Act No. 449/2001 Coll. Act on Hunting and on Amendments to Certain Acts, as amended. of regulations and implementing decree no. 244/2002 Coll. In order to ensure proper hunting management, game hunting, tracing of shot game, avoidance of game degradation, rescue of game, the criteria for obtaining the dog's fitness for use, the method and procedure for verification (testing) and evaluation are established. Act on Veterinary Care (Act no. 39/2007 Coll. as amended) is the only act, where the animals´ protection is regulated. Civil Code (Act no 40/1964 Coll. as amended) contains legislation in which a living animal has a special meaning and value as a living creature capable of being perceived by its own senses and having a special status in civil relations. The Slovak Penal Code (Act no 300/2005 Coll. as amended) allows to punish the perpetrator who abused the animal for imprisonment for 1 – 5 years. In the Czech Republic, during the tests, the "Animal Protection Regulations for Hunting Practice or Hunting Dogs" must be observed. The organizer must instruct persons who are actively participating in tests on the handling, transport, preparation of items or other equipment and acquaint them with the principles of ensuring the welfare and protection of animals under Act no. 246/1992 Coll., on protection of animals against cruelty. The organizer shall ensure that the animals are handled calmly and decisively and that the animals are in a state of welfare, they are not irritated and mistreated and the risk of injury is minimized. This Regulation does not replace the provisions and conditions laid down by law, by the Act no. 246/1992 Coll., on the protection of animals against cruelty, as amended, as well as by Act no. 166/1999 Coll., on veterinary care and amending some related acts (Veterinary Act).

MATHERIAL AND METHODS

We focused on some disciplines of hunting dogs in Slovakia and the Czech Republic, and we compared them from an ethical point of view in this work. We evaluated both the disciplines of work between the dog and the fox during the den trials, and we compared the discipline of "courage in wild boar´s fenced area" in Slovakia and "willingness to work on wild boar" in the Czech Republic. We compared the discipline of "duck´s scent on water surface", which is a part of special trials from water work and trials of bringing a small game by retrievers in

Slovakia. In the individual main trial of scent hounds, we found that they were also carried out on intentionally shot even-toed ungulates.

RESULTS AND DISCUSSION

We focused on observing ethical principles and welfare in hunting dog tests. The biggest problem is adherence to ethical principles during den trials in Slovakia, where the fox and the dog is in direct physical contact in the artificial lair throughout the trials and there is a fight between the dog and the fox. The fox may be expelled, or the dog may bark (report) the presence of the fox in the lair during the trial period or the fox may be bitten. It is evaluated which part of the body is bitten and grabbed: muzzle in muzzle, head, neck, hip, front or back body part. These bites can cause injury to dogs as well as foxes that sometimes end up fatally. During such den trials the fundamentals of ethics and welfare are not respected. In the Czech Republic, the trials are without direct contact in "test rules for non-contact standardization – beginners test" since the year 2000. There is an odour and sound permeable metal grid between the dog and the fox is in the artificial lair, which prevents direct contact. The Testing Rules for dogs' beginners in the Czech Republic are designed in such a way as to be as objective as possible, without contact with the fox, suited to assess the talents of earthdogs to work underground while complying with the principles of protection of animals against cruelty. Furthermore, we focused on the discipline of testing the courage in the wild boar fence, which is part of the tests of the hounds in Slovakia. The courage of each dog is tested separately in the wild boar fence. The wild boar fence must have The wild boar fence should be of adequate size with plenty of trees, thickset and glades; in the fencing should be kept already matured wild boar, which is sufficiently aggressive for dogs (preferred is wild sow) (Slovak Hunting Chamber, 2015). The dog is admitted to the wild boar fence, where it is in direct physical contact with the wild boar. The dog has to search for the game and when it stumbles the wild boar, it has to energetically challenge the wild boar and constantly bark. If the wild boar moves from the thickset, it should stay in constant contact with it. It is not a mistake if the dog is cautious and jumps away from the wild boar after chasing on the dog. The most important is the stamina of the dog. The courage is tested for 5 minutes after the dog had found the wild boar. In the Czech Republic, the discipline "willingness to work on wild boar" is tested on hound trials (for all breeds of hounds, hunting terrier and dachshunds) and individual hound testing of Slovakian Hound. These tests qualify a dog for hunting and tracing of hoofed game with a special focus on challenging hunting and tracing of the wild boar. In case that the dog will not be able to prove the stridency on the wild boar during the

hunting (trial), this discipline will be tested as a willingness to work on the wild boar according to the test rules for the hound. This discipline is tested in the wild boar fence area. The testing boar fence is double-walled, the inner part dimensions is about 3 x 3 m, the outer fencing is distant from the inner to 15 – 30 cm, to ensure work without contact. When reaching the outer sheathing of the wild boar fence, the dog should show a willingness to work with the signs of permanent teasing and continuous barking. Timeout is 5 minutes. In the case of harsh contact where there is a risk of injury to the dog, the referee must stop working before the time limit. In the Czech Republic, the hound trials qualify a dog for hunting and chasing of wild boar with a special focus on challenging hunting and tracing of wild boar. (Czech-Moravian Hunting Union, 2008). Another discipline where ethical principles are not observed is the duck´s scent on water surface (pointing dogs, retrievers, small breeds), which is part of the special tests of water work and retrievers tests of bringing small game in Slovakia. For water work, a wild duck or a domestic duck with a coloration of a wild one is used. The tested is based on a principle, that the wild duck with one flight feather modified to prevent it from flying, is released into reasonably dense thorns to allow the judges to follow the work of the dog. The flight feather must not be broken. The dog must follow the trail of the live duck and should bring it within 20 minutes or push it to free water surface (Slovak Hunting Chamber, 2015). We compared this discipline in the Czech Republic and have found that the discipline „duck´s scent on water surface“ is not part of the test rules: special trials of water work of hounds (also for flushing dogs, terriers, dachshunds), special trials of water work of retrievers. These trials are tested only on pre-killed game (Czech-Moravian Hunting Union, 2008). In Slovakia, the highest type of breeds scent hound is the individual main trial of scent hounds. The examination code states that the individual main tests of scent hound can only be done on a randomly shot deer, fallow deer and mouflon game, to be traced by the use of a dog – scent hounds (Slovak Hunting Chamber, 2015). In practice, the course of training, but also tests or competitions are often done on a deliberately shot even-toed ungulates, when the shotgun hit is not fatal for the game and allows the tracing of such injured game. In this way, ethical principles are not respected, and we therefore propose that such examinations should be governed by an order that ensures that ethical principles are respected. In the Czech Republic there are “individual scent hounds trials” that are tested on stricken deer, fallow deer, mouflon and wild boar. It is tested when hunting even-toed ungulates in case it is necessary to track a stricken or shot game (Czech-Moravian Hunting Union, 2008).

CONCLUSION

In order to comply with ethical principles and welfare, it is necessary to propose changes to the examination rules for den trials in Slovakia so that the dogs are evaluated as objectively as possible, without being in contact with the fox as well as assess the abilities of the earthdogs for underground working. From the welfare point of view, we propose to cancel the “duck´s scent on water surface” discipline on special trials from water work and retriever trials on the fetching of small game in Slovakia, in the same way as it is in the Czech Republic.

REFERENCES

Act No. 246/1992 Coll., On the Protection of Animals Against Cruelty as Amended

Act No.166/1999 Coll., On Veterinary Care as Amended

Act No. 449/2001 Coll. Act on Hunting as Amended

Act No. 300/2005 Coll. Penal Code

Act No. 39/2007 Coll., On Veterinary Care as Amended

Act No. 274/2009 Coll. On Hunting as Amended

Civil Code No. 40/1964 Coll as Amended

Czech-Moravian Hunting Union (2008): Dog and Hunting. Ed. Vega Praha 5, (v češtine), 231 pp, ISBN 80-86933-06-7

Decree No. 244/2002 Coll., Implementing Certain Provisions of the Act No. 449/2001 Coll., on Hunting

Decree No. 344/2009 Coll., Implementing the Hunting Act.

Slovak Hunting Chamber (2015): Test Rules and Breeding of Hunting Dogs in the Pocket. Publisher Epos, (v slovenčine), 192 pp. ISBN 978-80-562-0083-4

COMPARISON OF MINERAL CONTENT IN DOG FOODS AVAILABLE WITHIN THE CZECH REPUBLIC DOG FOOD MARKET

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ABSTRACT

The main intent of this study was to analyze the contents of selected minerals in dry complete for dogs which are available within the Czech Republic dog food market.

The outcomes were compared amidst themselves as well as to current guidelines which are declared by the European Pet Food Industry Federation 2018 and the Association of American Feed Control Officials 2014. Two accessible types of foods were selected – those grain containing, and those without any grain content. Samples were divided into two groups of twenty. Each group contained samples from five distinct manufacturers. Samples were also classified according to a dog's life stage (puppy, puppy large breed, adult, and senior). Chemical analysis was performed to assess the amounts of selected minerals, and sodium chloride. The amount of phosphorus was analyzed following the CSN 46 7092–11 and contents of other selected minerals were assessed utilizing the CSN EN ISO 6869. To evaluate the volume of sodium chloride we used the Volhard's Method of Argentometric Titration. The statistical evaluation of results proved differences in observed nutritional parameters in both groups. In the case of macroelements, the volume of magnesium was statistical very significant and the content of calcium, phosphorus, potassium, and sodium was not statistically significant. Concerning microelements, the analyses demonstrated highly significant statistical differences in the volume of iron, statistically significant outcome in copper and statistically nonsignificant in manganese, zinc, and sodium chloride. The majority of results, however, corresponded to the recommendations.

Keywords: dog nutrition; macroelements; microelements; calcium; phosphorus.

INTRODUCTION

The quality of commercial dog foods is in the center of interest of many pet owners and veterinarians. Different dog breeds and age categories have different nutritional demands and dog foods need to fit them. The manufactures should follow recommendations given by the European Pet Food Industry (FEDIAF) and the Association of American Feed Control Officials (AAFCO). Further declaration of the fundamental nutrients on the packaging is mandatory.

An optimal dietary intake of essential minerals in the right ratio is vital to keep good health and assures the nutritional stability of an organism. The eventual homeostatic disorder can be a result of a lack, or an excess of specific nutrients that can significantly affect individuals' health and welfare (Case et al. 2011). Inappropriate intake of certain minerals for a long time can evolve a series of clinical illnesses, including skeletal, neurological, and dermatological diseases (Davies et al. 2017). Feeding appropriate amounts of minerals should be a goal of all pet owners as well as feeding a balanced and quality diet (Case et al. 2011).

The main point of interest, as a result of this study, is to increase general awareness concerning the real content of mineral nutrients in foods. These foods are the only source of nutrition for the dog; so they play a vital role in its health and welfare.

There are several studies available evaluating the mineral composition of commercial dog foods. These studies compare their results to the stated recommendations and package facts provided by producers. One of them used 45 samples of dry dog food and performed an analysis of nutrients. Thirty-nine samples met the limits established by the American Association of Feed Control Officials in amounts of calcium, phosphorus, zinc, iron, mercury, manganese, and selenium. Some foods contained an excessive amount of calcium which may pose a risk in the growth of giant dog breeds (Gagné et al. 2013). An analysis of 177 samples of dry and wet dog and cat foods was performed in Britain. The results were compared to statements given by the European Pet Food Industry Federation. Some of the focused microelements, such as mercury did not come to the stated nutritional minimums. Only 30 out of 80 (i. e. 38%) met the recommendations.

MATERIAL AND METHODS

An analysis of chosen minerals in complete dog foods was performed. Elected dog foods remain available within the Czech Republic dog food market. Two accessible types of dog foods were selected – foods containing grain and foods not containing a grain (grain-free). Samples were divided into two groups according to their grain content. The foods containing grain gathered into the first group. The second group consisted of foods without any grain content. Samples were categorized according to main dog categories – puppy, puppy large breed, adult, and senior. Individual groups consisted of 20 samples, and five distinct foods went through testing. Dog foods were chosen randomly and all met the grain content terms. We classified the samples anonymously using Dog food 1 to 10 for labeling distinct dog foods. The dog age categories were labeled as Puppy 1 to 10, Puppy large breed 1 to 10, Adult 1 to 10, and Senior 1 to 10. The amounts of calcium, phosphorus, potassium, sodium, and magnesium were determined by chemical analysis. Microelements such as copper, iron, manganese, and zinc were also analyzed. The presence of sodium chloride in food samples was evaluated too. Spectrophotometer Helios α was applied to analyze the content of phosphorus. This method was consistent with the CSN 46 7092–11. The concentration values of other minerals such as calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were measured utilizing the atomic absorption spectrometer 200 Series AA following the CSN EN ISO 6869. Volhard's titration method was used to assess sodium chloride volume. We compared the results to the current standards for the nutrient composition of dry dog foods stated by the FEDIAF and AAFCO. The statistical evaluation involves the calculation of average (x), standard deviation (Sd), median, and coefficient of variation (Var). F-test and Student's t-test (Microsoft Excel) were used to compare the two groups of foods. The differences were evaluated according to statistical significance in levels $P > 0.05$ (statistically nonsignificant difference), $P \leq 0.05$ (statistically significant difference) and $P \leq 0.01$ (statistically highly significant difference).

RESULTS AND DISCUSSION

The tables 1 and 2 show contains of nutrients in individual foods provided on 100% dry matter basis.

Table 1 The content of selected macroelements and microelements and sodium chloride in foods not containing cereal

	Ca (g/kg)	P (g/kg)	K (g/kg)	Na (g/kg)	Mg (g/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	NaCl (g/kg)
Puppy 1	12.39	10.46	5.52	3.49	0.75	14.71	170.77	21.17	150.89	4.40
Puppy large breed 1	16.34	10.70	5.62	3.69	0.81	15.31	128.29	20.39	92.92	5.31
Adult 1	13.51	9.78	6.13	2.86	0.78	13.80	146.87	20.25	134.32	3.30
Senior 1	13.11	8.06	7.04	2.61	0.74	10.93	242.59	24.44	99.06	3.02
Puppy 2	20.23	14.13	5.35	3.90	0.88	17.85	282.93	42.31	100.82	2.87
Puppy LB 2	20.56	10.78	1.50	1.66	0.52	15.16	150.72	39.50	65.05	1.41
Adult 2	18.82	11.20	2.97	3.17	0.80	18.16	371.09	55.00	126.79	2.86
Senior 2	23.31	12.42	0.88	1.68	0.54	14.75	154.97	46.12	72.21	0.79
Puppy 3	11.53	7.63	7.13	4.57	0.78	11.26	167.05	22.16	167.31	2.47
Puppy LB 3	10.79	7.36	7.04	5.21	0.88	10.95	173.43	23.23	162.73	5.29
Adult 3	8.28	7.23	7.02	2.80	0.73	13.32	188.86	23.61	189.66	1.49
Senior 3	8.16	5.39	7.36	2.62	0.77	13.52	184.23	22.61	185.98	4.80
Puppy 4	15.49	13.55	7.11	3.82	1.01	19.72	260.74	65.78	201.77	5.99
Puppy LB 4	21.44	13.72	1.98	3.52	1.07	15.33	129.77	50.94	90.85	3.22

Adult 4	14.27	8.38	6.14	7.49	0.78	19.50	197.27	58.19	194.95	8.58
Senior 4	15.16	10.77	7.41	4.57	0.86	22.01	281.28	68.28	197.37	4.41
Puppy 5	14.33	11.81	5.12	3.36	0.73	18.33	333.93	86.14	158.32	0.56
Puppy LB 5	17.08	9.16	7.93	3.00	0.56	10.98	254.96	67.82	186.37	4.82
Adult 5	13.40	10.28	5.71	3.46	0.65	16.20	160.67	66.24	190.74	3.57
Senior 5	13.34	7.58	9.76	3.32	0.67	14.53	106.55	66.18	203.69	4.62
Average	**15.08**	**10.02**	**5.74**	**3.54**	**0.77**	**15.32**	**204.35**	**44.52**	**148.59**	**3.69**
Standard deviation	**4.169**	**2.412**	**2.289**	**1.277**	**0.140**	**3.166**	**72.365**	**21.267**	**47.011**	**1.929**
Median	**14.30**	**10.37**	**6.14**	**3.41**	**0.77**	**14.96**	**178.83**	**44.22**	**160.53**	**3.44**
Coefficient of variation	**27.649**	**24.069**	**39.901**	**36.079**	**18.234**	**20.672**	**35.413**	**47.773**	**31.638**	**52.296**

Legend: light orange – values does not come with one of recommended minimum nutrient levels, dark orange – the value does not come with more recommended minimum nutrient levels, light green – the value does not come with one of recommended maximum nutrient levels, dark green – the value does not come with more of recommended maximum nutrient levels LB – large breed

Table 2 The content of selected macroelements and microelements and sodium chloride in foods containing cereal

	Ca (g/kg)	P (g/kg)	K (g/kg)	Na (g/kg)	Mg (g/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	NaCl (g/kg)
Puppy 6	14.92	11.63	7.75	3.05	0.80	22.60	428.06	58.92	151.55	3.92
Puppy LB 6	12.02	9.86	6.06	3.75	0.74	93.38	343.95	47.25	273.15	5.06
Adult 6	19.13	14.73	7.97	2.82	0.94	19.52	399.87	57.09	97.53	1.31
Senior 6	9.74	9.73	6.44	3.13	0.79	21.25	410.93	47.68	247.19	3.82
Puppy 7	16.81	8.91	5.08	4.75	0.69	22.57	266.32	44.06	169.58	8.02
Puppy LB 7	17.15	11.77	5.48	5.03	0.62	21.85	280.77	45,98	172.31	2.17
Adult 7	23.86	11.68	3.89	3.12	0.66	22.81	340.92	45.25	130.27	1.20
Senior 7	23.41	12.30	4.11	3.22	0.77	23.60	330.26	48.79	139.13	4.14
Puppy 8	14.56	11.11	6.44	2.55	0.94	21.37	328.29	51.14	107.58	2.80
Puppy LB 8	14.99	8.46	6.64	2.63	0.99	22.82	327.91	53.78	119.64	2.48
Adult 8	15.15	12.92	7.08	2.65	0.96	24.36	437.03	57.62	94.17	1.70
Senior 8	15.03	13.50	7.11	2.52	0.88	23.77	432.42	62.72	87.08	2.31
Puppy 9	15.41	8.59	7.77	5.02	1.52	17.30	481.65	58.69	181.28	7.93
Puppy LB 9	20.99	12.00	4.53	3.59	1.61	19.51	595.42	83.92	150.99	6.61

Adult 9	16.80	8.73	6.94	3.17	1.71	25.89	616.52	111.26	213.20	6.81
Senior 9	18.61	11.87	4.40	3.24	1.74	15.26	581.45	59.03	81.73	3.97
Puppy 10	13.93	11.55	9.06	4.84	1.38	24.39	173.83	12.67	205.47	8.99
Puppy LB 10	17.86	9.01	8.91	4.65	1.34	20.49	168.97	12.45	210.42	9.18
Adult 10	13.94	9.04	8.62	4.70	1.27	19.12	194.08	14.18	196.74	7.66
Senior 10	15.44	13.02	13.75	3.95	1.25	8.01	155.23	16.44	213.71	9.40
Average	**16.49**	**85.21**	**6.90**	**3.62**	**1.08**	**24.49**	**364.69**	**49.45**	**162.14**	**4.97**
Standard deviation	**3.477**	**6.763**	**2.253**	**0.898**	**0.366**	**16.689**	**138.379**	**23.755**	**55.352**	**2.840**
Median	**15.42**	**84.38**	**6.79**	**3.23**	**0.95**	**22.21**	**342.44**	**49.97**	**160.57**	**4.05**
Coefficient of variation	**21.092**	**7.937**	**32.640**	**24.820**	**33.939**	**68.140**	**37.944**	**48.042**	**34.139**	**57.093**

Legend: light orange – values does not come with one of recommended minimum nutrient levels, dark orange – the value does not come with more recommended minimum nutrient levels, light green – the value does not come with one of recommended maximum nutrient levels, dark green – the value does not come with more of recommended maximum nutrient levels, LB – large breed

Table 3 The statistical evaluation of content of selected macroelements in foods containing and not containing cereal

	Ca (g/kg)	P (g/kg)	K (g/kg)	Na (g/kg)	Mg (g/kg)
f-test	0.436694	0.265453	0.945338	0.133854	0.000102
T-test	0.252592	0.149928	0.112957	0.822063	0.001477
Significance	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P ≤ 0.01

Legend: P > 0.05 (statistically nonsignificant difference), P ≤ *0.01 (statistically highly significant difference).*

Table 4 The statistical evaluation of content selected microelements and sodium chloride in foods containing and not containing cereal

	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)	NaCl (g/kg)
f-test	> 0.000001	0.006921	0.634553	0.483226	0.100282
T-test	0.025199	0.000081	0.493652	0.409398	0.102368
Significance	P ≤ 0.05	P ≤ 0.01	P > 0.05	P > 0.05	P > 0.05

Legend: P > 0.05 (statistically nonsignificant difference), P ≤ 0.05 (statistically significant difference) and P ≤ *0.01 (statistically highly significant difference).*

CONCLUSION

Some statistical differences were found in the analysis of complete dry dog foods. The differences were detected in both groups (with or without the content of cereal) and all life stage categories. A statistically highly significant difference was evaluated in the amount of magnesium. Concerning microelements, a statistically highly significant difference was evaluated in iron and a statistically significant difference was analyzed in the amount of copper. Majority of analyzed samples corresponded with the stated nutrient standards. For foods to be the most appropriate, it is recommended to follow the FEDIAF and AAFCO guidelines as much as possible.

ACKNOWLEDGEMENT

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REFERENCES

The sources of used literature are available at the author.

COMPARISON OF NUTRIENT AND ENERGY CONTENT IN DRY DOG FOODS FROM THE MARKET IN THE CZECH REPUBLIC

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ABSTRACT

The main aim of this study was to evaluate the amounts of fundamental nutrients and energy in complete dry dog foods, which are available within the dog food market in the Czech Republic, and compare the results admits the two chosen types of food – grain containing and not containing grain, called grain-free. Twenty samples from different manufacturers were collected from each group appointed to four dog age categories (puppy, puppy large breed, adult, and senior). The results were compared to present recommendations stated by the European Pet Food Industry Federation (2018) and the Association of American Feed Control Officials (2014) as well as to recommendations of the National Research Council norms (2006). The samples were analyzed for moisture content, crude protein, crude fiber, total fat, ash, and nitrogen-free extract. Estimated metabolizable energy contents were figured according to the prediction NRC (2006) equation. All analysis procedures follow the Commission Regulation (EC) no. 152/2009 and the present CSN EN ISO (Feed) and CSN 46 7092 Testing of feeding stuff. For statistical evaluation was used Microsoft Excel. Between both groups were analyzed statistical differences in the content of crude protein, nitrogen-free extract, ash, and crude fiber. In grain-free foods, statistically highly significantly higher values were assessed in the amount of crude protein and ash, statistically significantly higher in the presence of crude fiber. Statistically significantly lower amount was assessed in nitrogen-free extract in comparison with grain containing foods. All but one of the tested samples did correspond to present standards. This sample's crude protein content did not come the recommended minimum. This sample contained one of the grain-free food.

Keywords: grain-free dog food; crude proteinn; crude fiber; metabolizable energy

INTRODUCTION

A dog food quality assessment is one of the most discussed topics by pet owners who wish to assure their dogs' optimal nutrition and long-lasting health. Veterinarians also need up to date information so they could recommend quality dog food to their customers (Zicker, 2008). Recent studies in France and Germany showed a majority of dog owners feed commercial dog food at least partly. About one third to a half feed only those (Colliard et al. 2006; Becker et al. 2012). Complete dog foods are made to cover the nutritional demands of different dog breeds and age categories (Case et al. 2011). The main source of nutritional and energy needs information are the NRC (2006) norms. Manufacturers should follow the recommendations stated by the FEDIAF (2018) and AAFCO (2014). It is mandatory to declare the amounts of fundamental nutrients on the packaging. There are several types of foods for dogs within the dog food market. The dominant position is occupied by dry foods that are manufactured using the extrusion process. Dry dog foods are popular for their easy stocking and also because of the economics of feeding them (Daumas et al. 2014). Many scientific studies evaluate the amounts of nutrients. A study published by a French consumer association magazine (Chairopoulos et al. 2011) evaluated eight commercial dog foods for adult dogs according to different criteria. The laboratory analysis results of nutrient content were included. Hill et al. (2009) found out there are often strong differences between the analyzed content of nutrients and declared volumes. Rolinec et al. (2016) compared analyzed values of nutrients in dry dog foods to declared numbers on the packaging and inducted strong differences as well. Alvarado et al. (2008) evaluated the analytical content of nutrients and energy in dog foods designed for growing dogs. All the volumes of fundamental nutrients followed the standards stated by AAFCO. The contents of some amino-acids and minerals showed absence. Overall, only 23 % of analyzed dog foods followed the recommendations given by the AAFCO.

MATERIAL AND METHODS

Forty samples of dry extruded dog foods, accessible within the market of the Czech Republic, were purchased. A variety of 10 different local and foreign manufacturers were chosen. All dog food labels are easily accessible and widely used for feeding. The samples were divided into two groups. GROUP 1 consisted of foods with grain content. GROUP 2 was formed with foods without any grain content, grain-free. Each group held 20 samples from five various producers. Every sample was from different producers and samples were provided for each age category – puppy, puppy large breed, adult, and senior. The chemical analysis was conducted in a laboratory at the Department of Animal Nutrition by the University of Veterinary and Pharmaceutical Studies Brno, Faculty of Hygiene and Ecology. An analytical evaluation of moisture, crude protein, total fat, crude fiber, and ash was completed. The content of nitrogen-free extract (NFE) was calculated based on the results of an analysis. Moreover, the calculation of metabolized energy (ME) was done using the prediction NRC (2006) equation. All analysis procedures follow the Commission Regulation (EC) no. 152/2009 and the present CSN EN ISO (Feed) and CSN 46 7092 Testing of feeding stuff. The moisture content was measured using the gravimetry method of drying the sample to a constant weight at 103 ± 2 °C. The Kjeldahl method and Buchi analyzer were applied to indicate the volume of crude protein. The measured content of nitrogen was multiplied by factor 6,25. The Soxhlet method using petroleum ether extraction was utilized to determine the quantity of crude fat. Ash content was defined using the gravimetry after burning at 550 ± 20 °C in a muffle furnace. Using the gravimetry method (as a leftover after acid and alkaline hydrolysis) led by the ANKOM 220 Fiber Analyzer was applied to evaluate the crude fiber volume. The concentrations of analyzed nutrients and calculated values of NFE are given in g/kg on a dry matter basis (DM). The metabolized energy (ME) is cited in kJ/kg on a dry matter basis (DM). For statistical evaluation was used Microsoft Excel to evaluate an average (x), median, standard deviation (Sd) and coefficient of variation (Var). To appraise the conclusive difference of average values we used the Student's t-test, which F-test preceded. The difference between average values was evaluated using the Student's t-test, using significance $P \leq 0,01$ (statistically highly significant) a $P \leq 0,05$ (statistically significant).

RESULTS AND DISCUSSION

Table 1 GROUP 1 (grain-containing) - the content of crude protein, crude fat, crude fiber, NFE, crude ash and ME per kg of DM

dry matter (DM)	Protein (g/kg)	Fat (g/kg)	Fiber (g/kg)	NFE (g/kg)	Ash (g/kg)	ME (kJ/kg)
Puppy 1	305.80	188.60	12.70	425.60	67.30	4432.90
Puppy LB 1	302.50	174.40	14.20	430.10	78.80	4311.26
Adult 1	273.80	125.00	15.60	518.10	67.50	4098.24
Senior 1	266.22	122.99	22.72	523.72	64.35	4048.39
Puppy 2	298.10	165.50	10.20	433.30	93.00	4244.31
Puppy LB 2	312.00	133.00	13.90	466.60	74.40	4137.02
Adult 2	281.90	150.50	9.40	475.50	82.70	4209.67
Senior 2	278.46	109.18	28.99	502.12	81.25	3885.41
Puppy 3	294.40	164.90	6.90	464.50	69.30	4350.69
Puppy LB 3	289.70	140.40	22.00	483.60	64.30	4143.49
Adult 3	235.40	139.70	6.00	568.80	50.10	4283.70
Senior 3	202.88	130.29	17.71	597.70	51.42	4140.19
Puppy 4	354.20	202.70	15.80	343.90	83.40	4436.17

Puppy LB 4	314.40	121.60	19.00	461.80	83.20	4017.15
Adult 4	314.10	152.70	17.60	436.90	78.80	4189.29
Senior 4	286.50	124.78	8.63	516.55	63.54	4147.31
Puppy 5	342.10	180.40	12.20	385.00	80.30	4364.25
Puppy LB 5	328.30	154.00	20.10	418.30	79.30	4180.71
Adult 5	276.30	122.20	8.60	527.50	65.40	4141.89
Senior 5	286.50	124.78	8.63	516.55	63.54	4164.93
x	**292.18**	**146.38**	**14.54**	**474.81**	**72.10**	**4196.35**
median	292.05	140.05	14.05	471.05	71.85	4172.82
SD	34.16	25.91	6.05	61.39	11.18	138.65
Var	11.69	17.70	41.59	12.93	15.50	3.30

Legend: LB – large breed, x – average, SD – standard deviation, Var – coefficient of variation

Table 2 GROUP 2 (grain-free) - the content of crude protein, total fat, crude fiber, NFE, crude ash and ME per kg of DM

dry matter (DM)	Protein (g/kg)	Fat (g/kg)	Fiber (g/kg)	NFE (g/kg)	Ash (g/kg)	ME (kJ/kg)
Puppy 1	320.83	110.33	13.37	471.87	83.25	4003.46
Puppy LB 1	290.10	136.60	11.70	483.80	77.80	4148.78
Adult 1	269.52	121.65	21.48	493.10	94.25	3943.73
Senior 1	268.85	130.19	28.19	523.32	71.20	4098.28
Puppy 2	424.45	171.79	6.02	318.61	79.13	4406.38
Puppy LB 2	400.00	192.60	19.10	309.60	78.60	4398.14
Adult 2	163.17*	169.12	8.70	565.52	93.49	4216.13
Senior 2	342.35	181.81	22.78	371.20	81.86	4287.16
Puppy 3	381.29	192.40	16.59	323.77	85.94	4382.77
Puppy LB 3	300.80	136.80	26.20	451.30	84.90	4027.21
Adult 3	333.09	133.38	24.56	424.43	84.54	4035.74
Senior 3	354.78	142.47	32.54	380.83	89.38	4012.01
Puppy 4	446.00	215.36	8.30	240.66	89.68	4566.82
Puppy LB 4	459.50	166.30	22.40	250.60	101.20	4190.98
Adult 4	372.06	174.09	7.51	355.44	90.89	4341.65

Senior 4	306.92	118.47	37.27	449.31	88.03	3857.85
Puppy 5	421.93	185.54	27.87	284.53	80.13	4301.38
Puppy LB 5	409.05	142.74	19.42	345.57	83.23	4148.86
Adult 5	429.18	162.45	14.19	311.67	82.52	4290.33
Senior 5	410.00	165.02	32.74	308.02	84.22	4151.53
x	**355.19**	**157.46**	**20.05**	**383.16**	**85.21**	**4190.46**
median	363.42	163.73	20.45	363.32	84.38	4171.26
SD	74.61	28.67	9.17	93.89	6.77	181.50
Var	21.00	18.21	45.73	24.51	7.94	4.33

Legend: x – average, SD – standard deviation, Var – coefficient of variation, * the value does not come with recommended minimum nutrient level

Table 3 The statistical evaluation of content of crude protein, crude fat, crude fiber, NFE, crude ash and ME in grain containing (GROUP 1) and grain-free (GROUP 2) dog foods

	Protein (g/kg)	Fat (g/kg)	Fiber (g/kg)	NFE (g/kg)	Ash (g/kg)	ME (kJ/kg)
f-test	0.0013	0.6638	00777	0.0716	0.0342	0.2493
T-test	0.0020**	0.2078	0.0310*	0.0008**	0.0001**	0.9088
významnost	$P \leq 0.01$	$P > 0.05$	$P \leq 0.05$	$P \leq 0.01$	$P \leq 0.01$	$P > 0.05$

CONCLUSION

In between the GROUP 1 (samples with cereal content) and GROUP 2 (grain-free samples) were analyzed conclusive differences in contents of crude protein, nitrogen-free extract, ash, and crude fiber. In grain-free foods, the statistical highly significantly higher difference was examined in the measure of crude fiber and highly significantly lower content of nitrogen-free extract in comparison to samples with grain content. All but one sample of dog food corresponded to the recommendations declared by the FEDIAF (2018), AAFCO (2014), and the NRC norms (2016). This one grain-free food sample (GROUP 2, Adult 2) designed for adult dogs did not follow the standards. The quantity of crude protein was analyzed under the recommended minimum.

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REFERENCES

Used sources of literature are available at the author.

ULTRASOUND AND DOPPLER EVALUATION OF VASCULAR THROMBOSIS AND HAEMORRHAGIC INFARCTION IN THE SPLEEN IN A DOG – CASE REPORT

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ABSTRACT

Splenic vein thrombosis and splenic infarction are not a specific disease but is a concomitant symptoms in diseases that disrupt the regulatory mechanisms of coagulation. Splenic vein thrombosis and splenic infarction are sonographic findings of clinical importance, and dogs with splenic vein thrombosis and infarction can have one or more coexisting diseases. A 9-year-old, 36 kg, castrated male, Rhodesian Ridgeback was referred due to depletion of the clinical condition. The diagnosis of a splenic vein thrombosis and splenic infarction was based on the two-dimensional findings patterns of splenic infarction and absence Doppler signal of the splenic veins. Dog had a concurrent disease predisposing to thrombosis, including inflammation and hypoalbuminemie. Dog was submitted to total splenectomy. Splenic histopathology confirmed vascular thrombosis and haemorrhagic infarction. Moreover, a review of the splenic veins thrombosis and splenic infarction are discussed.

Keywords: spleen; thrombosis; infarction; ultrasound; Dopller ultrasound;

INTRODUCTION

The spleen of dogs is frequently affected by disorders that vary from local and systemic origin. Ultrasonography is the most common examination to detect splenic alterations (Nyland, 2002). Despite the characteristic of this method as non-invasive and effective in the evaluation of splenic parenchyma, the ultrasound appearance of focal and extensive lesions

usually does not allow a definitive diagnosis of benign or malignant lesion (Nyland, 2002; Fossum, 2007).

Splenic infarction (SI) has been described in dogs with bacterial endocarditis, hypercoagulable conditions secondary to liver diseases, renal diseases or hyperadrenocorticism, neoplasia, and thrombosis associated with cardiovascular diseases. SI has also been described in dogs with bacterial endocarditis, hypercoagulable conditions secondary to liver disease, renal disease or hyperadrenocorticism, neoplasia, and thrombosis associated with cardiovascular disease. Focal splenic infarcts occur secondary to embolism or thrombosis and may be associated with inflammatory diseases such as pancreatitis, endocarditis, septicemia, and neoplasia in humans. The appearance of infarcts is variable and depends on the time since the infarct occurred. Initially, infarcts appear as poorly marginated hypoechoic or complex lesions that cannot be distinguished from other focal splenic lesions on the basis of the ultrasonographic appearance alone (Schelling et al., 1988; Georg et. Al., 1990; Freeman et al., 2011).

Splenic veins thrombosis (SVT) is not an uncommon finding in dogs. It is often found incidentally, without clinical signs or accompanying splenic parenchymal changes. However, underlying causes of thrombosis should be investigated, such as splenic torsion, generalized vascular or clotting disorder, lymphoma, hyperadrenocorticism, mast cell disease, and other organ or systemic disease processes (Shirkhoda et. al., 1985; Hardie et. al. 1995). Doppler ultrasonoghraphy is helpful to evaluate splenic blood flow. Doppler ultrasonoghraphy has also been used to identify lack of blood flow in the splenic veins of dogs with splenic infarction and thrombosis, including dogs without visible thrombi. In addition, Doppler ultrasonography is helpful for diagnosing splenic vein thrombosis in abdominal inflammatory diseases or other pathologic conditions predisposing to hypercoagulability. The purpose of this study was to determine ultrasonographic patterns and blood flow in the splenic veins at the level of the splenic hilar border in dog with splenic infarction and vascular thrombosis (Brown, 1997; Nyland, 2002; Larson, 2013).

MATERIAL AND METHODS

Patient profile

A 9-year-old, 36 kg, castrated male, Rhodesian Ridgeback was referred due to depletion of the clinical condition. Referral history cited 3 days not eating, longer time underweight standard, lethargy, depression, without diarrhea and vomiting. Clinical, haematological and ultrasound examination has been performed. Hematological profil showed neutrophilic leukocytosis and anemia. Biochemical parameters showed elevated alkaline phosphatase, hypoalbuminemie and decreased creatinin. Dog was submitted to total splenectomy and sample from the splenic parenchyma and the splenic vessels was taken for the histological examination.

Ultrasound Examinations

Abdominal ultrasonographic examination was performed using commercially available diagnostic machines. The spleen was scanned along its entire length in longitudinal and transverse planes. Size and shape were subjective measurements based on operator experience with the proportion of splenic extension into the right or caudal abdomen serving as a useful indicator. Parenchymal appearance was based on instrument settings and comparative echogenicity relative to liver and kidney. Number, size, location, shape, margination, echogenic pattern, and acoustic characteristics were evaluated for focal parenchymal abnormalities. Splenic vasculature was evalueted with B-mode and color Doppler imaging. Doppler imaging parameters (spectral and color gains, pulse repetition frequency, frequency filtration) had been optimized for venous velocity flow.

RESULTS AND DISCUSSION

Due to the varied ultrasonographic appearance of the spleen with infarction and thrombosis, B-mode and Doppler assessment of the splenic vasculature becomes important (Hardie et. al., 1995). This study reports a case of splenic infarction and splenic vein thrombosis, highlighting the importance of the ultrasound examination.

Two previously unreported ultrasonographic patterns of splenic infarction are identified: (1) Focal, hypoechoic or isoechoic, circular, well marginated nodular masses with peripheral lesions causing deformation of the splenic margin.

(2) Diffuse hypoechoic or heteroechoic coarse/"Iacy" parenchymal pattern with no deformation of margin (Saunders et.al., 1998).

In our study sonographically appeared diffusely hypoechoic pattern with linear echoes separating large anechoic region as a coarse/ “lacy” pattern (Figure 1.), distinctly different from the hyperechoic (relative to liver) and fine pattern of normal splenic parenchym. A hypoechoic, wedge-shaped apperance with the base toward the splenic margin (Figure 2.) was observed with convex tranducer, this apperance has been described in humans (Nyland et.al., 2002).

Splenomegaly, decreased echogenicity of the parenchyma of the spleen and hypoechoic appearance or “lace” are suggestive sonographic findings, although the definitive diagnosis is given through exploratory laparotomy and histopathology (Silva et. al., 2015). Our ultrasonographic findings pattern of splenic infarction have been confirmed by histopathology and surgery.

Splenomegaly is often observed due to splenic vein thrombosis. Size of the spleen in longitudinal axis was 3,78 cm and in transversal view was 5,18 cm. This parameters schowed enlargement of spleen. Moreover, splenic extension was observed to the area of urinary bladder and splenic caput curved dorsally. The dilated splenic vein can be identified at the hilum (Figure 3.) and showing absence of colour for Doppler evaluation, may be employed to strengthen confidence in a diagnosis of splenic infarction and thrombosis, which can be confirmed by exploratory laparotomy. In this case thrombosis of the splenic vasculature was found at surgery (Figure 5). The use of color Doppler is necessary for hemodynamic assessment of the spleen, because color Doppler provides observable evidence of the absence of hemodynamic flow in cases of splenic infarction (Georg et.al., 1990; Nyland et.al., 2002). The ultrasonographic aspects observed during color Doppler evaluations of the patient corroborated the ultrasonographic aspects found in scientific literature. Because the sonographic aspects observed in two-dimensional examinations are merely suggestive of the presence of splenic infarction, Doppler ultrasonography is an important approach that contributes to the definitive diagnosis of this condition. Presence or absence of splenic vein blood flow was determined by Doppler evaluation of these vessels (Nyland et.al., 2002; Salgueiro et.al., 2017). We observed during Doppler evaulation absence of splenic vein blood flow (Figure 4).

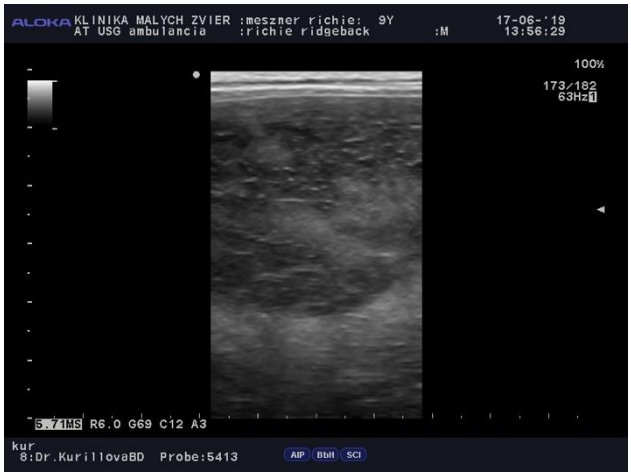

Figure 1 hypoechoic pattern with separating large anechoic region as a coarse/ "lacy" pattern

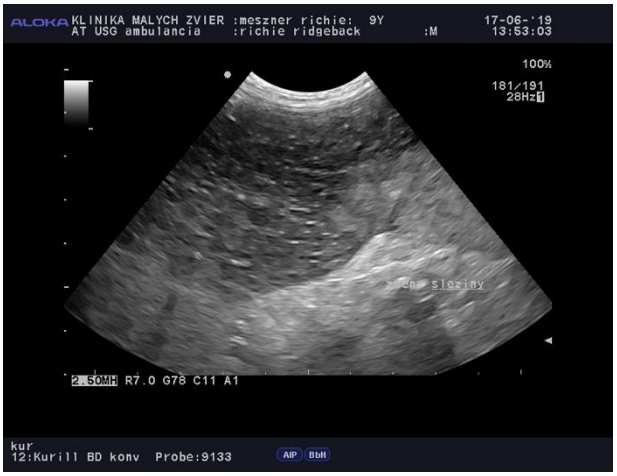

Figure 2 hypoechoic, wedge-shaped apperance with the base toward the splenic margin

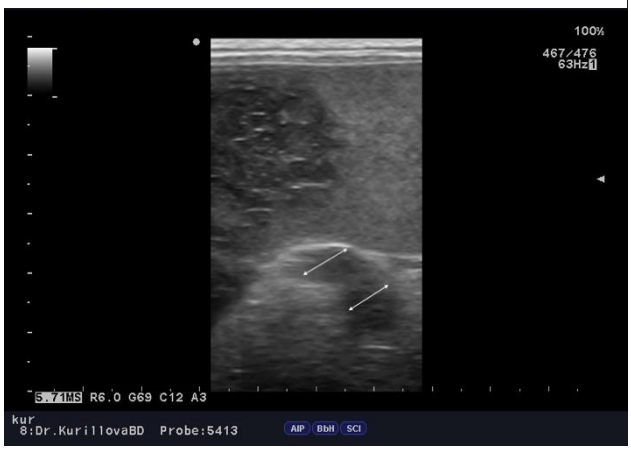

Figure 3 dilated splenic vein (arrows)

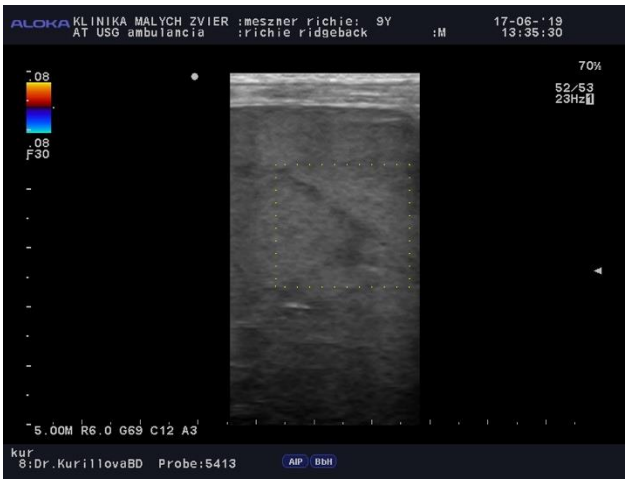

Figure 4 absence signal of color Doppler

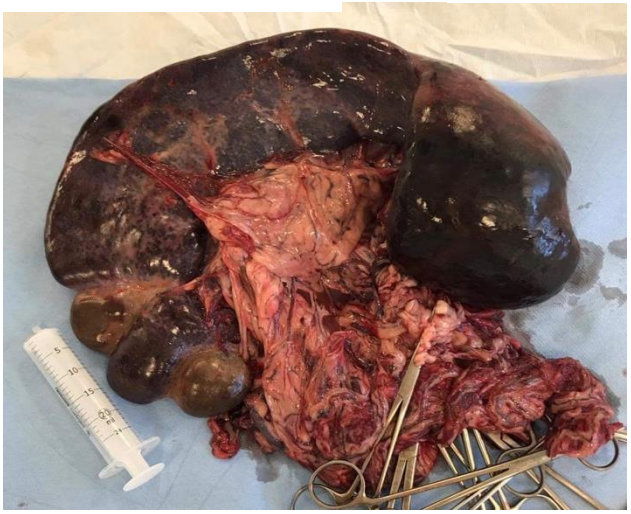

Figure 5 splenectomy performed: enlarged spleen, changes of shape and color

CONCLUSION

This report ilustrates the usefulness of ultrasound in detection and evaluation of splenic infarction and thrombosis in the dog. Ultrasonographic determination of alterations in splenic size, shape, extent, and distribution of parenchymal abnormalities correlated well with gross and histopathologic findings. This report characterizes the ultrasonographic patterns of splenic infarction and thrombosis in the dog as both a focal change in parenchymal echogenicity with a mass effect as well as a diffuse change in parenchymal pattern and echogenicity.

Serial examinations as well as additional correlative studies will be needed to better identify and determine the clinical significance of splenic infarction and thrombosis in the dog.

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REFERENCES

Brown JM, Quedens-Case C, Alderman JL, et al. Contrast enhanced sonography of visceral perfusion defects in dogs. J Ultrasound Med 1997;16:493–9.

Fossum, T. W. Surgery of the hemolymphatic system. In: Small animal surgery. 3th ed. St Louis: Mosby Elsevier, 2007. p. 617-634.

Freeman S.J. (2011). Spleen. In: Clinical Ultrasound. 324-347.

Goerg C, Schwerk WB. Splenic infarction: sonographic patterns, diagnosis, follow-up, and complications. Radiology 1990;174:803–7

Hardie E.M, Vaden SL, Spaulding K, et al. Splenic infarction in 16 dogs: a retrospective study. J Vet Intern Med 1995;9:141–8.

Larson M.M. (2013): The Liver and Spleen. In: Textbook of Veterinary Diagnostic Radiology. 6th edn. St. Louis: Saun-ders, pp.679-704.

Nyland, T. G.; Mattoon, J. S.; HerrgeselL, E. J.; Wisner, E. R. Spleen. In: Nyland, T. G.; Mattoon, J. S. Small animal diagnostic ultrasound. 2th ed. Philadelphia: W. B. Saunders Company, 2002. p. 41-42.

Salgueiro, N.B.M. (2017). Sonographic Aspects of Splenic Torsion Due to Abdominal Eventration in a Dog. Acta Scientiae Veterinariae. 45, 197.

Saunders H.M., Neath P.J. & Brockman D.J. (1998). B-mode and Doppler utrasound imaging of the spleen with canine splenic torsion: a retrospective evaluation. Veterinary Radiology and Ultrasound, 39, 349-353.

Shirkhoda A, Wallace S, Sokhandan M. Computed tomography and ultrasonography in splenic infarction. J Can Assoc Radiol (1985); 36,29–33.

Schelling G.S.(1988). Ultrasonic detection of splenic necrosis in the dog: 3 Case Reports of Splenic Necrosis Secondary to Infarction Veterinary Radiology, 29, 227-233.

Silva, E. O. da et al. (2015). Splenic lesions observed in 71 splenectomized dogs: a retrospective study. Semina: Ciências Agrárias, 5. 3181-3188

EVALUATION OF PROTEIN CONTENT AND QUALITY IN COMPLETE DRY DOG FOOD

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ABSTRACT

The aim of this study was to determine the content of crude protein and essential amino acids in complete dry foods for dogs bought in the Czech Republic's trading network and to compare the observed values between the two groups of food containing cereals and not containing cereals, so-called grain-free. Each group included 10 samples of foods from 5 different manufacturers, intended for each of the 2 different categories of dogs (puppies and adult dogs). The results were further compared with the current FEDIAF (2018) and AAFCO (2014) standards and the requirements of the NRC (2006). Analytical determination of moisture, crude protein and amino acids was carried out in samples of foods. All working procedures have been carried out in accordance with Commission Regulation (EC) No 152/2009 and the current CSN EN ISO (Feed) and CSN 46 7092 Testing of feeding stuff. Statistical evaluation of results was done in Microsoft Excel. Grain-free foods had higher average crude protein content and all essential amino acids. Statistically significant differences were found in the amino acid content of threonine, valine, isoleucine, histidine, lysine, arginine and in sum of essential amino acids. In the grain containing group, 4 samples had a methionine content below the recommended minimum, two samples of the grain-free food had a reduced methionine content and one sample the phenylalanine content. This sample was also the only crude protein content below the recommended minimum. On sample of food for puppies from the cereal group and three grain-free food samples had a higher lysine content than the NRC (2006) recommended for growing dogs.

Keywords: dog food; crude protein; essential amino acid; methionine; lysine

INTRODUCTION

The production of pet food is a dynamically growing economic sector with a growth rate of approximately 4% per year (Animal Protein Producers Industry, 2015). Dry dog and cat food (extruded or baked) accounts for approximately 70% of the market for the dominant proportion of foods produced by the extrusion process (Pet Food Institute, 2012). Manufacturers of food for dogs are guided by the recommendations of the European Pet Food Industry Federation (FEDIAF 2018) and the Association of American Food Control Officials (AAFCO 2014), which sets out the minimum standards for all necessary nutrients that food must meet in order to be marked as complete. Protein quality is determined by its digestibility and amino acid composition, primarily by the representation of essential amino acids (Case et al. 2011). Proteins are found in animal food and in plant raw materials, and proteins of animal origin are considered to be fully-fleded. Most animal proteins have a higher digestibility and more favorable representation of essential amino acids than vegetable proteins. In foods, proteins are determined on the basis of the total nitrogen content of the food, multiplied by a factor of 6.25. However, it is not possible to assess the quality of proteins, to assess the quality of the proteins represented, an analysis of amino acids or animal experiments (Case et al. 2011) is necessary. A number of scientific studies have dealt with the evaluation of protein content and quality in commercial dog food. Donadelli et al. (2019) made comparisons of amino acids and protein quality using the PER method on chickens for various egg by-products, poultry meal and vegetable proteins used in the production of dog and cat foods. Hendriks et al. (2015) dealt with the study of the bioavailability of crude protein and amino acids in commercial food for adult dogs. Alvarado et al. (2008) studied the nutritional quality of dry fodder for growing dogs available on the market in Chile. Samples of 26 brands from three different batches were used for detailed chemical analysis and found an adequate amount of crude protein and all essential amino acids in all samples, with the exception of the lower tryptophan content for several samples.

MATERIAL AND METHODS

20 samples of extruded dry dog food were purchased from 10 different Czech and foreign producers in the Czech Republic's business network. These were commonly available and often sold brands of foods. The samples were divided into two groups for foods containing

cereals (GROUP 1) and food without cereals, the so-called grain-free (GROUP 2). In each group there were 10 samples of foods from 5 producers, the category of food for puppies and adult dogs was always included in each producer.

Chemical analysis of all samples of foods was carried out in the laboratory of the Department of Animal Nutrition by the University of Veterinary and Pharmaceutical Studies Brno, Faculty of Hygiene and Ecology. Analytical determination of moisture content, crude protein and essential amino acids. All working procedures have been carried out in accordance with Commission Regulation (EC) No 152/2009 laying down the methods of sampling and laboratory testing for the official control of foods, and with the current ČSN EN ISO (foods) and ČSN 46 7092 methods of testing foods. The moisture content was determined gravimetrically by drying the sample to constant weight at a temperature of 103 ± 2 °C. The crude protein content was determined by the Kjeldahl method on the Buchi analyser, when the nitrogen content observed was multiplied by a factor of 6.25. The amino acid content was determined on the Ingos AAA 400 analyser using medium-pressure liquid chromatography with an ion-exchange column, ninhydrin derivatization and photometric detection. The observed concentrations of all the nutrients analysed are expressed in g/kg of dry matter (DM). For statistical evaluation of results, Microsoft Excel was used, where arithmetic mean (x), median, standard deviation (SD) and coefficient of variation (Var) were calculated. To assess the relevance of the difference in average values, the Student's t-test was used, for which the F-test is required. The difference between the averages was tested using the Student's t-test at the materiality level $P \leq 0.01$ (statistically highly significant) and $P \leq 0.05$ (statistically significant).

RESULTS AND DISCUSSION

Table 1 shows the observed content of crude protein and essential amino acids in grain containing foods, table 2 shows the content of the same indicators in grain-free food.

Table 1 GROUP 1 (grain containing) - the content of crude protein and essential amino acids in g/kg of DM

g/kg of DM	CP	Thr	Val	Met	Ile	Leu	Phe	His	Lys	Arg	EAA
Puppy 1	305,80	11,00	16,00	4,90	14,20	25,70	13,60	9,60	20,70	21,80	137,50
Adult 1	273,80	10,80	13,10	6,20	11,30	21,90	10,60	8,20	16,80	18,70	117,60
Puppy 2	298,10	10,10	13,00	2,50	10,00	20,90	10,60	7,60	14,90	20,70	110,30
Adult 2	281,90	9,90	14,60	1,40	10,20	16,10	11,00	4,70	8,70	17,60	94,20
Puppy 3	294,40	10,50	15,10	1,90	11,50	24,00	12,20	5,90	10,30	17,10	108,50
Adult 3	235,40	8,10	11,00	1,70	9,10	21,40	9,70	5,30	12,30	12,80	91,40
Puppy 4	354,20	10,40	12,60	5,20	11,50	24,00	13,00	7,60	16,50	18,20	119,00
Adult 4	314,10	12,60	16,20	4,90	13,50	22,60	16,70	8,40	19,70	16,90	131,50
Puppy 5	342,10	11,70	17,00	10,40	13,60	28,90	15,40	8,50	16,90	21,30	143,70
Adult 5	276,30	10,50	14,20	5,70	10,90	22,00	12,30	7,70	15,60	17,70	116,60
x	**297,61**	**10,56**	**14,28**	**4,48**	**11,58**	**22,75**	**12,51**	**7,35**	**15,24**	**18,28**	**117,03**
median	296,25	10,50	14,40	4,90	11,40	22,30	12,25	7,65	16,05	17,95	117,10
SD	34,43	1,18	1,87	2,75	1,69	3,34	2,23	1,56	3,84	2,62	17,13
Var	11,57	11,14	13,09	61,43	14,60	14,68	17,86	21,17	25,18	14,32	14,64

Legend: x – average, SD – standard deviation, Var – coefficient of variation, CP – crude protein, EAA - sum of essential amino acids

In the grain-containing group (GROUP 1), a lower methionine content was observed compared to the minimum of FEDIAF (2018) and AAFCO (2014) for 4 samples of adult dog foods and puppies from 2 producers (Puppy 2, Adult 2, Puppy 3 and Adult 3). In 1 sample food for puppies (Puppy 1), a higher lysine value was found than the NRC (2006) standards recommended for growing dogs, i.e. < 20 g/kg of dry matter.

Table 2 GROUP 2 (grain-free) - the content of crude protein and essential amino acids in g/kg of DM

g/kg of DM	CP	Thr	Val	Met	Ile	Leu	Phe	His	Lys	Arg	EAA
Puppy 1	320,83	13,70	16,90	5,10	14,50	25,30	14,30	9,70	19,10	22,00	140,60
Adult 1	269,52	11,10	12,60	4,00	12,20	21,60	10,80	8,30	18,70	19,80	119,10
Puppy 2	424,45	15,40	21,90	3,80	16,30	28,50	11,00	7,80	17,80	24,70	147,20
Adult 2	163,17	5,50	7,70	1,60	5,40	10,70	4,20	3,20	7,20	10,20	55,70
Puppy 3	381,29	16,40	20,30	4,10	17,20	30,20	18,70	10,70	25,20	26,60	169,40
Adult 3	333,09	14,60	18,30	4,00	15,40	27,80	17,40	9,20	21,80	22,60	151,10
Puppy 4	446,00	16,50	21,00	8,40	16,90	31,50	15,90	12,40	26,30	25,60	174,50
Adult 4	372,06	14,00	17,70	7,70	14,30	27,10	12,30	11,00	23,30	22,70	150,10
Puppy 5	421,93	18,60	22,20	9,00	19,80	34,80	20,70	12,30	33,60	30,00	201,00
Adult 5	429,18	16,00	19,80	0,90	17,50	30,50	13,80	12,00	29,10	26,50	166,10
x	**356,15**	**14,18**	**17,84**	**4,86**	**14,95**	**26,80**	**13,91**	**9,66**	**22,21**	**23,07**	**147,48**
median	376,68	15,00	19,05	4,05	15,85	28,15	14,05	10,20	22,55	23,70	150,60
SD	88,07	3,65	4,56	2,73	3,95	6,70	4,72	2,79	7,23	5,37	39,04
Var	24,73	25,74	25,53	56,26	26,41	24,99	33,92	28,89	32,57	23,26	26,47

Legend: x – average, SD – standard deviation, Var – coefficient of variation, CP – crude protein, EAA - sum of essential amino acids

In the grain-free group (GROUP 2), a lower methionine content was observed compared to the minimum of FEDIAF (2018) and AAFCO (2014) for 2 samples of adult dog foods from 2 producers (Adult 2 and Adult 5). One sample of adult dog food (Adult 2) was also crude protein and phenylalanine content below the recommended minimum. In 3 sample food for puppies (Puppy 3, 4, 5), a higher lysine value was found than the NRC (2006) standards recommended for growing dogs, i.e.<20g/kg of dry matter.

Table 3 The statistical evaluation of content of crude protein and essential amino acids in grain-containing (GROUP 1) and grain-free (GROUP 2) dog foods

	CP	Thr	Val	Met	Ile	Leu	Phe	His	Lys	Arg	EAA
f-test	0,001	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,014	0,001	0,000
T-test	0,075	**0,013**	**0,041**	0,760	**0,029**	0,110	0,412	**0,038**	**0,018**	**0,025**	**0,043**
významnost	P > 0,05	P ≤ 0,05	P ≤ 0,05	P > 0,05	P ≤ 0,05	P > 0,05	P > 0,05	P ≤ 0,05	P ≤ 0,05	P ≤ 0,05	P ≤ 0,05

Legend: CP – crude protein, EAA - sum of essential amino acids

CONCLUSION

There were statistically significant differences in the amino acid content of threonine, valine, isoleucine, histidine, lysine, arginine and in sum of essential amino acids between the grain-containing (GROUP 1) and grain-free (GROUP 2) foods, with higher amino acid content in the grain-free foods. The grain-free foods also had a higher average crude protein content and all of the remaining essential amino acids, but the difference was not statistically significant. In the grain-containing group, 4 samples had a methionine content below the recommended minimum given by the FEDIAF (2018) and AAFCO (2014) standards, in the grain-free group two samples had a reduced methionine content and one sample had a reduced phenylalanine content. This sample of adult dog food also had as the only crude protein content lower than the recommended minimum. One sample of food for puppies from the grain-containing group and three samples of the grain-free group, on the other hand, had a higher lysine content than recommended by the NRC (2006) standards for growing dogs.

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REFERENCES

Used sources of literature are available at the author.

ANIMAL MODEL OF ULCEROSE COLITIS

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ABSTRACT

The aim of this study was to obtain an animal model of ulcerous colitis (UC) induced chemically by dextran sodium sulphate (DSS). The experiment was carried out on 126 specific-pathogen-free (SPF) mice of BALB/c line divided to three experimental groups AM1, AM3, AM5, and one control group K. The first phase of the experiment involved 5-day decontamination of mice by administration of antibiotics (amoxicillin, ciprofloxacin). In the second phase, on the basis of observation of pathogenesis of ulcerous colitis induced chemically by various concentrations of DSS (1, 3 and 5 %), we obtained an optimum animal UC gnotomodel. The optimum 5 % concentration used for chemical induction of UC resulted in higher than 10 % total weight loss of mice that was accompanied by dehydration and mild to moderate bleeding from the rectum. Microscopic examination confirmed atrophic changes in the colon mucus with leukocyte infiltration in the intestine.

Keywords: animal gnotomodel, dextran sulphate sodium, microbiota, ulcerative colitis,

INTRODUCTION

Intestinal microbiota is the largest and the most diverse community of microorganisms in the human body. The population of intestinal microorganisms represents only a part of the complexity of the intestinal microbiome, which includes a diverse range of microbial genes

and intestinal microbiota gene products. The intestinal microbiome undisturbed by the pathological process exhibits symbiotic action, produces vitamins, suppresses the expansion of pathological microorganisms and facilitates digestion of food substrates while constantly interacting with the host's immune system. The intestinal microbiota is a changing ecosystem that is heavily burdened by many factors such as unbalanced diet, stress, the use of antibiotics or necessity to overcome a disease. Disruption of this fragile ecosystem between the host and the microbiota can impair the development of the immune system, which can subsequently lead to disease states. Its adverse quantitative and qualitative changes referred to as dysbiosis lead to the development of disease (Nishida at all, 2018). Idiopathic inflammatory bowel diseases (IBDs) refer to a group of chronic inflammatory diseases of the gastrointestinal tract with alternating periods of relapse and remission. Crohn's disease (Crohn's Disease, CD) and ulcerative colitis (UC) are two major manifestations of IBD. These two forms of IBD differ mainly by the localization of inflammatory lesions in the digestive tract and the extent of histopathological changes in the intestinal wall. The high morbidity, severe early and late complications of the disease with potential disability, shortening of the patient's life and significantly lowering its quality are the reasons for seeking new possibilities of prevention, rational diagnosis and treatment of IBD. Gnototechnology is involved in the study of the mechanism of action of diets, antibiotics, environmental toxins and host genotypic variations on microbiota with subsequent clinical manifestations of the disease (Turnbaugh at all., 2009). Experimentally induced IBD model involving gnotobiotic animals enables systematic manipulation with variable factors and verify or refute hypotheses (Ramos and Papadaki, 2019).

The aim of our study was to obtain an animal gnotomodel of ulcerative colitis by means of chemical induction by DSS (dextran sodium sulphfate).

MATERIAL AND METHODS

The presented experiment with protocol number 4073/18-221/3, approved by SVPS SR and in accordance with the regulations of the Ethics Committee UVLF Košice, was performed on 126 SPF female mice of BALB/c line from breeding facility Velaz s.r.o. (Prague, Czech Republic). The mice were transported in special transport containers to the accredited

Laboratory of Gnotobiology, of the Institute of Microbiology and Gnotobiology, UVLF Košice, Slovakia (SK U 16016). Prior to placing the laboratory animals into a gnotobiotic rodent isolator for THF 3271IE 101/97 rodent breeding and a two-sleeve CBC breeding isolator (CBC, Ltd, Madison, Wisconsin), the surface of vessels was disinfected with peracetic acid. This was followed by a thorough venting of the peracetic acid vapours and the mice could be moved into breeding polypropylene containers 7-8 mice per container of dimensions of3665 x W207 x v140mm. The laboratory animals were fed *ad libitum* with complete mixed feed intended for mice in ST-1 breeding (Velaz s.r.o., Prague, Czech Republic), with continuous access to autoclaved water in glass bottles. The supply and exhaust air filtration was provided by HEPA filter cartridge in a PP housing, designed as a one-way filter (H13 according to EN 1822). The isolators were equipped with a set of sensors that regularly recorded air temperature, relative humidity and pollutant gases AP2 (hydrogen sulphide, toluene, ethanol, ammonia and hydrogen). The optimum relative humidity was maintained at 45-65 % and the optimum temperature was 20–24 ^{o}C. Illumination of the gnotobiotic isolator for mice was provided by outer neon lighting fixtures and natural illumination that ensured regular rhythm of light and darkness. At the end of the quarantine period lasting 5 days, the SPF mice were assigned to 3 experimental groups AM1, AM3, AM5 (111 mice) and one control group K (15 mice). The animals were treated with selective antibiotics (amoxicillin, ciprofloxacin) for decontamination. The second phase of the procedure involved chemical induction of ulcerative colitis by oral administration of DSS (dextran sodium sulphate) according to the dosage schedule (Table 1).

Table 1 Procedure – administration schedule

Group	Administration of antibiotics (every 12 hours for 5 days)	Recovery	IBD induction (chemically DSS)
K (n=15)	**Amoxicillin** potentiated by potassium clavulanate at a dose of 0.2 ml orally (with an active ingredient concentration of 387.11 mg/kg/mouse) + **Ciprofloxacin** at 0.1ml s.c. (with active ingredient concentration 19.60 mg/kg/mouse)	**10 days** in a microbiologically controlled environment of a gnotobiotic isolator	-
DSS/1 (n=37)			**DSS 1%** (in water at 1% concentration for 5 days)
DSS/3 (n=37)			**DSS 3%** (in water at 3% concentration for 5 days)
DSS/5 (n=37)			**DSS 5%** (in water at 5% concentration for 5 days)

Health of the mice and the consistency of their faeces was observed and recorded twice a day. Faecal samples during the first phase of the procedure were collected prior to the ATB administration and subsequently on the first, second, fifth and fifteenth day after decontamination. In the second phase of the procedure, faecal samples were collected on null day before chemical induction of ulcerative colitis, and on first, third and fifth day after UC induction. Weight of individual animals was recorded daily at the time of DSS application and the observed weight loss was evaluated in %. In order to obtain samples for microbiological and histological analysis, the gnotobiotic mice from each group were sacrificed humanly with a sodium pentobarbital euthanasia (86 mg /kg body weight), followed by cervical dislocation at null, fifth, fifteenth and twentieth day of the procedure.

RESULTS AND DISCUSSION

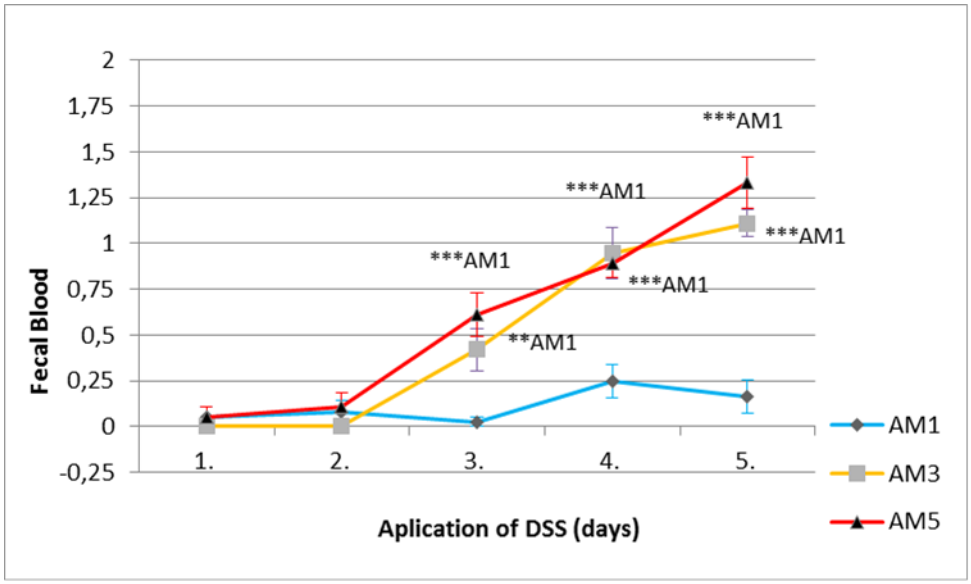
Intestinal sterilization through oral administration of antibiotics facilitates the study of the physiology of nutritionally important relationships between the intestinal microbiota and the host. Temporary intestinal sterilization may involve absolute or selective elimination of microbiota (Johnson at all, 2004). Various antibiotic combinations have been shown to completely or selectively sterilize the gastrointestinal tract of mice and rats (Schlegel at all,

2000; Wiesner at all, 2001). In the present study, we decontaminated the digestive tract of SPF mice of the BALB/c line with selective ATB in the first phase of the procedure based on our previous study (Gancarčíková et all, 2018) so the health of the animals thus obtained was not altered. Orally administered amoxicillin potentiated by potassium clavulanate at a dose of 387.11 mg/kg in Amoksiklav (Sandoz, Slovenia) and subcutaneously administered Ciprofloxacin 19.60 mg/kg in Ciloxan (Alcon, Spain) were used to decontaminate the mice. The animals were raised in a well-defined environment of gnotobiotic isolators. Only few research studies compared decontamination with ATB combination based on cultivation with decontamination with routinely used ATBs. In our study, we chose ATB decontamination by culture-based selection as recommended by Johnson et all (2004), in view of the exclusion of ATB with a significant negative impact on animal health. There are also differences in the length of the ATB administration. In our study, based on previous procedures (Popper and Gancarčíková, 2014), we chose a 5-day administration due to a negative culture recovery of bacteria from faeces on day 3 of ATB administration. After 10-day convalescence, we recovered colonies from the cultures of faeces and the content of the caecum and by means of biochemical tests and sequencing of 16S rDNA we identified them as *E. coli* and *Enterococcus* species. Using a flow cytometry, we observed a decreased survival of microorganisms in faeces which differed significantly ($p < 0.01$) between the first and second day (28.33%) after and prior to ATB administration (60.58%) Johnson et al. (2004) used a very similar method (BacLight ™ Live/Dead viability kit). They monitored inactivation of antibiotics through the viability of bacteria in faeces samples stained with a fluorescent dye. The mean proportion of live bacteria in faeces in their study, expressed as a percentage of the total bacterial count, decreased from 13.86% prior to administration of ATB to 0.37% after ATB treatment. After administration of Baytril the percentage bacterial viability was significantly reduced to less than 1% of the original level ($p < 0.05$). Only few decontamination studies in mice were engaged in observation of the overall health of these animals after administration of ATBs or their combinations. The liver, as the main organ of detoxification of various xenobiotics derived from the external environment of the organism, plays an essential role in displacement of ATB from the macroorganism. For this reason, we chose it as a reliable indicator of the overall change in the health of mice. We monitored the activity of hepatic enzymes and evaluated histological findings in liver tissues of mice. The AST and ALT activities were increased after five days of antibiotic administration. Similarly,

an increase in the specific liver isoenzyme LDH-5 was reported following decontamination. This enzyme catalyzes the reversible conversion of lactate to pyruvate and reaches the circulation with minimal tissue damage. These results were consistent with histological finding which showed structural changes in all decontaminated animals (presence of necrotizing hepatocytes, vacuolization and damaged sinusoids, multinucleated cells and fat infiltration). However, after a period of recovery, the LDH-5 isoenzyme activity returned back to the prior treatment levels. Convalescence of decontaminated animals under gnotobiotic conditions for ten days prevented restoration of species diversity in mouse microbiota and sufficed to bring the metabolic and morphological values to the physiological range. Induction and subsequent progression of intestinal inflammation is a complex of multifactorial interactions between the host and the environment (Jiminez et all, 2015). In particular, the physiological state of the host plays an important role in the origin and severity of the disease and thus the products such as antibiotics may contribute to the emergence of transient pathogenic bacteria involved in acute inflammation. Currently, a variety of animal models can be used to study the processes involved in intestinal inflammation, but rodent models and especially genetically engineered mice are the primary models used to study acute and chronic intestinal inflammation. As with the selection of the best animal model to investigate specific aspects of intestinal inflammation, the choice of the most effective chemicals and biological agents to stimulate inflammation must be carefully considered. The formation of spontaneous, long-term intestinal inflammation can be a protracted process, so it is often necessary to use chemical and bacterial stimuli to accelerate the process. More than fifty animal models are currently available. Many studies have used inducible models of colitis where mice were administered agents such as DSS (dextran sodium sulphate), TNBS (2,4,6-trinitrobenzenesulfonic acid), oxazolone or peptidoglycan polysaccharide (Neurath, 2012). These models have the advantage of causing inflammation in inbred mouse strains with a normal immune system. In the second phase of our procedure, we examined the pathogenesis of ulcerative colitis at various concentrations (1, 3 and 5%) of its chemical induction by DSS in an inbred mouse model of the BALB/c line. Clinical evaluation of inflammation included daily monitoring of body weight and general health. In the present study, we observed the lowest total body weight on day 5 after UC induction in the 5% DSS animal group at significantly lower weight ($p < 0.01$) on day 4 compared to day 2 of DSS administration. In the observed group (AM5) the highest total weight loss, ranging from 5-

14%, was confirmed. The inflammatory score or degree of inflammation was characterized by monitoring scores of droppings consistency, rectal bleeding, and total weight loss (Table 2).

Table 2 Rectal bleeding after chemical induction of ulcerative colitis by 1, 3 and 5% DSS in gnotobiotic BALB/c line mice

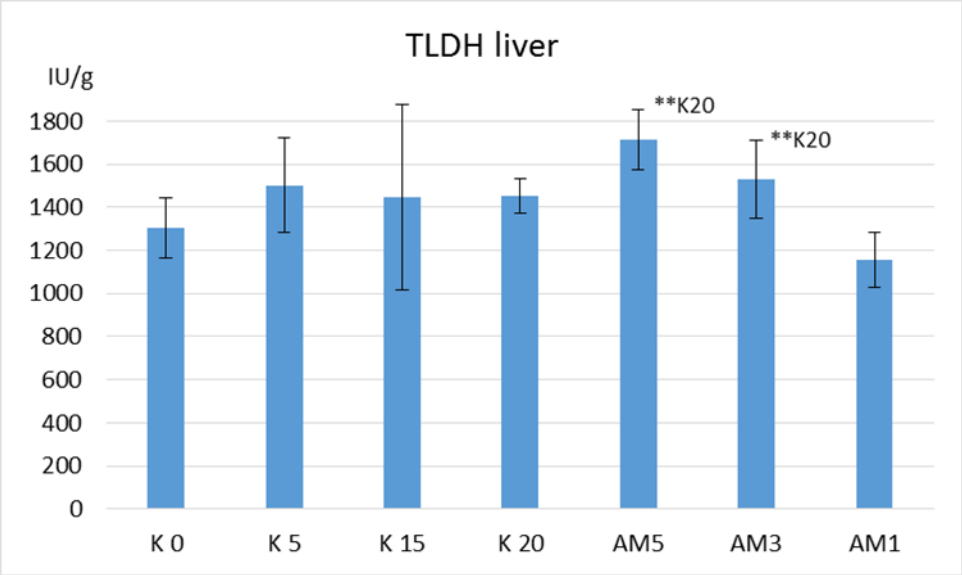


AM1 - 1% DSS administration, AM3 - 3% DSS administration, AM5 - 5% DSS administration,
degree of bleeding: 0- no traces of blood, 1-slight traces of blood, 2-slight bleeding, 3-severe bleeding,
**** - p <0.001 (inter-group statistics).*

The inflammatory score in the AM5 group gradually increased depending on the level of inflammation and the highest score on the 10-point scale at level 5-6 indicated mild to moderate inflammation of the intestinal mucosa of the caudal section of the gastrointestinal tract (colon). Observation of colon villus cross-section by light microscopy showed that the cross-section of villi in the 5% DSS (AM5) animal group was significantly lower ($p < 0.01$; $p < 0.001$) compared to groups exposed to 3 and 1% DSS application, respectively. At the same time, we observed the smallest, statistically significant circumference of the villi, the height of the villi as well as the lowest depth of crypts ($p < 0.001$) in the same intestinal section of the group (AM5). Histopathological analysis confirmed changes that were manifested by the presence of submucosa oedema, oedematous alterations in *lamina propria*, disruption of crypt architecture, mild erosion or ulceration of the epithelium, infiltration of polymorphonuclear leukocytes into submucosa and *lamina propria*. The release of lactate dehydrogenase (LDH) from cells (hepatocytes, monocytes, lymphocytes) into the environment is used as one of the

markers of cytotoxicity, tissue and cell damage under *in vitro* conditions (Myllyluoma et all, 2008). LDH is a highly sensitive but nonspecific marker of cell membrane disruption and cell viability (Legrand et all, 1992), therefore its increased activity indicates deterioration of cell membrane integrity. In the first phase of our procedure, we observed an increased activity of the total enzyme TLDH in the liver parenchyma of the control group of animals (K5) confirming the hepatotoxic effect of 5-day selective SPF decontamination of animals with antibiotics. The second significant increase in liver TLDH activity was detected after five days of chemical induction of DSS, with significantly higher activities of the enzyme of interest ($p < 0.01$) in groups exposed to 5% and 3% DSS compared to animals without DSS (K20).

Table 3 Specific activity of liver TLDH in BALB/c gnotobiotic mice during the decontamination by antibiotics and after chemical induction of ulcerative colitis with 1, 3 and 5% DSS



K0 - control on day 0, K5 - control after 5 days of decontamination with selective ATB, K15 - control after 10 days convalescence, K20 - control on day 20 of the procedure without DSS application,
AM1 - 1% DSS administration, AM3 - 3% DSS administration, AM5 - 5% DSS administration,
*TLDH - total lactate dehydrogenase, *** - p <0.01 (inter-group statistics).*

CONCLUSION

On the basis of decontamination by selective antibiotics and the observed pathogenesis of ulcerative colitis at various concentrations of its chemical induction, we obtained an optimal

animal gnotomodel of ulcerative colitis, manifested by atrophic changes in the caudal intestinal mucosa, with leukocyte infiltration in the intestine, accompanied by dehydration. The optimum 5% DSS concentration utilised for chemical induction of UC will then be used in faecal microbiota transplantation procedures in patients with IBD to gnotobiotic mice.

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REFERENCES

Gancarčíková S., Nemcová R., Popper M. Hrčková G., Sciranková Ľ., Maďar M., et al. (2018): The influence of feed-supplementation with probiotic strain *L. reuteri* CCM 8617 and alginite on intestinal microenvironment of SPF mice infected with *S.* Typhimurium CCM 7205. Probiotics and Antimicrobial Proteins, 11, 2, 493-508.

Legrand C., Bour J.M., Capiaumont J., Martial A., Marc A., Wudtke, M., et al. (1992): Lactate dehydrogenase activity of the number of dead cells in the medium of cultured eukaryotic cells as marker. Journal of Biotechnology, 25, 3, 231-243.

Myllyluoma E., Ahonen A.M., Korpela R., Vapaatalo H., Kankuri, E. (2008): Effects of multi species probiotic combination on Helicobacter pyroli infection *in vitro*. Clinical and vaccine immunology, 15, 9, 1472-1482.

Jiminez J.A., Uwiera T.C., Douglas Inglis G., Uwiera R.R. (2015): Animal models to study acute and chronic intestinal inflammation in mammals. Gut Pathogens, 7, 29.

Neurath F.M. (2012): Animal Models of Inflammatory Bowel Diseases: Illuminating the Pathogenesis of Colitis, Ileitis and Cancer. Digestive Diseases, 30, 91–94.

Nishida A., Inoue R., Inatomi O., Bamba, S., Naito, Y., Andoh, A. (2018): Gut microbiota in the pathogenesis of infammatory bowel disease. Clinical Journal of Gastroenterology, 11, 1–10.

Ramos M. D., Papadakis M.D. (2019): Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clinic Proceedings, 1, 155-165.

Turnbaugh P.J., Hamady M., Yatsunenko T., Cantarel B.L., Duncan A., Ley R.E., Sogin M.L., Jones W.J., Roe B.A., Affourtit J.P., Egholm M., Henrissat B., Heath A.C., Knight R., Gordon J.I. (2009): A core gut microbiome in obese and lean twins. Nature, 457, 480–484.

Popper M., Gancarčíková S. (2014): Dekontaminácia laboratórnych SPF myší a ich využitie v gnotobiotických štúdiách. Vedecké práce doktorandov Zborník zo seminára doktorandov venovaného pamiatke akademika Boďu, IX. ročník, Košice, 9. a 10. september, 92-95.

Johnson S.A., Nicolson S.W., Jackson S. (2004): The effect of different oral antibiotics on the gastrointestinal microflora of a wild rodent (Aethomys namaquensis). Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology, 138, 4, 475-483.

Schlegel L., Coudray-Lucas C., Barbut F., Le Boucher J., Jardel A., Zarrabian S., Cynober L. (2000): Bacterial dissemination and metabolic changes in rats induced by endotoxemia following intestinal E-coli overgrowth are reduced by ornithine alpha-ketoglutarate administration. Journal of Nutrition, 130, 12, 2897-2902.

Wiesner S.M., Jechorek R.P., Garni R.M., Bendel C.M., Wells C.L. (2001): Gastrointestinal colonization by Candida albicans mutant strains in antibiotic-treated mice. Clinical and Diagnostic Laboratory Immunology, 8, 1, 192-195.

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