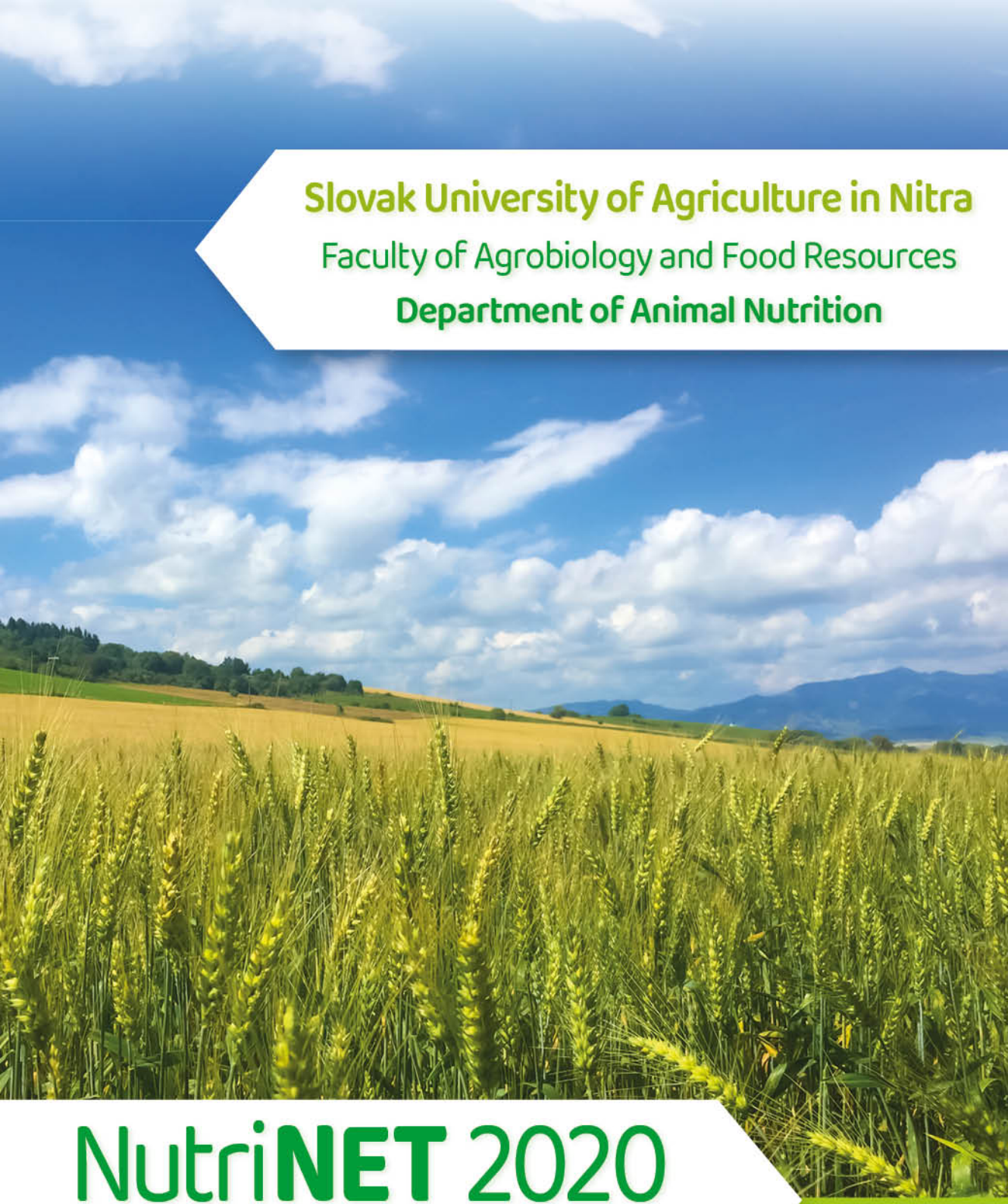
Slovak University of Agriculture in Nitra
Faculty of Agrobiology and Food Resources
Department of Animal Nutrition



NutriNET 2020

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CONTENT

Animal Nutrition PhD. Science

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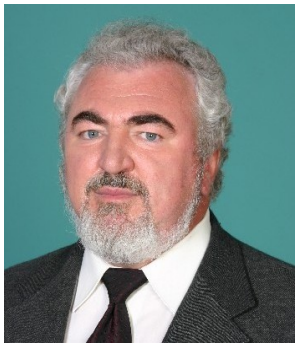

Narodil sa 21. júla 1950 v Čakajovciach, okres Nitra. Na SPU v Nitre, pracuje nepretržite od ukončenia štúdia na vtedajšej AF VŠP v roku 1974 v odbore zootechnickom. Počas celého obdobia pôsobil na Katedre výživy zvierat AF resp. FAPZ, kde absolvoval vedeckú prípravu (1982), habilitáciu (1994) a za profesora v odbore Všeobecná zootechnika bol menovaný v roku 2001. Viac ako 46-ročná pedagogická a vedecko-výskumná činnosť prof. Bíra je orientovaná na výživu. Významne sa podieľal na kreovaní študijného odboru Výživa, ktorý po jeho akreditácii aj garantoval. Pôsobil ako predseda odborovej komisie doktorandského štúdia a garant pre habilitácie a inaugurácie. Úspešne vybudoval vlastnú vedeckú školu s uplatnením absolventov na vlastnom pracovisku a v rôznych pozíciách rezortu, výskume a v praxi. Vyškolil 11 doktorandov a viac ako 150 diplomantov. Patrí medzi uznávaných odborníkov v poľnohospodárskej praxi. Vo vedecko-výskumnej činnosti sa venoval výžive prežúvavcov, konzervovaniu krmív a využívaniu netradičných zdrojov krmív. Ako zodpovedný riešiteľ a spoluriešiteľ sa podieľal na riešení viacerých národných a medzinárodných výskumných a vzdelávacích grantových úloh a projektov. Publikoval viac ako 200 pôvodných vedeckých prác s ohlasmi vo viac ako 400 citáciách. Je autorom a spoluautorom 7 vysokoškolských učebníc, z toho 2 vydaných v zahraničných vydavateľstvách, 29 titulov vysokoškolských skrípt a učebných textov, 8 monografií a 4 odborných knižných publikácií. Je resp. bol členom vedeckých rád u nás a v zahraničí, členom redakčných rád domácich a medzinárodných vedeckých časopisov a riadnym členom Slovenskej akadémie pôdohospodárskych vied. Dve funkčné obdobia pôsobil ako člen pracovnej skupiny Akreditačnej komisie Vlády SR pre poľnohospodárske a lesnícke vedy. Na pracovisku v rokoch 1994-1997 zastával funkciu vedúceho katedry, v rokoch 1997-2000 a 2003-2006 funkciu prodekana Agronomickej fakulty, resp. Fakulty agrobiológie

a potravinových zdrojov a v rokoch 2006-2015 funkciu dekana Fakulty agrobiológie a potravinových zdrojov SPU v Nitre. Z ocenení za jeho prácu možno vybrať Zlatú medailu Rektora SPU a medaily sesterských univerzít v Košiciach, v Brne, v Prahe, Českých Budějoviciach. Ďalej mu bola udelená Zlatá medaila Ministra pôdohospodárstva SR za transformáciu výsledkov výskumu do praxe a Ministrom školstva SR Veľká medaila Sv. Gorazda za dlhoročnú riadiacu, tvorivú a pedagogickú prácu vo vysokom školstve. V roku 2013 udelila Mendelova univerzita v Brne prof. Bírovi čestný titul Doctor honoris causa za významný prínos k rozvoju náuky o výžive zvierat a vzájomnej spolupráci.

THE EFFECT OF UREA ADDITION ON MINERAL PROFILE OF GRAPE POMACE SILAGE

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ABSTRACT

The grape pomace (*Vitis vinifera* L.) variety *Pinot Gris* was ensilaged in two variants: Control (without additives) and Urea (with urea addition at a dose 2 kg/t). The grape pomace matter of both variants (n=3) was stored in plastic silage units. After 6 weeks of storage, average samples of grape pomace silages were taken for chemical analysis. The macroelement (Ca, P, Na, K, Mg) and microelement content (Mn, Zn, Cu, Fe) was determined in grape pomace silages. Urea addition influenced the macroelement profile of grape pomace silage with statistically lower P, K and higher Na content. The application of urea nonsignificantly influenced the content of all analyzed microelements in grape pomace silage. The results confirmed that the addition of urea influenced the mineral profile of grape pomace silage, mainly values of macroelements.

Keywords: additive, grape pomace, macroelements, microelements, silage

INTRODUCTION

The grape pomace can be fed either fresh, ensiled or dried, it is even used as a silage additive (Li et al., 2017). The grape pomace is a feed with a higher content of crude fiber and acid detergent lignin, although is valuable for their high content of bioactive substances (Gálik et al., 2019; Ivanišová et al., 2019). It is a suitable feed especially for ruminants (Bíro et al. 2019; Rolinec et al., 2019). The many factors affect the quality of silage, including the addition of additives (Jambor, 2003; Lád et al., 2006; Mlynár et al., 2006; Jendrišáková, 2010; Jančová et al., 2011; Plachý et al., 2014; Kudrna et al., 2019; Loučka et al., 2019). In ruminants, urea is added to the feed ration or used as a silage additive (Sousa et al., 2008; Doležal et al., 2012; Elis et al., 2016; Klop et al., 2016; Morais et al., 2017; Bíro et al., 2020). The urea as nutritive additive affects the silage fermentation and increases nutritional value of their content (mainly crude protein and PDIN content) (Phesatcha and Wanapat, 2016; Doležal et al., 2017; Kang et al., 2018). The aim of the work was to determine the effect of urea addition on the mineral profile of grape pomace silage.

MATERIAL AND METHODS

The grape pomace (*Vitis vinifera* L.) variety *Pinot Gris* in cooperation with University farm Kolíňany, farm Oponice was ensilaged. The experiment consisted of two variants: Control (without additive) and Urea (addition of urea at a dose 2kg/t). The silage matter was stored in plastic silage units and then they were placed in a barrel and covered with sand. After 6 weeks, average samples of grape pomace silage were taken for chemical analysis. In grape pomace silage, the dry matter, ash, macro and microelements content was determined. Nutrients were

analysed by standard analysed methods and procedures: dry matter by drying at 103±2 °C (POL-EKO APARATURA oven, Poland), ash by complete combustion in a muffle furnace (LM 212; VEB Elektro, Germany), mineral profile (Ca, Na, K, Mg, Mn, Zn, Fe, Cu) by atomic absorption spectrophotometry (ContrAA 700, Germany), phosphorus (P) by colorimetrically (Spectrophotometer 6400 JENWAY, United Kingdom). Statistical parameters using SPSS Statistics 20.0 (IBM) (ANOVA-Tukey test) and independent samples of T-test were evaluated.

RESULTS AND DISCUSSION

The grape pomace (GP) silage with urea had in comparison with silage without additive significantly ($P<0.05$) higher content of dry matter (Table 1). Monitoring the levels of minerals in blood and feed avoid metabolic and production disorders (Pavlata et al., 2014; Hric et al., 2019; Skalická et al., 2019). The content of ash was significantly ($P<0.05$) higher in the control silage. Hanušovský et al. (2019) found ash content in grape pomace of Pinot blanc variety from 39.06 to 39.72 g/kg DM, that was in comparison with our results lower. Generally, grape pomace silage had lower ash content than alfalfa silage (Juráček et al., 2018). Statistically nonsignificant differences were detected in content of Ca. The P content was significantly ($P<0.05$) lower by 2.73% in silage with additive. The ratio of Ca/P was 1.36/1 for the control silage and 1.39/1 for silage with additive. The values of Ca were lower in comparison with the Feedipedia database (6.10 g/kg DM) in grape pomace silage with comparable dry matter content (37.00 g/kg) (Heuzé and Tran, 2017). The grape pomace silage had lower content of Ca and P in comparison to grape skin (11.50 g Ca and 5.1

g/kg DM of P) (Petrikovič et al., 2000). The results confirmed that GP silage is characterized by a higher Ca content than maize silage (Mitrík, 2018; Juráček et al., 2018). Compared to traditional silage (Skalická et al., 2013), grape pomace silage, had a higher P content (maize silage 2.26 g, alfalfa silage 3.11 g and grass silage 3.62 g/kg DM from 2012). The content of Mg was in both variants very similar with nonsignificant differences. The average value of Mg in the silages of both variants was higher than reported by Feedipedia (1.20 g/kg DM) (Heuzé and Tran, 2017). The GP silage with urea had significantly ($P<0.05$) higher content of Na (by 23.08%), but significantly ($P<0.05$) lower content of K (by 15.82%). The ratio of K/Na was 41.18/1 for control silage and 28.17/1 for silage with urea. The Na content in both variants was higher compared to average value 0.20 g/kg DM published in Feedipedia database (Heuzé and Tran, 2017). In the experiment, the lower content of K in comparison with the Feedipedia database (19.40 g/kg DM) (Heuzé and Tran, 2017) was found. Tayengwa and Mapiye (2018) found higher values of the Na and K (0.70 g Na and 20.40 g K per kilogram of dry matter). The application of urea nonsignificantly ($P>0.05$) influenced the content of Cu, Fe and Mn in GP silage (higher values by 8.84%; 4.66% and by 1.78%). The Feedipedia database (Heuzé and Tran, 2017) shows an almost twice as high Cu content (34.00 mg/kg DM) compared to the results of the implemented experiment. The average content of Zn in GP silage was nonsignificantly ($P>0.05$) lower by 3.69% in comparison with the control silage. Šimko et al. (2019) observed Zn average concentrations 16.42 and 28.99 mg/kg DM from two different locations in grape pomace samples of Pinot blanc variety. The determined values of Zn in a recent study were in the stated range.

Table 1. Mineral profile of grape pomace silages

Nutrients	Parameter	Control	Urea
Dry matter g/kg	$\bar{x}$	365.20*	384.34*
	S.D.	7.951	9.035
Ash g/kg DM	$\bar{x}$	47.64*	44.7*
	S.D.	0.695	0.224
Ca g/kg DM	$\bar{x}$	5.50	5.45
	S.D.	0.334	0.068
P g/kg DM	$\bar{x}$	4.03*	3.92*
	S.D.	0.083	0.083
Mg g/kg DM	$\bar{x}$	1.29	1.33
	S.D.	0.038	0.008
Na g/kg DM	$\bar{x}$	0.39*	0.48*
	S.D.	0.109	0.026
K g/kg DM	$\bar{x}$	16.06*	13.52*
	S.D.	1.896	1.034
Cu mg/kg DM	$\bar{x}$	16,97	18.47
	S.D.	0.641	2.076
Fe mg/kg DM	$\bar{x}$	107.33	112.33
	S.D.	6.501	8.287
Mn mg/kg DM	$\bar{x}$	14.07	14.32
	S.D.	0.388	0.319
Zn mg/kg DM	$\bar{x}$	24.63	23.72
	S.D.	3.535	2.048

*values with the same index in the line are statistically significant at $P < 0.05$, Control: grape pomace silage without additive, Urea: grape pomace silage with urea

CONCLUSION

In grape pomace silage, from analysed macroelements (Ca, P, Na, K, Mg) the highest content of K and from microelements (Cu, Fe, Mn, Zn) the highest content of Fe regardless of the effect of the additive was detected. Urea addition influenced the macroelement profile of grape pomace silage with statistically lower P, K and higher Na content. The content of macroelements Ca and Mg was not affected by treatment. The application of urea nonsignificantly influenced the content of all analyzed microelements in grape pomace silage. The results confirmed that the addition of urea as nutritive additive influences the mineral profile of grape pomace silage, mainly values of macroelements.

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THE EFFECT OF DIETARY INCLUSION OF WHITE LUPINE SEEDS ON HEMATOLOGICAL PROFILE IN DWARF LOP BREEDING FEMALES

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ABSTRACT

The aim of the study was to evaluate effect of feeding complete diets with various component composition on selected haematological indicators in the Dwarf Lop rabbit females. The control group received a commercial diet, while the experimental group was fed the diet containing a considerable share of the white lupine seed meal (250g/kg of the diet). Except for the leukocyte count, the haematological indicators assessed were not affected by a diet. Breeding does fed with the experimental diet showed a lower leukocyte count ($P < 0.05$). However, further studies focusing on examination of specific leukocyte subpopulations would be carried out to broaden existing knowledge.

Keywords: companion rabbit; nutrition; *Lupinus albus*; haematology

INTRODUCTION

Recently, there is an obvious interest for off-production husbandry of rabbits. The rabbit has become a famous companion animal and pet rabbits have shown stable rearing popularity. The strategy of the nutrition and feeding of pet rabbits clearly differ from that of the medium-sized meat-type and laboratory rabbits. Because of strictly off-production purposes of dwarf rabbits, their long lifespan linked by good health must be taken in consideration when the diets are formulated (Prebble, 2014). An inclusion of the lupine meal in diets intended for broiler rabbits showed beneficial effects on the growth characteristics and health state of the kits and also on the milk production of the does (Volek et al., 2014). Our previous findings revealed that the use of white lupine seeds in complete rabbit diets affects some blood indicators in the young, growing dwarf rabbit kits (Šimek et al., 2018). It can be expected that similar effects may exist also in the adult breeding dwarf rabbit does. Therefore, the aim of this study was to evaluate effects of the dietary use of the white lupine seeds meal on the selected haematological indicators in the breeding Dwarf Lop does.

MATERIAL AND METHODS

Animals and housing. The study was performed on a total of 24 Dwarf Lop breeding does. These females originated from a common hobby stock that focus on exhibition purposes. The does were housed in outside hutches with partially regulated microclimatic factors.

Experimental design and nutrition. The breeding does were subdivided into 2 dietary groups - the control group (*n=11*) and the experimental group (*n=13*). The control groups received a commercial

foreign complete pelleted diet designed for dwarf rabbits (Berkel-Futter Light 6008, Germany); its analyzed chemical composition (g/kg) was follow: crude protein (CP) 146.5, ether extract (EE) 24.5, crude fibre (CF) 158.1, acid detergent fibre (ADF) 213.2, neural detergent fibre (NDF) 383.3, acid detergent lignin (ADL) 48.2, starch (St) 138.6, and ash 78.7. The does of the experimental group were fed by complete pelleted diet with a noticeable share of the white lupine seed meal (25% of the diet) specially formulated in our department. The analyzed chemical composition (g/kg) of the experimental diet was follow: CP 182.1, EE 42.8, CF 145.9, ADF 228.2, NDF 314.2, ADL 51.2, St 162.8, and ash 79.1 Both the control diet (CD) and the experimental lupine diet (LD) were offered to the respective groups of breeding does from 1 week prior to kindling and feeding of the diets lasted till the weaning of kits. The rabbit does were fed once a day and received the respective pelleted diets at the rate of 25-30g/kg of their live weight (LW).

Blood collection and haematological examination. The experimental procedures were approved by Animal Welfare Committee of the University of Veterinary and Pharmaceutical Sciences Brno. One day prior to weaning, rabbit does were weighed and then the collection of the does' blood was performed using *v. saphaena lateralis*. The heparinized whole blood was manually examined in the department laboratory. Counts of the red blood cells (RBC) and white blood cells (WBC) were determined by haemocytometer with specific solutions. The haematocrit value (HCT) was determined using micro-haematocrit method. The haemoglobin concentration was determined spectrophotometrically according the Drabkin method.

Statistical analyses. The one-way ANOVA was used to determine differences in the LW and haematological indicators between the dietary groups. When ANOVA showed the significant differences between the groups, Fisher's LSD test was used. The differences were considered significant at $P < 0.05$ (*).

RESULTS AND DISCUSSION

The Table 1 presents the LW and values of haematological examination in dwarf does of the present study. It can be noted that the different diet composition had a significant effect only on the count of the WBC. The counts of the RBC in both dietary groups of the present study are consistent with the recommended reference range (4.6-7.0 × 10^{12}/l) mentioned for adult crossbred dwarf rabbits (Yeh et al., 2018). Typically, dwarf rabbit breeds tend to display rather lower values of the RBC as compared to the medium-sized rabbit genotypes (Šimek et al., 2017). The observed value of the HCT in does of the present study is in concordance with reference range (0.27-0.41 l/l) mentioned by Yeh et al. (2018). As for the haemoglobin concentration, its values found in does of the present study are consistent with those stated by Harcourt-Brown (2002) and Yeh et al. (2018). Concerning the proved dietary effect on the WBC in the present study, does of the LD group showed the significantly lower WBC value as compared to those fed with the control diet. Earlier, Šimek et al. (2018) also observed the non-significant WBC-lowering effect in young kits of the Dwarf Lop breed when fed with the diet containing white lupine. On the other hand, Alharbi et al. (2014) found a WBC elevation when broiler rabbits were fed lupine monodiet. It is possible that the partial dietary inclusion of the lupine seed may affect also leucocytes population in dwarf rabbits.

The usual WBC range for rabbits is 5.0-12.0 × 10^9 cells/l (Harcourt-Brown, 2002). Šimek et al. (2017) and Yeh et al. (2018) point out that dwarf rabbit breeds tend to display rather lower WBC values as compared to other rabbit genotypes. In both dietary groups of the present study, the WBC values observed can be considered as physiological. However, it is necessary to perform other profiled studies in order to broaden knowledge about dietary effects of white lupine seeds not only on the total WBC count but also on the specific WBC subpopulation in rabbits.

Table 1. Live weight and selected haematological indicators of the Dwarf Lop does fed with different diets.

Item	Unit	Dietary group				*P*
		CD (*n* = 11)		**LD** (*n* = 13)		
		mean	SEM	mean	SD	
LW	**g**	1797.3	42.45	1780.4	29.52	NS
RBC	**10^{12}/l**	5.0	0.28	5.4	0.10	NS
HCT	**l/l**	0.4	0.02	0.3	0.01	NS
HGB	**g/l**	116.2	3.89	116.0	3.95	NS
WBC	**10^9/l**	6.0	0.49	4.6	0.46	*

CD, control diet; LD, experimental lupine diet; LW, live weight; RBC, red blood cells; HCT, haematocrit value; HGB, haemoglobin concentration; WBC, white blood cells; NS = not significant.
* = means within a row differ at ($P < 0.05$)

CONCLUSION

Based on the found results it can be concluded that the WBC count in adult breeding does of the Dwarf Lop breed was significantly affected by a diet, while does fed with a share of the white lupin seed meal displayed its lower value. However, further studies dealing with examination of specific leukocyte subpopulations would be carried out to broaden existing knowledge.

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EFFECT OF DIETARY ACIDIFIERS ON THE DIGESTIBILITY OF NUTRIENTS AND FERMENTATION ACTIVITY IN THE LARGE INTESTINE OF WEANED PIGS

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ABSTRACT

The objective of this study was to examine the effect of feeding diets containing dry organic acid blend (lactic acid – ammonium formate – ammonium propionate – citrate – sorbate) in weaned pigs on the digestibility of nutrients and the fermentation activity in the large intestine. In the experiment, 12 weaned pigs (Slovak Large White×Landrace; initial weight 8.84 ± 0.33 kg) were allotted into two groups. Pigs were fed a control diet (control group) or a diet supplemented with dry organic acid blend, 0.6g / 100g (test group). The duration of the experiment was 28 days. For fecal digestibility determination ash which is insoluble in hydrochloric acid was used as marker. During the 4-week post-weaning period, significantly higher ($P < 0.05$) coefficient of digestibility of ash was observed in pigs fed dry organic acid blend, while no statistically significant differences were observed in the fecal digestibility of organic nutrients. Although, compared with the control group, feeding dietary acidifiers improved

the digestibility of organic nutrients and the values of digestibility coefficients were higher in the test group; crude protein +2.3%, crude fat +3.6%; crude fibre +0.8% and nitrogen-free extract +1.8%. The results of the fermentation process analysis indicated that the butyrate concentration increased ($P < 0.05$) and the pH decreased ($P < 0.05$) in the test group compared to the control group. Statistical analysis of the results also confirmed lower level of crude protein in the dry matter basis of faeces in animals of the test group ($P < 0.05$).

Keywords: acidifiers; digestibility; fermentation; nutrients; piglets

INTRODUCTION

Acidifiers have been widely used for decades in livestock feeds, due to their preservative and nutritional qualities (Spratt, 1985; Partanen and Mroz, 1999). Weaning is a time of nutritional and environmental stress. Inclusion of dietary organic acids as an alternative to antibiotic addition was evaluated in several studies using weaned pigs and in growing and finishing swine (Eisemann and van Heugten, 2007). Organic acidifiers have been widely used for weaning pigs' diets for years and most common organic acidifiers contain fumaric, citric, formic or lactic acid (Kil et al, 2011). The probable mode of actions of organic acids includes reducing the digesta pH value in the gastrointestinal tract (Ravindran and Kornegay, 1993), regulating the balance of microbial populations in the gut, stimulating the secretion of digestive enzyme (Thaela et al., 1998). More recently, a number of studies have highlighted the potential effects of organic acids in improving digestion, nutrient digestibility and the promotion of growth performance in pigs (Luise et al., 2017; Long et al., 2018).

This fact motivated us to determine the dietary effects of dry organic acid blend in weaned pigs on the digestibility of nutrients and fecal indicators of fermentation processes.

MATERIAL AND METHODS

At weaning (d 28 ± 2), 12 pigs (Slovak Large White×Landrace; initial weight 8.84±0.33 kg) were divided in two groups (six pigs in control group and six pigs in test group). The duration of the experiment was 28 days. The test group diet was supplemented with dry organic acids blend in an amount of 0.6g/100g. The characteristics of the applied organic acid blend were the following: lactic acid, ammonium formate, ammonium propionate, citrate, sorbate; ash 39%. Nutritional analysis of test and control diet are shown in Table 1. The diets used in this experiment were formulated to meet NRC (2012).

Table 1. Chemical composition (%; as fed basis) of diets

Diet	**control**	**test**
DM %	89.42	89.20
CP %	19.55	19.95
Fat %	3.49	3.48
CF %	3.25	3.30
Ash %	4.93	5.65
NFE %	58.20	56.82
ME (MJ/kg)	13.66	13.54

DM – dry matter; CP - crude protein; CF - crude fiber; NFE – nitrogen free extract; ME – metabolizable energy

For apparent fecal digestibility determination acid-insoluble ash was used as marker. Feed and fecal samples were analyzed for dry matter (DM), crude protein (CF), fat, crude fiber (CF), ash as well as ash which is insoluble in hydrochloric acid according to the EC

Commission Regulation 152/2009. The nitrogen free extract (NFE) was mathematically calculated from DM, CP, fat, CF and ash. The faeces were taken directly from the rectum at the end of the investigation. The quantitative determination of the short-chain fatty acids (SCFAs) was done by the method of isotachophoresis employing a two-capillary analyser EA100 (VILLA LABECO, Slovakia). The samples of faeces were analysed for pH from extract (4hours / 2g fresh faeces plus 20 ml distilled water). The differences between means were determined, according to the unpaired t-test using GraphPad Prism 6 software.

RESULTS AND DISCUSSION

Acidifiers have been commonly targeted for weanling pigs (Menegat et al., 2019). It is generally known that dietary acidifiers lower gastric pH, resulting in increased activity of proteolytic enzymes, improved protein digestibility (Kim et al., 2005).

Compared to the control group, pigs fed diet supplemented with dry organic acid blend tended to increase ($P < 0.05$) the apparent fecal digestibility of ash (Table 2). It may be due to higher content of ash in the test diet after dry organic acids blend administration.

Table 2. Coefficients of nutrient digestibility in weaners (Mean ± SD)

	control	**test**
CP %	78.90 ± 2.22	81.20 ± 1.78
Fat %	66.20 ± 3.91	69.80 ± 3.32
CF %	32.80 ± 3.09	33.60 ± 2,59
Ash %	38.90 ± 3.18	44.20 ± 2.92*
NFE %	88.60 ± 1.95	90.40 ± 1.08

SD – standard deviation; CP – crude protein; CF – crude fiber; NFE – nitrogen free extract; significant difference: *P < 0.05.

The apparent fecal digestibility of CP, fat, CF and NFE was also improved (crude protein +2.3%, crude fat +3.6%, crude fibre +0.8% and nitrogen-free extract +1.8%) in pigs supplemented with diet containing mixed of organic acids compared to the control group. Our findings showed a tendency for an improved of the apparent fecal digestibility of organic nutrients after supplementation of diet with mixed organic acids in weaned pigs. However, differences for the fecal digestibility of CP, fat, CF and NFE were not statistically significant in our study.

In our work, we recorded the increase of short chain fatty acids concentrations (significantly for butyric acid, $P < 0.05$) in the test group. The results of the faeces analysis also indicated that the pH and the crude protein (DM) decreased ($P < 0.05$) in the test group compared to the control group (Table 3).

Table 3. Indicators of fermentation process and nitrogen excretion determined in the faeces of weanlings (as dry matter) (Mean ± SD)

	control	**test**
Acetate g/kg	19.68 ± 2.58	21.29 ± 2.41
Propionate g/kg	13.61 ± 1.56	14.04 ± 1.92
Butyrate g/kg	6.69 ± 0.56	7.70 ± 0.58*
Total SCFAs g/kg	39.97 ± 4.47	43.05 ± 4.71
pH	6.56 ± 0.28	6.19 ± 0.27*
CP g/kg	233.6 ± 11.9	213.6 ± 12.5*

SD – standard deviation; CP – crude protein; SCFAs – Short-chain fatty acids; significant difference: *P < 0.05.

In the review Kil et al. (2011), who assessed the 11 experiments measuring apparent total tract digestibility (ATTD) of protein, the feeding of dietary acidifiers improved the ATTD of protein, on average, by 1% compared with the control group. Partanen et al. (1998)

reported that organic acids can lower the microbial proliferation and then reduce the competition of nitrogen between the microflora and the host and therefore improve nitrogen retention in pigs.

Short-chain fatty acids (SCFAs) can stimulate the intestinal cell proliferation (Marsman and McBurney, 1996) and have previously been reported to be associated with organic acid supplementation in diets, resulting in improved mucosal growth, motility and proliferation of the epithelial cell lining (Mroz, 2005). Among all the SCFA, butyric acid, produced by fermentation of some carbohydrates in large intestine, has a positive effect on epithelial cell growth, blood flow, and absorptive functions in pigs (Canani et al., 2012).

Our findings are consistent with the results published by Partanen et al. (2002), where faecal pH was higher in diets without additives than in those supplemented with organic acids (formic acid; formic acid–sorbate blend) in grower and finisher diets. On the contrary, other researchers have observed that concentration of SCFAs was reduced in the colon digesta in piglets fed diets with organic acids (fumaric and lactic acid) compared with those fed unsupplemented diets (Zentek et al., 2013).

CONCLUSION

The present study demonstrated dietary dry organic acid blend supplementation in weaned pigs could improve the nutrient digestibility and improves the concentrations of total short-chain fatty acids in faeces. In this study, the supplementation of diets for weanling pigs with mixed organic acids affected the concentrations of butyrate in the faeces.

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DETERMINATION OF DEOXYNIVALENOL IN FEED CEREALS BY ELISA TEST

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ABSTRACT

Cereals are frequently contaminated by microscopic filamentous fungi which are spread in soil and in various organic substrates. Many of these species are capable, under certain conditions, of synthesizing secondary metabolites, mycotoxins. Deoxynivalenol is a product of *Fusarium* fungi and may cause alimentary mycotoxicosis in humans and animals. The 114 cereal samples were analysed. Deoxynivalenol was detected in 35 samples. The average concentration in positive wheat samples was 0.08 mg/kg and in barley 0.002 mg/kg. The highest incidence of deoxynivalenol (55.6%) was determined in samples of maize with average value of concentrations 0.688 mg/kg. The highest deoxynivalenol concentration, 1.925 mg/kg, was determined in a maize samples.

Keywords: mycotoxin; contamination; wheat; barley; maize

INTRODUCTION

Feed cereals are an important component of a balanced diet in human and animal nutrition. The cereals are included in the grass family *Poaceae*. Cereal grains contain carbohydrates, proteins and an important source of B vitamins and minerals. During the growth, cereals are often contaminated with microscopic filamentous fungi. It is estimated that 25% of the total cereal production is degraded by mycotoxins. Cereals represent a suitable nutrition substrate for various micromycetes. They are mostly contaminated by fungi of the genus *Fusarium* and *Alternaria*. The phytopathogens *Fusarium graminearum* and *Fusarium culmorum* can infect crops on the field and cause a disease called *Fusarium* head blight or scab (McMullen et al., 2012). The presence of microscopic filamentous fungi in cereals can lead to various negative effects: modification of nutritive value, development of mycotoxicosis and allergy agents (Bennett and Klich, 2003). In addition to degrading the plant and grain, fungi of the genus *Fusarium* are capable of producing mycotoxins. The most common mycotoxin of the genus *Fusarium* is deoxynivalenol (Mostrom, 2011). Deoxynivalenol (syn. vomitoxin, DON) is mainly contaminant of wheat, barley, corn, oats, rice, sorghum and millet (Tian et al., 2016). The acute toxicity of deoxynivalenol is manifested by gastrointestinal disorders (nausea, vomiting, gastroenteritis). Pigs are the most sensitive to the effects of the deoxynivalenol, the less sensitive are poultry and cattle. In poultry was observed a reduced weight of the egg, a deterioration of the quality of the eggshell and residues remain in the egg after feeding the DON-containing feed (Herzig and Suchý, 2005). In cattle, deoxynivalenol causes a reduced fat content in milk and also

reduces milk yield (Rajčáková and Mlynár, 2004). In horses, poisoning is manifested by altered reflexes, meningitis, hyperesthesia, neurological as well as encephalomyelitis damage. Deoxynivalenol can also cause acute intoxications in dogs and cats. Symptoms of acute mycotoxicoses include: vomiting, tachycardia, diarrhea, hemorrhage, edema, skin necrosis, hemorrhagic inflammation of the gastric and colonic mucosa, damage to the hematopoietic system, leukopenia, thrombocytopenia and hemorrhage in the brain. In addition, animals are more susceptible to infectious diseases and reduced performance has been reported (Eriksen and Pettersson, 2004). The aim of this work was detected deoxynivalenol contamination of feed grains.

MATERIAL AND METHODS

The presence of deoxynivalenol was examined in 33 samples of wheat (*Triticum aestivum*), in 36 samples of barley (*Hordeum sativum*) and in 45 samples of maize (*Zea mays* spp. *Mays*). A total number of samples was 114 pieces and they were obtained from storage company Tajba, a.s., Čaňa. Preparation of samples was performed according to the Veratox protocol (Veratox for deoxynivalenol; Neogen corporation). To 10 g of the ground sample was added 100 ml of distilled water. The samples were mixed on a horizontal shaker (Orbital Shaker - Biosan) for 3 minutes and then filtered through Whatman 1 filter paper. The filtrates were diluted 1:2 with distilled water and prepared for the quantitative determination of DON by ELISA and evaluated by ELISA reader (Dynex Technologies Inc., Virginia, USA).

RESULTS AND DISCUSSION

The occurrence of deoxynivalenol in the analysed cereal samples is presented in Table 1. In wheat, the presence of DON was confirmed in 8 samples of 33 (24.2%) with concentrations range 0.021 – 1.307 mg/kg. Out of 36 barley samples, deoxynivalenol was detected only in 2 samples (5.6%), with concentrations 0.019 and 0.052 mg/kg. In maize, the incidence of deoxynivalenol was 55.6%, with concentrations from 0.109 to 1.925 mg/kg. In all the analysed samples, the concentration of DON did not exceed the permitted maximum levels (8 mg/kg) (Commission recommendation, 2006/576/CE).

Tab. 1 The incidence of deoxynivalenol

Cereals	n/n*	I (%)	Concentration of DON (mg/kg)	Average value (mg/kg)
Wheat	33/8	24.2	0.021 – 1.307	0.080
Barley	36/2	5.6	0.019 – 0.052	0.002
Maize	45/25	55.6	0.109 – 1.925	0.688

n – the total number of cereal samples, n* – the number of positive samples, I – incidence

The occurrence of deoxynivalenol in cereals varies from country to country. The highest incidence of deoxynivalenol (100%) was found out in the cereals in Czech Republic and Lithuania (Hajšlová et al., 2007; Mankevičienė et al., 2007). In Romania, the presence of deoxynivalenol in the cereals reached 79% (Tabuc et al., 2009) and in Netherlands up to 89% of examined samples (Tanaka et al., 1990). Similarly to Slovak Republic, also in Poland was recorded the highest deoxynivalenol contamination in samples of maize (75%) (Krysińska-Traczyk et al., 2007).

A relatively low incidence of DON was recorded for cereals in Serbia (34.5%) (Jajić et al., 2008) and in Norway (29.4%) (Van Der Fels-Klerx et al., 2012). In Spain and Argentina, the incidence of deoxynivalenol in maize was less than 30%, while in Spain, DON concentrations in maize ranged from 0.026 to 0.131 mg/kg (Broggi et al., 2007; Castillo et al., 2008). In Slovakia, the occurrence of deoxynivalenol was found in individual cereal species in the following average concentrations: 0.048 mg/kg in barley, 0.070 mg/kg in wheat and 0.075 mg/kg in maize (Remža and Matušová, 2011).

CONCLUSION

Occurrence of fungi and their secondary metabolites cannot be completely prevented. One of the way, how to guarantee the quality of food for humans and the safety of animal feed is the regular monitoring of contaminants. To prevent the mycotoxin occurrence, various substances are used for their elimination such as adsorbents, antioxidants and various biological active substances. The possibility of food-borne mycotoxicosis through contaminated food and feed should not be forgotten in the differential diagnosis of various human and animal diseases.

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APPARENT DIGESTIBILITY OF DRY MATTER AND CRUDE PROTEIN OF BROILER CHICKENS FED DIETS WITH HUMIC SUBSTANCES

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ABSTRACT

The objective of the study was to investigate the effects of dietary intake of humic substances (HS) on the digestibility coefficient (dc) of dry matter (DM) and the apparent assimilable mass coefficient of crude protein (CP) corrected for protein catabolism (AMC_N) of broiler chickens. One-day-old broiler chickens (Ross 308, n=120, 38.86±6.71 g), were divided into 4 equal groups (A, B, C / negative control) used in the experiment. Chickens were fed with mixtures (HYD1 197.79, HYD2 194.03, HYD3 183.70 g/kg CP for 37 days. The humic substances were added into feeds: A: 0.7% Humac nature (HN), B: 0.7% Humac nature monogastric (HNM), C: 0.5% HNM. The applied additives HN/HNM (Humac Ltd., Slovak Republic) contained humic acids min. 650/570 and fulvic acids min. 50/50 g/kg. The body weights and feed consumption were assessed once a week. The excreta was sampled directly from the cloaca and used for the quantification of DM, CP, ash and ash insoluble in HCl on days 17, 24 and 31. The addition of humic substances caused the increase ($P<0.05$) of dc of DM(%) of the

experimental group C compared to control 66.04/50.90 or 61.14/46.29 on day 17 and 24, respectively. Similarly, in the case of B group the enhancement 71.29/50.56 was observed on day 31. AMC_N was higher ($P < 0.05$) in the same experimental groups: in C 0.69/0.54 or 0.64/0.50 on day 17 alternatively 24 as well as in B 0.73/0.54. The average weight gains were A: 97.27 ± 15.93, B: 98.56 ± 11.55, C: 94.33 ± 17.02 and control: 94.11 ± 19.82 g/day in week 5. The positive effects were observed on the production parameters after dietary intake of HNM at the concentration of 0.5% and 0.7%.

Keywords: gut of poultry; humates; dry matter; crude protein; excreta

INTRODUCTION

Humic substances (HS) are longer used in the plant production than in animal production because of the positive effect on the rates of seed germination, the transfer micronutrients for plants, the improvemen of water retention and the enhancement of microbial composition in soil. These natural materials are composed from humic acids (HA), humin, ulmic acids, fulvic acids and other minerals (Peña-Méndez et al., 2005; Arif et al., 2016). According to the International Humic Substances Society (2007), HS are major components of the natural organic matter in the soil, water and in geological organic deposits such as lake sediments, peats, brown coals and shales. These are responsible for the characteristic brown colour of decaying plant debris and contribute to the brown or black colour in surface soils. As for formation, HS are complex and heterogeneous mixtures of polydispersed materials created by biochemical and chemical reactions during the decay and transformation of plant and microbial remains in the process called

humification. The important components of this process are plant lignin, its transformation products, polysaccharides, melanin, cutin, proteins, lipids, nucleic acids and fine char particles. HA, as components of HS, are created in the process of decoposition of organic matter. These compounds are characteristic with a long molecular chain, molecular weights 5-100 kDa and pH below 2 (Islam et al., 2005). The strong base (NaOH or KOH) are used for the extraction of HA and FA from the soil and other solid phase sources. Because of the insolubility of HA at low pH, they are precipitated by adding strong acid. On the other hand, HS are used for the preparation of animal diets and are important growth promoters of poultry (Mutus et al., 2006; Arafat et al., 2017). The mechanisms of HS in the process of the performance improvement of poultry are not completely certain.

Abdel-Mageed (2012) and Taklimi et al. (2012) mentioned that HS are enhancing the growth by modifying partitioning of nutrient metabolism. HS are typical with their ability for the alteration of the intestinal microflora with the favourable effects on the counts of beneficial bacteria and the reduction of pathogenic bacteria in intestine (Schepetkin et al., 2003). According to the study of Taklimi et al. (2012), HA had a positive effect on the crypt depth in the jejunum villi of broiler chickens. Therefore, HA probably have the important impact on the performance of poultry via microbial ecosystems in the gastrointestinal apparatus tract. The scientific hypothesis for the experiment was based on the positive effects of HS on the enzymatic activities in the gastrointestinal apparatus which have potential to improve digestion of DM, CP, starch and cellulose in the gut of poultry.

The study aimed to investigate the effects of dietary intake of

preparations containing HS on the digestibility coefficient (dc) of DM and the apparent assimilable mass coefficient of CP corrected for protein catabolism (AMC_N) of broiler chickens.

MATERIAL AND METHODS

Chickens and diets

One hundred and twenty, one-day-old broiler chickens of hybrid Ross 308 (average weight 38.86 ± 6.71 g), were delivered from a commercial hatchery. They were divided at random into 4 groups of 30 animals (control / A, B, C). The chickens were housed in four-floor pens located in one experimental hall of the University of Veterinary Medicine and Pharmacy in Košice (Slovak Republic) with constant access to feed and water. The pens were identical concerning the same direction and the same area (0.12 m^2 per broiler chicken). All groups were fed with mash diets (Agrocass, Ltd., Slovak Republic; HYD1 – 197.85, HYD2 – 194.03, HYD3 - 183.70 g/kg CP in the time of 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 (HYD1) and grower (HYD2) feed mixtures only.

HS (Humac Ltd., Slovak Republic) were added into feeds as follows: A – 0.7% Humac natur (HN), B – 0.7% Humac natur monogastric (HNM), C – 0.5% Humac natur monogastric (HNM) and control without these additives for the whole experiment. The characteristics of the applied preparations HN/HNM were the following: the size of particles up to 100 μm, 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 DM. The body weights (BW) of chickens and the feed consumption were assessed once a week.

Feed analysis

The samples of diets were analysed (Table 1) according to the official methods of the Association of Official Analytical Chemists (Cunniff, 1995). The analyses were conducted for the determination of DM, CP, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF) and ash. Fibres were analysed by common methods (Van Soest et al. 1991). The mineral composition of the feed (Ca, Mg, Na, K, Cu, Zn, Mn) was determined by atomic absorption spectrophotometry (AAS) (Van Loon, 1980). The quantitative determination of phosphorus was performed spectrophotometrically (Carvalho et al. 1998). The insoluble portion of ash in HCl was determined in the feed mixture as the residue of ash, after dissolving ash in diluted hydrochloric acid by weighing (Daněk et al. 2005).

Check of digestibility

The digestibility was checked on 17, 24 and 31 days of age. The excreta was sampled directly from the cloaca into sterile glass containers on the designated day. The quantification of DM, CP, ash and portion of insoluble ash in HCl was performed according to above-mentioned methods. The dc and (AMC_N) were calculated according to the methods described by Gugliemo and Karasov (1993) and modified by Marcin et al. (2016).

Statistical analysis

The data are expressed as means ± standard deviation (SD) of single values (IBM SPSS Statistics, Version 24). 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 $P < 0.05$.

RESULTS AND DISCUSSION

The addition of HS had a positive effect on the increase of dc of DM (Table 2) in the experimental groups. The higher values of DM were observed ($P < 0.05$) in the C or B groups after the intake of 0.5% or 0.7% HNM in feed compared to control by 22.93%, 24.29% and 28.86% on days 17, 24 and 31. Similarly, AMC_N was higher in the experimental groups as well (Table 3). Whereas, the significant increase ($P < 0.05$) was observed in the C or B groups in comparison to the negative control group by 20.89%, 21.57% or 25.97% at the same sampling time.

According to published information, the HA added into a feed of poultry improved digestibility and the utilization of nutrients in several studies (Kocabagli et al., 2002, Taklimi et al., 2012, Ozturk et al., 2014). Kocabagli et al. (2002) evaluated of the dietary effects of humate on the feed utilization in broilers which resulted in the information that the addition of humate (2.5 kg/Ton) into feed improved body weight gains as the effect of the increased nutrient utilization. Ozturk et al. (2014) observed the effect of HS (7.5, 15 and 22.5 g/kg) on performance and the utilization of nutrients in Ross chicks. They concluded that 15 and 22.5 g/kg of HS significantly increased the digestibility of nutrients.

Demeterová et al. (2009) determined the improvement of the feed conversion ratio and the European efficiency index of broiler chickens on day 41 as the effect of the dietary addition of HS combined with the

probiotic strain *Enterococcus faecium.* However, an improvement in FCR was observed which is believed to be due to the significant increase of indices of metabolic activity of phagocytes, caused by HS when combined with. E. faecium DSM 7134.

Positive effect on enzymatic activities in the gastrointestinal apparatus of sheep was observed too. The addition of HS combined with urea increased the amylolytic and cellulolytic activities in the forestomachs of sheep (Marcin et al., 2020). The positive effect on AMC_N was demonstrated by Terry et al. (2018). This parameter was linearly increased with the increasing dietary HS concentration.

The average finishing body weights of chickens in the groups were the following A: 2487.55 ± 367.72, B: 2523.68 ± 292.20, C: 2465.39 ± 326.51 and control: 2480.01 ± 320.88 g. The average weight gains were A: 97.27 ± 15.93, B: 98.56 ± 11.55, C: 94.33 ± 17.02 and control: 94.11 ± 19.82 g/day in week 5. The average daily feed intakes were A: 180.57, B: 188.36, C: 177.83 and control: 161.22 g/day during week 5.

CONCLUSION

The addition of humic substances in the preparation HNM caused the significant increase of the digestibility coefficient (%) of DM and the values of the apparent assimilable mass coefficient of CP corrected for protein catabolism in the experimental groups B and C compared to control. The positive effects were observed on the production parameters after dietary intake of HNM at the concentration of 0.5% and 0.7%.

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Table 1. The analysed nutrient composition of experimental diets

	Experimental diets		
Ingredients (g/kg)	HYD1	HYD2	HYD3
Dry mater	873.13	882.93	877.10
Crude protein	197.85	194.03	183.70
Crude fat	28.18	72.70	59.40
ND fibre	100.03	111.53	113.0
AD fibre	45.40	56.85	59.70
Ash	55.13	54.68	45.70
Ash insoluble in HCl	2.43	1.93	2.10
Starch	407.88	392.15	393.50
Calcium	4.30	6.38	6.50
Magnesium	2.79	2.73	2.73
Sodium	2.41	1.31	1.40
Potassium	8.08	8.10	7.45
Phosporus	5.20	7.58	4.50
Copper	0.02	0.06	0.05
Zinc	0.03	0.04	0.12
Manganese	0.08	0.16	0.126
Metabolizable energy (MJ/kg)*	12.93	12.44	12.21

*Calculation based on Kirchgesner and Roth (1983)

Table 2. The digestibility coefficient (dc) of DM of broiler chickens after intake of HS (n = 6; mean ± SD)

	dc (%)		
Group	day 17	day 24	day 31
A	56.82 ± 6.057	56.83 ± 4.356	55.56 ± 8.891
B	54.59 ± 5.327	58.58 ± 4.501	71.29 ^{b} ± 9.441
C	66.04 ^{b} ± 2.700	61.14 ^{b} ± 2.689	61.50 ± 7.715
control	50.90 ^{a} ± 2.525	46.29 ^{a} ± 8.368	50.56 ^{a} ± 6.054

HS – humic substances, means with different superscript letters differed significantly: ^{a,b} $P < 0.05$

Table 3. The apparent assimilable mass coefficient of CP corrected for protein catabolism (AMC_N) of broiler chickens after intake of HS (n = 6; mean ± SD)

Group	day 17	day 24	day 31
A	0.60 ± 0.081	0.60 ± 0.087	0.58 ± 0.129
B	0.58 ± 0.079	0.62 ± 0.072	0.73 ^{b} ± 0.038
C	0.69 ^{b} ± 0.057	0.64 ^{b} ± 0.018	0.64 ± 0.104
control	0.54 ^{a} ± 0.038	0.50 ^{a} ± 0.025	0.54 ^{a} ± 0.046

HS – humic substances, means with different superscript letters differed significantly: ^{a,b} $P < 0.05$

EVALUATION OF PROTEIN TRANSFORMATION IN DAIRY COWS

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ABSTRACT

In breeding conditions of dairy farms in the 1st stage of lactation was confirmed the effect of increased content of crude protein (CP) content, in farms with low content of CP (<150 g/kg dry matter), optimum (150-170 g/kg dry matter) and high content of CP (>170 g/kg dry matter), to increase the level of ammoniacal nitrogen (NH_3-N)in the rumen (17.4 ± 1.7; 19.6 ± 2.3; 22.8 ± 3.4 mg/100 ml), to the concentration of urea in the blood (22.8 ± 3.7; 27.8 ± 4.8; 35.1 ± 6.6 mg/dl) and to the concentration of urea in the milk (18.25 ± 2.6; 24.8 ± 3.9; 33.3 ± 6.7mg/dl). With increasing content of CP was confirmed according to regression equations to increasing of environmental load by the excretion nitrogen in the urine (139.4 ± 19.8; 189.5 ± 30.4; 254.7 ± 51.3 g N /day) and by the emission of ammonia (68.1 ± 6.1; 83.7 ± 9.4; 103.8 ± 15.9 g /day). The highest efficiency of N utilization was confirmed at a CP content of 150-170 g /kg dry matter of TMR at the level of 31.1 ± 1.4 %.

Keywords: nitrogen, milk urea, urine, urine nitrogen

INTRODUCTION

Increasing milk production in the current conditions on farms puts great emphasis on nutritional requirements, especially the protein requirements of dairy cows with high production. At the level of the farms, the challenge remains for production groups of cows to determine the optimal amount of protein in terms of the need to increase milk and milk protein production and effective utilization of nitrogen (EUN) (Hristov et al., 2011) by optimizing the amount of metabolizable proteins and fermentable carbohydrates (Schwab, 2010). Protein overfeeding in dairy cows, in addition to loss N in urine, increases energy requirements (Milano et al., 2000) and negatively affects reproductive parameters (Ferguson and Sklan, 2005). Increasing the N content in TMR reduces the EUN and increasing the excretion of N in the form of urea nitrogen in the urine -UUN (Hristov and Huhtanen, 2008). The level of urea in milk (MUN) and blood (BUN) is affected by the rumen and metabolic nitrogen balance. Urea is the final metabolite of nitrogen excretion, which includes -*ammoniacal nitrogen* absorbed from the rumen derived from rumen degradable protein of TMR, -*metabolic nitrogen* formed by deamination of part of the absorbed amino acids (AA), which are not included in proteosynthesis due to imbalance of essential AAs. Increased supply of crude protein resp. rumen-degradable protein increases the level of NH_3 in the rumen and content of blood urea, increases the energy needs of metabolism and liver function load (Hammon et al., 2005). With an average daily production of 1 kg of milk protein (157 g N per day), 4 g of N (ranging from 2 to 9 g / d) is usually excreted in milk urea, which is almost 40 times more N in the form of milk protein compared to milk urea

(Wattiuax and Ranathunga, 2016). Recycling of N should also be part of the nutritional models to avoid excessive protein feeding and UUN excretion into the environment (Lapierre and Lobley, 2001) and the release of ammonia into the environment (Hristov et al., 2011). Monitoring MUN for the determination of the efficiency of N utilization and UUN excretion in urine can be effectively used as a tool for nutritional and environmental management and as a control of protein nutrition.

The aim of the work was to evaluate the relations of the metabolic transformation of CP, by analysis of NH_3 in the rumen, urea in blood and milk as metabolic indicators of protein level in dairy cows with the possibility of use for evaluation of protein nutrition in conditions of the farm. Based on the content of milk urea, re-evaluate the effective utilization of N and the environmental burden at the protein overfeeding.

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.

Determination of rumen metabolites by analysis of the amount and proportion of VFA, pH values, NH_3 in rumen contents samples 4-6 hours after feeding with the addition of thymol. The concentration of NH_3 in contents of rumen was determined by a Kjeldahl-N using a 2300 Kjeltec Analyser Unit (Foss Tecator AB, Hoganas, Sweden).

Determination of metabolites of intermediary metabolism for the assessment of protein metabolism was determined photometrically with a diagnostic kit using a biochemical analyzer "Ellipse".

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.

Evaluation of nutritional composition and milk production. In feed trials on a total of 27 farms and 216 individual dairy cows in the first lactation phase, daily feed intake in the group and the nutritional composition of TMR were intensively monitored. Days in milk (DIM) of evaluated dairy cows in the 1st phase of lactation was 88 ± 17 on average with a minimum 60 and a maximum 130 in the holding. 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 and rapeseed meal) fed as TMR. The average concentration of nutrients in TMR and the production characteristics of dairy cows at the

evaluated farms are presented in Table 1. The parameters of milk production and composition of TMR showed significant differences in chemical dietary composition against actual production and composition of milk on evaluated farms.

Table 1. Nutritional composition of TMR and composition of milk

	Average	SD	Min	Max
Nutrients in TMR (% of Dry Matter)				
Crude protein	16.59	1.5	12.57	19.61
NEL	6.64	0.2	6.20	7.13
NDF	34.48	3.6	28.04	41.82
ADF	21.29	1.8	18.33	24.98
Starch	23.35	3.5	16.93	30.21
NFC	36.52	3.3	29.45	43.88
NEL/CP	0.40	0.04	0.34	0.50
Starch/CP	1.43	0.3	1.02	1.91
NFC/CP	2.22	0.3	1.73	2.79
Production and composition of milk				
Milk yield kg/d	35.20	7.1	23.00	52.90
Milk protein %	3.12	0.2	2.70	3.55
Milk fat %	3.66	0.4	2.91	4.91
Yield of milk protein kg/d	1.09	0.2	0.69	1.48
Yield of milk fat kg/d	1.27	0.3	0.76	1.78
Milk urea mg/dl	27.20	7.1	15.53	43.50
Milk urea nitrogen mg/dl	12.69	3.3	7.25	20.30

Statistical processing of results. The results achieved were processed by mathematical-statistical methods involving statistical program GraphPad Prism4. We evaluated values and their differences using the test Tukey-HSD at significant levels of $P \leq 0.01$ and $P \leq 0.05$, respectively. Each parameter was presented by its average (x), standard deviation (SD), descriptive statistics respectively.

RESULT AND DISCUSSION

1. Evaluations of effect different CP content on metabolism and transformation of protein in cows.

The analyzed content of CP in the evaluated farms was on average 165.9 ± 15.0 g/kg DM of TMR with significant fluctuations of values from 125.7 to 196.1 g/kg DM. The evaluation of the influence of the level of protein nutrition in dairy cows in the first phase of lactation on the transformation of proteins through selected indicators is summarized in Table 2 where farms divided with low content of CP <150 g/kg (3 farms), optimal 150-170 g/kg (14 farms) and high content of CP >170 g/kg DM (10 farms). In the monitored farms, the analyzed level of NH_3-N fluctuated depending on the intake of protein and energy in TMR from 12.75 to 22.0 mg/dl with an average value of 16.85 ± 2.6 mg/dl. Excessive protein intake is the cause of increased NH_3 production in the rumen. Microbial proteins are 80 % composed of NH_3 resp. NH_3-N of ammoniacal N (Bach et al., 2005), where the minimum level of NH_3-N required for their synthesis is 9.2 mg/dl (Reynal and Broderick, 2005). An important marker in the evaluation of protein transformation is the concentration of urea in the blood serum. The analyzed serum urea averaged 29.94 ± 6.8 mg/dl with fluctuations of values from 20.53 to 47.0 mg/dl. Synthesis of urea ranges from 40 to 70% depending on CP uptake in TMR, the ratio of carbohydrate to protein in TMR (Recktenwald, 2010). 30 - 60% of the urea formed is recycled to the rumen and converted to NH_3 to promote microbial protein synthesis (Van Amburgh et al., 2010), allowing rumen microorganisms to overcome asynchronous nutrient supply (e.g., CP <12%). The amount of recycled urea is not considered when

formulating the feed ration, which results in an excessive content of CP in the feed ration of dairy cows (Reynolds and Kristensen, 2008).

Table 2. Metabolism and transformation of protein according to CP content in TMR

	CP<150g/kg	CP 150-170g/kg	CP >170g/kg
Daily ration			
Crude protein g /kg DM	139.0±11.8^{a,b}	161.8±6.3^{a}	179.7±8.5^{b}
Starch g/kg DM	217.6±14.2	242.1±44.3	230.8±22.9
NDF g/kg DM	394.8±20.5	343.1±37.9	341.1±28.0
Rumen			
NH3 mg/100ml	17.4±1.7	19.6±2.3	22.8±3.4
NH_3-N mg/100ml	14.3±1.4	16.1±1.9	18.7±2.8
pH	6.47±0.2	6.55±0.4	6.39±0.4
Σ VFA mmol/l	118.7±20.9	118.5±19.6	129.9±23.1
Blood			
Total proteins g/l	80.5±5.1	80.9±2.0	74.8±2.0
Urea mg/dl	22.8±3.7	27.8±4.8	20.5±6.6
BUN mg/dl	10.7±1.7	13.0±2.3	9.6±3.1
AST μkat/l	1.69±0.2	1.68±0.2	1.59±0.1
Production and composition of milk			
Milk yield kg/d	29.7±5.9	37.9±7.8	33.2±4.7
Milk protein %	3.21±0.2	3.04±0.2	3.22±0.2
Milk urea mg/dl	18.25±2.6^{c}	24.8±3.9	33.3±6.7^{c}
Milk urea nitrogen mg/dl	8.52±1.2^{d}	11.57±1.82	15.54±3.12^{d}
EUN %	29.8±1.5	31.1±1.4	29.0±1.6
Environmental load			
UN g N/day	139.4±19.8^{e,f}	189.5±30.4^{e}	254.7±51.3^{f}
Emission of NH_3 g/day	68.1±6.1^{g,h}	83.7±9.4^{g}	103.8±15.9^{h}

b,c,d,f,h: $P < 0.001$; *a,e,g:* $P < 0.01$

Significant relationships were observed in the monitored dairy farms divided according to content of CP (Table 2), where not only the markers NH_3-N and serum urea (blood urea nitrogen -BUN) were evaluated, but also other indicators for a more accurate evaluation of protein transformation. An important indicator in this evaluation in the monitoring farms was the concentration of urea in milk resp. urea nitrogen in milk (MUN), which is also affected by the content and

intake of proteins from the feed ration. Based on a positive correlation between serum urea and milk urea (Broderick and Clayton 1997), it is used as a biomarker of efficiency of N utilization for milk production (Huhtanen et al., 2015), but also to assess physiological dependences between intake and excretion of N in urine (Nousiainen et al., 2004, Burgos et al., 2007) and for the emission of ammonia on dairy farms (Van Duinkerken et al., 2011). To optimize protein nutrition, the amount of CP in TMR is required that, on the one hand, prevents production losses at low intake of CP and, on the other hand, does not lead to metabolic load and N loss of N in urine when the content of CP in TMR increases. In farms with a low content of CP (<150 g/kg DM of TMR), was analyzed the content of CP in the average 139.0 ± 11.8 g/kg DM with an average NH_3-N content of 14.3 ± 1.4 mg/100 ml, the concentration of blood urea nitrogen (BUN) 10.7 ± 1.7 mg/dl and the concentration of milk urea nitrogen (MUN) 8.52 ± 1.2 mg/dl. The efficiency of N utilization (EUN) in these farms was found on average 29.8 ± 1.5% (28.2 - 31.2 %), and with lower CP content in TMR, also lower excretion of N in urine (139.4 ± 19.8 g N/day) and lower emission of ammonia (68.1 ± 6.8 g /day). In farms with a high content of CP at a level >170 g/kg DM was found higher content of NH_3-N, BUN and MUN, but also higher excretion of N in urine (254.7 ± 51.3 g N/day) and higher emission of ammonia (103.8 ± 15.9 g /day). The level of excreted N in the urine was directly related to the increase in the content of CP in the feed ration in the monitored farms. High intake of N causes the accumulation of NH_3 in the rumen and increases its absorption with higher production of urea in the liver (Reynolds and Kristensen 2008), which increases the content of blood urea and milk urea and is directly related to increased excretion of N in urine and

reduces utilization of N to milk protein (Recktenwald et al., 2014). This process is directly affected by the content of CP and the ratio of starch to CP and less by the content of starch in the TMR. The emission of ammonia is dependent on the excretion of urea nitrogen in the urine (UUN). Excreted N in urine is higher in the form of UUN, which upon contact with urease is rapidly converted to ammonium and gaseous ammonia (Burgos et al., 2007), which is a potential source of environmental and water contamination.

Inefficient utilization of N per milk protein is the reason why dairy cows in agriculture are considered to be a major environmental problem and the largest source of ammonia emissions (Powell et al., 2014). The low efficiency of N utilization is directly related to the high content of CP in the feed ration (Aguilar et al., 2012). Correction of the daily intake of N reduces the levels of excreted N in the urine and leads to improved efficiency of utilization of N per milk protein and optimal concentration of urea in milk. Optimal values of milk urea in the range from 17 to 26 mg/dl resp. MUN 7.9 -12.1 mg/dl (Ishler, 2017), confirm the optimal level of ammonia for the synthesis of microbial proteins and the efficient transformation of N to milk protein, where the amount of excreted urea in the urine does not cause an environmental load.

These values are characteristic for the protein content in the feed ration at the level of approximately 16 %, which is confirmed by the results from the monitored farms in our conditions with the content of CP from 150-170 g/kg DM of TMR with the highest EUN on average 31.1 ± 1.4 % (28.6 - 33.4 %), with a MUN concentration of 11.57 ± 1.82 and a BUN concentration of 13.0 ± 2.3 mg/dl. The content of CP in this range is in our conditions depending on the soil-climatic conditions, the production of protein forages, and their inclusion in the TMR. In TMR

with lower content of CP compared to higher CP content, Colmenero and Broderick (2006) did not observe a difference in milk and production of milk protein, but a significantly lower excretion of N in urine, which is comparable to our evaluation.

CONCLUSION

Monitoring of urea levels in milk and their evaluation for the production group in relation to the nutrients content of TMR and dry matter intake allows an objective evaluation of the protein nutrition of dairy cows. Change in content of milk urea is a risk factor for metabolic and reproductive disorders, and it is useful in evaluating herd health programs and ecological burden, by the evaluation of the efficiency of nitrogen utilization and excretion N in the urine.

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THE EFFECT OF GRAPE POMACE INTAKE ON BLOOD OF DOGS

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ABSTRACT

Previous study shows negative effect of grapes or raisin feeding on renal function of dogs, which was followed with other clinical signs and changes in blood parameters. Therefore, the aim of this study was to determine the effect of dry pomace feeding on selected parameters of dog blood. Three bitches were fed either with the control diet or diet supplemented with 1% of dried grape pomace (on dry matter basis). Selected blood parameters were analysed. After intake of diet with dried grape pomace the lymphocytosis was detected. In addition, intake of diet with dried grape pomace increased the white and red blood cells count, haemoglobin and glucose concentration and decreased platelet count, compared to feeding with the control diet. Creatinine content was high after feeding with booth diets (the control and grape pomace). According to these results, it can be concluded, that the feeding with dried grape pomace in an amount of 1% of diet dry matted is from health point of view questionable and further research is needed.

Keywords: dog, diet, grape pomace, blood cells, blood biochemistry.

INTRODUCTION

In recent years, interest has grown in the antioxidant and antimicrobial properties of a number of polyphenols found in different plants. The by-products of the wine industry (grape pomace, skin and seeds) and wine polyphenol extracts contain a wide range of bioactive compounds (Brenes et al., 2016). Gálik et al. (2018) characterised grape by-products as a source of many potential bioactive compounds, which have a potential to be positive in federation for nutrient utilisation increasing point of view. In addition, Brindza et al. (2015) concluded, that plant antioxidants are beneficial for improving health. However, studies of Mazzaferro et al. (2004), Morrow et al. (2005) and Eubig et al. (2005) reported acute renal failure in dog after consumption of grapes, raisin or both. Vomiting, diarrhoea, lethargy and anuria were common clinical signs. On the other hand, Martineau et al. (2016) concluded, that long-term consumption of a pet specific blend of a polyphenol-rich extract from grape and blueberry (PEGB; from the Neurophenols Consortium), was not associated with renal or hepatic injury. They considered this extract as safe. Nowadays a research project aimed on by-products from grape processing as a bioactive substances source in animal nutrition is solved on the Department of Animal Nutrition (SUA in Nitra). Therefore, we aimed to determine the effect of dried grape pomace ingestion by dogs on selected blood parameters.

MATERIAL AND METHODS

This study included three bitches of American Staffordshire terrier breed at the age of 2.67 year and weight 25.4 kg on average. Bitches were housed as companion dogs. Bitches were fed twice a day, 50% of daily diet at morning and 50% at evening. As feed a commercial complete feed mixture was used in calculated amount, daily diet meets all bitches' nutritional requirements. Whole experiment contained 6 time periods (weeks), from which first two weeks were as preparatory periods and last four weeks were experimental periods. Blood samples were collected at the end of third, fourth, fifth and sixth week, always morning before feeding change to other diet. Feeding scheme and nutritional characteristic of commercial feed mixture and dried grape pomace is shown in Table 1. Blood samples were collected from *vena cephalica antebrachii* by the veterinarian. For blood cells count analysis an uncoated blood samples were taken in to EDTA K3 tube (Sarstedt, Germany). Parameters like total white blood cell x 10^9/l (WBC), lymphocytes count x 10^9/l (LYM), medium size cells count x 10^9/l (MID), granulocytes count x 10^9/l (GRA), red blood cell count x 10^{12}/l (RBC), haemoglobin g/l (HGB), haematocrit % (HCT), mean corpuscular haemoglobin pg (MCH), platelet count x 10^9/l (PLT), platelet percentage % (PCT) were analysed using haematological analyser Abacus Junior Vet (Diatron, Austria). For blood biochemistry analysis, blood samples were taken into tube without anticoagulant and after coagulation were centrifuged 10 minutes (3000 rpm). Gained blood serums were analysed for lipase (µkat/l), glucose (mmol/l), cholesterol (mmol/l), total protein (g/l), albumin (g/l), creatinine (µmol/l), calcium (mmol/l) and phosphorus (mmol/l) content in a specialised laboratory (Alphamedical, Slovakia). Gained results were

statistically processed in IBM SPSS v 20.0, a descriptive statistic for each experimental week as well as for average of 3rd and 5th week, and average of 4th and 6th week was calculated. Significance between weeks was tested with Tukey HSD test, a $P<0.05$ was considered significant.

Table 1. Nutritional characteristic of feeds and feeding scheme

	Commercial complete dry feed fixture for dogs	Dried grape pomace	Diet labeling
Diet dry matter composition according to week of experiment			
1st week preparatory	100%	-	
2nd week preparatory	99%	1%	
3rd week experimental	100%	-	C diet
4th week experimental	99%	1%	GP diet
5th week experimental	100%	-	C diet
6th week experimental	99%	1%	GP diet
Nutritional characteristic (% of feed dry matter)			
Dry matter	92.8	94.2	
Crude protein	18.6	9.9	
Ether extract	9.7	8.4	
Crude fibre	3.3	18.4	
Ash	7.0	4.0	
Nitrogen free extract	61.4	59.3	

RESULTS AND DISCUSSION

Results of detected blood cells count parameters, as well as of selected biochemical parameters are shown in Table 2. The increase of WBC ($P<0.05$) and LYM ($P<0.05$), as well as slight insignificant increase of RBC and HGB was detected between consumption of C diet and GP diet on average. Closer look at development of WBC, LYM, RBC and HGB from 3rd to 6th week revealed similar trend: increase between 3rd and 4th week, followed by decrease between 4th and 5th week and then once again increase between 5th and 6th week.

Table 2. Blood indices of dogs according to group and sampling time

Mean ±S.D.	Average weeks with		Third week	Fourth week	Fifth week	Sixth week
	C diet	GP diet	C diet	GP diet	C diet	GP diet
Weight	26.2^{bc} ±0.45	25.8^{ab} ±0.39	26.6^{c} ±0.10	25.4^{a} ±0.10	25.8^{ab} ±0.10	26.1^{abc} ±0.10
WBC	9.26^{ab} ±0.50	12.0^{cd} ±1.24	8.86^{a} ±0.35	11.0^{bc} ±0.58	9.65^{ab} ±0.21	13.1^{d} ±0.48
LYM	3.56^{ab} ±0.59	5.82^{c} ±0.37	3.03^{a} ±0.17	6.05^{c} ±0.34	4.09^{b} ±0.06	5.58^{c} ±0.25
MID	0.56 ±0.19	0.43 ±0.07	0.68 ±0.20	0.45 ±0.03	0.44 ±0.11	0.41 ±0.10
GRA	5.13^{ab} ±0.19	5.78^{ab} ±1.52	5.15^{ab} ±0.07	4.50^{a} ±0.30	5.11^{ab} ±0.30	7.06^{b} ±0.87
RBC	8.78^{ab} ±0.27	8.94^{ab} ±0.19	9.02^{a} ±0.09	9.09^{a} ±0.08	8.54^{b} ±0.11	8.80^{ab} ±0.16
HGB	201^{ab} ±7.00	206^{a} ±2.65	207^{a} ±3.51	209^{a} ±0.58	195^{b} ±1.53	204^{ab} ±1.00
HCT	58.9^{ab} ±1.75	59.9^{ab} ±0.95	60.4^{a} ±0.68	60.4^{a} ±0.43	57.4^{b} ±0.85	59.4^{ab} ±0.99
MCH	22.9 ±0.23	23.1 ±0.34	23.0 ±0.31	23.0 ±0.21	22.9 ±0.17	23.2 ±0.46
PLT	423 ±7.83	407 ±19.06	424 ±7.51	409 ±5.13	422 ±9.64	405 ±29.46
PCT	0.45 ±0.02	0.46 ±0.04	0.45 ±0.01	0.46 ±0.03	0.45 ±0.03	0.46 ±0.05
CB	67.5^{ab} ±1.88	67.4^{ab} ±4.86	68.9^{ab} ±1.65	71.9^{a} ±0.10	66.1^{ab} ±0.10	63.0^{b} ±0.10
ALBU	37.2^{ab} ±1.72	36.2^{ab} ±2.18	38.7^{a} ±0.10	38.2^{a} ±0.10	35.6^{ab} ±0.10	34.2^{b} ±0.10
KREA	129^{ab} ±1.50	121^{bc} ±6.85	128^{ab} ±1.00	127^{ab} ±1.00	130^{a} ±1.00	114^{c} ±1.00
GLUK	5.38^{dc} ±0.05	5.50^{a} ±0.02	5.42^{bc} ±0.01	5.51^{a} ±0.01	5.33^{d} ±0.01	5.48^{ab} ±0.01
CHOL	7.24^{a} ±0.07	7.15^{ab} ±0.21	7.30^{a} ±0.01	7.34^{a} ±0.01	7.18^{ab} ±0.01	6.95^{b} ±0.01
Lipase	0.43^{ab} ±0.04	0.41^{a} ±0.02	0.47^{b} ±0.01	0.42^{ab} ±0.01	0.39^{a} ±0.01	0.39^{a} ±0.01
Ca	2.53^{ab} ±0.04	2.55^{ab} ±0.07	2.56^{ab} ±0.01	2.61^{a} ±0.01	2.49^{b} ±0.01	2.48^{b} ±0.01
P	1.36^{ab} ±0.13	1.29^{ab} ±0.09	1.48^{a} ±0.01	1.20^{b} ±0.01	1.24^{b} ±0.01	1.37^{ab} ±0.01

^{abcd} – means within the row bearing different superscript are significant different at $P<0.05$.

The same results showed also glucose concentration, with higher concentration after weeks with intake of diet supplemented with dried grape pomace ($P<0.05$). Opposite progress revealed platelet count, weeks with intake of diet supplemented with dried grape pomace had lower values, compared to control diet (weeks 3 and 5). We cannot say that all detected blood parameters were in reference interval for dogs. LYM count in blood of dogs after GP diet intake was in both experimental weeks (4^{th} and 6^{th} week) higher than upper value of reference interval published by Harvey (2012) 3.4 x 10^9, and by manufacturer of haematological analyser Abacus Junior Vet, 4.8 x 10^9. Increase of LYM within reference interval can be considered as immune system stimulation, however, increase above reference interval not. Similar conclusions can be assigned also to effect of grape pomace on RBC count and HGB concentration. RBC and HGB were after feeding with C diet on the upper value of reference interval, and the feeding with GP diet even raised both parameters above reference interval. Other parameters of blood cells count, as well as biochemical parameters were within published reference intervals for dog (Slanina et al., 1991; Harvey, 2012; Laboklin, 2020). Eubig et al. (2005) published a retrospective evaluation of 43 dogs with clinical or health problems after the ingestion of grapes or raisins. They found these findings: decreased urine input, ataxia, weakness. In general hypercalcemia and hyperphosphatemia were presented in 90% and 62% of dogs respectively. None of these findings were detected in this study (Table 2.). Also Morrow et al. (2005) detected canine renal pathology associated with grape or raisin ingestion. In that study, dogs were exposed to different amount of raisin (3 to 30 g/kg of feed dry matter) and grapes (4 g/kg of feed dry matter to unknown amount). Dogs in

study of Morrow et al. (2005) have detected creatinine in interval from 189 to 982 µmol/l, which indicate serious failure in renal function. Creatinine concentration detected in this study is in interval from 114 to 130 µmol/l, which is above upper limit of physiological optimum (35 to 106 µmol/l) of dogs published in Laboklin (2020). Results of this study suggest that feeding of dogs with dried grape pomace is not so dangerous like feeding with grapes and raisin as concluded Eubig et al. (2005) and Morrow et al. (2005). Nevertheless, also the dose of 1% of dried grape pomace in the diet increased some blood parameters above the physiological range. On the grounds of differences published in articles Eubig et al. (2005); Morrow et al. (2005) and results of this study it can be hypothesized that the physical form (grapes, dried grape pomace, raisin) plays very important role in manifestation of clinical signs and blood parameters. At any rate, the feeding of dogs with grape-by products (dried grape pomace) is questionable and further research in this field is necessary.

CONCLUSION

Feeding of dogs with dried grape pomace in an amount of 1% of diet dry matter is not so dangerous like feeding of grapes or raisins. Nevertheless, intake diet with 1% of dried grape pomace significantly increased white blood cells and lymphocytes count and glucose concentration. Lymphocytes count was in experimental group fed with GP diet always above physiological range. Red blood cells count and haemoglobin concentration was after feed with control diet high and the feeding with GP diet even increased these values over physiological range. Intake of GP diet decreased the thrombocyte count, compared to control diet. Creatinine concentration was in all samples above the

physiological range, but was less than compared to dogs with renal pathology associated with grape or raisin intake. According to these results can be concluded, that physiological form of grapes fed to dogs plays important role. The feeding with dried grape pomace in an amount of 1% of diet dry matter is questionable and further research in this area is needed.

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THE FULL FAT MEALWORMS MEAL AS A BROILER CHICKEN´S FEED

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ABSTRACT

The aim of this study was to evaluate whether mealworms meal is appropriate feed for fast growing chickens. The experiment was carried out with 96 Ross 308 male chickens. The trial was performed from day 12 to day 38 of chick's age. Broilers were divided into three equal groups. The two experimental groups received feed mixtures containing 2% (MM2; n = 32) and 5% (MM5; n = 32) of yellow mealworms (*Tenebrio molitor* L.) meal (MM). The third group (MM0; n = 32) was a control group which had 0% of mealworms meal in diet. The broilers from mealworms meal free group achieved higher live weight at the end of trial, higher feed consumption and higher breast meat yield compared to groups with 2% and 5% mealworms in diet. From the chicken growth point of view the inclusion of mealworms meal does not seem to have a positive effect on their growth.

Keywords: *Tenebrio molitor*; poultry nutrition, carcass yield, blood biochemistry

INTRODUCTION

To produce 1 kg of animal products, 2-15 kg of plant material is required, depending on the species and breeding conditions. Generally, 40-50% of the global cereal harvest is used for feed production (Profetas, 2018). The cost of conventional feed sources (such as soybean meal and fishmeal) is very high and, in addition, their availability appears to be limited in the future. By 2050, an increase in the consumption of animal products by 60 to 70% is expected, correlating with the increase in the human population, which is estimated to increase to 9 billion people. This increase in food consumption will require huge resources, with feed being the most demanding in terms of limited availability of natural resources, continued climate change and food-feed-fuel competition (Makkar *et al*., 2014).

Tenebrio molitor L. larvae (mealworms) it´s easy to breed, feed and it have a valuable protein profile. The crude fat content is approximately 31-43% on dry matter basis (Makkar *et al*., 2014; Ghaly and Alkoaik, 2009).

The aim of this study was to evaluate whether mealworms meal is appropriate feed for fast growing chickens.

MATERIAL AND METHODS

The dried yellow mealworms used in our experiment was obtained from Underground food (Czech Republic). The experiment was carried out with 96 Ross 308 male chickens. Broilers were fed with starter feed

mixture until 10 days of age. From the tenth to twelfth day of the chickens a preparatory period was carried out. Chickens were fed with experimental and control feed mixtures in this period. The trial was performed from day 12 to day 38 of chick's age. Broilers were divided by body mass into three equal groups with 4 replicates per treatment, i.e. there were 8 animals per replicate pen. The two experimental groups received feed mixtures containing 2% (MM2; n = 32) and 5% (MM5; n = 32) of yellow mealworms (*Tenebrio molitor* L.) meal (MM). The third group (MM0; n = 32) was a control group which had 0% of mealworms meal in diet. Mealworms meal replaced the appropriate proportion of soybean extracted meal. Table 1 shows composition and proximate analyses of used diets. The rations were calculated according to the Broiler nutrition specifications (Aviagen group, 2019) as an isonitrogenous and isocaloric. The mash form diets were offered to broilers. The chickens had free access to feed and water. The chemical composition of nutrient content of diets were determined for dry matter, crude protein, ether extract, crude fibre, and ash according to (Commision regulation (EC) 152, 2009). The conventional deep litter system with wood shavings were used. Room temperature, humidity and lighting regime were controlled according to requirement for actual age of chickens (Aviagen group, 2018). Health status was evaluated daily and live weight measured every week during the trial.

Table 1. Nutrient and chemical composition of diets

Components (g/kg)	**MM0**	**MM2**	**MM5**
Soybean meal	335.4	311.6	275.4
Wheat	287.0	289.0	291.4
Maize	271.4	277.9	289.9
Rapessed oil	50.0	45.5	37.6
Premix*	30.0	30.0	30.0
Yellow mealworms	0.0	20.0	50.0
L-Lysine	8.1	8.1	8.1
Monocalcium phosphate	7.2	6.6	5.9
L-Threonine	5.0	5.0	5.0
DL-Methionine	3.6	3.5	3.5
$CaCO_3$	2.4	2.8	3.2
Chemical analyses of diets (in dry matter)			
AME_N ($MJ \cdot kg^{-1}$)	12.65	12.66	12.65
Crude Protein (%)	20.89	20.71	21.61
Ether extract (%)	6.48	6.50	6.72
Crude fibre (%)	2.77	3.12	2.80
Ash (%)	6.23	5.63	5.61

*Premix contains (per kg): lysine 70 g; methionine 75 g; threonine 31 g; calcium 190 g; phosphorus 33 g; sodium 46 g; copper 500 mg; iron 2,500 mg; zinc 3,300 mg; manganese 3,300 mg; iodine 34.20 mg; selenium 12 mg; tocopherol 1,500 mg; calciferol 165,000 international units (IU); phylloquinone 44 mg; thiamine 135 mg; riboflavin 280 mg; pyridoxine 200 mg; cobalamin 960 mg; biotin 6 mg; niacinamid 1,200 mg; folic acid 55 mg, calcium pantothenate 445 mg; choline chloride 6,000 mg; butylhydroxyanisol 41 mg; butylhydroxytoluen 182 mg.
MM0 – 0% mealworms meal; MM2 – 2% mealworms meal; MM5 – 5% mealworms meal.

At the end of the experiment six birds were selected randomly from each group, weighed and slaughtered. Feathers were removed and chickens were eviscerated. Carcass yield was calculated. Breast and thigh meats without skin were separated from carcasses after cooling. All visible external fat was removed from sample meats. The breast and thigh meat were weighed, and their percentage of carcass weight was calculated.

Blood was collected into heparinized tubes at slaughter. It was centrifuged for 10 minutes at 3,000 rpm till 2 hours after collection. The separated blood plasma was frozen (-20 °C) until biochemical examination. The following parameters were determined using standardized biochemical methods using Erba Lachema (Czech Republic) commercial sets on the Ellipse automatic biochemical analyzer (AMS Spa, Italy) in blood plasma samples: Enzymes activity AST – aspartate aminotransferase (AST/GOT 500), GGT – gamma-glutamiltranserase (GGT 250), ALT – alanine aminotransferase (ALT/GPT 500), ALP – alkaline phosphate (ALP AMP 500), LD – lactate dehydrogenase (LDH-L 100), triglycerides (TG; TG 250), cholesterol (CHOL 250), creatine kinase (CK – 100, no. 10004494 Erba Lachema, Czech Republic), uric acid (UA; UA 500, no. 10010225 Erba Lachema, Czech Republic), TP – total protein (TP 500).

The data were processed by Microsoft Excel (USA) and StatSoft Statistica version 12.0 (USA). A one-way analysis of variance (ANOVA) was used to determine the differences between groups. To ensure evidential differences, Scheffé´s test was applied and $P < 0.05$ was regarded as a statistically significant difference.

RESULTS AND DISCUSSION

The used dried mealworms meal had 53.25% crude protein, 29.35% ether extract, 6.21% crude fibre and 3.90% ash. In experiment (Ramos-Elorduy *et al*., 2002) mealworms grew on low-nutritive waste products and fed these to broiler chickens. The mealworms were able to transform the low nutritive waste products to a high protein diet.

Broilers from control group had higher ($P < 0.05$) live weight compared to MM2 group from 31st day of age. This trend preserved to the end of the trial. See Table 2. On the other hand, Bovera *et al*. (2016) do not found significant influence on growth performance parameters and carcass traits and chemical and physical properties of broilers meat.

Table 2. Broilers live weight (mean /g/ ± standard error)

	MM0	**MM2**	**MM5**
n	31	29	29
12th day	266 ± 4.81	269 ± 2.82	265 ± 2.85
17th day	525 ± 9.68	531 ± 6.29	533 ± 5.19
24th day	1,064 ± 19.17	992 ± 27.77	1,059 ± 17.64
31st day	1,710 ± 35.46^{b}	1,472 ± 72.24^{a}	1,613 ± 43.28^{ab}
38th day	2,379 ± 61.70^{b}	2,052 ± 115.00^{a}	2,222 ± 85.56^{ab}

MM0: 0% mealworms meal; MM2: 2% mealworms meal; MM5: 5% mealworms meal. n – number of cases.
$^{a:b}$ – different letters in one line means statistically significant differences $P < 0.05$.

Feed intake was higher in control group of chickens compared to the MM2 group. Higher feed intake correlates with higher live weight of chickens in control group. Feed conversion ratio was without differences among groups.

Table 3. Average feed intake (g) and average feed conversion ratio per trial (mean ± standard error)

	n	Feed intake	Feed conversion ratio
MM0	4	3,444 ± 98.85[b]	1.45 ± 0.03
MM2	4	3,004 ± 105.98[a]	1.46 ± 0.03
MM5	4	3,195 ± 92.28 [ab]	1.46 ± 0.08

MM0: 0% mealworms meal; MM2: 2% mealworms meal; MM5: 5% mealworms meal. n –number of cases (number of repetitions of one group).
[a:b] – different letters in one row means statistically significant differences $P < 0.05$.

The carcass yield and thigh meat yield (Table 4) were without changes among all three experimental groups. Chickens from control group had higher ($P < 0.05$) breast meat yield compared to MM5 group. Carcass composition of Ross 308 broilers was not influenced with the addition of mealworm meal (in doses 0%, 2%, 4%, 8% and 10.48%) in diets, which indicated this feed did not disturb the carcass yield characteristics (Elahi *et al*., 2020).

Table 4. Mean carcass yield (mean ± standard error)

Skupina	n	Live weight (g)	Carcass yield (%)	Breast meat from carcass (%)	Thigh meat from carcass (%)
MM0	6	2,830 ± 99	73.68 ± 0.77	31.98 ± 0.67 [b]	21.74 ± 0.37
MM2	6	2,515 ± 102	71.42 ± 0.66	30.90 ± 1.27 [ab]	21.78 ± 0.44
MM5	6	2,516 ± 85	72.00 ± 0.66	27.05 ± 1.28 [a]	22.76 ± 0.45

MM0: 0% mealworms meal; MM2: 2% mealworms meal; MM5: 5% mealworms meal.
[a:b] – different letters in one row means statistically significant differences $P < 0.05$.
n – number of cases.

The blood biochemical examination does not prove any statistically significant differences between control and experimental groups. Our results are in agreements with Elahi *et al*. (2020) who did not found

differences among groups in hematological characteristics of broiler chickens fed on the mealworm meal diets.

Table 5. Blood biochemical parameters (mean ± standard error)

	MM0	MM2	MM5
n	10	10	10
ALT (µkat/l)	0.27 ± 0.03	0.39 ± 0.04	0.27 ± 0.03
AST (µkat/l)	3.27 ± 0.47	3.53 ± 0.29	3.50 ± 0.47
GGT (µkat/l)	0.36 ± 0.02	0.31 ± 0.02	0.36 ± 0.03
ALP (µkat/l)	26.62 ± 1.47	24.28 ± 2.13	22.58 ± 2.21
LD (µkat/l)	75.54 ± 8.20	67.81 ± 6.27	61.53 ± 6.80
CK (µkat/l)	599.76 ± 79.87	519.01 ± 39.14	503.05 ± 75.88
UA (µmol/l)	398.93 ± 44.19	331.48 ± 29.67	320.20 ± 29.02
TP (g/l)	31.79 ± 0.74	32.09 ± 1.06	34.42 ± 1.56
Chol (mmol/l)	3.51 ± 0.20	3.76 ± 0.14	3.34 ± 0.11
TG (mmol/l)	0.37 ± 0.02	0.48 ± 0.05	0.40 ± 0.07

MM0: 0% mealworms meal; MM2: 2% mealworms meal; MM5: 5% mealworms meal. $P > 0.05$.

ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; GGT – Gamma-glutamyltransferase; ALP – Alkaline phosphatase; LD – Lactate dehydrogenase; CK – Creatine kinase; UA – uric acid; TP – Total protein; Chol – cholesterol; TG – Triglycerides.

CONCLUSION

The broilers from mealworms meal free group achieved higher live weight at the end of trial, higher feed consumption and higher breast meat yield compared to groups with 2% and 5% mealworms in diet. From the chicken growth point of view the inclusion of mealworms meal does not seem to have a positive effect on their growth.

So, a few more studies are needed to prove or disprove suitability of mealworms meal as a broilers feed. In further studies, it would be useful to focus more deeply on the metabolism, gut microbiome and broiler meat quality.

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Animal Nutrition PhD. Science

EFFECT OF ZINC PHOSPHATE-BASED NANOPARTICLES ON CULTURABLE BACTERIA IN PIG

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ABSTRACT

High doses of zinc oxide (ZnO) added to the diet for piglets for the prevention of diarrhoea can increase the growth rate and contaminate the farms and the surrounding environment. Therefore, there is a need to find a replacement of dietary ZnO with an equally effective alternative. In this study, we compared the effect of two formulations of zinc phosphate-based nanoparticles (ZnA and ZnC NPs) on growth performance. A total of 100 weaned piglets were divided into 10 equal groups with the base diet (control) or the base diet supplemented with ZnA, ZnC, or ZnO at concentrations 500, 1000, and 2000 mg Zn per kilogram of diet. Supplements were given to animals for 10 days. Fecal samples were collected on day 0, 5, 10 and 20. At the end of the treatment (day 10). Comparing to that of control, the significantly higher piglet weight gain was observed in all piglet groups fed with

ZnA ($P < 0.05$). Differences in the total aerobic bacteria and coliform counts in piglet feces after NPs supplementation compared to that of control and ZnO groups were also found ($P < 0.05$). The majority of aerobic culturable bacteria from the feces represented Escherichia (28.57–47.62%), Enterococcus (3.85–35.71%), and Streptococcus (3.70–42.31%) spp.

Keywords: microbiome; pig weaning; diet.

INTRODUCTION

Zinc is an essential trace element for animals as it plays an important role in nutrition, growth, and immunity. Due to its efficiency and a reasonable price, zinc in the form of zinc oxide (ZnO) has been commonly used in high doses (2000 to 3000 mg/kg diet) for weaned piglets as an alternative to antibiotics to prevent intestinal inflammation and increase weight gain (Poulsen et al.,1995 and Hu et al., 2012). However, the European Union will ban feeding zinc in such high concentrations in 2022 (SCVMP, 2017, EMA, 2017). The high zinc doses (2500 mg/kg diet) affect the intestinal microbiota, and there is also evidence for co-selection of the antibiotic resistance traits (Bednorz et al., 2013, Rensing et al, 2018). From our previous study it has been proved that zinc nanoparticles (ZnNPs) can be an alternative to high doses of zinc, in terms of higher pharmacokinetic efficiency, especially when used against coliform bacteria in other mammals as mentioned in our previous study (Horky et al.,). ZnNPs (450 mg/kg diet) significantly reduced the Escherichia coli population in the small and large intestine in weaned piglets. Furthermore, it leads to much lower excretion of zinc in the feces in comparison to that of the same dose of ZnO (Pei et al., 2019). It has been demonstrated that ZnNPs in

pig nutrition can reduce the zinc use up to 60% while maintaining the same positive effect on the intestinal microbiome (Wang et al., 2018). Nevertheless, there are many questions remaining about the safety of nanoparticles and their metabolites for animals and their fate and the effect on the environment.

MATERIAL AND METHODS

Chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA) and Penta (Prague, Czech Republic) of p.a. purity, unless noted otherwise. The pH was measured using inoLab Level 3 (Wissenschaftlich-Technische Werkstatten GmbH; Weilheim, Germany). ZnNPs synthesis The phosphate-based ZnNPs were synthesized according to the procedure published in our previous study [11]. The experiment was performed with the approval of the Ethics Committe at the Faculty of AgriSciences, Mendel University in Brno, Czech Republic in accordance with the Act No. 246/1992 Coll. on the protection of animals against cruelty. Proposed experiment was conducted on an accredited experimental farm of the Research Institute of Animal Production in Prague (Czech Republic). The experiment was carried out on weaned piglets divided into 10 groups with 10 animals in each group. The sex ratio in the group was 50:50 (castrates: females). The first group served as control, where zinc intake was not manipulated in the diet. The second, third, and fourth group were supplemented with zinc in the form of ZnO at a dose of 500, 1000, and 2000 mg of Zn per kilogram of diet. The experiment started on the weaning day of the piglets (day 28 of the animal age), and the Zn feeding lasted for 10 days. Different forms of zinc were mixed into the piglet mixture. The total content of phosphorus in the diet was 6.49

g/kg respectively 130,52mg - calculated by the feed program Agrokonzulta (Czech Republic). The piglets had a feed and water available *ad libitum*. The animal husbandry complied with Decree No. 208/2004 Coll. (Decree on minimum standards for the protection of farm animals). Piglets were weighed again at the end of the experiment on day 20. Quantitative and qualitative analysis of the microbiome in piglet feces was realised.

Fresh fecal samples were collected from individual piglets immediately before the start of the zinc treatment (day 0) and then on day 5, 10 and 20. The fresh feces were sampled into sterile collection tubes, kept on wet ice and processed within 4h. Determination of the total aerobic microorganisms and the total coliforms count was performed. In addition, qualitative identification of the major bacterial groups was conducted. Both analyses were carried out separately for each sample. The fecal samples were homogenized in sterile 0.85% saline (1:9 w/v), and the homogenate was then serially diluted. Subsequently, 1.0 mL of diluted suspension was pour plated on the Plate Count agar (PCA) and MacConkey agar (Sigma-Aldrich) in duplicates. All colonies from PCA and from MacConkey agar were counted after 24 h at 37 °C. The results are expressed as log (CFU/g) of feces. For identification of the major groups of bacteria, the fecal samples were applied on selective agars designed for growth of E. coli and coliforms (HiCrome Chromogenic Coliform HiCynth agar, Himedia, India), streptococci, and enterococci (Columbia blood agar with Streptococcus selective supplement, Oxoid, UK; Mitis Salivarius agar, Sigma-Aldrich). Representative individual colonies were picked from each agar plate and streaked on 5% blood sheep agar and incubated at 37 °C for 24 h. Individual isolates were identified by the

matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) Bruker ultrafleXtreme (Bruker Daltonik GmbH, Bremen, Germany) using MALDI BioTyper™ Compass Explorer 4.1.90 analysis software equipped with MBT 8468 MPS library. Bacterial isolates were then stored at − 80 °C for further analysis.

In each Zn treatment group, analysis of variance (ANOVA) test was used to detect significant differences in microbial growth based on Zn concentrations. Pair- wise comparisons based on the weighted average of "Studentized" ranges were done using the Dunnett's C post hoc test for unequal variances. The paired T-test was used to study significant differences in microbial growth between time periods and day zero.

RESULTS AND DISCUSSION

The diversity of culturable bacteria was assessed from the same fecal samples taken for evaluation of bacterial concentrations. The results were analysed as a relative abundance of cultivable bacterial taxa for every piglet group (Fig. 1). Escherichia sp. were present in all piglet groups at all-time points. Staphylococci were found in piglet feces from all ZnO treatment groups. The ZnA group also showed a higher prevalence of Streptococcus sp. and Lactobacillus sp. Also, Enterococcus, Aerococcus and Corynebacterium taxa were observed in most of the groups and time points to some extent.

On the day 0, the piglets were weaned and fed with diet containing ZnO and ZnNPs. At that time, the majority of bacteria belonged to Escherichia (28.57–47.62%), Entero-coccus (3.85–

35.71%), Streptococcus (3.70–42.31%), Lacto-bacillus (0–14.71%) and Corynebacterium (0–27.27%) taxa, but Staphylococcus, Aerococcus and Proteus taxa were also present in low abundance. On the 10th day of the experiment Lactobacillus sp. (0–50.00%) increased, moreover, the group with the highest concentration of ZnC also showed Lactococcus sp. (3.85%). High abundance of Staphylococcus sp. (37.50–50.00%) was found in all ZnO concentrations on the 20th day. There were two cases of piglets where Yersinia enterocolitica was found (ZnA 2000 mg/kg, and ZnC 500 mg/kg).

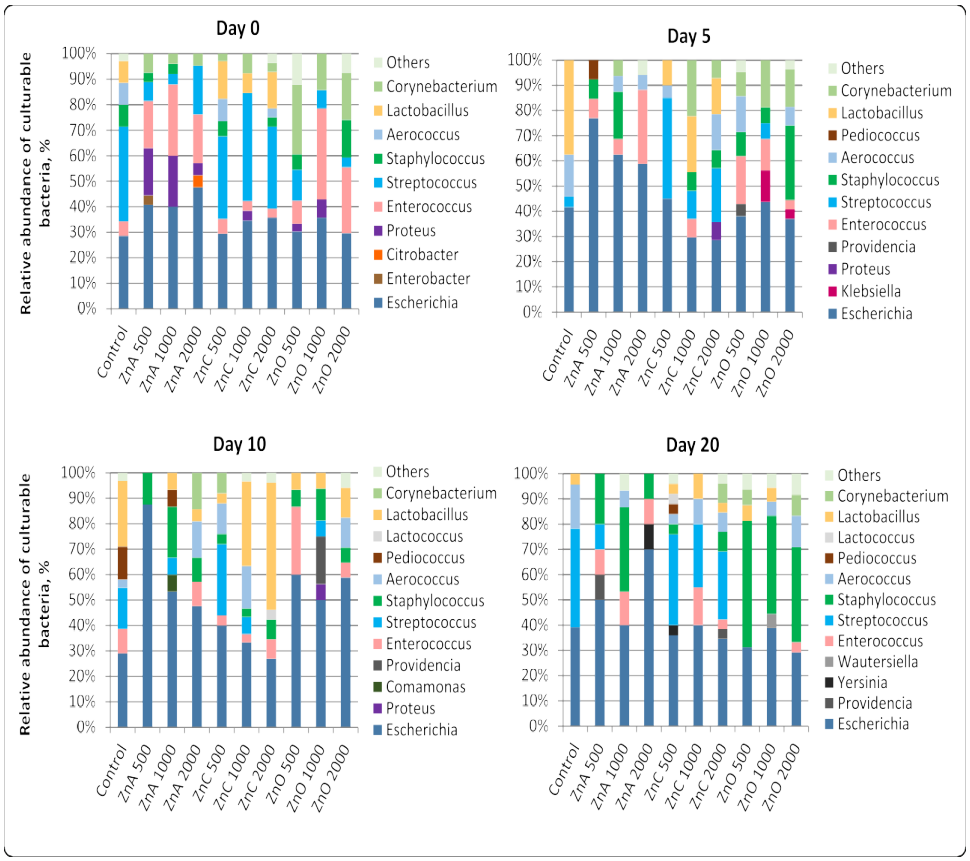

Fig. 1. The occurrence of cultivable bacterial genera in piglets feces (identified by MALDI-TOFMS).

Piglet growth

Piglets had weight between 6.0 and 8.1 kg in different groups at the start of the experiment (Table 1). The control group had a weight gain of 2.0 kg after 20 days. At a dose of 500 mg Zn/kg diet, the highest weight increase was observed in the group of piglets with ZnA NPs (3.7 kg). Piglets fed a Zn dose of 1000 mg/kg diet had the highest weight increase in the ZnA group (3.7 kg). At the dose of Zn 2000 mg/kg diet, the highest weight increase was also observed in the ZnA group (4.4 kg). Overall, significantly higher weight gains ($P < 0.05$) were observed in ZnA piglet groups compared to that of the control group at all Zn concentrations. The increase was 2.5 kg, 2.7 kg, and 3.4 kg for Zn dose 500, 1000, and 2000 mg Zn/kg diet, respectively. The lowest body weight gain was observed in the control group (2.0 kg). In addition to body weight increase, Zn NPs are able to positively affect the feed conversion ratios as result of the study (Zhao et al., 2014). It has been shown that a dose of Zn (as ZnO) of 1200 mg/kg diet is sufficient to improve intestinal integrity and consequently increase the weight gain (Wang et al., 2019). In our study, a significant positive effect of ZnA in all concentrations was observed on the weight of weaned piglets.

Table 1 Piglet weight during the experiment

	Day	**0**	**10**	**20**	**WG**
	Control	6.6±1.3	7.7±1.0	8.6±1.5	2,0
500 mg Zn/kg	ZnO	7.1±0.4	8.6±1.1	9.9±1.1	2,8
	ZnA	7.4±0.9	8.6±1.1	11.1±0.9*	3,7
	ZnC	6.0±0.9	8.0±1.1	9.2±1.1	3,2
1000 mg Zn/kg	ZnO	7.9±1.2	9.0±1.4	11.5±0.9*	3,6
	ZnA	7.6±1.7	7.5±1.3	11.3±1.0*	3,7
	ZnC	6.8±1.8	9.1±1.7	9.8±1.0	3,0
2000 mg/kg Zn/kg	ZnO	7.0±0.9	8.6±1.1	9.9±1.3	2,9
	ZnA	7.6±1.5	8.9±1.5	12.0±1.8*	4,4
	ZnC	8.1±0.9	10.3±0.9*	10.9±1.0	2,8

*indicates significant differences ($p<0.05$) between the control and the treated group

WG-weight gain (difference between average weight on 0 and 20 day)

CONCLUSION

Two formulations of Zn phosphate-based NPs were tested as alternatives to ZnO, which is traditionally used as a dietary supplement in livestock productions. The extensive use of high-dose dietary ZnO may result in a long-term adverse effect on the environment, therefore the application of lower-doses ZnNPs with the same effect on swine

production could be a potential solution. We demonstrate that dietary supplementations with ZnA NPs significantly increased piglet weight gain. ur results indicate that NPs are a promising alternative to high pharmacological doses of conventional ZnO.

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USE OF FEED ADDITIVE IN NUTRITION OF BROILER CHICKENS

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ABSTRACT

The effect of the administered feed additive on animal health, production and some blood parameters was monitored in the experiment on broiler chickens of the Cobb 500 breed. The feed additive (Humac Natur) was added to the complete feed mixture of the experimental group at a dose of 7 g per 1 kilogram. At the end of the experiment, the average live weight of broilers was 2291.7 g in the control group and 2311.9 g in the experimental group. The use of Humac Natur in 0.7% concentrations in the feed ration had an effect on the overall feed conversion ratio, which was 1.51 kg in the control group and 1.65 kg in the experimental group. The serum concentrations of calcium, phosphorus and chlorides in the experimental group were significantly lower compared to the control group ($P < 0.001$). Differences in serum magnesium levels were not statistically significant among groups.

Key words: poultry, humic acids, health, production, minerals

INTRODUCTION

Humic substances are characterized as soil components, occurring in sediments, peat, brown coal, lignite and other natural materials. 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 acid shows anti-inflamatory effects in animal, and improves the immune system. It also has positive effect on live functioning, ultimately reduces mortality and increases growth in poultry (Islam et al., 2005; Vaško et al., 2012). Humic acids inhibit bacterial and fungal growth, thus decrease levels of mycotoxins in feed. Some studies investigated the effect of using humic acid as growth promoter in poultry and obtained positive results (Sahin et al., 2016; Arafat et al., 2017). It could enhance the bird immunity and reduce various kinds of stress (Cetin et al., 2011; Nagaraju et al., 2014; Salah et al., 2015; Mudroňová et al., 2018; Mudroňová et al., 2020). At present, humic substances have a relatively wide range of uses in agriculture, various industries, environmental protection and pharmacology. We observed the effect of humic substances on the health, production and selected serum mineral parameters in the experiment with the broiler chickens.

MATERIAL AND METHODS

Sixty pieces of broiler chickens hybrid COBB 500 with an average weight of 50 g/chick were included in the experiment. The animals were housed in the experimental premises of the breeding establishment, under the conditions laid down for the fattening of broiler chickens. At the beginning in the experimental pens, the temperature was 30.9 ºC with a gradual decrease to 21 ºC on day 23

and subsequently this temperature was maintained until the end of the experiment. The average relative humidity was 60.93 %. The chickens were equally divided into two groups of 30. The control group (C) was fed a commercial complete feed mixture. The broilers were fed with commercial feed mixture HYD 01 - starter diet for fattening broilers within 10 days of age, HYD 02 - diet for next 14 days growing period and HYD 03 - final fed mixture until slaughter. The experimental group (E) of broiler chickens was fed in the same way, but in this group Humac Natur was added to the feed mixture at a dose of 7 g per 1 kilogram. The characteristic of the applied supplement was the following: content of humic acids min. 65% in dry matter (DM); fulvic acids min. 5% in DM; complex of minerals and microelements. The diets used in this experiment were formulated to meet Zelenka et al. (2007). During fattening, chickens were allowed access to feed and water *ad libitum*. During the experiment, mortality was monitored; chickens and consumed feed mixture were weighed at weekly intervals; live weight, feed consumption, average daily gain and feed conversion ratio were calculated. Mineral elements were determined in the blood of brooded broilers at the end of the experiment. Serum concentrations of calcium (Ca), phosphorus (P), magnesium (Mg) and chlorides were determined were determined using a fully automatic random access benchtop analyser Ellipse (Italy).

Comparison of the observed parameter values between groups was done using the t-test in Prism Free Trial software (GraphPad Software, USA).

RESULTS AND DISCUSSION

The effect of humic acids on production parameters and health was studied in an experiment on broiler chickens of the Cobb 500 breed. In the control group, the death of 1 chicken was registered in the second week of experimental period and one chicken was culled for significantly lagging behind in growth. No deaths were registered in the experimental group, but in the second and fourth weeks after weighing, 2 chickens were eliminated for a significant lag in growth. From individual measurements of broiler weight, performed at weekly intervals, the results are shown in Table 1. From an average weight of 50 g at the beginning of the experiment in both groups, on day 35, the chickens reached an average live weight of 2291.7 g in the control group and 2311.9 g in the experimental group.

Table 1, Average weight in control (C) and experimental (E) group (g/1pc)

week/group	C	E
0	50.0	50.0
1	191.7	182.1
2	514.8	488.9
3	985.5	963.1
4	1633.0	1571.5
5	2291.7	2311.9

Karaoglu et al. (2004) in an experiment on poultry feeding humates at concentrations of 0.1, 0.2 and 0.3% in the compound feed, did not find significant differences in final weight after 49 days of fattening. Average daily weight gains were 51.8, 49.8, 52.9 and 49.9 g

respectively. The humate supplementation had statistically no significant effects on all production parameters. There was not observed dead chick in the experimental group, while 1.8 % of mortality was in the control group.

The consumption of the feed mixture (FM) fed in the control and experimental group is given in table 2. A total of 97.56 kg FM was fed in the experimental group and 97.37 kg in the control group. The feed conversion ratio, calculated from the total FM consumption and the achieved weight of the broilers was 1.51 kg in the control group and 1.65 kg in the experimental group. Feed conversion ratio in the experimental group was higher than in the control group, mainly due to a significant increase in feed consumption per 1 kg gain in the last week of the experiment.

Table 2, Amount of fed compound feed in the control and experimental groups during fattening

week	Consumption of compound feed (in kg)	
	C	E
1	5.45	5.48
2	12.92	12.68
3	20.80	20.00
4	27.60	27.80
5	30.60	31.60
Total amount	**97.37**	**97.56**

On day 35 of fattening, the chickens in the experimental group reached a higher average live weight +20.2 g compared to the chickens from the control group. Demeterová and Šamudovská (2011) in the case of the

use of sodium humate in their experiment on broilers, recorded minimal differences in weights between the groups. The weight of the chickens in the control group on day 37 was 2476.6 g and in the experimental group 2481.5 g. Marcinčáková et al. (2018) in the experiment with the addition of 0.8% humic acids found a minimal effect on feed conversion ratio (control group 1.64 and experimental group 1.63).

Concentrations of calcium, phosphorus, magnesium and chlorides in broiler blood were determined to evaluate the mineral profile (Table 3). The serum concentration of calcium, phosphorus and chlorides was statistically significantly lower in the experimental group ($P<0.001$) compared to the values measured in the control group. There was no statistically significant difference in the magnesium serum level between the control and the experimental group (1.09 and 1.13 $mmol.l^{-1}$, respectively). Avci et al. (2007) found in an experiment on quails supplemented with humic acids at concentrations of 360, 480 and 600 mg per kilogram, that there were no statistically significant differences in phosphorus, potassium, iron, copper and zinc contents between the experimental and control groups. Calcium levels were significantly increased in the experimental groups compared with the control groups. Jaďuttová et al. (2019) found a statistically significantly lower blood calcium content in the group of broilers, which received 0.8% addition of humic acids, compared to the control group and also in the group with the addition of 1% humic acids in the diet. For phosphorus and magnesium, no statistically significant differences were found between the groups. A significantly higher content of calcium and lower content of phosphorus was found in the bones of experimental animals.

Table 3, Mineral concentrations in the blood of broilers (in mmol.l^{-1})

		Ca	P	Mg	Chlorides
Control group (n=12)	Average	**1.98***	**1.79***	**1.09**	**104.25***
	SD±	0.08	0.17	0.10	5.44
Experimental group (n=12)	Average	**1.19**	**1.16**	**1.13**	**89.63**
	SD±	0.20	0.27	0.12	7.71

* P<0,001; SD = standard deviation

CONCLUSION

To improve the economic parameters of breeding and productive health, feed supplements are used in livestock nutrition. Among preparations of this nature we also include humic substances with different content of humic and fulvic acids. In the experiment on broilers after application of humic preparation in 0.7% concentrations in the feed mixture, we did not find statistically significant differences in the monitored production parameters. Differences were found in the evaluation of the mineral profile in the blood of broilers. Statistically significantly lower concentrations of calcium, phosphorus and chlorides were registered in the experimental group with the addition of humic substances. Preliminary results also indicate a positive effect of humic acid administration on product quality and stimulation of the immune system of broiler chickens.

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USABILITY SELECTED FEED SUPPLEMENTS FOR PREVENTION AND CARE FOR THE HEALTH OF CALVES DURING MILK NUTRITION

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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 186 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 28 days of age. Compared to the control, the significant influence of applied feed supplements was found in the *Lactobacillus sporogenes* group in body weight after birth ($P<0.01$) 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 significant positive influence on the growth increasing. Use of *Ascophyllum nodosum* had a positive influence on the growth increasing but not significant.

Keywords: calves; probiotics; prebiotics; health; nutrition

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). As it was proved by many studies (Svenson and Huldgren 2008; Svensson et al. 2003; Kamal et al. 2014), the growth of live body weight and occurrence of calf diarrhoeas are influenced in dairy calves also by parity of mother and season of the year of the birth.

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).

The regular application of probiotics may help to create the stable and balanced intestinal microflora that will improve the calf health (Soto et al. 2011). Probiotics are viable microorganisms exerting a favourable effect on the host's health by improving its intestinal microbial balance (Kaur et al. 2002). For pathogenic microorganisms probiotics are competitors in the utilization of intestinal space and nutrients, they reduce intestinal pH by the production of organic acids, release bacteriocins and hydrogen peroxide and stimulate the host's immunity system. Probiotics may reduce the risk of infections and intestinal disorders (Ewaschuk et al. 2004). To maintaince the changeless and high level of probiotics in the digestive tract of calves by the administration frequency of these products should be as so long as possible (Ohashi et al. 2009). The inclusion of a probiotic in the feed ration decreases the amount of pathogenic strains of *Escherichia coli* by 36% in the feces of sheep and heifers (Braden et al. 2004). After the application of a probiotic to grazing dairy cows in the summer season the temperature of their body surface decreases, which contributes to an alleviation of the heat stress of animals (Pompeu et al. 2011).

Prebiotics are selectively fermented components facilitating specific changes in the large intestine, both in the composition and growth and in the activity of bacteria in the digestive tract. The large intestine is one of the metabolically most active organs in the body; that is why the intake of prebiotic products has a significant influence on its function (Wang 2009). The use of prebiotics showed a positive influence on the production of short-chain fatty acids in the intestinal microflora (Scheid et al. 2013).

Feed supplements will have a positive effect on reducing the incidence of diarrheal disease and improved health status of the organism of calves. The aim of this study was to investigate hypotheses that the growth and health in calves are affected by the probiotical and prebiotical feed supplements with antidiarrhoeic effect, in relation to sex, period of the birth, and number of mother's lactations.

Material and Methods

186 Holstein calves (62 in *Lactobacillus sporogenes* group, 62 in *Ascophyllum nodosum* group and 62 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 experiment was conducted from February 2018 to March 2019.

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 10 (Analytical Software, Tallahasee, FL, USA). There were evaluated factors of treatment group (1 – *Ascophyllum nodosum*, N=62, 2 -

Lactobacillus sporogenes, N=62; and 3 – control, N=62); sex (male, N=87; female, N=99), season of the birth (1 - spring, N=35; 2 - summer, N=61; 3 – fall, N=53; and 4 – winter, N=37), and number of mother's lactations (first lactation, N=58; 2 - second and higher lactation, N=128). The normality of data distribution was evaluated by the Wilk-Shapiro/Rankin Plot procedure. All data conformed to a normal distribution. Significant differences between groups were tested by Comparisons of Mean Ranks. Values are expressed as means ± SD.

Results

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).

Table 1: The influence of applied supplements on the growth and morbidity 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)	186	41.49±5.11	42.11±5.28	40.99± 4.70	0.4642	
BW in 28th day (kg)	186	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)	186	0.33±0.10	0.39±0.09	0.30± 0.10	0.0000	2:3**, 1:2*
Number of diarrhoeas, Week 1	186	0.19±0.40	0.15±0.36	0.31 ±0.46	0.0813	
Number of diarrhoeas, Week 2	186	0.19±0.40	0.16±0.37	0.24 ±0.43	0.5311	
Number of diarrhoeas, Week 3	186	0.05±0.22	0.03±0.18	0.02 ±0.13	0.6006	
Number of diarrhoeas, Week 4	186	0.00±0.00	0.00±0.00	0.00 ±0.00	M	
Duration of diarrhoea (day)	186	1.71±3.46	1.27±3.04	2.40 ±3.61	0.1743	
Total number of diarrhoeas	186	0.23±0.42	0.16±0.37	0.34 ±0.48	0.0657	

*$P < 0.05$; **$P<0.01$; ***$P<0.001$; SD = standard deviation; ADG = average daily gains; BW = body weight; *P* = significance; N = number (1 – *Ascophyllum nodosum*, N=62, 2 - *Lactobacillus sporogenes*, N=62; and 3 – control, N=62); M = missing value;

At the present study were found significant effects of gender and season of the birth on growth intensity of calves (Tables 2 and 3). The males were the heviest at the birth and the 28th day of life than females (43.00±5.05 kg vs 40.24±4.67 kg, P<0.001; 53.13±6.22 kg vs 50.50±4.94 kg, P<0.01). The calves born during the summer period had the lowest average daily gains for all observed period from the first week to the terminating of experiment (0.30±0.08 kg against 0.36±0.13 kg, 0.36±0.11 kg, and 0.36±0.11 kg; P<0.05).

Table 2: The influence of sex on the growth and morbidity of calves

Variables	N	Sex		min	max	*P*
		male	female			
		$\bar{x}$± SD	$\bar{x}$± SD			
BW at birth (kg)	186	43.00±5.05	40.24±4.67	27.00	53.00	0.0002***
BW in 28th day (kg)	186	53.13±6.22	50.50±4.94	34.00	68.00	0.0016**
ADG from birth to 28th day (kg)	186	0.34±0.12	0.34±0.09	0.10	0.80	0.7651
Number of diarrhoeas, Week 1	186	0.25±0.44	0.18±0.39	0.00	1.00	0.2416
Number of diarrhoeas, Week 2	186	0.25±0.44	0.15±0.36	0.00	1.00	0.0849
Number of diarrhoeas, Week 3	186	0.01±1.10	0.05±0.22	0.00	1.00	0.1344
Number of diarrhoeas, Week 4	186	0.00±0.00	0.00±0.00	0.00	0.00	M
Duration of diarrhoea (day)	186	1.99±3.45	1.63±3.35	0.00	16.00	0.4686
Total number of diarrhoeas	186	0.28±0.45	0.21±0.41	0.00	1.00	0.3137

*P<0.05; **P<0.01; ***P<0.001; SD = standard deviation; ADG = average daily gains; BW = body weight; P = significance; N = number (male, N=87; female, N=99), M = missing value; min = minimum; max = maximum

Table 3: The influence of birth season on the growth and morbidity of calves

Variables	Birth season				*P*	Significance
	1	2	3	4		
	$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD		
BW at birth (kg)	41.91±5.22	41.50±4.99	40.99± 5.01	42.00±5.07	0.7706	
BW in 28th day (kg)	52.86±6.58	50.39±5.16	51.83±4.90	52.74±6.52	0.1147	
ADG from birth to 28th day (kg)	0.36±0.13	0.30±0.08	0.36±0.11	0.36±0.11	0.0012**	2:3**, 1:2*, 2:4*
Number of diarrhoeas, Week 1	0.11±0.32	0.25±0.43	0.23±0.42	0.24±0.44	0.4518	
Number of diarrhoeas, Week 2	0.17±0.38	0.20±0.40	0.19±0.40	0.24±0.44	0.8855	
Number of diarrhoeas, Week 3	0.09±0.28	0.03±0.18	0.02±0.14	0.00±0.00	0.194	
Number of diarrhoeas, Week 4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	M	
Duration of diarrhoea (day)	1.40±3.24	1.77±3.30	2.00± 3.74	1.92±3.24	0.8701	
Total number of diarrhoeas	0.17±0.38	0.26±0.44	0.25±0.43	0.27±0.45	0.7453	

*P<0.05; **P<0.01; ***P<0.001; SD = standard deviation; ADG = average daily gains; BW = body weight; 1 = spring, 2 = summer, 3 = fall, 4 = winter; P = significance; N (1 - spring, N=35; 2 - summer, N=61; 3 – fall, N=53; and 4 – winter, N=37), M = missing value;

Calves born to primiparous showed significantly lower live body weights at the birth, also at the last weighing in the 28th day than calves from older cows (40.10±4.80 kg vs 42.18±5.01 kg, P<0.01; 49.99±5.64 kg vs 52.52±5.59 kg, P<0.01) (Table 4).

Table 4: The influence of the number of mother's lactations on the growth and morbidity of calves

Variables	N	Number of lactations		min	max	*P*
		1	2			
		$\bar{x}$± SD	$\bar{x}$± SD			
BW at birth (kg)	186	40.10±4.80	42.18±5.01	27.00	53.50	0.0084**
BW in 28th day (kg)	186	49.99±5.64	52.52±5.59	34.00	68.00	0.0049**
ADG from birth to 28th day (kg)	186	0.33±0.10	0.34±0.11	0.10	0.80	0.3686
Number of diarrhoeas, Week 1	186	0.24±0.43	0.20±0.40	0.00	1.00	0.5589
Number of diarrhoeas, Week 2	186	0.17±0.38	0.21±0.40	0.00	1.00	0.5446
Number of diarrhoeas, Week 3	186	0.02±0.13	0.04±0.19	0.00	1.00	0.438
Number of diarrhoeas, Week 4	186	0.00±0.00	0.00±0.00	0.00	0.00	M
Duration of diarrhoea (day)	186	1.78±3.31	1.80±3.44	0.00	16.00	0.9573
Total number of diarrhoeas	186	0.24±0.43	0.24±0.43	0.00	1.00	0.9906

*P<0.05; **P<0.01; ***P<0.001; SD = standard deviation; ADG = average daily gains; BW = body weight; 1= primaparous, 2= multiparous; P = significance; N = number (and number of mother's lactations (first lactation, N=58; 2 - second and higher lactation, N=128); M = missing value; min = minimum; max = maximum

The interaction between Treatment*Sex (P<0.05) and between Treatment*Season of the birth (P<0.01) were calculated in the average daily gains.

The using of *Ascophyllum nodosum* (treatment group 1) nor *Lactobacillus sporogenes* (treatment group 2) had not the influence on

scour incidences measured of more parameters. It did not affect either one parameter (number of diarrhoeas in calves in each week and totally, and duration of scours (Table 1). Neither calf died or was culled for bad health. The faeces had liquid consistency during the first weeks, then normal firm. In the first week colour was yellow, later on green.

Discussion

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).

At the present study were found significant effects of gender and season of the birth on growth intensity of calves. The males were the heviest at the birth and the 28th day of life than females.

In their study Kertz et al (1997) reported different weight gains in young bulls and heifers; they were higher by 8.5% in young bulls than in heifers and similar results were found out also by Dhakal et al. (2013).

The calves born during the summer period had the lowest average daily gains for all observed period from the first week to the terminating of experiment. The main advantage of the hutch rearing of calves is the minimized risk of disease transfer from calf to calf. However, the temperature stress is generally disregarded (Coleman et al. 1996; Spain and Spiers 1996). Our research has confirmed the findings of many authors that high air temperature can cause stress also in calves (Mader and Davis 2004; Broucek et al. 2009).

Growth of live body weight of calves was also influenced by the factors of birth seasonality and number of mother's lactations. The interaction between Treatment*Sex and between Treatment*Season of the birth were recorded in the average daily gains evaluation. It does means that treatment by probiotics can be positive or negative influenced by gender of treated calves, also by season of the birth.

The using of *Ascophyllum nodosum* (treatment group 1) nor *Lactobacillus sporogenes* had not the influence on scour incidences measured of more parameters.

Calves originated from young mothers displayed lower live body weights than calves from older cows. Dhakal et al. (2013), Kertz et al. (1997) and Svensson et al. (2003) identically reported higher weight gains in calves born from mothers on the second and higher lactation.

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 sporogenes*) 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. Action and effect of this feed supplement may be affected by season of the birth and gender of calves.

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GRAPE POMACE IN EQUINE NUTRITION: EFFECT ON APPARENT DIGESTIBILITY OF MINERALS

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ABSTRACT

The aim of this study was to analyze dried grape pomace (DGP) as a possible source of minerals in equine nutrition, as well as the effect of dietary inclusion of DGP on the apparent digestibility of selected minerals (Ca, P, Mg, K, Cu, Mn and Zn). Digestibility analysis was carried out by total faeces collection method. Twelve adult Slovak warmblood sport horses were divided into 3 groups: control group (C, without supplementation), experimental group 1 (E1, feed rations + 200 g of DGP) and experimental group 2 (E2, feed rations + 400 g of DGP).

The mineral profile analysis of studied DGP revealed an interesting content of Ca, K and Zn. A tendency of higher digestibility of K and Cu in both experimental groups compared to C group was detected (P>0.05). Moreover, an increased Mg digestibility was recorded in E2 group in comparison with C group (P>0.05). The digestibility of P was practically not affected by DGP consumption. On the other hand, lower digestibility coefficients for Ca, Mn and Zn in both groups with DGP supplementation were observed (P>0.05). Although differences between the groups were not significant, these results suggest that DGP could be used in horse diets in the adequate dose a possible digestibility improvement of some minerals. However, to confirm the indicated positive trend for digestibility, further experiments with additional levels of DGP in horses are needed.

Keywords: grape by-product; horse; minerals; nutrition; utilization

INTRODUCTION

Wine industry generates large quantities of by-products with problematical disposal, which can lead to serious environmental problems (Dwyer et al, 2014, Bekhit et al., 2016). The use of these by-products in feed industry as substantial source of certain nutrients and biologically active compounds has been widely studied recently (Teixeira et al., 2014, Chamorro et al., 2015, Domingues et al., 2016, Kerasioti el al., 2017, Chedea et al., 2018, Kollathova et al., 2020). According to Bennemann et al. (2016) wine pomace contains an interesting amount of Cu, Mn and Fe. Chikwanha et al. (2018) state, that this by-product contains moderate amount of some minerals (K, Ca, P, Cu, Fe and S), thus makes it a potentially good source of these nutrients. High concentration of macrominerals (Ca, P, K) and

microminerals (Cu, Fe) in grape pomaces was reported by Hanusovsky et al. (2019) and Kollathova et al. (2019). Mineral profile analysis of grape pomaces carried out by Zairati et al. (2017) revealed a high Zn, Cu, K, Ca, Mg, Na, P and Fe contents. Therefore this study aims to investigate dried grape pomace as a possible source of minerals for horses, as well as the effect of dried grape pomace on digestibility of selected minerals.

MATERIAL AND METHODS

The experiment took place in cooperation with The Riding Centre of the Department of Animal Husbandry (Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Nitra) and lasted 30 days. Twelve clinically healthy sport horses were used in the study (Slovak warm blood, six geldings and six mares, average age 9±4 years, average body weight of 577±34 kg, medium level of exercise). Animals were stabled individually in boxes with built-in feeder and automatic waterer. Horses were divided randomly into three groups; control group (C) and two experimental groups (E1 and E2). Feed rations, formulated individually depending on daily requirement of nutrients (NRC, 2007), consisted of crimped barley and oat (in a ratio 1:1), meadow hay, and feed mixture (müsli form). The daily amount of forage was divided in two parts (50 % in the morning, 50 % in the evening) and concentrates were fed three times per day (25 % in the morning, 25 % at midday, 50 % in the evening). Feed rations in experimental groups were enriched by 200 g (E1) and 400 g (E2) of dried grape pomace (DGP, *Vitis vinifera* variety *Pinot gris*) added to the evening portion of concentrates. Grape pomace was obtained from the University Experimental Farm of the Slovak University of

Agriculture in Kolíňany (Slovakia). In the context of digestibility analysis, in the last 5 days of the trial (1st, 2nd and 3rd) collection of daily amount of faeces was carried out individually from each animal. Average samples of feeds and faeces were transferred to the Laboratory of the Quality and Nutritive Value of Feeds (Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra) and processed according to standard laboratory methods and procedures (EC No 152/2009). The content of mineral nutrients was determined by High Resolution Continuum Source Atomic Absorption Spectrometer contrAA 700 (ANALYTIK JENA) and 6400 Spectrophotometer. The determination of individual elements content was based on the absorptions measured at the following wavelengths: Ca at 422.7 nm, P at 666 nm, Mg at 285.2 nm, K at 766.5 nm, Cu at 324.7 nm, Mn at 279.5 nm, Zn at 213.9 nm, Cu at 324.7 nm and Zn at 213.9 nm. The mineral profile of feeds used in the experiment is shown in Table 1.

Table 1. Content of minerals in feeds used in the experiment

	DGP	Meadow hay	Barley:Oat 1:1	Müsli
	Mean±Standard Deviation			
Ca[1]	5.25±0.14[a]	3.38.±0.04[b]	0.50±0.01[c]	13.50±0.71[d]
P[1]	2.91±0.00[a]	2.05±0.08[b]	4.64±0.17[c]	9.16±1.92[d]
Mg[1]	1.16±0.03[a]	2.62±0.04[b]	1.53±0.01[c]	3.76±0.75[b]
K[1]	14.77±0.16[a]	15.75±0.37[a]	8.35±0.01[b]	10.29±1.47[b]
Cu[2]	13.48±0.30[a]	5.63±0.08[b]	7.60±0.01[c]	33.97±0.52[d]
Mn[2]	11.27±0.38[a]	143.69±0.75[b]	20.44±0.17[c]	169.48±0.09[b]
Zn[2]	32.03±0.01[a]	24.50±1.05[b]	37.44±1.85[a]	210.70±1.59[c]

[1] in $g.kg^{-1}$, [2] in $mg.kg^{-1}$, DGP - dried grape pomace. Values followed by different letters within a row are significant at the level 0.05.

Apparent digestibility of minerals was evaluated by total collection of faeces method. Digestibility coefficients were calculated according to formula:

% D = (Nutrient Intake – Faecal Excretion) / Nutrient Intake x 100

Results were statistically evaluated with IBM SPSS v. 20.0. Descriptive statistics using one-way ANOVA were generated. Then, statistical significance of results were separated using Tukey test.

RESULTS AND DISCUSSION

Grape pomace used in the experiment was characterized by interesting concentration of Ca, K and Zn (Table 1.). Higher content of the mentioned minerals in grape pomaces was also recorded by Ziarati et al. (2017), Chikwanha et al. (2018) and Hanusovsky et al. (2019). The mineral profile of these by-products may vary depending on extrinsic factors such as edaphoclimatic conditions and viticultural practices, as well as intrinsic factors such as variety and maturity of the grapes (García-Lomillo, Gonzáles-San José, 2017). Therefore, their composition should be determined on a case-by-case basis (Ziarati et al., 2017). Effect of DGP on apparent digestibility of selected minerals is shown in Table 2. A tendency of higher digestibility of K and Cu in both experimental groups compared to C group was detected ($P>0.05$). Moreover, an increased Mg digestibility was recorded in E2 group in comparison with C group ($P>0.05$). The digestibility of P was practically not affected by DGP consumption. On the other hand, lower digestibility coefficients for Ca, Mn and Zn in both groups with DGP supplementation were observed ($P>0.05$). Polyphenols in grape pomace can positively affect the digestibility of nutrients (Makkar, 2003,

Viveros et al., 2011, Lichovnikova et al., 2015, Foiklang et al., 2016), however too many tannins in feed rations can result in digestibility decrease (Nistor et al., 2014, Ishida et al., 2015, Vinyard and Chibisa 2019).

Table 2. Effect of dried grape pomace on apparent digestibility of minerals (%)

	C group	E1 group	E2 group
	Mean±Standard Deviation		
Ca[1]	56.42±18.27	52.78±6.05	44.73±37.52
P[1]	36.76±22.40	33.43±6.53	35.45±22.45
Mg[1]	67.27±12.02	65.40±4.27	68.68±9.22
K[1]	78.47±11.30	80.95±2.54	86.07±6.21
Cu[2]	28.11±46.59	40.01±1.90	39.98±25.10
Mn[2]	67.61±17.06	58.77±5.34	64.65±14.96
Zn[2]	46.78±28.53	31.90±7.85	21.27±23.50

[1] in $g.kg^{-1}$, [2] in $mg.kg^{-1}$, C group - without supplementation, E1 group - feed rations + 200 g DGP, E2 group - feed rations + 400 g DGP.

CONCLUSION

Due to interesting mineral content grape pomace could be used as an alternative source of these nutrients in horse diets (Ca, K and Zn in this case). But variability within grape cultivars must be taken into account. Feeding grape pomace to horses up to 400 g had no significant impact on digestibility of selected minerals. However, a tendency of improved K, Cu and Mg utilisation was detected. In the future additional research is required to determine the optimal dose of grape pomace in horse feed rations and confirm the indicated trends.

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INCREASING THE NUTRITIONAL VALUE OF BROILER CHICKEN MUSCLE FAT USING LUPIN MEAL IN THEIR DIET

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ABSTRACT

The aim of the study was to determine whether the replacement of soybean extracted meal (50% and 100%) with meal from white lupin seeds (Zulika variety) affected the quality (fatty acid content) of breast and thigh muscle fat in hens and cockerels of fattened chickens (ROSS 308) in comparison with the control group, where the feed mixtures were based on soybean products only. After the administration of diets containing lupin meal, a statistically significant ($P \leq 0.05$) decrease in fat in their breast and thigh muscles was demonstrated in hens. This dependence has not been demonstrated for cockerels. The thigh muscle showed up to three times higher fat content compared to the breast muscle.

In terms of muscle fat quality, evaluated on the basis of the representation of individual groups of fatty acids (in g/100 g fat), saturated (SFA), monounsaturated (MUFA), polyunsaturated n-6 FA and n-3 FA, it can be concluded that the SFA content increased in the

breast muscle in hens and cockerels with the increasing content of lupin meal in the diet, on the contrary, it decreased in the thigh muscle.

Similarly, in MUFA, the mean values in the breast muscle of hens and cockerels were statistically significantly higher ($P \leq 0.05$) in the experimental groups compared to the control; no statistically significant differences were found in the thigh muscle. In the group of n-6 FA fatty acids, despite the fact that statistically significant differences were demonstrated between some means in individual groups of n-6 FA, based on our results, the influence of lupin diets on their content in breast and thigh muscle could not be clearly demonstrated. On the contrary, in the group of n-3 FA fatty acids, the effect of the administered experimental diets on the content of n-3 FA in the fat of breast and thigh muscle was clearly statistically significant ($P \leq 0.05$), both in hens and cockerels.

The results show that with the increasing dose of lupin meal in the diet, their content in the muscle fat of fattened chickens also increases. Based on the increased content of n-3 FA in muscle fat in experimental chickens, it can be stated that the administration of feed mixtures based on lupin meal in fattened chickens leads to an increase in the nutritional value of their muscle which is one of the important foods in human nutrition.

Keywords: poultry; muscle; protein feed; quality of animal foods; fatty acids; MUFA; PUFA

INTRODUCTION

At present, the task of the European agriculture is to produce as much domestic protein feed as possible as an alternative to the imported soybeans and soybean products, mostly from overseas countries. Most protein components for the production of compound feeds are needed for the nutrition of monogastric animals, especially pigs and poultry. In practice, this need mainly by soybean extracted meal. Therefore, more and more efforts are being made to replace it with domestic protein feeds.

One of the ways to obtain domestic protein feeds is to increase the production of legumes, including lupins, whose seeds contain a high proportion of protein, in some varieties comparable to the protein content of soybeans, which is characterized by a high content of arginine. Therefore, lupin seeds have found application in feed mixtures for poultry nutrition (Jeroch et al., 2016), especially in the fattening of broiler chickens (Geigerová et al., 2017; Chládek et al., 2017).

White lupin seeds also contain high-quality oil characterized by a high content of polyunsaturated fatty acids, which led us to the hypothesis that the replacement of soybean extracted meal with lupin seed meal in diets for broiler chickens may positively affect the quality of fat in chicken muscle and thus the nutritional value of the muscle as an important food for human nutrition.

MATERIAL AND METHODS

The aim of the work was to determine the effect of lupin meal (substitute for soybean extracted meal) in feed mixtures fed to broiler chickens ROSS 308, on the composition of fatty acids of breast and

thigh muscle of hens (F) and cockerels (M). For experimental monitoring, 6 groups of 40 chickens, 2 control groups K0% (F and M) and 4 experimental groups (E) were set up: 2 groups E50% (F and M) and 2 groups E100% (F and M). The K0% group received feed mixtures containing only soybean extracted meal, in the experimental groups the soybean extracted meal was replaced by lupin seed meal (Zulika variety), namely 50% in the E50% groups and 100% in the E100% groups. The chickens were fattened on deep litter under a light regime of 23 hours light + 1 hour dark.

During fattening, the chickens were given granulated complete feed mixtures BR 1 (1st - 14th day of fattening), BR 2 (15th - 29th day of fattening) and BR 3 (30th - 35th day of fattening). At the end of the fattening, 10 chickens from each group were slaughtered, from which the pectoral and thigh muscles were obtained and the fat content was determined and subsequently the individual fatty acids were determined. Fat was determined by fatty acid extraction using a GAS CHROMATOGRAF GC-2010 analyzer from Shimadzu. The fat content of the muscle was expressed in g/kg of muscle dry matter, the fatty acid content in g FA per 100 g fat.

The achieved results were processed by mathematical-statistical methods using the statistical program Unistat 5.6. The mean values and their differences were evaluated by multiple comparisons using the Tukey-HSD test, at the significance level $P \leq 0.05$. Each indicator is represented by a mean value (x) and a standard deviation (± SD).

RESULTS AND DISCUSSION

Performance indicators

The aim of the study was to determine whether the feeding of lupin meal, as a substitute for soybean extracted meal in feed mixtures, will affect the quality of muscle fat in broiler chickens. The fattening lasted 35 days, when the chickens of individual groups reached the average live weight - in hens F K0% 2.37 ± 0.216 kg, F E50% 2.33 ± 0.205 kg and F E100% 2.19 ± 0.283 kg; in cockerels M K0% 2.41 ± 0.290 kg, M E50% 2.55 ± 0.276 kg and M E100% 2.46 ± 0.301 kg.

There were no statistically significant differences between the mean values between the control and experimental groups, except for hens, where a lower weight was demonstrated ($P \leq 0.05$) in the group F E100% compared to the control group chicken K0%. The stated weight was reached when the conversion of feed mixtures in hens F K0% was 1.43 kg, F E50% 1.44 kg and F E100% 1.48 kg; in cockerels M K0% 1.43 kg, M E50% 1.43 kg and M E100% 1.46 kg.

Fat content in dry matter of chicken muscle (g/kg)

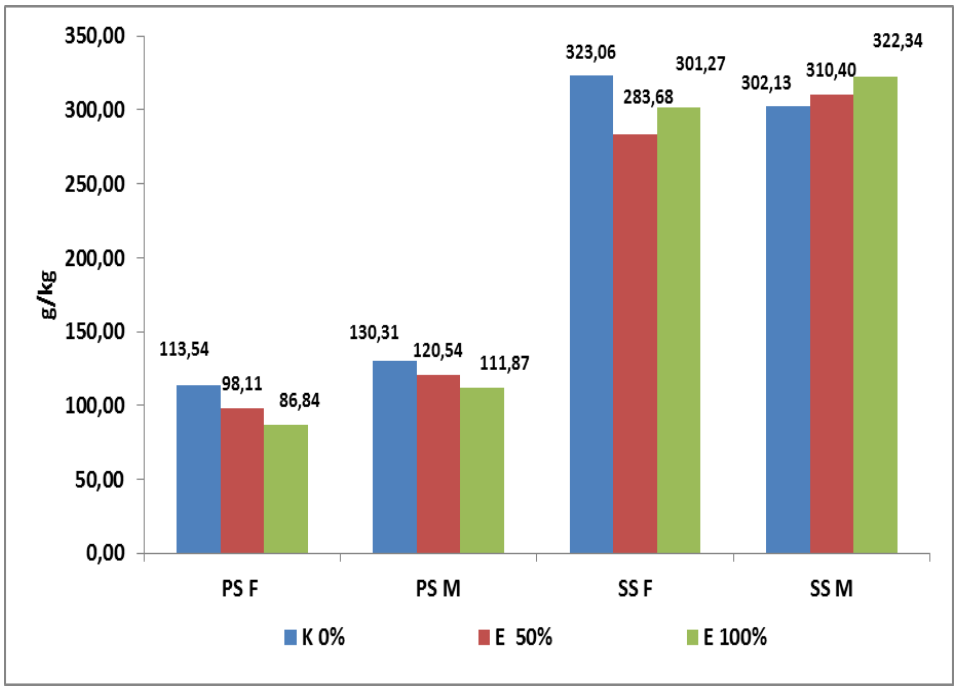
The results given in Table 1 show the general fact that the thigh muscle of chickens contains up to 3 times more fat compared to breast muscle, regardless of gender and the diet fed to the chickens. In the breast muscle, a gradual decrease in fat content was observed in relation to the content of lupin meal in the diet, a statistically significant difference $P \leq 0.05$ was only between the control (PS F K0%) and 100% replacement (PS F E100%). In the thigh muscle, this dependence was

observed only in hens between the control and the SS F E50% group. In contrast, in the case of cockerels, with the increasing content of lupin meal in the diet, the content of muscle fat increased inconclusively, as documented in Figure 1.

Table 1. Fat content (g/kg) in dry matter of chicken muscle (PS breast muscle, SS thigh muscle, F hens, M cockerels, x arithmetic mean, SD standard deviation, ab $P \leq 0.05$, K0% control, E50%, E100% experimental groups)

Fat	x	SD	Fat	x	SD
PS F K0%	**113.54**[a]	20.150	**SS F K0%**	**323.06**[a]	27.299
PS F E50%	**98.11**	14.361	**SS F E50%**	**283.68**[b]	19.487
PS F E100%	**86.84**[b]	14.806	**SS F E100%**	**301.27**	19.581
Fat	**x**	**SD**	**Fat**	**x**	**SD**
PS M K0%	**130.31**	26.924	**SS M K0%**	**302.13**	27.656
PS M E50%	**120.54**	15.868	**SS M E50%**	**310.40**	31.215
PS M E100%	**111.87**	25.302	**SS M E100%**	**322.34**	26.965

Figure 1. Graphical representation of the average fat content (g/kg) in the dry matter of the chicken muscle (PS breast muscle, SS thigh muscle, F hens, M cockerels, K0% control, E50%, E100% experimental groups)



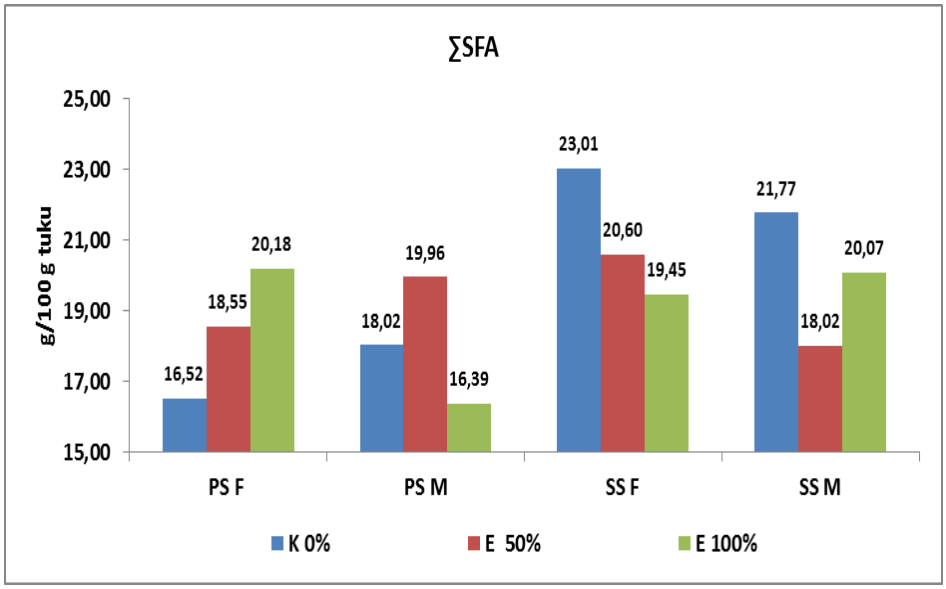
Saturated fatty acid (FA SFA) content of muscle fat in g / 100 fat

As can be seen from Table 2, the effect of lupin-based compound feed on saturated fatty acid (SFA) content cannot be unambiguously confirmed, although statistically significant differences were demonstrated between some average SFA values. In hens, a trend was observed that the SFA content in the breast muscle increased in the experimental groups, while it decreased in the thigh muscle. Between the mean value in the control and experimental groups, the difference in SFA was tested as a statistically significant at $P \leq 0.05$.

Table 2. Saturated fatty acid content (SFA) in g / 100 g chicken muscle fat (PS breast muscle, SS thigh muscle, F hens, M cockerels, x arithmetic mean, SD standard deviation, ab $P \leq 0.05$, K0% control , E50%, E100% experimental groups)

∑ SFA	x	SD	∑ SFA	x	SD
PS F K0%	**16.518^{b}**	1.826	**PS M K0%**	**18.024**	2.820
PS F E50%	**18.547^{a}**	1.198	**PS M E50%**	**19.956^{a}**	1.352
PS F E100%	**20.176^{a}**	1.905	**PS M E100%**	**16.385^{b}**	1.764
∑ SFA	**x**	**SD**	**∑ SFA**	**x**	**SD**
SS F K0%	**23.011^{a}**	1.609	**SS M K0%**	**21.769^{a}**	0.942
SS F E50%	**20.595^{b}**	1.697	**SS M E50%**	**18.016^{b}**	3.231
SS F E100%	**19.454^{b}**	1.352	**SS M E100%**	**20.072**	1.133

Figure 2. Graphical representation of the average content of saturated fatty acids (∑ SFA) in the fat of chicken muscle (PS breast muscle, SS thigh muscle, F hens, M cockerels, K0% control, E50%, E100% experimental groups)



The dominant fatty acid from the SFA group in chicken fat was C16:0 (Table 3), regardless of the sex of the chickens and the type of muscle tissue (breast, thigh). The same conclusions were reached by Sbihi et al. (2014). The proportion of C16: 0 of the total ∑ SFA content ranged from 62.01% to 81.01% in muscle fat. In general, regardless of the diet administered, the C16:0 content was higher in thigh muscle fat compared to breast muscle.

Table 3. The content of the predominant fatty acid from the SFA group (g/100 g fat) and its percentage (%) in the ∑ SFA group (PS breast muscle, SS thigh muscle, F hens, M cockerels, x arithmetic mean, SD standard deviation , ab, cd $P \leq 0,05$, K0% control, E50%, E100% experimental groups)

C16:0	x	SD	%	C16:0	X	SD	%
PS F K0%	**13.130^{b}**	1.561	**79.49**	**SS F K0%**	**18.534^{a}**	1.389	**80.54**
PS F E50%	**14.510**	0.954	**78.23**	**SS F E50%**	**16.389^{b}**	1.369	**79.58**
PS F E100%	**15.917^{a}**	1.572	**78.89**	**SS F E100%**	**15.571^{b}**	1.140	**80.04**
C16:0	**x**	**SD**	**%**	**C16:0**	**X**	**SD**	**%**
PS M K0%	**14.270**	2.149	**62.01**	**SS M K0%**	**17.420^{a}**	0.928	**80.02**
PS M E50%	**15.873^{a}**	1.142	**77.07**	**SS M E50%**	**14.595^{b}**	2.638	**81.01**
PS M E100%	**13.091^{b}**	1.426	**67.29**	**SS M E100%**	**16.164**	1.045	**80.53**

Content of monounsaturated fatty acids (∑ MUFA) in muscle fat in g/100 fat

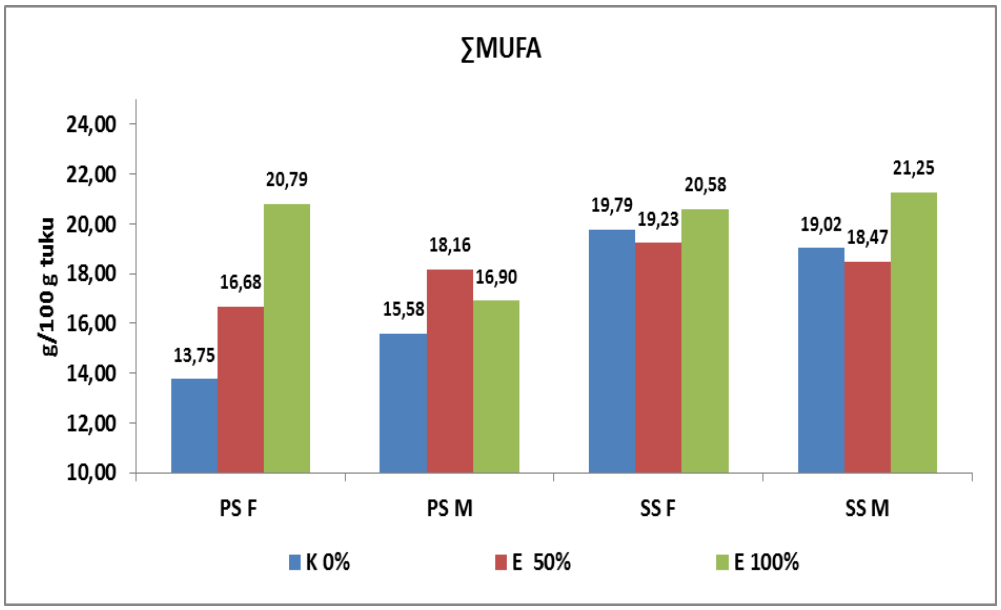
The results for the total content of monounsaturated fatty acids (∑ MUFA) are shown in Table 4 and Figure 3. It is clear from the results that the content of ∑ MUFA in muscle fat in experimental chickens was mostly higher compared to the control, which means that diets based on

lupin meal can increase MUFA in muscle fat. Statistically significant ($P \leq 0.05$) changes were demonstrated mainly in the breast muscle, especially in the breast muscle fat of hens. In cockerels, breast muscle also had a higher ∑ MUFA content in the experimental groups, but a significant difference was confirmed only between the PS M K0% and PS M E50% groups. For the thigh muscle, higher mean values of ∑ MUFA in muscle fat were also recorded in hens and cockerels, but these differences were tested as statistically inconclusive.

Table 4. Monounsaturated fatty acid content (MUFA) in g/100 g fat in chicken muscle (PS breast muscle, SS thigh muscle, F hens, M cockerels, x arithmetic mean, SD standard deviation, ab, cd $P \leq 0.05$, K0% control, E50%, E100% experimental groups)

∑ MUFA	X	SD	∑ MUFA	x	SD
PS F K0%	**13.754^{bd}**	2.281	**PS M K0%**	**15.582^{b}**	2.388
PS F E50%	**16.681^{bc}**	1.108	**PS M E50%**	**18.163^{a}**	1.396
PS F E100%	**20.791^{a}**	1.923	**PS M E100%**	**16.899**	1.866
∑ MUFA	**X**	**SD**	**∑ MUFA**	**x**	**SD**
SS F K0%	**19.785**	1.741	**SS M K0%**	**19.021**	1.562
SS F E50%	**19.231**	1.170	**SS M E50%**	**18.465**	3.942
SS F E100%	**20.584**	1.832	**SS M E100%**	**21.251**	1.408

Figure 3. Graphical representation of the average content of monounsaturated fatty acids (∑ MUFA) in chicken muscle fat (PS breast muscle, SS thigh muscle, F hens, M cockerels, K0% control, E50%, E100% experimental groups)



Of the total content of ∑ MUFA, C18:1n9 was the most represented acid in muscle fat, both in hens and cockerels in their breast and thigh muscles. Its share within the ∑ MUFA group was around 70%. Regarding C18:1n9, a trend of its higher content in muscle fat was also observed, regardless of the sex and type of muscle tissue. The results achieved by us are in agreement with the authors Bhardwaj et al. (2004).

Table 5. Dominant fatty acid content from the MUFA group (g/100 g fat) and its percentage (%) in the ∑ MUFA group (PS breast muscle, SS thigh muscle, F hens, M cockerels, x arithmetic mean, SD standard deviation , ab $P \leq 0.05$, K0% control, E50%, E100% experimental groups)

C18:1n9	X	SD	%	C18:1n9	x	SD	%
PS F K0%	**9.911^{b}**	1.512	**72.06**	**SS F K0%**	**13.776**	0.853	**69.63**
PS F E50%	**12.045^{b}**	0.715	**72.21**	**SS F E50%**	**13.908**	0.764	**72.32**
PS F E100%	**14.862^{a}**	1.506	**71.48**	**SS F E100%**	**14.736**	1.199	**71.59**
C18:1n9	**X**	**SD**	**%**	**C18:1n9**	**x**	**SD**	**%**
PS M K0%	**10.714^{b}**	1.738	**68.76**	**SS M K0%**	**13.048^{b}**	0.829	**68.60**
PS M E50%	**12.693^{a}**	1.049	**69.88**	**SS M E50%**	**11.880^{b}**	1.921	**64.34**
PS M E100%	**11.964**	1.480	**70.80**	**SS M E100%**	**14.989^{a}**	0.,955	**70.53**

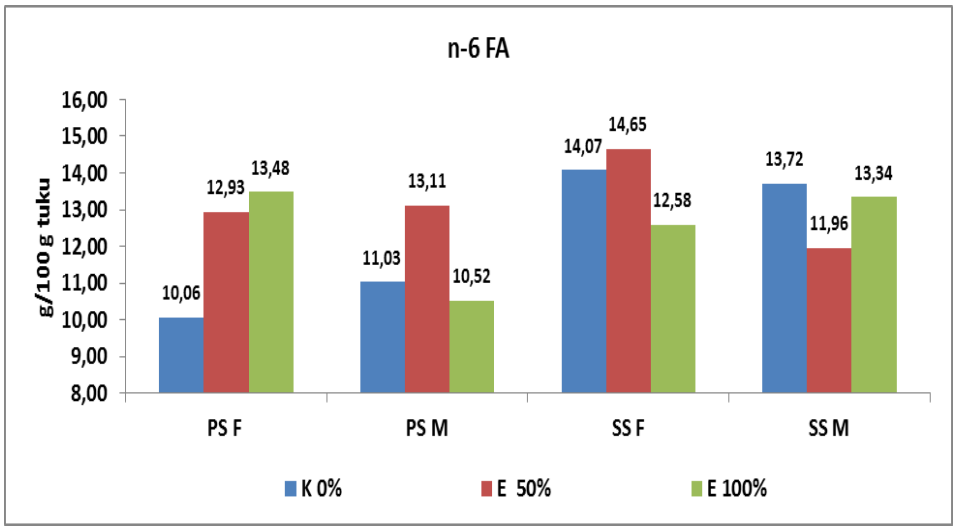
Content of polyunsaturated n-6 fatty acids (∑ n-6 FA) in muscle fat in g / 100 fat

As shown in Table 6 and documented in Figure 4, the effect of the administered lupin meal based diets on the content of polyunsaturated fatty acids from the n-6 FA group cannot be clearly demonstrated, even though there were statistically significant differences between some groups.

Table 6. Content of polyunsaturated fatty acids from group n-6 FA (g/100 g fat) in chicken muscle (PS breast muscle, SS thigh muscle, F hens, M cockerels, x arithmetic mean, SD standard deviation, ab, cd P ≤ 0.05, K0% control, E50%, E100% experimental groups)

∑ n-6 FA	x	SD	∑ n-6 FA	x	SD
PS F K0%	**10.063^{b}**	1.301	**PS M K0%**	**11.028^{b}**	2.117
PS F E50%	**12.931^{a}**	1.067	**PS M E50%**	**13.111^{a}**	0.877
PS F E100%	**13.481^{a}**	1.321	**PS M E100%**	**10.518^{b}**	1.023
∑ n-6 FA	**x**	**SD**	**∑ n-6 FA**	**x**	**SD**
SS F K0%	**14.072^{c}**	0.859	**SS M K0%**	**13.715**	0.895
SS F E50%	**14.646^{a}**	1.344	**SS M E50%**	**11.958**	2.425
SS F E100%	**12.576^{bd}**	1.225	**SS M E100%**	**13.344**	1.678

Figure 4. Graphical representation of the average content of polyunsaturated fatty acids ∑ n-6 FA in chicken muscle fat (PS breast muscle, SS thigh muscle, F hens, M cockerels, K0% control, E50%, E100% experimental groups)



The dominant fatty acid from group n-6 FA was C18:2n6, as documented in Table 7. Within the group ∑ n-6 FA, it represented more than 90%.

Table 7. Content of dominant fatty acid from n-6 FA group (g/100 g fat) muscle and its percentage (%) in group ∑ n-6 FA (PS breast muscle, SS thigh muscle, F hens, M cockerels, x arithmetic mean, SD standard deviation, ab P ≤ 0.05, K0% control, E50%, E100% experimental groups)

C18:2n6	X	SD	%	C18:2n6	x	SD	%
PS F K0%	**9.098^{b}**	1.223	**90.41**	**SS F K0%**	**13.094**	0.687	**93.05**
PS F E50%	**11.765^{b}**	0.932	**90.98**	**SS F E50%**	**13.747^{a}**	1.247	**93.86**
PS F E100%	**12.176^{a}**	1.290	**90.32**	**SS F E100%**	**12.230^{b}**	1.190	**97.25**
C18:2n6	**X**	**SD**	**%**	**C18:2n6**	**x**	**SD**	**%**
PS M K0%	**10.246**	1.984	**92.91**	**SS M K0%**	**12.868**	0.829	**93.82**
PS M E50%	**12.004^{a}**	0.775	**91.56**	**SS M E50%**	**11.216**	2.220	**93.79**
PS M E100%	**9.713^{b}**	0.906	**92.35**	**SS M E100%**	**12.502**	1.533	**93.69**

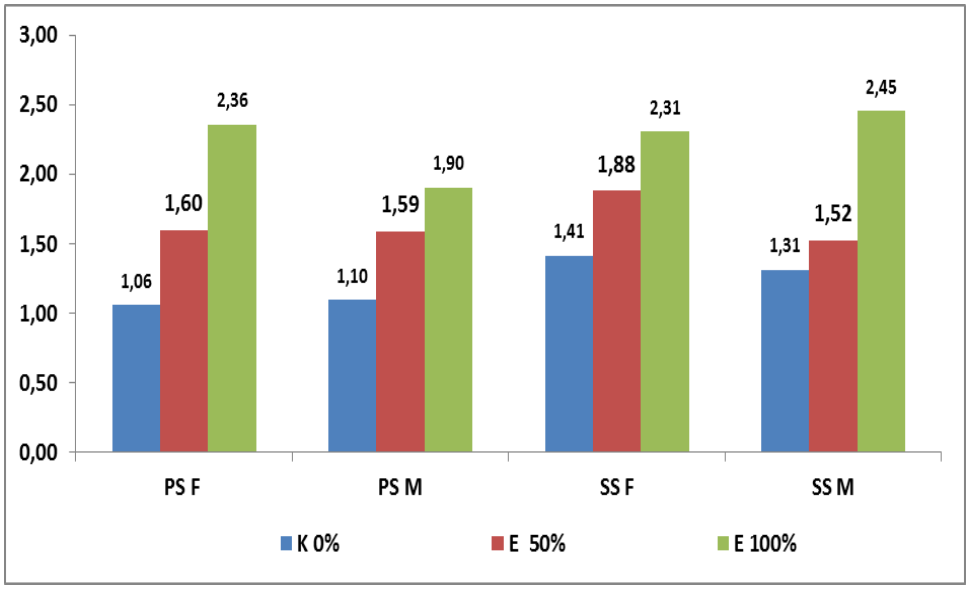
Content of polyunsaturated n-3 fatty acids (∑ n-3 FA) in muscle fat in g/100 fat

Table 8 and Figure 5 show the results of the total polyunsaturated n-3 FA content. Higher average levels of n-3 FA in the muscle fat of chickens were statistically demonstrated (P ≤ 0.05) in the experimental groups, regardless of the sex and type of muscle tissue of the chickens. The results show that with increasing share of lupin meal in diets, the content of ∑ n-3 FA in chicken muscle fat also increased significantly (P ≤ 0.05).

Table 8. Content of polyunsaturated fatty acids from n-3 FA group (g/100 g fat) in chicken muscle (PS breast muscle, SS thigh muscle, F hens, M cockerels, x arithmetic mean, SD standard deviation, ab, cd $P \leq 0.05$, K0% control, E50%, E100% experimental groups)

∑ n-3 FA	x	SD	∑ n-3 FA	x	SD
PS F K0%	**1.058^{bd}**	0.132	**PS M K0%**	**1.095^{bd}**	0.241
PS F E50%	**1.598^{bc}**	0.152	**PS M E50%**	**1.591^{bc}**	0.130
PS F E100%	**2.358^{a}**	0.245	**PS M E100%**	**1.902^{a}**	0.179
∑ n-3 FA	**x**	**SD**	**∑ n-3 FA**	**x**	**SD**
SS F K0%	**1.410^{bd}**	0.114	**SS M K0%**	**1.312^{b}**	0.102
SS F E50%	**1.878^{bc}**	0.195	**SS M E50%**	**1.523^{b}**	0.288
SS F E100%	**2.306^{a}**	0.210	**SS M E100%**	**2.453^{a}**	0.332

Figure 5. Graphical representation of the average content of polyunsaturated fatty acids (∑ n-3 FA) in chicken muscle fat (PS breast muscle, SS thigh muscle, F hens, M cockerels, K0% control, E50%, E100% experimental groups)



The dominant fatty acid from the group ∑ n-3 FA can be considered C18:3n3, which represented more than 90% of ∑ n-3 FA in muscle fat. C18:3n3 acid was also shown to have a statistically significant ($P \leq 0.05$) increase in muscle fat of experimental chickens, regardless of sex and muscle tissue type. Also in this case, with a higher content of lupin meal in the diet, its content increased, as documented in Table 9.

Table 9. Content of dominant fatty acid from n-3 FA group (g/100 g muscle fat) and its percentage (%) in ∑n-3 FA group (PS breast muscle, SS thigh muscle, F hens, M cockerels, x arithmetic mean, SD standard deviation, ab, cd $P \leq 0.05$, K0% control, E50%, E100% experimental groups)

C18:3n3	X	SD	%	C18:3n3	x	SD	%
PS F K0%	**0.931^{bd}**	0.127	**88.00**	**SS F K0%**	**1.328^{b}**	0.092	**94.18**
PS F E50%	**1.525^{bc}**	0.143	**95.43**	**SS F E50%**	**1.825^{b}**	0.190	**97.18**
PS F E100%	**2.134^{a}**	0.245	**90.50**	**SS F E100%**	**2.237^{a}**	0.202	**97.01**
C18:3n3	**X**	**SD**	**%**	**C18:3n3**	**x**	**SD**	**%**
PS M K0%	**1.005^{bd}**	0.181	**91.78**	**SS M K0%**	**1.272^{b}**	0.096	**96.95**
PS M E50%	**1.520^{bc}**	0.129	**95.54**	**SS M E50%**	**1.480^{b}**	0.276	**97.18**
PS M E100%	**1.758^{a}**	0.162	**92.43**	**SS M E100%**	**2.304^{a}**	0.309	**93.93**

The results achieved by us are in accordance with the work of the authors Suchý et al. (2011), Jeroch et al. (2016), Chláhek et al. (2017).

CONCLUSION

It can be concluded from the obtained results that the replacement of soybean extracted meal in feed mixtures of broiler chickens with lupin

meal had a positive effect mainly on the content of polyunsaturated fatty acids in muscle fat of chickens from n-3 FA groups. With the increasing dose of lupin meal in the diet, their share in muscle fat increased. This increased the nutritional value of muscle fat as well as the nutritional value of the muscle, which is an important food for human nutrition.

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THE ANTIMICROBIAL ACTION OF PALM OILS CONTAINING MEDIUM-CHAIN FATTY ACIDS AGAINST GRAM-POSITIVE BACTERIA ASSOCIATED WITH MASTITIS IN DAIRY CATTLE: *IN VITRO* STUDY

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ABSTRACT

Gram-positive bacteria belonging among ubiquitous group of pathogens that are able to induce mastitis in dairy cattle herds are potential threat for not only animals, but also human communities since they have been known for their increasing resistance to commonly used on-farm-disinfections and antibiotics. Thus, the need for alternatives is necessary and naturally occurring substances, such as palm oils rich in medium-chain fatty acids (MCFAs), which are known for their antibacterial activity, can serve as a substitution. Coconut, palm kernel,

and tucuma oil are the regular constituents of both human and animal nutrition. Therefore, the aim of this study was to evaluate the *in vitro* inhibitory activity of palm oils rich in MCFAs, after cleavage by an exogenous lipase from *Mucor javanicus*, against five strains of Gram-positive bacterial pathogens proven to cause mastitis in dairy cattle (*Staphylococcus aureus* and *Streptococcus agalactiae*) by the broth microdilution method. Tested palm oils showed growth-inhibitory effect against all tested bacterial strains in the range of 64–8192 µl/ml. *Str. agalactiae* has been determined as more sensitive species, when compared to *S. aureus*. The results of the present study propose the idea that palm oils rich in MCFAs can serve as an alternative approach within the predip and postdip procedures in bovine mastitis control, but further *in vivo* studies are needed to confirm the findings for their possible practical usage.

Keywords: antibacterial; bovine; *Staphylococcus*; *Streptococcus*; vegetable oil

INTRODUCTION

Bovine mastitis is defined as an inflammation of the mammary gland in dairy cows caused by the invasion and breakdown of milk-producing tissues by pathogenic microorganisms (Tremblay et al., 2014). The economic impact of this disease is enormous. The annual losses have been estimated to be approximately 2 billion dollars in the USA (Hossain et al., 2017). Mastitis can be manifested in two different ways - subclinical, with no visible symptoms and clinical, with visible various symptoms including mild (flakes in milk, slight swelling of infected quarter) or severe ones (abnormal milk secretions, hot swollen quarter/udder, fever, rapid pulse, loss of appetite, depression and death)

(Schroeder, 2012). There are over 250 microorganisms that may be the cause of mastitis in bovines (Bhuvana and Shome, 2013) consisting of two different groups of bacteria according to the origin of microbial pollution: contagious pathogens that live on the cow's udder and teat skin and transfer from affected cow (or quarter) to unaffected during milking (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis*); and environmental pathogens which are present in the housing and bedding and which can transfer during milking or between milkings, when the cow is loafing, eating or lying down (*Escherichia coli*, *Streptococcus uberis*, *Streptococcus dysgalactiae*) (Dufour et al., 2019).

Bovine mastitis represents significant problem for milk producers worldwide due to the disadvantages associated with the usual treatment of this disease, meaning antibiotics (betalactams, macrolides, and lincosamides) (Barkema et al., 2006), which include low cure rate, increasing occurrence of bacterial resistance, and the presence of antibiotic residues in milk (Gomes and Henriques, 2016). The modern approaches to treat and prevent mastitis in dairy herds try to decrease the negative impact of antibiotics within the animal production cycle and include nonsteroidal anti-inflammatory drugs (Breen, 2017) and intramammary teat seals (Kromker et al., 2014). Nevertheless, the mastitis in dairy cattle is still of serious concern and is a reason for seeking an alternative treatment in current research especially because of the increasing bacterial resistance towards antibiotics which can possibly pervade other areas, not only dairy industry.

In many cases, it is necessary to look for an alternative in nature such in case of plants that grow in extreme conditions (deserts, rainforests, hot

springs etc.) (Gohel et al., 2006). Nowadays, natural substances still play an important role in medicine and serve as a primary source in drug discovery processes (Harvey, 2008; Newman and Cragg, 2012). Natural products have been shown to be a source of compounds with antibacterial activity. Approximately 66% of approved antibacterial drugs are of natural origin or derivatives of its products (Brown et al., 2014).

According to their known activity in controlling bacterial growth and their ability to promote animal production, organic acids are promising alternatives to antibiotics (Polycarpo et al., 2017). Unbranched saturated fatty acids with medium-chain lengths of carbons, namely caproic ($C_{6:0}$), caprylic ($C_{8:0}$), capric ($C_{10:0}$) and lauric ($C_{12:0}$) acids, are naturally occurring e.g. in cow milk (Legrand, 2008). Above mentioned fatty acids pose antimicrobial activity against various pathogens, including Gram-positive bacteria (Hovorkova et al., 2018). The seeds of tropical palms such as tucuma (*Astrocaryum vulgare*), coconut (*Cocos nucifera*), and African oil (*Elaeis guineensis*) palm are one of the most economically important sources of plant oils, and are known to contain mainly MCFAs, with a prevalence of $C_{12:0}$. It has been proven by previous research that the antibacterial effect of palm oils rich in MCFAs may be exerted only after their cleavage (Hovorkova et al., 2018). Palm oils are essential components of nutrition that beside fatty acids contain various biologically active constituents such as carotenoids, tocopherols (Chiu et al., 2009), and coenzyme Q10 (de Souza Guedes et al., 2017). Moreover, in contrast with free fatty acids, palm oils stand out with enhanced sensory properties and lower prices. Unfortunately, no studies dealing with the antibacterial effects of palm oils rich in MCFAs against bovine mastitis-causing bacteria exist.

The presented study was carried out in order to investigate the possible *in vitro* activity of palm oils rich in MCFAs with the aim of decreasing the undesirable bacterial colonization of udders of dairy cows, especially within the milking process.

MATERIAL AND METHODS

Chemicals

Three different palm oils were analysed, namely tucuma (*A. vulgare*) oil, which was purchased from Natural Sweet Botanicals (USA), and coconut (*C. nucifera*) and palm kernel (*E. guineensis*) oil, which were obtained from Sigma-Aldrich (USA). According to the standardised methodology of microdilution antimicrobial susceptibility tests for bacteria that grow aerobically (CLSI, 2013), every oil was analysed in three individual experiments, each of which was carried out in triplicate. Prior testing, the oils were prepared as described by Hovorkova et al. (2018). Briefly, the oils were dissolved in dimethyl sulfoxide (DMSO) and emulsified by Tween 80 (both Sigma-Aldrich) to ensure sufficient dispersion into an emulsion with a final concentration of 819 200 µg/ml. The final concentration of solvents in the tested samples did not exceed 1%; thus, the bacterial viability could not be influenced (Wadhwani et al., 2009). Appropriate volumes of emulsions were diluted in tryptic soy broth (TSB) (Oxoid, UK) or TSB enriched with yeast extract (TSB-YE) (Oxoid) to reach a final concentration of 8192 µg/ml. The emulsion of oil in medium was then supplemented with a lipase from *Mucor javanicus* (Sigma-Aldrich), at 2.73 mg/ml (according to its lipolytic activity). The solution was then shaken in a water bath heated to 37 °C for 1 h to release MCFAs from triglycerides and to facilitate their antibacterial action. Penicillin G

(Sigma-Aldrich) was used to control the growth of the bacterial cultures.

Bacterial cultures and their maintenance

Five strains of bacteria, that were proven to be mastitis causatives in dairy cows, were chosen to determine the antibacterial activity of tested palm oils rich in MCFAs. Bacterial strains were obtained from two different sources (Czech Collection of Microorganisms, CCM; and from the German Collection of Microorganisms and Cell Cultures, DSM; see Bacterial strains and their specification – Table 1. Aliquots of bacterial cultures were stored at -80 °C in 20% glycerol until use in TSB or TSB-YW. Stock cultures of microorganisms were cultivated in medium at 37 °C for 24 h prior to testing. Negative and positive control of bacterial growth was included into the experiment's design on microtiter plates.

Determination of the minimum inhibitory concentrations

Minimum inhibitory concentrations (MICs) were analysed following the protocol of the Clinical and Laboratory Standards Institute (CLSI, 2013) to determine the antibacterial activity of coconut, palm kernel, and tucuma oil against chosen Gram-positive bovine mastitis-causing strains of bacteria. *In vitro* broth microdilution method was performed in 96-well microtiter plates. The initial concentration of palm oils to test their antibacterial activity was 8192 μg/ml.

Plates were inoculated by bacterial suspension with a final density of 5×10^5 CFU/ml, which was achieved by a McFarland Densitometer Biosan DEN 1 (BioTech, Czech Republic), and incubated at 37 °C for 24 h. The "before/after incubation" bacterial growth was measured

spectrophotometrically by a Tecan Infinite® 200 PRO microplate reader (Tecan Group Ltd., Switzerland) at a wavelength of 405 nm.

Table 1. Bacterial strains and their specification

Bacterium	Strain	Specification	Other designation
Staphylococcus aureus subsp. *aureus* Rosenbach 1884[AL]	CCM 4442	bovine mastitis isolate (CZ); production of ß-haemolysin; atypical strain: phosphatase and clumping factor negative	P. Benda M 27/92
	CCM 6188	bovine mammary gland isolate; loss of haemolysins production	B. Skalka K 126
	DSM 6732	bovine udder isolate; murein: A11.3; no toxin genes present (PCR)	ATCC 25178
Streptococcus agalactiae Lehmann and Neumann 1896[AL]	CCM 6187	bovine mammary gland isolate (CZ); test organism for CAMP-test, production strain for CAMP-factor; control strain for PYRAtest	J. Smola 3767
	DSM 6784	bovine udder infection isolate; Lancefield group B; beta-haemolytic; recommended as reference strain for cAMP test	ATCC 27956

MICs were expressed as the lowest concentrations of palm oils that inhibited the bacterial growth by at least 80% compared to the oil-free growth control. Moreover, a susceptibility of all strains to penicillin G was also tested using MICs determination, alike in case of palm oils. The final MICs of tested compounds were determined as the mode of all measured values. During the experiment, antibacterial effect of palm oils was observed only after lipase cleavage. Therefore, the results mentioned below apply only to oils after cleavage by a lipase from *M. javanicus*.

Statistical analysis

As mention above, the MICs were determined as mode of measured values calculated in Microsoft Excel editor same as average values for MICs of tested oils and penicillin G against all bacterial strains displayed in Table 2.

Differences between MICs of oils were analyzed by ANOVA procedure, the effect of bacterial genus on the MICs of oils was analyzed by Welch's test for unequal variances, both calculated in Statistica 12 software. Significant differences were evaluated on a probability level 0.05.

RESULTS AND DISCUSSION

The MICs displayed in Table 2 show antibacterial activity of the chosen palm oils against all tested Gram-positive bovine mastitis proven-causative bacterial pathogenic strains ranging from 64 (*Str. agalactiae*) to 8192 µg/ml (*S. aureus*). The most sensitive of the tested bacterial strains according to the lowest measured modal MIC value was *Str. agalactiae* CCM 6178 (64 µg/ml in the case of palm kernel oil), followed by *Str. agalactiae* DSM 6784 (128 µg/ml for tucuma oil, respectively). According to the inhibitory activity, viewed as the average of the MIC values for each oil tested against all strains, the most effective showed itself to be palm kernel oil (average MIC 2112 µg/ml), followed by tucuma (average MIC 2656 µg/ml), and coconut oil (average MIC 3942 µg/ml). *Str. agalactiae* species was determined as more sensitive to antibacterial action of tested palm oils than *S. aureus* species with an average MIC value of 331 µg/ml for all tested oils, compared to *S. aureus* that exerted MIC 4324 µg/ml for tested oils. *Str. agalactiae* was also more sensitive species to antibiotic control, which was evaluated by penicillin G (average MIC 0.00037 µg/ml), than *S. aureus* (average MIC 0.00138 µg/ml).

According to the statistical analysis, there was no significant difference between MICs of tested oils on a probability level 0.05, but it revealed, that bacterial genus significantly influence the MICs of MCFAs rich oils ($P \leq 0.05$).

Table 2. Minimum inhibitory concentrations of tested palm oils (µg/mL)[1]

Strain		modus MIC [µg/mL]				average MIC of oils	average MIC of penicillin G
		coconut oil	palm kernel oil	tucuma oil	penicillin G		
S. aureus	CCM 4442	2048	2048	2048	0.001953	4324	0.00138
	CCM 6188	8192	4096	4096	0.001953		
	DSM 6732	8192	4096	4096	0.000244		
Str. agalactiae	CCM 6187	1024	64	256	0.000244	331	0.00037
	DSM 6784	256	256	128	0.00049		
total average MIC		3942	2112	2656	0.00082		

[1]modus of triplicates of three independent experiments

MCFAs are known for its *in vitro* antibacterial activity towards various pathogens, but to the best of our knowledge, there is no evidence about the antibacterial properties of palm oils rich in MCFAs against staphylococci and streptococci that have been proven to cause mastitis in dairy cattle. Batovska et al. (2009) tested the antibacterial effect of MCFAs against three different *S. aureus* strains by the agar well diffusion method and observed inhibitory activity of capric acid towards all three strains at concentrations of 250–500 µg/ml, and of lauric acid inhibiting one *S. aureus* strain at a concentration 125 µg/ml.

These inhibitory concentrations towards *S. aureus* are approximately 10× lower than in the case of the palm oils observed in this study (2048–8192 µg/ml). Palm oils are diverse mixture of not only MCFAs, but also various biologically active compounds (Srivastava et al., 2016), interacting with each other and thus probably reducing the antibacterial activity of the oils when compared to free fatty acids. MICs might be decreased also due to probable incomplete release of fatty acids from triglycerides during the cleavage by lipase. In accordance with our results, Nair et al. (2005) observed inhibition of growth of 15 clinical isolates of mastitis-causing pathogens including *S. aureus*, *Str. agalactiae*, *Str. dysgalactiae*, and *Str. uberis* by caprylic acid and monocaprylin.

Udder clearness and hygiene are crucial when controlling mastitis in dairy herds (Schreiner and Ruegg, 2003), so a variety of pre- and post-milking teat disinfection procedures are used on farms. The most applied after milking substances are iodinebased (iodophors) and chlorhexidine-based; however, both types of disinfectants currently struggle with increasing bacterial resistance (Behiry et al., 2012). Thus, the antibacterial properties of plant oils rich in MCFAs can serve as a tool in controlling contamination by mastitis-causing pathogens. Not only their antibacterial activity, but also the positive effect on skin conditions may be the advantage of plant oils rich in MCFAs (Oyedeji and Okeke, 2010). Therefore, using plant oils rich in MCFAs as a part of teat preparation and treatment during the milking process can be beneficial.

CONCLUSION

With rising bacterial antibiotic resistance, the search for new alternatives in treatment of animal pathogenic strains is current aim of worldwide research and spread of resistant bacteria through animal production into human communities is undesirable. In presented study, the antibacterial activity of palm oils rich in MCFAs, namely coconut, palm kernel, and tucuma oil (after cleavage with a lipase from *Mucor javanicus*), was tested against five mastitis causative bacterial strains using *in vitro* microdilution method. The results offer an alternative approach to mastitis prevention in cattle herds since oils rich in MCFAs are valuable antibacterial remedies suitable for use in teat preparation and treatment.

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SUSCEPTIBILITY OF *ENTEROCOCCUS* SPP., *SALMONELLA* SPP. AND *STAPHYLOCOCCUS* SPP. TO ORGANIC ACIDS

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ABSTRACT

The aim of this study was to evaluate *in vitro* antibacterial effect of 17 mixtures additives in various concentrations, specifically medium-chain fatty acids (MCFAs), myristic acid, cinnamic acid and citric acid, on selected bacteria, especially *Staphylococcus aureus* (ATCC 29213, ATCC 43300), *Salmonella enteritidis* (147/7F4), *Salmonella typhimurium* (K3), *Enterococcus faecium* (CCM 6226) and, *Enterococcus cecorum* (CCM 4285), by the standardized microdilution method in a 96-well microtitration plates. The minimum inhibitory concentrations (MICs) of tested additives were determined as the lowest concentration limiting the growth of bacteria in wells compared to a positive control of ≥80%. The additives were the least effective in

inhibition of Gram-negative strains of *Salmonella* (*S. enteritidis*, MICs: 2048-≥4096 µg/ml; *S. typhimurium*, MICs: ≥4096 µg/ml). Both strains of *S. aureus* were sensitive to the presence of all additives. The lowest MIC for *S. aureus* ATCC 29213 was 56 µg/ml using C12/C14 (70/30). The remaining additives inhibited growth at concentrations 64-2048 µg/ml. Interestly, *S. aureus* ATCC 43300 was the most sensitive to C12/C14+GML+CitA (24,5/10,5/35/30) at MIC 64 µg/ml. Other additives showed MICs in range 64-2048 µg/ml. Gram-positive bacteria, *E. cecorum* and *E. faecium* were the most sensitive to C12/C14 (70/30) (MIC 32 and 64 µg/ml). The remaining values of MICs ranged from 128 to ≥4096 µg/ml. Compared to Gram-negative bacteria, antibacterial effect of MCFAs, GML, citric and cinnamon acid was observed mostly to Gram-positive bacteria.

Keywords: feed; additive; antibiotics; inhibition; antibacterial

INTRODUCTION

Proper nutrition of livestock play the crucial role in maintaining optimal growth, reproduction and quality of production. To stimulate production performance, feed additives are commonly added to animal diets. Moreover, the feed additives are frequently also aimed to improve animal health and welfare, especially during stressful periods of life (Van der Aar et al., 2017). The European Feed Standard Agency (EFSA) describes feed additives as products used in animal nutrition for purposes of improving the quality of feed and the quality of food from animal origin, or to improve the animals´ performance and health (Pirgozliev et al., 2019). Antimicrobial additives, as different types of antibiotics, were extensively used in order to reduce harmful

microorganisms in the intestinal microbiota of livestock, especially broiler chickens (Lesson, 2007). Recently, replacing antibiotics in animal feedstuffs with biologically active substances has become a very current topic, especially after the ban of in-feed antibiotics as growth promoters in European Union in 2006 (van der Aar et al., 2017). The trend of banning the use of antibiotics, coccidiostats and other medical growth promoters in animal feedstuffs has developed because of the possibility that these compounds can contribute to the emergence of resistant bacteria. These can then be transferred to humans through the food chain or direct contact causing serious illnesses. The emerging and steady increase of the occurrence of bacteria that are resistant to multiple antibiotics, so-called multiresistance, has become a global public problem in human as well as in veterinary medicine (Chambers and Deleo, 2009; Edo et al., 2015). The pathogenic and zoonotic potential of multiresistant bacteria (MRB), i.e., the multiple ways of transmission to humans by direct and indirect contact and vice versa, as well as the economic implications altogether account to the huge public health impact of MRB in livestock (Dahms et al., 2014). As both concern and legislative action in terms of antibiotic use in food animals are increasing, equally the search for new tools to counter pathogens is rising in the scientific word (Liu et al., 2018).

Besides such concepts as vaccines, therapeutic drugs and immune enhancers, some other alternatives are currently studied. Among them, organic acids, including medium-chain fatty acids (MCFAs) take place. They have been known for their positive benefits as feed additives in improving animal health, production, and feed digestibility (Baltić et al., 2017). This group of chemicals consists of caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0) and lauric acid (C12:0).

MCFAs are saturated and unbranched six to twelve carbon fatty acids, which occur naturally as medium-chain triglycerides in milk fat of many animal species (mouse, rabbit, rat etc.) and various plant materials (coconut, palm and *Cuphea* oil etc.) (Dierick et al., 2003; Marten et al., 2006; Zentek et al., 2011). Importantly, free fatty acids and monoglycerides have strong antibacterial effect, especially against Gram-positive bacteria, such as *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Clostridium perfringens* etc. (Bergsson et al., 2002; Skrivanova et al., 2014; Lalouckova et al., 2019). The combined health-promoting and pathogen-mitigating functions of MCFAs and monoglycerides are particularly significant, primarily in the swine and poultry industry (Lamot et al., 2016, Sánchez-Cordón et al., 2018). Over the past few years, there has been progress in evaluating the effectivity of free MCFAs and monoglycerides as feed additives, and also in proven capability of mitigating some feedborne pathogens (Jackman et al., 2020).

In addition to MCFAs, other organic acids (OA), may be a suitable possible alternative to antibiotics, as a growth promoters. The use of OA, such as citric, lactic, and cinnamic acid has shown improved digestibility, improved mineral absorption thus beneficial effect in feed efficiency in animals (Yilmaz et al., 2018). These organic acids, together with myristic acid, have also strong antimicrobial properties (Dibner and Buttin, 2002; Liu and Huang, 2012).

The present research was carried out to evaluate antibacterial effect of commercial feed additives for monogastric animals (especially poultry), consisting of mixtures of various concentrations of MCFAs,

myristic acids, cinnamic acid and citric acid against selected non/pathogenic bacteria.

MATERIAL AND METHODS

Bacterial strains and culture media

The type bacterial strains from culture collections ATCC and CCM were obtained from the American Type Culture Collection (Manassas, USA) and Czech Collection of Microorganisms (Brno, CZ). K3 and 147/7F4 were obtained from University of Chemistry and Technology (Prague, CZ).

The inhibitory activity of tested additives was determined against *Staphylococcus aureus* (ATCC 29213, ATCC 43300), *Salmonella enteritidis* (147/7F4), *Salmonella typhimurium* (K3), *Enterococcus faecium* (CCM 6226), *Enterococcus cecorum* (CCM 4285) grown and maintained in Müller-Hinton broth (MHB) (Oxoid; Prague, CZ). The bacterial cultures were incubated at 37 °C for 24 h under aerobic conditions.

Tested additives

Tested additives (Table 1) were purchased from Daa Vision (Netherlands).

Preparation of samples for microdilution test

Respective tested additives were weighted and diluted in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Prague, CZ) and MHB was added to reach a final concentration of 409 600 µg/ml of each potentially

active compound. The final concentration of DMSO did not exceed 0.5%, and thus it did not influence the activity of tested compounds.

Table 1. List of tested additives

Additive	Specific acids, namely
C8/C10	Caprylic a./Capric a.
C12	Lauric a.
C14	Myristic a.
C12/C14 (70/30)	Lauric a./Myristic a.
C12/CitA (50/50)	Lauric a./Citric a.
C12/CitA (70/30)	Lauric a./Citric a.
C12/CinA (50/50)	Lauric a./Cinnamic a.
C12/CinA (70/30)	Lauric a./Cinnamic a.
C12/C14 + CitA (35/15/50)	Lauric a./Myristic a.+Citric a.
GML (90)	Monolaurin
GML/CinA (50/50)	Monolaurin/Cinnamic a.
GML+CitA (50/50)	Monolaurin+Citric a.
GML+CitA (70/30)	Monolaurin+Citric a.
C12/C14+GML	Lauric a./Myristic a.+Monolaurin
C12/C14+GML (35/15/50)	Lauric a./Myristic a.+Monolaurin
C12/C14/GML+CitA (24,5/10,5/35/30)	Lauric a./Myristic a./Monolaurin + Citric a.
C12/C14+GML+CinA (24,5/10,5/35/30)	Lauric a./Myristic a.+Monolaurin+Citric a.

Determination of inhibitory effect *in vitro*

The inhibitory activity of the tested compounds was evaluated *in vitro* by the broth microdilution method using 96-well microtiter plates, modified according to the recommendations proposed for more effective assessment of the anti-infective potential of natural products (Cos et al. 2006, Hecht et al. 2007). Seven two-fold dilutions were carried out from the initial solution dilutions of each compound prepared in MHB.

The bacterial inoculum were standardized to achieve a density of 5 x 10^5 CFU/ml using the McFarland scale and inoculated into wells (10 µl). Microplates were incubated at 37 °C for 24 h under aerobic conditions.

The growth of microorganisms was assessed as the turbidity determined by an Infinite 200® PRO microplate reader (Tecan, Switzerland) at 405 nm. The minimum inhibitory concentrations (MICs) were related to the density of the growth control and expressed as the lowest compound concentrations that resulted in an 80% growth reduction compared to that of the compound-free growth control.

All samples were tested as three independent experiments, each carried out in triplicate.

RESULTS AND DISCUSSION

Complete data of antibacterial effects of tested compounds with calculated mean values are presented in Table 2.

Following the European ban of antibiotic growth promoters in 2006, the use of OA in animal nutrition has gained significant importance in the feed industry. OA are widely distributed in nature as constituents of herbal and animal tissues. Their primary bacteriostatic action against many microbial species occurs by reducing pH of the diet, efficacy is higher in acidic conditions (Cherrington et al., 1991). The antimicrobial activity is caused by the ability of OA to dissociate (Partanen, 2001).

In our research, the most effective additive was C12/C14 (70/30), a mixture of these compounds showed low MICs against three bacteria, *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecium* (CCM 6226), *Enterococcus cecorum* (CCM 4285). All the mentioned types of bacteria are Gram-positive bacteria. Preuss et al. (2005) state that lauric acid has a higher antibacterial potential than caprylic, capric and myristic acid. The antibacterial effect of lauric acid could potentially be enhanced by *in vivo* tests due to the conversion of lauric acid to monolaurin with higher biological activity (Lieberman et al., 2006; Schlievert and Peterson, 2012). A relationship between chain length of OA and its antimicrobial effect was observed in the past (McGaw et al., 2002).

The addition of organic acids in diet can have a beneficial effect on the performance of poultry by decreasing pathogenic bacteria. The most common bacteria that can affect the intestinal health of poultry are *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* which can be controlled by supplementation of an OA in diet (Van lmmerseel et al., 2006). *Salmonella* spp., as a Gram-negative pathogen, can multiplay in the gastrointestinal tract of birds and potentially be excreted in the faeces during growing phase (Kusar et al., 2010). In our

research, the MICs of strains *S. enteritidis* (147/7F4) and *S. typhimurium* (K3) were high, ranging from 2048 to >4096 µg/ml. Skrivanova et al. (2007) tested three strains of *Salmonella* spp., MICs were higher than 5000 µg/ml, which is consistent with this study.

The Gram-positive pathogen *S. aureus* has the characteristic ability to rapidly develop resistence to any antibiotic drugs. Resistance to methicillin was first reported in methicillin-resistant *S. aureus* (MRSA) in 1961 (Pantosti et al., 2007). MRSA (ATCC 43300) was the most sensitive to C12/C14+GML+CitA (24,5/10,5/35/30) at MIC 64 µg/ml. On the contrary, *S. aureus* (ATCC 29213) was the most sensitive to C12/C14 (70/30) at MIC 56 µg/ml. MRSA (ATCC 43300) appears to be a potentially dangerous strain, compared to *S. aureus* (ATCC 29213), because it was resistant to seven tested additives. It could be related to the resistance gens of MRSA (Utsui and Yokota, 1985).

Interestingly, strains of *Enterococcus* spp. showed different MICs. The tested additives showed lower MICs (64-1024 µg/ml) against bacterium *E. faecium* (CCM 6226), which was the probiotic culture. *E. cecorum* (CCM 4285) reported higher MICs (32-4096 µg/ml). *E. faecium* was the most sensitive culture. The results could not be compared, because this is the first report focused on susceptibility of this probiotic strain of bacterium to organic acids. Its probably due to the lower proportion *E. faecium* in gastrointestinal tract (GIT). Only 10,1% *E. faecium*, of all enteroccal bacteria, is present in digestive tract of birds (Stępień-Pyśniak et al., 2016).

In summary, organic acids possess significant antimicrobial effect, primarily to *S. aureus* (ATCC 29213, ATCC 43300) and *E. faecium* (CCM 6226).

Table 2. Minimum inhibitory concentrations of tested additives against *S. aureus* (ATCC 29213, ATCC 43300), *S. enteritidis* (147/7F4), *S. typhimurium* (K3), *E. faecium* (CCM 6226) and *E. cecorum* (CCM 4285) (µg/ml)

	Minimum inhibitory concentrations (µg/ml)					
Type of bacteria / Type of additive	*Staphylococcus aureus* ATCC 29213	*Stahylococcus aureus* ATCC 43300	*Salmonella enteritidis* 147/7F4	*Salmonella typhimurium* K3	*Enterococcus faecium* CCM 6226	*Enterococcus cecorum* CCM 4285
C8/C10	2048	2048	>4096	>4096	512	1024
C12	128	128	>4096	>4096	128	32
C14	256	256	>4096	>4096	1024	32
C12/C14 (70/30)	56	256	>4096	>4096	64	32
C12/CitA (50/50)	128	128	2048	4096	128	61
C12/CitA (70/30)	128	512	>4096	4096	128	32
C12/CinA (50/50)	128	512	2048	>4096	160	64
C12/CinA (70/30)	128	512	4096	>4096	128	32
C12/C14+CitA (35/15/50)	512	512	>4096	>4096	128	>4096
GML (90)	64	128	>4096	>4096	64	>4096
GML/CinA (50/50)	128	128	2048	>4096	128	2048
GML+CitA (50/50)	128	128	4096	>4096	128	4096
GML+CitA (70/30)	128	128	>4096	>4096	128	4096
C12/C14+GML	64	128	>4096	>4096	64	>4096
C12/C14+GML (35/15/50)	64	256	>4096	>4096	64	>4096
C12/C14/GML+CitA (24,5/10,5/35/30)	128	128	>4096	>4096	256	>4096
C12/C14+GML+CinA (24,5/10,5/35/30)	64	64	4096	>4096	128	>4096

CONCLUSION

The results confirm that feed additives containing organic acids, especially MCFAs have an inhibitory effect to *Staphylococcus aureus* (ATCC 29213, ATCC 43300), *Enterococcus faecium* (CCM6226) and *Enterococcus cecorum* (CCM 4285). Lauric acid had the highest inhibitory potential of all tested additives, especially in combination with myristic acid (70/30). It is obvious that MCFAs, monolaurin, citric and cinnamic acid, are suitable additives to animal feed and possible alternative to antibiotic treatment.

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EFFECT ON MACROELEMENTS IN THE BLOOD OF SOWS BY FEEDING DRIED GRAPE POMACE

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ABSTRACT

The aim of this study was to analyse mineral profile of gestating and lactating sows after 7 days long intake of dried grape pomace. Experiment was realized with 14 sow's Landrace x Duroc crossbreed divided to two groups control (CON) and dried grape pomace (DGP). Sows in DGP group received during last week of pregnancy diet containing dried grape pomace in amount of 1%, whereas CON group received basal diet. Blood samples before start of experiment and during first day after parturition were taken, transported to laboratory, centrifuged and gained serum were until analysis stored at -18°C. Blood serum was analysed on calcium (Ca), phosphorus (P), magnesium (Mg) and chlorides (Cl^-) concentration. The results indicate increase in P levels in the DGP group ($P<0.05$). P increase also in CON group, but was not statistically significant. The chlorides Cl^- concentration was above the reference values in both groups at the beginning of the experiment. After parturition the level of Cl^-

decreased. Decreases in Ca and Mg were recorded only in DGP. This could be affected by the physiological process in the sow's body during and after parturition. In CON, the concentrations of Ca and Mg increased, but the increase was not statistically significant. No negative effects of DGP feeding on macroelements profile of sow's blood serum have been demonstrated, they can be fed as a supplement to the feed ration, but further studies are needed to more accurately determine the doses for each animal category.

Keywords: grape pomace; nutrition; swine; macroelements

INTRODUCTION

Pigs have a dietary requirement for many inorganic elements. They are necessary and irreplaceable for the formation of tissues, cells and the body system and important for the body's metabolism (NRC, 2012). In general, calcium and phosphorus are most often lacking in feed diet for pigs (Zeman et al, 2004). Calcium and phosphorus are important to gestating sows for development of fetuses and integrity of the sow's skeleton (Trottier et al., 2000). According to several authors, grape pomace is a good source of nutrients like Ca, P, Na, Mg, K, Cu, Fe (Simko et al., 2019, Galik et al., 2019, Hanusovsky et al.,2019, Chikwanaha et al., 2018, Guerrera-Rivas et al., 2016, Teixeira et al., 2014). In recent years, there has been a growing interest of feeding farm animals with innovative feeds, especially those rich in antioxidant compounds. The inclusion of agricultural by-products in farm animals nutrition is also considered important due to the possible ecological pollution of the ecosystem during storage (Kafantaris et al., 2017, Brenes et al., 2016). Thus, the aim of this study was to investigate the

effects of feed supplemented with GP on macro-mineral profile in blood serum of sow's during last week of pregnancy and after parturition.

MATERIAL AND METHODS

This experiment was conducted at the Pig Farm in Dubovany (SPD Veselé) and in compliance with the European Union Guideline on animal care (Directive 2010/63/EU, European Union, 2010). 14 healthy sows were included in the experiment. Before the start of feeding dried grape pomace (DGP), sows were moved to the farrowing stall, placed into farrowing crates and randomly divided into control (CON) and experimental groups (DGP). Feeding of DGP began 7 days before the expected parturition. The experimental group received an addition of 1% DGP to the basal diet. Both feeds were analysed in Laboratory of Nutritional Value and Feed Quality (Department of Animal Nutrition, SUA in Nitra). Nutrients were determined according to standard laboratory methods (AOAC, 2000). Basal and dried grape pomace nutritional characteristic of the diet components is shown in Table 1. Blood samples were taken 7 days before the expected parturition, which was immediately before start of feeding with dried grape pomace addition, and 1 day after parturition. Blood was taken from the *vena cava cranialis* by the veterinarian from Pig Farm in Dubovany. After collection, the samples were cooled and transported to the laboratory for analysis. Subsequently, the blood was processed into blood serum. Blood serum was analyzed for individual macroelements using the Labkit (Chemelex, S.A., Barcelona). Absorbance of final solutions was measured by spectrophotometer Helios (Thermo Electron Corporation).

Table 1. Nutritional characteristic of control and experimental diet

Nutrients are expressed on feed dry matter basis	Complete feed for lactating sow	Dried grape pomace
Dry matter ($g.kg^{-1}$)	893.25	941.4
Crude protein ($g.kg^{-1}$)	174.50	92.8
Ether extract ($g.kg^{-1}$)	20.75	79.25
Crude fiber ($g.kg^{-1}$)	46.70	172.6
Ash ($g.kg^{-1}$)	56.20	37.25
NFE ($g.kg^{-1}$)	595.10	559.5
Organic Matter ($g.kg^{-1}$)	837.05	904.15
Starch ($g.kg^{-1}$)	408.70	14.3
Total sugar ($g.kg^{-1}$)	41.30	176.5
NFS ($g.kg^{-1}$)	508.00	299.05
ME_{Pig} ($MJ.kg^{-1}$)	14.39	12.69
Ca ($g.kg^{-1}$)	8.78	4.27
P ($g.kg^{-1}$)	6.24	3.03
Mg ($g.kg^{-1}$)	2.59	1.08
Na ($g.kg^{-1}$)	2.97	0.25
K ($g.kg^{-1}$)	9.37	12.18
Cu ($mg.kg^{-1}$)	25.85	11.05
Fe ($mg.kg^{-1}$)	354.5	65.9
Mn ($mg.kg^{-1}$)	84.7	10.8
Zn ($mg.kg^{-1}$)	182	13.75

ME_{Pig} was calculated according to (Noblet and Perez, 1993)

The determination of individual element content was based on the absorption measured at the following wavelengths: Ca content was detected at 650 nm, P at 340 nm, Mg at 520 nm and Cl at 480 nm. The

results were statistically evaluated with program IBM SPSS v. 26.0. The analytical data were compared by variance analysis using One-way ANOVA. The level of statistical significance was set at $P < 0.05$ (Independent samples T-Test and Tukey test).

RESULTS AND DISCUSSION

The mineral profile of feed dried grape pomace is given in the Table 1. The results of the monitored parameters of the mineral profile of sow's blood serum are shown in the Table 2. The concentrations of Ca, P and Mg found in the CON and DGP groups, before the start of the experiment and even during first day after parturition, were within the reference values published by several authors (Merck 2015; Slanina et al., 1991; Vrzgula et al., 1990;). A statistically significant increase was recorded only for phosphorus within the experimental group. Phosphorus values correspond to the reference values published in the study for gestating (1.5-3.2 $mmol.l^{-1}$) and lactating (0.9-4.0 $mmol.l^{-1}$) sows (Verheyen et al., 2006). Conversely, a decrease was observed for chlorides. According Vrzgula et al. (1990) chlorides are excreted in the milk of lactating animals, which could also cause a decrease in the blood serum in both groups. Chedea et al. (2019) had also recorded an increase in phosphorus value and a decrease in Ca and Mg value after feeding grape pomace by piglets. This decrease may be due to the polyphenols content of grape pomace as published by Chamorro et al. (2013) and Fiesel et al. (2015).

Table 2. Average macroelements concentration of sows serum during experiment

	CON before	DGP before	CON after	DGP after	SEM	P-value
P mmol.l^{-1}	2.07^{a}	2.16^{a}	2.34^{ab}	2.71^{b}	0.07	0.128
Ca mmol.l^{-1}	2.36	2.91	2.45	2.87	0.09	0.006
Mg mmol.l^{-1}	0.88	0.98	0.94	0.86	0.02	0.747
Cl mmol.l^{-1}	113^{ab}	116^{b}	108^{a}	110^{ab}	1.05	0.247

P - value indicated the effect of the blood sampling time on the parameter
^{ab}- different letters within the row indicate significant difference at the P<0.05 (Tukey Test). CON – Control group. DGP – Dried grape pomace group. SEM – Standard error of mean.

CONCLUSION

Dried grape pomace can be considered as an alternative source of nutrients for sows. No negative effects on the status of macroelements in blood serum of the experimental animals were found, with the level of dried grape pomace incorporation in the feed diet in an amount of 1%. The effect of feeding dried grape pomace on the mineral profile of sow's was statistically proven only for phosphorus. During the experiment, the chlorides level decreased in both groups, and thus reference values for this indicator were reached. However, further experiments are necessary to confirm the present results and try to determine the maximum incorporation rates for the dried grape pomace in balance feeds. The effect of feeding in other categories of animals should also be monitored.

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THE EFFECT OF FEED PARTICLE SIZE IN LAYING HENS ON THE DIGESTIVE TRACT MORPHOLOGY – A PRELIMINARY REPORT

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ABSTRACT

The influence of feed particle size on laying hens' parameters were evaluated. Laying hens of LOHMANN BROWN CLASSIC were divided into 2 different experimental groups – coarse group and fine group. The differences between the diets was in particle size. The structure of diets was evaluated by dry sieving using a separator Retch AS 200 Control. Geometric Mean Diameter (1,339.49 vs. 564.24, respectively) and Geometric Standard Deviation (1,369.11 vs. 393,56, respectively) of diets were calculated. The different feed mixtures (coarse and fine) feeding had no influence ($P > 0.05$) on live body weight, blood biochemical parameters, feed consumption and most of digestive tract section of lying hens. The statistically significant difference ($P < 0.05$) was found on the weight of proventriculus. The bigger proventriculus was found in fine group compared to coarse group.

Keywords: Lohman Brown Classic, Geometric Mean Diameter, Geometric Standard deviation, Retch AS 200 Control, proventriculus, gizzard, hammer mill

INTRODUCTION

The physical structure of feed mixture is determined by the size and shape of the particles of these compounds. Size can be defined as the average particle size distribution of individual components of feed or more simply as fineness of feed grinding (AMERAH *et al*., 2007). The specific size was described by WOLF *et al*. (2012) in table 1. According to SVIHUS (2011), poultry diets should contain at least 20% of cereal particles more than 1,500 to 2,000 µm or 30% of particles larger than 1,000 µm in size to stimulate foregut development and functionality.

Table 1. Particle size distribution (mm)

Designation	**Size of particles**
Coarse	> 1.4 mm
Medium	0.8 – 1.4
Fine	0.4 – 0.8
Very fine	< 0.4 mm

The particle size is usually determined by dry sieving of representative sample (BAKER and HERRMAN, 2002). The sample is sieving for 10 minutes on the set of sieves. The amount of particles retained on individual sieves is determined and calculate the GMD (Geometric Mean Diameter, also d_{gw}) and GSD (Geometric Standard Deviation, also S_{gw}) (LENTLE *et al*., 2006). GMD indicates the trough particle size (in mm or µm) and GSD indicates particle size uniformity.

The lower GSD means the higher uniformity of particles (AMERAH *et al*., 2007).

GMD and GSD can be calculated using these formulas (ASABE, 2008):

$$d_{gw} = log^{-1}\left[\frac{\sum_{i=1}^{n}(W_i \log \bar{d}_i)}{\sum_{i=1}^{n} W_i}\right]$$

$$S_{log} = \sqrt[2]{\left[\frac{\sum_{i=1}^{n} W_i(\log \bar{d}_i - \log d_{gw})^2}{\sum_{i=1}^{n} W_i}\right]} = \frac{S_{ln}}{2.3}$$

$$S_{gw} \approx \frac{1}{2} d_{gw}\left[10^{S_{log}} - \frac{1}{10^{S_{log}}}\right]$$

where:

d^i is nominal sieve aperture size of the i^{th} sieve, mm

d_{i+1} is nominal sieve aperture size in next larger than i^{th} sieve (just above in a set), mm

d_{gw} is geometric mean diameter or median size of particles by mass, mm
or is geometric mean diameter or median size of particles on i^{th} sieve, mm
or is $\sqrt[2]{d_i * d_{i+1}}$, which is $\bar{d}_i$

S_{log} is geometric standard deviation of log-normal distribution by mass in ten-based logarithm, dimensionless

S_{ln} is geometric standard deviation of log-normal distribution by mass in natural logarithm, dimensionless

S_{gw} is geometric standard deviation of particle diameter by mass, mm

W_i is mass on i^{th} sieve, g

n is namber of sieves + 1 (pan)

SAFAA *et al*. (2009) noticed the higher intake in laying hens of coarse feed mixture in comparison with fine feed mixture. AMERAH *et al*. (2007) found that broiler chickens fed a mixture of fine particles

of wheat had lower weight gains and feed intake than groups fed mixtures with a higher proportion of medium or coarse particles in diets.

Feed particle size may also influence the gastrointestinal tract. Several studies observed feeding with coarse mixture increase the relative gizzard weight (NIR *et al*., 1994 a, c; ENGBERG *et al*., 2002; PÉRON *et al*., 2005; AMERAH *et al*., 2007). A large, well-developed gizzard improves intestinal motility (FERKET, 2000) and increase level of cholecystokinin (SVIHUS *et al*., 2004). This stimulates secretion of pancreatic enzymes and gastroduodenal reflux (DUKE, 1992). EGE *et al*. (2019) found that the relative weight of the gizzard and pancreas in laying hens was higher when fed roughly ground cereals.

MATERIAL AND METHODS

Animals and experimental conditions

In 6 replicates, a total of 120 laying hens of LOHMANN BROWN CLASSIC aged 16 weeks were randomly divided into 2 different experimental groups (in total 60 hens per feeding group) with 10 birds per pen each representing a single experimental unit. The lighting program was set according to the technological instruction (LOHMANN TIERZUCHT, 2019). The temperature was maintained at 20 ± 2 °C during the entire trial. After adapting period (19 weeks of age) hens received two different experimental diets – the coarse and the fine diet. The feed intake of each groups was recorded daily. The laying hens were fed *ad libitum*. The body weight was regularly noticed. The experiment lasted for 5 weeks (from 19 until 24 weeks of hens age). At the end of trial, one hen aged 24 weeks, of each groups

was slaughtered and blood was collected and evaluated. Selected digestive tract section and liver were weighed.

Experimental diets

In the trial the commercial feed mixture was used. It was chosen one with the coarse particle size (coarse group). For to be sure to have the nutritional same diets differing only in particle size, the part of the bought feed mixture was ground on the hammer mill using the 3 mm sieve. This is how the finely ground feed mixture was formed (fine group). Both coarse and fine feed mixture comprised corn, wheat, calcium carbonate, soybean extracted meal (toasted), barley, dark distilled offal, peeled sunflower extracted meal, rapeseed extracted meal, peas, wheat bran, corn extract, vinasse, animal fat, vegetable fat and oil, sodium chloride and calcium dihydrogen phosphate. Chemical composition of used diets is shown in table 2.

Table 2. Chemical composition of used diets in dry matter

	Coarse	**Fine**
Crude protein (%)	16.37	16.84
Crude fibre (%)	3.46	3.48
Ether extract (%)	3.41	3.55
Ash (%)	14.58	14.78

The structure of diets was evaluated by dry sieving using a separator Retch AS 200 Control. A representative sample (100 g) of each diet was passed through the set of sieves with different mesh sizes (3 mm, 2 mm, 1.5 mm, 1 mm, 0.3 mm) for 10 min at an amplitude 1.8 mm/g. After the shaking process, the amount of particles retained on each screen was determined by subtracting the weight of the sieve

and the retained feed from the blank weight of the sieve. The GSD and GMD were calculated.

Sample collection

Blood was collected into heparinized tubes. It was centrifuged for 10 minutes at 3,000 rpm till 2 hours after collection. The separated blood plasma was frozen (-20 °C) until biochemical examination. The following parameters were determined using standardized biochemical methods using Erba Lachema (Czech Republic) commercial sets on the Ellipse automatic biochemical analyzer (AMS Spa, Italy) in blood plasma samples: Enzymes activity AST – aspartate aminotransferase (AST/GOT 500), GGT – gamma-glutamiltransferase (GGT 250), ALT – alanine aminotransferase (ALT/GPT 500), ALP – alkaline phosphate (ALP AMP 500), LD – lactate dehydrogenase (LDH-L 100). As other markers of hepatic metabolism, fat and nitrogen metabolism, as well as kidney activity, was determined concentrations of the total bilirubin – Bili (BIL T JG 350), TG triglycerides (TG 250), cholesterol (CHOL 250), urea (UREA, no. UR 107; Randox, United Kingdom), creatine kinase (CK – 100, no. 10004494 Erba Lachema, Czech Republic), creatinine (kreat – CREA 500, no: 1010227 Erba Lachema, Czech Republic), TP – total protein (TP 500) and albumin (Alb 500). Consequently, the content of globulins (TP minus albumin) and albumins to globulins ratio were calculated.

The digestive tract parts were weighed and calculated per kg of live weight.

Statistical analysis

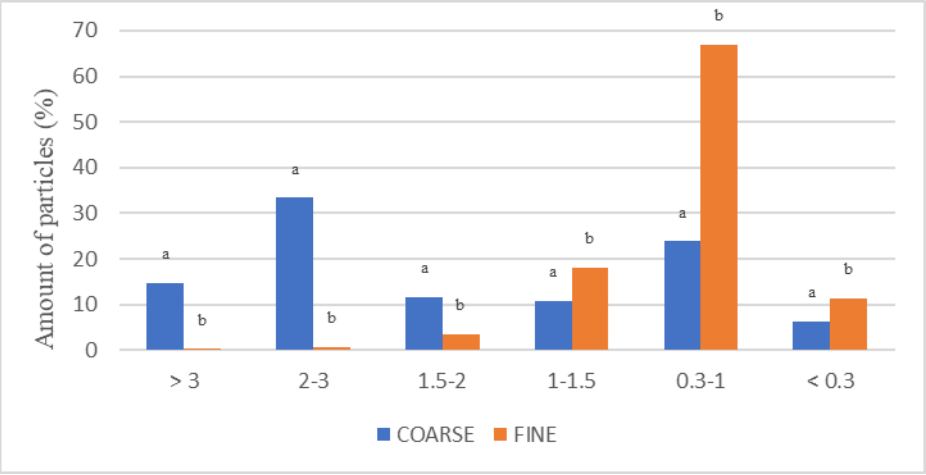
Data has been processed by Microsoft Excel (USA) and StatSoft Statistica (USA). It was used one-way analysis of variance (ANOVA).

For evaluate statistically differences between groups was used the Sheffé´s test and $P < 0.05$ was regarded a level of statistically significant difference.

RESULTS AND DISCUSSION

The differences between particle size distribution in coarse and fine mixture are shown in graph 1.

Graph 1. Particle size distribution



[a,b] means statistically significant differences ($P < 0.05$)

It is obvious that the coarse feed mixture contained almost 60% of particles bigger than 1.5 mm and about 30% of particles smaller than 1 mm. On the opposite, the fine feed mixture was mostly formed from particles not bigger than 1 mm, specifically 77%. Particles bigger than 1.5 mm was only about 4%. The statistically significant differences between coarse and fine feed mixture in amount of particles on sieves were found on each sieve ($P < 0.05$). Values for GMD and GSD were calculated based on the weight fraction on the individual sieves. Results are shown in table 3.

Table 3. GMD and GSD of diets

	n	**GMD (µm)**	**GSD (µm)**
		Mean ± SE	
Coarse	7	1,339.49 ± 21.74[a]	1,369.11[a]
Fine	7	564.24 ± 9.36[b]	394.56[b]

[a,b] means statistically significant differences ($P < 0.05$); n – number of cases; GMD – Geometric Mean Diameter; GSD – Geometric Standard Deviation; SE – standard error

Values of GMD correspond to values from other experiments dealing with the similar issue. For example, EGE *et al*. (2019) calculated values 707 vs. 1096 µm. There were found statistically significant differences between coarse and fine feed mixtures ($P < 0.05$). In case of GSD, which indicates the homogeneity of the mixture, there were found statistically significant differences as well ($P < 0.05$). The fine feed mixture had a lower GSD (394.56 µm) what means that fine feed mixture is more homogeneous than the coarse one (1,369.11 µm).

Table 4. Feed consumption

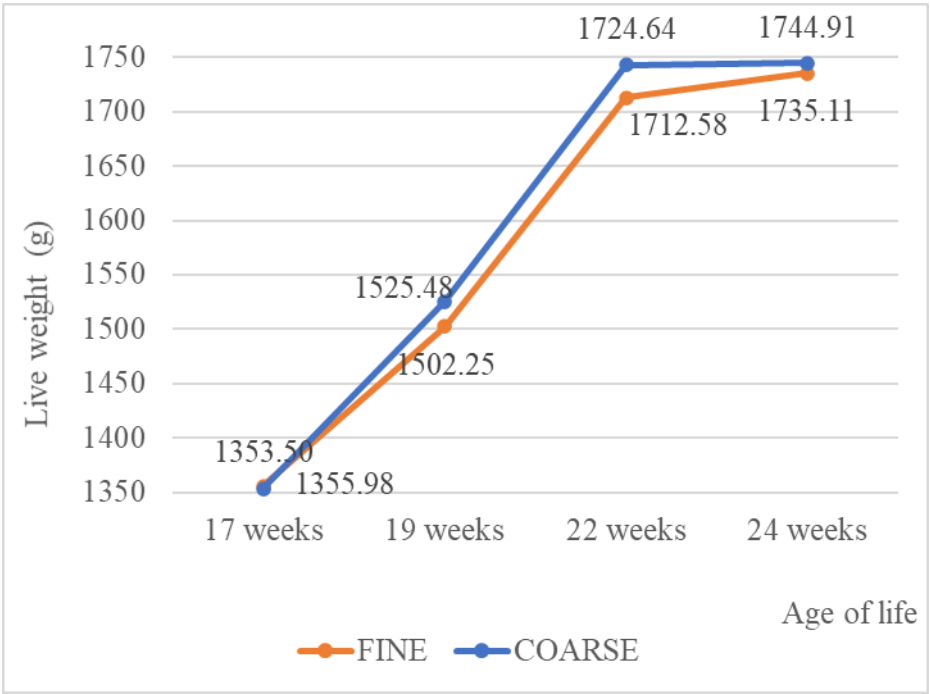
	n	**Feed consumption**	
		(g/bird/trial)	(g/bird/day)
		Mean ± SE	
Fine	6	3,435.52 ± 30.36	98.16 ± 0.87
Coarse	6	3,495.52 ± 24.23	99.87 ± 0.69

$P > 0.05$; n – number of cases; SE – standard error

The average daily feed intake corresponds to the instructions for LOHMANN BROWN CLASSIC which is at the beginning

of the laying 90-100 g (LOHMANN TIERZUCHT, 2019). There were found no statistically significant differences (P > 0.05).

Graph 2. Average live weight



P > 0.05

The technological instructions noticed that the average live body weight in 24 weeks should be 1,865 g (LOHMANN TIERZUCHT, 2019). In graph 2 is shown that hens in the trial had about more than 100 g lower weight. There were no statistically significant differences between the experimental groups.

The physical structure of the compound feed did not affect the blood biochemical parameters (P > 0.05). Results are shown in table 5. REZAEIPOUR and GAZANI (2014) who examined the structure of the mixtures for broilers also did not show changes in blood biochemical parameters.

Table 5. Blood biochemical parameters

	Coarse (n = 6)	**Fine** (n = 6)
	Mean ± SE	
ALT (µkat/l)	0.18 ± 0.02	0.16 ± 0.01
AST (µkat/l)	3.05 ± 0.03	2.92 ± 0.37
GGT (µkat/l)	0.83 ± 0.02	0.73 ± 0.01
ALP (µkat/l)	4.81 ± 1.42	6.97 ± 2.54
LD (µkat/l)	62.23 ± 11.90	54.95 ± 5.51
CK (µkat/l)	97.13 ± 19.45	92.03 ± 23.48
TB (µmol/l)	7.88 ± 1.23	6.98 ± 1.61
Glu (mmol/l)	9.10 ± 1.38	10.55 ± 1.02
Urea (mmol/l)	2.76 ± 1.36	3.02 ± 2.25
Creat (µmol/l)	31.88 ± 3.21	33.83 ± 3.36
TG (mmol/l)	15.25 ± 2.08	15.15 ± 1.58
Chol (mmol/l)	3.266 ± 1.02	3.10 ± 0.73
TP (g/l)	57.52 ± 4.31	54.87 ± 2.83
Alb (g/l)	26.71 ± 1.42	26.04 ± 0.93
A/G	0.87 ± 0.08	0.91 ± 0.06

P > 0.05; n – number of cases; SE – standard error; ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; GGT – Gamma-glutamyltransferase; ALP – Alkaline phosphatase; LD – Lactate dehydrogenase; CK – Creatine kinase; TB – Total bilirubin; Creat – Creatinine; TG – Triglycerides; Chol – cholesterol; P – phosphorus; Ca – calcium; TP – Total protein; Alb – albumin; A/G – Albumin/Globulin

The physical structure of the compound feed did not affect the blood biochemical parameters (P > 0.05). REZAEIPOUR and GAZANI (2014) who examined the structure of the mixtures in broilers also did not show changes in blood biochemical parameters.

Table 6. Weight of chosen parts of digestive tract and liver

	Coarse	**Fine**
n	6	6
(g/kg of BW)	Mean ± SE	
Crop	3.43 ± 0.64	3.57 ± 0.33
Proventriculus	3.38 ± 0.16[a]	3.94 ± 0.48[b]
Gizzard	18.07 ± 2.16	19.54 ± 1.88
Duodenum	5.62 ± 0.53	5.84 ± 0.42
Jejunum	9.69 ± 1.17	9.48 ± 0.88
Ileum	8.31 ± 0.83	8.03 ± 1.41
Colon	3.08 ± 0.49	2.65 ± 0.48
Cecum	3.66 ± 0.95	4.07 ± 0.31
Liver	21.15 ± 1.86	21.85 ± 0.87

[a,b] means statistically significant differences (P < 0.05); n – number of cases; SE – standard error; BW – body weight

According to the table 6, the influence of particle size had an effect (P < 0.05) on weight of proventriculus. Other examined digestive tract parts were without significant changes. NIR *et al*. (1994 a, b) found that larger particles in compound feed for poultry positively affects the development of the muscular stomach, especially its weight. In these experiments found out that groups of poultry fed coarse and intermedium coarse physical structure of the feed mixture had the gizzard about 26% and about 41%, respectively larger.

CONCLUSION

The influence of feed particle size on laying hens' parameters were evaluated. The diets with statistically significant different particle size (coarse and fine) had no influence ($P > 0.05$) on live weight, biochemical blood parameters, feed consumption and most of the parts of digestive tract of lying hens. The statistically significant ($P < 0.05$) higher weight of proventriculus was found in fine group of hens compared to coarse group. It can be concluded despite the different structure of feed mixtures, no effect on the monitored markers was recorded before the laying hens peak.

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THE USE OF HUMIC ACIDS IN BROILER DIET AND EFFECT ON NUTRITION AND MEAT QUALITY

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ABSTRACT

The aim of this study was to investigate the effect of the supplementation of humic substances on live and carcass yield, chemical composition, physicochemical parameters of breast meat of broiler COBB 500. Broiler chickens (90) were divided into 3 groups (30pcs). Control group was fed only with commercial diet without any supplements. Experimental group 1 was supplemented with 0.5 % Humac Natur Monogastric (HNM) and experimental group 2 with 0.7 % Humac Natur (HN). Our results suggest that addition of humic acids into broilers diets increased and significantly influenced live weight ($p < 0.001$) and carcass weight ($p < 0.01$). Supplementation of humic acids had significant positive effects on ageing processes in breast muscles, however don´t have significant effect on chemical composition of breast meat.

Keywords: humic substances, broiler, nutrition, breast muscle

INTRODUCTION

In livestock farming, nutrition is an important factor in achieving economic efficiency of breeding and the quality of the resulting products. Different types of additives substances used in animal nutrition can contribute to a more rational use of the feed ration, improving production parameters and often to positively affect the quality of the resulting animal products (Esenbuğa et al., 2008).

As one of the alternative of feed additives are humic acids. Humic substances are the result of the decomposition of organic matter, especially plants, and are naturally found in water, soil or brown coal. Humic substances are the most common natural complexing ligands found in nature. They are among the strongest chelating agents among natural organic substances. Humic substances can be divided into three components: fulvic acids, humic acids and humin. The most important and most researched part of humic substances is humic acids. They form a group of similar aromatic polyfunctional compounds, brown color, medium to high molecular weight, which are soluble in alkaline solvents (Trckova et al., 2005). At present, there is insufficient evidence of the mechanism by which humic substances promote growth. They are thought to increase the utilization of nitrogen, phosphorus and other nutrients due to their chelating properties. Supplementation of diets for poultry with humic substances leads to improved production indicators. They have a positive effect on poultry growth, feed conversion, egg production, shell strength and reduce farm mortality. Due to their chemical composition such as proteins, vitamins, antioxidants, digestive components, water solubility, content

of antibacterial, antiviral and antifungal substances and substances stimulating immunity, humic acids are commonly used in poultry industry. Humic substances lower the pH value of the intestine, affect the contractile activity of smooth muscle, improve nutrient utilization and feed conversion, increase the permeability of cell membranes and thus facilitate the transport of minerals from the blood to the cells metabolites. It also helps stop excessive water loss through the intestine (Aristimunha et al., 2020).

MATERIAL AND METHODS

In the experiment, 90 broiler chicks COBB 500 (Mach Drubež Ltd. Litomyšl, Czech Republic) at the age of one day, were randomly divided into a control (30pcs) and 2 experimental groups (60 pcs). The broilers were fed with commercial feed mixture BR1 - starter diet for fattening broilers within 10 days of age, BR2 - diet for growing from day 11 to day 30 and BR3 - final fed mixture (Agrocass plus, Ltd. Čaňa, Slovak Republic) from day 31 to 37 (Table 1).

Table 1. Chemical analysis (as dry matter basis) of diets

Diet	BR1	BR2	BR3
CP, g/kg	254.61	222.16	209.14
CFa, g/kg	56.24	55.17	60.09
CF, g/kg	38.20	37.98	36.62
Starch, g/kg	454.38	497.13	504.23
Ca, g/kg	11.43	9.84	9.05
P, g/kg	6.52	5.89	5.59
ME, MJ/kg	14.16	14.38	14.49

CP - crude protein; CFa – crude fat; CF - crude fiber; ME – metabolizable energy

The control group (C) was fed with standard feed mixtures without any supplement. The experimental group 1 (HNM) was supplemented with 0.5 % Humac Natur Monogastric and the experimental group 2 (HN) was added with 0.7 % Humac Natur (Humac Ltd., Slovak Republic). The characteristics of the applied supplements were the following: Humac Natur (content of humic acids min. 65% in dry matter (DM); fulvic acids min. 5% in DM; minerals and microelements: Ca 42.28, Mg 5.11, Fe 19.05 g/kg; Cu 15, Zn 37, Mn 142, Co 1.24, Se 1.67 as well as Mo 2.7mg/kg DM); Humac Natur Monogastric (content of humic acids min. 60% in DM; fulvic acids min. 5% in DM; formic acid 3.24% in DM; complex of minerals and microelements).

During fattening chickens have access to water and feed *ad libitum.* The broiler chicks were reared on deep litter and microclimatic conditions complied with the requirements for fattening of broilers. Broilers were reared under a conventional temperature regiment at 21 ^{0}C. The relative humidity was maintained between 60 – 70 %.

After the end of the fattening, the average weight of the chickens were calculated. To determine the yield of chickens, the broilers were weighed before and after slaughter. Carcass weight was determined as a percentage of the body weight of the broilers after slaughtering and weighing before slaughter. To determine the percentage of breast muscle to the total weight of the carcass, these parts were separated from the body and then weighted. The percentage of breast muscle in the carcass was calculated as the weight of the individual parts and the body weight after the slaughter.

After fattening, the animals were stunned and killed by cervical dislocation. Breast muscle samples were taken for further physico –

chemical analysis. AOAC (1990) methods were used for moisture, dry matter and fat content of breast samples.

The pH values of breast muscles and the concentrations of lactic acid and phosphates were measured at 24 hours after killing and on 3rd a 7th day after killing. The samples were stored at 4 ºC until use. The pH values of meat samples were analyzed with a digital pH meter (inoLab pH720, WTW, Weilheim, Germany) with glass electrode in the water extract of the meat. The concentrations of lactic acid and phosphates were analyzed by use Electrophoretic analyzer EA 102, with conductive detector (Villa Labeco, Slovak Republic). Results were gradually processed by ITPPro 32 (Kas-Comp, Bratislava, Slovakia).

The results of the experiment were statistically analyzed using Graph Pad Prism 5. One-way ANOVA test (Tukey comparison, $p < 0.05$) was used to compare individual results between groups.

RESULTS AND DISCUSSION

In our study, the live body weight of broilers (g) were higher in both experimental groups than in control group (Table 2). There were statistically significant increase ($p > 0.001$) in live weight in experimental group HN compared to C group. There were also noted statistically significant increase ($p > 0.01$) in HN group compared to HNM group. According Skalická and Koréneková (2016), the live body weight in broilers were not affected by sodium humate. Similar results were found by Marcinčáková et al., (2015), using humic acid supplementation. On the other hand, Pistová et al. (2016) observed positive effect of humic substances and garlic on live body weight of broiler chicken.

There was found statistically significant increase in carcass weight in C group ($p < 0.01$) and HNM group ($p < 0.05$) compared to HN group. Significant increase ($p < 0.01$) in weight of breast muscle was also recorded in the HN group compared to the control group.

Table 2. Weights of carcass and body parts

	C	HNM	HN	P values
Live weight (g)	2309.3±90[b]	2357.5±118.8[b]	2524.5±148[a]	P< 0.001
Carcass weight (g)	1698.8±71,4[b]	1766.5±133.9[b]	1894.4±142.8[a]	P< 0.01
Carcass yield (%)	73.6±1.6	74.9±2.8	75.0±1.9	P > 0.05
Breast (g)	519.1±49.3[b]	506.5±67.5[b]	604.2±66.1[a]	P < 0.01
Breast yield (%)	30.6±2.9	28.6±2.8 [a]	32.0±3.5[b]	P > 0.05

a, b – values are statistically significant

Table 3, indicated, that feed supplementation used in this study had effect on the decrease of content fat in both experimental groups HN and HNM and increase the content of proteins in group HN in meat.

Table 3. The results of chemical composition of breast meat (%)

	C	HNM	HN	P value
Dry matter	25.46±0.11	26.09±0.34	25.31±0.54	P > 0.05
Fat	2.94±0.1	2.41±0.86	2.28±0.01	P > 0.05
Water	74.2±0.38	4.04±0.25	75.00±0.05	P > 0.05
Proteins	21.48±0.1	21.25±0.60	22.03±0.52	P > 0.05

The results of physicochemical parameters like pH values, concentration of lactic acid and phosphates during *post-mortem* processes in chicken breast muscle are given in Table 4. The dynamics of lactic acid over the entire time interval of the maturing process in meat reflects the quantitative conversion of glycogen to lactic acid. It is

lactic acid that greatly affects the pH values of meat (Scheffler et al. 2013). There were observed not only in the concentration of lactic acid, phosphates and pH, but also in the dynamics of these parameters.

Table 4. The results of physicochemical analysis of breast meat

	Day	C	HNM	HN	P value
	1	1.17 ± 0.42 $^{1.2}$	1.34± 0.16 ^{1}	1.59 ± 0.33 $^{1.2}$	P > 0.05
LA	3	1.58 ± 0.11 $^{a\ 1}$	1.76 ± 0.20 $^{ab\ 2}$	1.93 ± 0.32 $^{b\ 1}$	P > 0.05
	7	1.04± 0.21 $^{a\ 2}$	1.58 ± 0.15 $^{b\ 1,2}$	1.36 ± 0.36 $^{ab\ 2}$	P < 0,01
	1	0.83± 0.36 ^{a}	1.20 ± 0.09 ^{ab}	1.47 ± 0.34 $^{b\ 1}$	P < 0.01
PO	3	0.82 ± 0.15 ^{a}	1.18 ± 0.11 ^{b}	1.23 ± 0.27 $^{b\ 1.2}$	P < 0.01
	7	0.61 ± 0.15 ^{a}	1.14 ± 0.19 ^{b}	1.01 ± 0.24 $^{b\ 2}$	P < 0.001
	1	5.96 ± 0.07 ^{a}	5.81 ± 0.08 ^{b}	5.80 ± 0.09 ^{b}	P < 0.01
pH	3	5.91 ± 0.12 ^{a}	5.71 ± 0.08 ^{b}	5.75 ± 0.11 ^{b}	P < 0.05
	7	5.96 ± 0.08 ^{a}	5.79 ± 0.12 ^{b}	5.79 ± 0.07 ^{b}	P < 0.05

The values in the rows with different designations (a, b, c) and the values in the columns with different designations (1,2,3) are statistically different at the significance levels $p \leq 0.05$. The rows compare the individual parameters (especially lactic acid (LA), phosphates (PO), pH values (pH) between the control group (C) and the experimental groups (HNM 0.5% and HN 0.7%). compared values between 1st, 3rd and 7th day.

The concentration of lactic acid increased in the breast muscle the control group during the first three days. Statistically significant decrease ($p < 0.05$) was observed on day 7 after killing of broilers. The concentration of phosphates gradually decreased and did not change statistically significantly. The pH values decreased slowly during the first three days and then increased again to the original value on day 7. The dynamics of the concentrations lactic acid, phosphates

and pH values in the experimental groups had a similar course as in the control group. In the experimental group (HNM) with the addition of 0.5% humic acids a significant increase ($p < 0.01$) in the concentrations of lactic acid was observed on day 3 after killing.

Within 24 hours after killing, we did not observed statistically significant intergroup differences in meat concentrations. The highest concentrations of lactic acid were analysed in the experimental group HN on day 3 of the storage meat. On this day, statistically significant increase ($p > 0.05$) were noted in experimental group HN compared to C group. Statistically significant increase ($p>0.01$) were also recorded on day 7 in the experimental group HNM in compare with compared to C group. The phosphate content was highest in the experimental group HN, at the time 24 hours after killing. There were found statistically significant increase ($p > 0.01$) in HN group compared to the C group. Statistically significant increase were noted on day 3 ($p < 0.01$) and on day 7 ($p > 0.001$) in group HNM compared to C group. There were also recorded statistically significant increase on day 3 ($p < 0.01$) and on day 7 ($p > 0.001$) in HN group compared to C group. The pH vales was the lowest 3th day *post mortem* in HNM group. There were found statistically significant decrease in pH values on day 1 ($p < 0.01$), day 3 ($p < 0.05$) and 7 ($p < 0.05$) in HNM group compared to C group. There were recorded also statistically significant decrease in pH values on day 1 ($p < 0.01$), day 3 ($p < 0.05$) and day 7 ($p < 0.05$) in HN group compared to C group.

CONCLUSION

Based on the results of the experiment, it is possible to reveal, that the application of humic acid as a supplement in the harvested broiler

chickens of the Cobb 500 breed has its justification in combination with complete ingredients. Addition of humic substances significantly positively affected on live/carcass weight of broilers and also physicochemical changes of breast muscle during process of maturing.

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DIGESTIBILITY OF ORGANIC MATTER IN SELECTED VARIETIES OF SORGHUM

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ABSTRACT

The alternation of warm and dry periods results in slowing down of vegetation growth, drying of soils, which leads to high losses of plant production. Consequently, it is so difficult to ensure sufficient production of the required amount of feed. One of the many alternatives is to use plants tolerating dry conditions, such as sorghum in the sowing procedures. Sorghum can be considered as an alternative, complementary and drought-resistant feed. The practical part of the article is focused on the comparison of 8 varieties of sorghum, which were determined for digestibility parameter of organic matter *in vitro* pepsin by cellulase method. From our achieved results, we evaluated that the highest digestibility of organic matter had Nutri Honey BMR and Sweet Caroline (49.99%). These 2 varieties were recommended as a suitable feed supplement for animal nutrition.

Keywords: sorghum, nutritional value, *in vitro* method, organic matter digestibility

INTRODUCTION

In terms of worldwide production, sorghum is the fifth most widespread cereal in the world just behind wheat, corn, rice and barley (Ratnavathi *et al*., 2012). In the arid and semi-arid regions of the world, sorghum is the leading cereal. Sorghum plants belong to the C4 type of plants, especially adapted to drought-prone areas with hot semi-arid conditions (Vittal *et al*., 2010). According to Doležal *et al*. (2009) traditional varieties are replaced by new hybrids showing more suitable agrotechnical and nutritional properties.

Based on the digestibility of organic matter in the later stages of plant development, forage varieties of sorghum can be divided into traditional varieties and BMR (Brown Mid Rib) varieties. BMR varieties have a reduced lignin content in their cell walls and thus they are characterized by increased nutrient digestibility in later vegetation stages. BMR forms are created by intensive breeding of Sudanense grass or *Sorghum bicolor* x *Sorghum sudanense* hybrids (Hermuth *et al*., 2012).

MATERIAL AND METHODS

Experimental location characteristic

Samples of sorghum were grown in two experimental localities Obora and Písky, which are located in field station in Žabčice in the

South Moravian Region. The average annual rainfall is 480 mm and the average temperature of this area is 9.2 °C. The average time of sunshine is 1,800–2,000 hours per year. Obora is characterized by heavier soils with higher soil moisture, which is caused by high groundwater levels. On the other hand, soil in Písky is characterized by lighter sandy and dry soils.

Sorghum hybrids and varieties

The following sorghum varieties were used for the experiment: *Sorghum bicolor* x *Sorghum sudanense* (KWS Freya, Nutri Honey, Nutri Honey BMR), *Sorghum bicolor* x *Sorghum bicolor* (KWS Tarzan, Ruzrok). Sorghum grains varieties included Sweet Susana, Sweet Caroline and Express. In this experiment there were tested 8 varieties of sorghum. Seeds of these varieties were provided by SEED SERVICE and KWS companies.

Experimental design and used laboratory methods

The harvest date was determined based on the value of the average dry matter from partial samples of plants (28% of dry matter and above). Samples of the monitored varieties were harvested and grinded. This individual samples were pre-dried in dryer (65 °C for 24 hours). Then these samples were analysed for *in vitro* organic matter digestibility (OMD) by using the pepsin cellulase method with Daisy incubator.

The principle of digestibility of organic matter of feed by using *in vitro* pepsin cellulase method is incubation of the sample in an acidic

solution of pepsin, hydrolysis of starch at elevated temperature and subsequent incubation in buffered solution of cellulose.

1.5 L of pepsin solution heated on 40 °C was added into heated incubation bottle containing the samples in a Daisy incubator. Then the incubation process was done (24 hours). After that the starch was removed by putting incubation bottle into water bath (80 °C, 30 minutes). Subsequently, the solution in bottle was removed and the samples were washed 3x times with distilled water. Then the samples in incubation bottles were put in the Daisy incubator. After the incubation, 1.5 L of cellulose solution was added into these bottles and incubated again (24 hours). Then the samples were rinsed with distilled water until the water was clear. The excess of water in these samples was removed using filter paper and then dried in an oven at 103 °C to constant weight. They were the cooled in a desiccator and then weighed.

Statistical analyses

The results of the experiment were evaluated using Microsoft Excel and STATISTICA 12. Gathered values were tested using by analyses of variance (ANOVA) and post-hoc Sheffé´s test, where $P < 0.05$ was regarded as statistically significant difference.

RESULTS AND DISCUSSION

Nutri Honey BMR and Sweet Caroline varieties had the highest digestibility of organic matter (49.99%) see Table 1. The lowest ($P < 0.05$) digestibility of organic matter was observed in Ruzrok variety

with value 36.18%. Digestibility of organic matter for the rest of the tested sorghum variates was above 40% except KWS Freya, where the digestibility was moving around 39.33%. The average digestibility of sorghum organic matter according to Přikryl (2014) should be in the range of 45 – 60%. Our results in Express, Nutri Honey BMR, Sweet Caroline and Sweet Susana varieties are agreement with Přikryl (2014). The Ruzrok variety has been shown much lower digestibility of organic matter. In comparison with corn, the value of digestibility of organic matter is around 66 – 72%. This is also confirmed by Phipps and Wilkinson (1985) who reported corn digestibility of organic matter about 72%, depending on the used hybrid. Gurbuz and Davies (2010) also found that sorghum grains with lower levels of condensed tannins have higher digestibility of organic matter.

Table 1 Achieved values of digestibility of organic matter for individual varieties

Varieties	Digestibility of organic matter		
	n	Mean	Standard deviation
Express	14	47.16 ^{bcd}	8.075
KWS Freya	17	39.33 ^{ab}	6.675
KWS Tarzan	20	41.65 ^{abe}	3.824
Nutri Honey	16	44.19 ^{bcd}	3.363
Nutri Honey BMR	14	49.99 ^{cd}	5.428
Ruzrok	18	36.18 ^{a}	7.694
Sweet Caroline	14	49.99 ^{d}	5.001
Sweet Susana	19	48.14 ^{cde}	5.413

$^{a, b, c, d, e}$ – different letters in row means statistically significant differences ($P<0.05$)

CONCLUSION

A statistically significant difference ($P < 0.05$) was found among selected varieties in digestibility of organic matter *in vitro* by pepsin cellulase method. The highest digestibility of organic matter was achieved with the varieties Nutri Honey BMR (49.99%) and Sweet Caroline with the same potential. The lowest value of digestibility was observed in the Ruzrok variety, where the digestibility of organic matter was 36.18%.

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MONITORING THE EFFECT OF DIFFERENT CALCIUM LEVELS ON ORGANISM OF HENS IN THE FIRST LAYING PERIOD

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ABSTRACT

The aim of the study was to evaluate some aspects of calcium metabolism in hens in the beginning of laying period. The study was conducted on 38 hen's hybrid Lohman Brown Classic. Birds were kept in individual cages and divided to two groups of 19 hens. Hens were fed by standard feeding mixture for hens with different contain of calcium (2,1% and 3,7% - L and H, respectively) and with 0.5 percent chromium oxide added as an indigestible indicator. The experimental period lasted from 17th to 24th weeks of age. A statistically significant difference ($P < 0.05$) was also recorded in the weight of the animals. Hens with 2.1% Ca in diet had higher live weight at the end of monitored period. In the monitored blood parameters was the statistically significant different ($P < 0.05$) between L group and H group in the ALP parameter. Egg production was monitored daily and egg quality was analyzed once per week. The statistically significant difference ($P < 0.05$) between L group and H group was recorded for eggshell weight, % shell and shell thickness.

Keywords: eggshell, calcium, hens, egg quality

INTRODUCTION

Calcium is one of the main nutrients for laying hens. Two to three grams are exuded during shell calcification per day. Transport of calcium is mediated by vitamin D_3. Hormonal form of vitamin D_3 [1.25 (OH) $2D_3$] regulates biosynthesis of Ca-binding protein. The organism adapts to different calcium supply ratio with delay (Kleyn, 2013). Calcium has many important roles in the body. It participates in bone mineralization, maintaining acid-base balance, blood coagulation, co-ordinating neuromuscular irritation, and plays an important role in the formation of shells and consequently influencing their quality (Kleyn, 2013, Zelenka, 2017). The quality of the shells is very important because it significantly affects the economic side of production (Hamilton et al., 1979; Dunn et al., 2011). It is up to 99% present in bone tissue (Kleyn, 2013, Zelenka, 2017). Calcium is most concentrated in the palisade layer in eggs (Arpášová et al., 2010). The organism adapts, with some delay, to the different calcium supply in the ration (Zelenka, 2014).

MATERIAL AND METHODS

Thirty-eight hen's hybrid Lohman brown classic were divided into 2 groups. The first group was fed by standard feeding mixture for hens with 2.1 % contant of calcium (L). The second group received diet contain more calcium than first group, specifically 3.7% (H). Feed mixture was granulated. Animals had free access to feed and water. The experimental period lasted from 17^{th} to 24^{th} weeks of age.. Feed consumption was evaluated daily. Hens were kept in individual balance

cages. Room temperature, humidity and lighting was set and controlled by technological instructions Lohman brown classic (Lohmann, 2013). Health status was evaluated daily during the trial. The animals were weighed individually once a week.

Egg production and individual weight were monitored and individual eggs were analyzed once a week. Were evaluated the qualitative parameters of the eggs - eggs strength, shell weight, % shell, shell thickness. The egg weight and shell weight were determined using a laboratory scale. Egg shell strength was determined by machine Egg Force Reader. The eggshell thickness was monitored in 3 places: in the center of the eggshell and on the blunt and sharp of the peak shell. From this data was calculated arithmetic mean. Six birds were randomly selected from each group to receive blood from the right-wing vein for monitored some blood parameters at 24 weeks of hens age.

Blood was collected into heparinized tubes. It was centrifuged for 10 minutes at 3,000 rpm till 2 hours after collection. The separated blood plasma was frozen (-20 °C) until biochemical examination. The following parameters were determined using standardized biochemical methods using Erba Lachema (Czech Republic) commercial sets on the Ellipse automatic biochemical analyzer (AMS Spa, Italy) in blood plasma samples: Enzymes activity AST – aspartate aminotransferase (AST/GOT 500), GGT – gamma-glutamiltranserase (GGT 250), ALT – alanine aminotransferase (ALT/GPT 500), ALP – alkaline phosphate (ALP AMP 500), LD – lactate dehydrogenase (LDH-L 100). As other markers of hepatic metabolism, fat and nitrogen metabolism, as well as kidney activity, was determined concentrations of the total bilirubin – Bili (BIL T JG 350), TG triglycerides (TG 250), cholesterol (CHOL

250), urea (UREA, no. UR 107;Randox, United Kingdom), creatine kinase (CK – 100, no. 10004494 Erba Lachema, Czech Republic), creatinine (kreat – CREA 500, no: 1010227 Erba Lachema, Czech Republic), TP – total protein (TP 500) and albumin (Alb 500). Consequently, the content of globulins (TP minus albumin) and albumins to globulins ratio were calculated.

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). Was used one-way analysis ov variance (ANOVA). To ensure evidential differences Scheffé´s test was applied and $P < 0.05$ as regarded as statistically significant difference.

RESULTS AND DISCUSSION

The average feed consumption ranged from 4.34 kg (H) to 4.85 kg (L) without statistical significant difference ($P > 0.05$) between group. The same result without a statistically significant difference in feed consumption between groups with different calcium levels is reported in study by Atteh and Leeson (1982). Many authors study different levels of calcium (Alone, 1969, Hurwitz and Bar, 1965; Gilbert et al., 1981; Clunies et al., 1992).

Table 1. Weight of hens

Group	L		H	
Week of age	n=19	Mean ± standard error	n=19	
18	1.455 ±	0.0253	1.412 ±	0.0247
19	1.590 ±	0.0296	1.508 ±	0.0293
20	1.687 ±	0.0275 [a]	1.596 ±	0.0268 [b]
21	1.731 ±	0.0275 [a]	1.637 ±	0.0268 [b]
22	1.702 ±	0.0480	1.661 ±	0.0308
23	1.756 ±	0.0320 [a]	1.651 ±	0.0285 [b]
24	1.791 ±	0.0327 [a]	1.704 ±	0.0260 [b]

[a,b] – different letters on one line - statistically significant differences ($P < 0.05$). n means number of cases.

Table 1 show average weight of hen´s. Statistically significant difference (P <0.05) between group L and group H are recorded in the twentyeth, twenty-first, twenty-third and twenty-fourth week of age, , hens. The weights of the hens ranged from 1.455 kg to 1.791 kg in group L and from 1.412 kg to 1.704 kg in group H. There were not statistically significant differences between the groups in the number of eggs laid or in the weight of individual eggs. Evaluate of the optimal calcium level in the feed ration for laying hens is very important because, for example Reddy *et al*. (1968) wrote that dietary calcium levels greater than 3.85% appeared to be detrimental to egg production and next authors Herbert *et al*. (1977) reported that 4.05% dietary calcium reduced the performance of laying hens. In our study was the average egg laying for group L was 24 eggs with an average weight of 52 g and for group H 23 with an average weight of 49 g without statistically significant. The same result is result by Clunies et al. (1992) which wrote that calcium levels (2.5, 3.5 and 4.5%) hadnt significant effect on egg production or egg weight in their trail. Statistically significant difference ($P < 0.05$) between the groups we can see in Table 3 for the evaluated parameters - shell weight, % shell and shell thickness.

Table 3. Qualitative parameters of eegs

Group; n = 19	L		H	
Parameters	Mean ± standard error			
Strength (N)	38.545 ± 0.5260		38.748 ± 0.5806	
shell weight (g)	5.025 ± 0.0587	a	5.237 ± 0.0685	b
% shell	9.749 ± 0.1107	a	10.564 ± 0.1116	b
shell thickness (mm)	0.371 ± 0.0037	a	0.394 ± 0.0029	b

[a,b] – different letters on one line - statistically significant differences ($P < 0.05$). n means number of cases.

We evaluated these parameters, because they are important for quality eggs, for example the shell strength is one of the most important qualities of shell. Bain (1997) describes that the calcium content of the ration and its retention affects egg quality. In Table 4 we can see a tendency for higher content of calcium in plasma of group H, which was fed a mixture with higher calcium levels but without statistically significant difference ($P > 0.05$) opposite to Pavlík, Lichovníková and Jelínek wrote, that plasma calcium was significantly increased from the beginning to the end of their study.

Atteh and Leeson (1982) describes significantly ($P < 0.01$) increased with increasing dietary calcium and significantly decreased with increasing dietary magnesium content in their study. See Table 4. Statistically significant differences ($P < 0.05$) between group L and H was found in the ALP parameter in our study.

Table 4. Blood biochemical parameters (n = 6).

Group	L		H	
Parameters	Mean ± standard error			
AST	2.700 ± 0.1382		2.642 ± 0.0552	
GGT	0.100 ± 0.0183		0.133 ± 0.0201	
ALP	23.127 ± 4.8799	a	6.133 ± 1.2233	b
ALT	0.252 ± 0.1126		0.155 ± 0.0249	
LD	3.480 ± 0.4038		2.880 ± 0.0720	
CK	17.392 ± 3.6696		13.822 ± 2.1485	
Tbili	8.833 ± 3.8002		5.567 ± 0.7940	
Glu	15.010 ± 0.3931		15.340 ± 15.3400	
Chol	5.693 ± 0.7365		5.205 ± 0.2751	
TG	15.185 ± 2.6195		16.593 ± 0.6923	
TP	50.483 ± 2.5804		52.250 ± 1.1732	
Alb	24.967 ± 0.8500		22.417 ± 1.5523	
Glob	25.517 ± 2.2045		29.833 ± 1.3601	
Alb/Glob	1.026 ± 0.1154		0.769 ± 0.0835	
Urea	1.155 ± 0.1491		1.215 ± 0.0718	
Kreat	39.733 ± 3.6150		42.817 ± 2.0122	
UA	434.100 ± 62.3262		575.817 ± 69.0918	
Ca	2.723 ± 0.2564		3.108 ± 0.2286	
Mg	1.233 ± 0.0957		1.285 ± 0.0779	

[a,b] – different letters on one line - statistically significant differences ($P < 0.05$). n means number of cases. ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; GGT – Gamma-glutamyltransferase; ALP – Alkaline phosphatase; LD – Lactate dehydrogenase; CK – Creatine kinase; TB – Total bilirubin; Creat – Creatinine; TG – Triglycerides; Chol – cholesterol; P – phosphorus; Ca – calcium; TP – Total protein; Alb – albumin; A/G – Albumin/Globulin.

Alkaline phosphatase (ALP) has activity in the blood serum represents isoenzymes from several tissues Fishman and Ghosh, (1967) and Posen (1967) wrote that bone, intestine, and liver are considered the sources of the majority of normal serum ALP activity animals and humans. From the Table 4 we can see that higher values are in group with low contain calcium, which identically describes Hurwitz and Griminger (1961) in their study, that that plasma ALP activity increased when the diet was deficient in calcium. The same result

Garlich (1973) achieved in his study, when the ALP activity of the experimental group increased in response to calcium deficiency in the feed mixture. Harr (2002) wrote that increases in plasma ALP activity to be specific for osteoblasticactivity. Accord to Jiang *et al*. (2013) showed a negative correlation between ALP activity and bone stiffness.

CONCLUSION

It is important to perform studies on appropriate calcium levels in the feed ration of laying hens, as calcium levels affect both egg production and hen health. Calcium levels affect the serum ALP content of laying hens and calcium metabolism and affect egg quality and laying hen strength egg-shell in our study.

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INCREASING THE NUTRITIONAL VALUE OF EGG YOLK FAT USING LUPIN MEAL IN LAYING HEN DIET

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ABSTRACT

The aim of the experiment was to find out how the content of lupin meal in the diet for the nutrition of laying hens affects the quality of fat in egg yolk. The results of the experiments using lupin seeds (unpeeled and peeled) with the substitution of 50% and 100% soybean extracted meal in compound feeds confirmed the positive effect of lupin meal-based diets on the egg yolk fat composition. Although the diets in the experimental groups of laying hens did not affect the fat content in the egg yolk, there were qualitative changes in its quality demonstrated during laying. These changes in egg yolk fat, compared to the control, were characterized by a decrease in saturated fatty acids (SFA) in the experimental groups, in some groups by a slight decrease in monounsaturated fatty acids (MUFA) and a significant increase in polyunsaturated fatty acids from omega 6 and omega 3 groups. It can

be concluded based on the results that the use of lupin meal in compound feeds for laying hens increases the quality of eggs produced.

Keywords: laying hens; protein feed; quality of food of animal origin; fatty acids; MUFA; PUFA

INTRODUCTION

Current trends in the European Union in agricultural production are focused on the production of protein feed. The important reason is to reduce the import of expensive soybean products, which are practically an integral part of all compound feed for livestock nutrition. One of the ways to increase the production of protein feeds is to increase the cultivation of legumes.

Legume seeds are a rich source of protein. Some species, such as lupins, have a comparable or even higher protein content than soybeans (Straková et al., 2006; Saastamoinen et al., 2013; Suchý et al., 2016). From this point of view, seeds of some varieties from the group of white and yellow cultivated lupins seem to be promising. Their advantage is the fact that thanks to tuberous root bacteria they enrich the soil with nitrogen, their powerful root system leaves a lot of organic matter in the soil and from the point of view of their use in nutrition it is not negligible they do not belong to genetically modified organisms.

Another advantage of seeds of cultivated varieties of lupins is that they contain the least antinutritional substances compared to other types of legumes (peas, soybeans, beans). For these reasons, the seeds of white lupin varieties are of great interest as an alternative source of dietary protein in livestock feed. Lupin seeds have found great application in

feed mixtures for poultry nutrition (Jeroch et al., 2016), especially in the fattening of broiler chickens (Geigerová et al., 2017; Chládek et al., 2017) or laying hens.

One of the positive effects was that the color of the egg yolk and the spectrum of fatty acids in the yolk were improved in laying hens (Drażbo et al., 2014). Lupin seeds contain high-quality protein, which is characterized by a high content of arginine (an essential amino acid for poultry). Lupin seeds of white varieties also contain high-quality oil characterized by a high content of polyunsaturated fatty acids. These results led us to determine whether the content of lupin meal in the diet does not positively affect the quality of fat in the products, in this experiment the composition of fatty acids in the egg yolk fat.

MATERIAL AND METHODS

The aim of the work was to determine the effect of lupin meal as a substitute for soybean extracted meal in feed mixtures fed to laying hens Isa Brown, on the fatty acid composition of egg yolk fat. Laying hens at the age of 18 weeks were placed individually in a three-storey cage technology, with manual feeding (ad libitum) and automatic drinkers. In total, there were of 5 groups with 70 laying hens in each group - one control group (K 0%) and 4 experimental groups (N 50%, N 100%, L 50% and L 100%). Fat content in feed mixtures was 19.7 $g.kg^{-1}$ (control group), 35.4 $g.kg^{-1}$ (N 50%), 46.4 $g.kg^{-1}$ (N 100%), 33.0 $g.kg^{-1}$ (L 50%), 51.5 $g.kg^{-1}$ (L 100%). During the laying period, laying hens were given three types of feed mixtures (N1 starter, N1 and N2, composition of fatty acids in feed mixtures is in Table 1).

The control group received a commercially produced feed mixture, for the experimental groups feed mixtures of similar component and

nutrient composition were prepared, with the difference that in the experimental mixtures, 50% and 100% soybean extracted meal was replaced by lupin meal, from unpeeled (N) or peeled (L) lupin seeds of the Zulika variety. During the laying period, 10 eggs were taken at 8-week intervals (5 times in total) from each group in which the fat content in the yolk was determined and fatty acid analyzes were performed.

Table 1: Content of fatty acids in feed mixtures (only FA > 0.1 g)

g of fatty acid per 100 g of fat	**K**	**N 50%**	**N 100%**	**L 50%**	**L 100%**
C14:0 (myristic acid)	0.20	0.13	0.14	0.19	0.11
C16:0 (palmitic acid)	14.59	10.24	10.06	12.81	9.50
C17:0 (margaric acid)	0.15	0.11	0.11	0.12	0.10
C18:0 (stearic acid)	2.21	1.80	1.85	2.20	1.79
C20:0 (arachidic acid)	0.26	0.57	0.76	0.59	0.83
C24:0 (lignoceric acid)	0.19	0.41	0.55	0.42	0.56
∑SFA	**17.60**	**13.26**	**13.47**	**16.33**	**12.89**
C16:1 (palmitoleic acid)	0.21	0.24	0.31	0.30	0.32
C18:1n9 (oleic acid)	18.82	17.05	23.07	18.05	25.28
C20:1n9 (gadoleic acid)	0.54	2.67	3.82	2.67	4.36
∑MUFA	**19.57**	**19.96**	**27.20**	**21.02**	**29.96**
C18:2n6 (linoleic acid)	27.09	34.38	31.08	21.89	27.34
C20:2n6 (eicosadienoic acid)	0.06	0.13	0.18	0.14	0.20
∑n-6 PUFA	**27.15**	**34.51**	**31.26**	**22.03**	**27.54**
C18:3n3 (α-linolenic acid)	4.16	5.68	7.16	6.28	7.20
C20:5n3 (eicosapentaenoic acid)	0.32	1.57	2.00	1.39	2.30
C22:5n3 (docosapentaenoic acid)	0.61	1.36	1.81	1.36	1.83
C22:6n3 (docosahexaenoic acid)	0.21	0.10	0.09	0.18	0.05
∑n-3 PUFA	**5.30**	**8.71**	**11.06**	**9.21**	**11.38**

Fat was determined by extraction, fatty acids using a GAS CHROMATOGRAF GC-2010 analyzer of Shimadzu company. The fat

content in the egg yolk was expressed as a percentage, the fatty acid content in g per 100 g of fat. The achieved results were processed by mathematical-statistical methods using the statistical program Unistat 5.6. The mean values and their differences were evaluated by multiple comparisons using the Tukey-HSD test, at the significance level $P \leq 0.05$. Each indicator is represented by a mean value (x) and a standard deviation (± SD).

RESULTS AND DISCUSSION

During the laying period, a sample of 10 eggs was taken from each group 5 times at 8 week intervals, i.e. in the 8th, 16th, 24th, 32nd and 40th weeks of laying (a total of 50 eggs per group). A yolk was taken from the egg samples, in which the fat content was determined and its percentage content was calculated. The results are shown in Figure 1. In general, regardless of the diet administered, the percentage of fat in the yolk ranged from a relatively narrow range of 24.59% to 25.68%.

The results show that lupin-based diets did not affect the percentage of fat in the yolk. Evidence of this statement is the fact that the average fat in the yolk in the control group did not differ statistically significantly from the average values of the experimental groups.

Changes in the composition of fatty acids in egg yolk during the laying period

Saturated fatty acids (SFA)

The results shown in Figure 2 show that the laying hens fed the lupin meal diets showed a significantly lower proportion of SFAs in the egg yolk fat compared to the control. By far the lowest content of SFA in

the yolk fat was demonstrated in the experimental group of laying hens N 100%, which were fed a feed mixture with 100% replacement of soybean meal with lupin from unpeeled lupin seeds. It can be concluded based on the results lupin meal has the effect of reducing the SFA content in egg yolk fat.

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

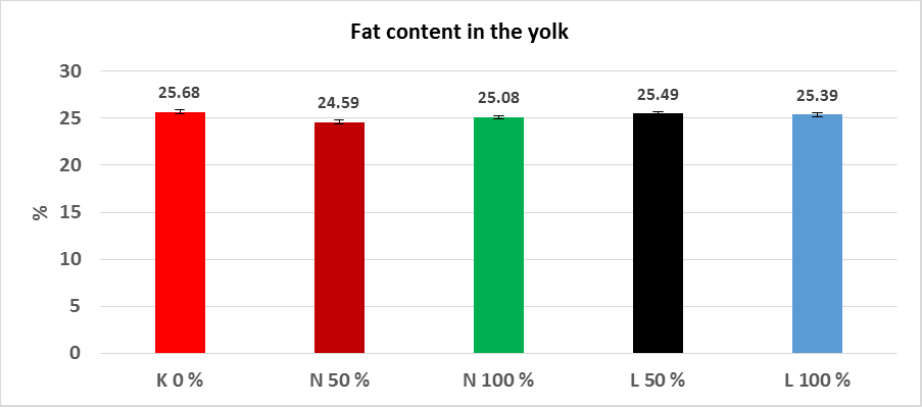
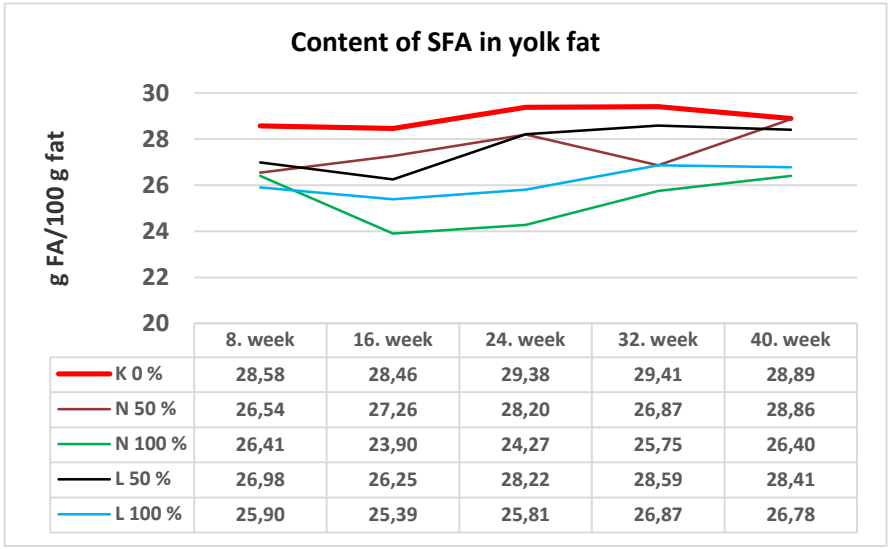


Figure 2 Average SFA content in yolk fat in the control and in the experimental groups

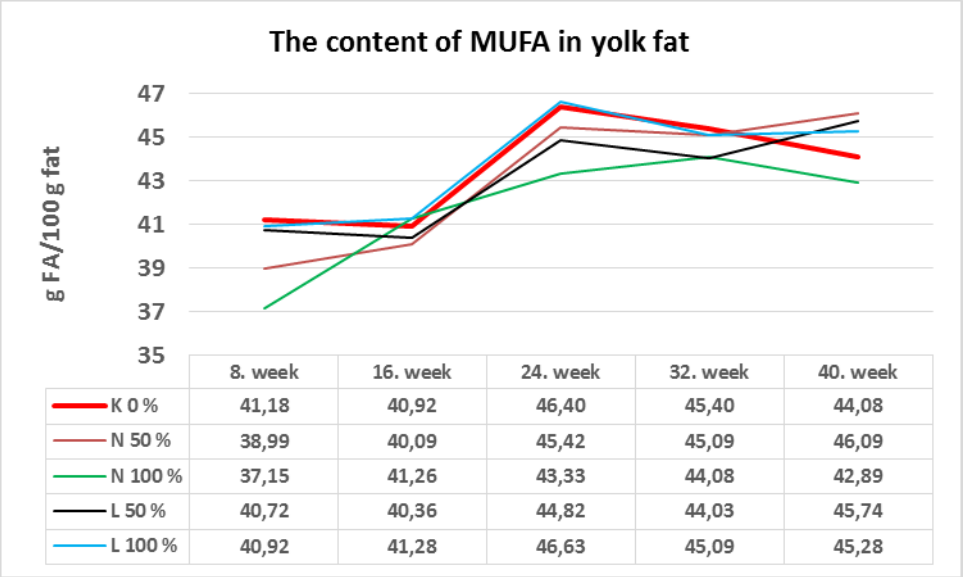


	8. week	16. week	24. week	32. week	40. week
K 0 %	28,58	28,46	29,38	29,41	28,89
N 50 %	26,54	27,26	28,20	26,87	28,86
N 100 %	26,41	23,90	24,27	25,75	26,40
L 50 %	26,98	26,25	28,22	28,59	28,41
L 100 %	25,90	25,39	25,81	26,87	26,78

Monounsaturated fatty acids (MUFA)

Ambiguous conclusions are provided by the results of the MUFA analysis, as documented in Figure 3. Compared to the control, the average MUFA content in the yolk fat during the laying period was comparable or slightly lower in the experimental groups, and even higher at the end. By far the lowest, the average MUFA content in fat was confirmed in the experimental group N 100%.

Figure 3 Average MUFA content in egg yolk fat in the control and in the experimental groups



	8. week	16. week	24. week	32. week	40. week
K 0 %	41,18	40,92	46,40	45,40	44,08
N 50 %	38,99	40,09	45,42	45,09	46,09
N 100 %	37,15	41,26	43,33	44,08	42,89
L 50 %	40,72	40,36	44,82	44,03	45,74
L 100 %	40,92	41,28	46,63	45,09	45,28

Polyunsaturated fatty acids (n-6 FA)

It can be concluded from the results shown in Figure 4 that the lupin-based feed mixtures administered to the experimental groups of laying hens had, in comparison with the control, a significant effect on the increase of n-6 FA in the yolk fat. The highest content of n-6 FA was in the egg yolk in experimental laying hens N 100%, with 100% replacement of soybean meal with lupin in the diet. The dynamic changes of n-6 FA in egg yolk fat can also be considered interesting.

Without differentiation of individual groups, a gradual decrease of n-6 FA in yolk fat can be observed in all groups during the laying period until the 24th week of laying. From the week 24, the level of n-6 FA was stabilized and until week 40 it only fluctuated in a relatively narrow range of values.

Polyunsaturated fatty acids (n-3 FA)

Compared to the control group, the effect of lupin meal in compound feeds on the increase of n-3 FA in egg yolk fat was clearly confirmed throughout the observed laying period, similarly to n-6 FA.

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

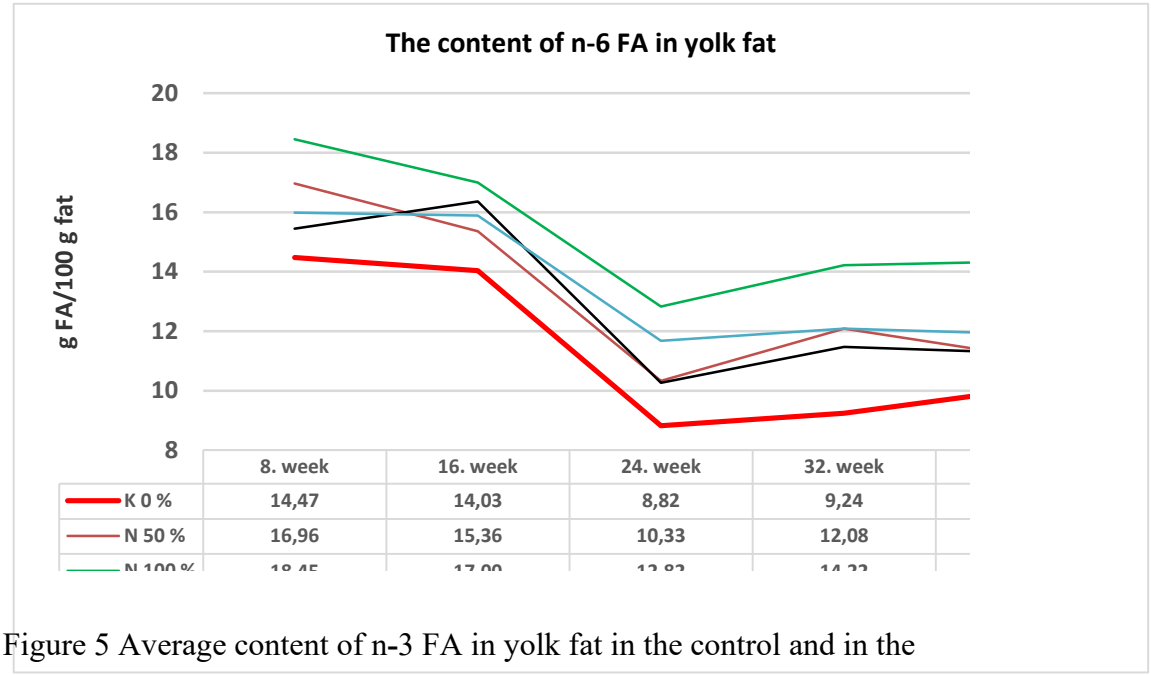
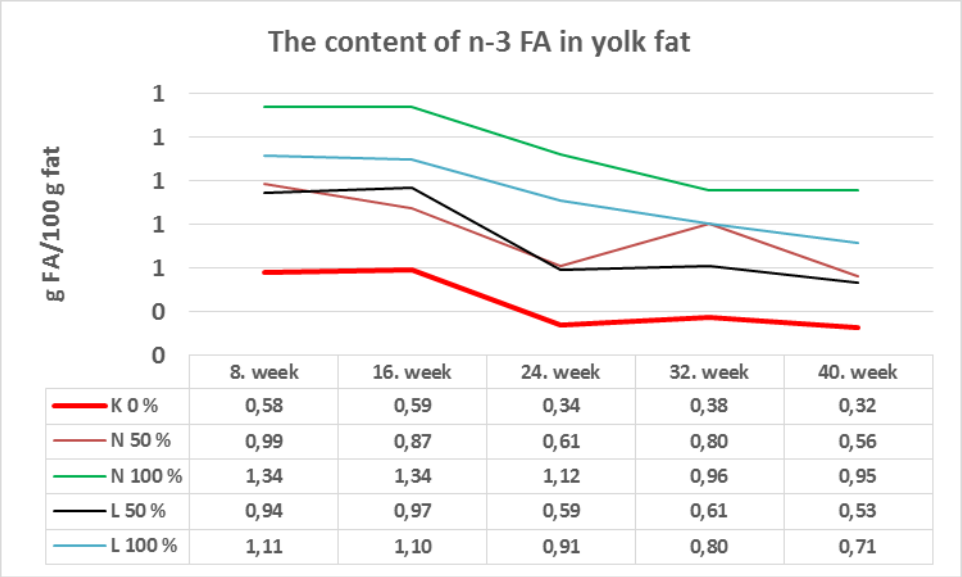


Figure 5 Average content of n-3 FA in yolk fat in the control and in the experimental groups



	8. week	16. week	24. week	32. week	40. week
K 0 %	0,58	0,59	0,34	0,38	0,32
N 50 %	0,99	0,87	0,61	0,80	0,56
N 100 %	1,34	1,34	1,12	0,96	0,95
L 50 %	0,94	0,97	0,59	0,61	0,53
L 100 %	1,11	1,10	0,91	0,80	0,71

The content of n-3 PUFA in eggs can be increased by supplementing the diets of laying hens with certain dietary supplements, such as oils (most often fish oil), meals (linseed meal) or algae (Baiao and Lara 2005) or lupin meal as shown the results of this study. Ceylan et al. (2011) evaluated the effect of dietary supplementation of soybean oil, rapeseed oil, and linseed oil on two levels (15 g/kg and 30 g/kg diet) for 12 weeks in laying hens. They concluded no changes in egg production and egg weight, but hens receiving sunflower oil produced eggs with yolks of fair colour, which customers considered as unintended effect. In despite of this, the results of changes in fatty acid composition after the addition of sunflower oil showed that their composition was significantly ($P < 0.01$) affected by the treatment. In this longer time study, feeding lupin meal helped to increase the values of UFA in yolks comparing with a feeding soybean meal without any other sideways outcomes (production, health of the layers). Optimal changes in egg yolk should be lower levels of SFA and LA, and higher levels of ALA

and DHA – results in conformity with change soybean protein for lupin protein.

Fish is the richest dietary source of EPA and DHA, but a large part of the population all over the world consumes little or no fish, mostly from the countries without access to the seashore (Welch et al. 2011). Therefore, other dietary sources of EPA and DHA are being sought. Food enrichment with long chain n-3 PUFA is probably the best long-term solution to boost their intake (Molendi-Coste et al. 2011). An interesting route is n−3 PUFA enrichment of eggs through dietary supplementation of laying hens. An important benefit of this advance is their wide acceptability as human food and food component. People consume eggs worldwide, without restriction by, for example religion.

CONCLUSION

In conclusion, the use of lupin meal-based diets as a substitute for soybean meal has a positive effect on the fatty acid composition of egg yolk fat, which is due to:

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

It can be concluded from these results that the use of lupin meal leads to an increase in the nutritional value of eggs as one of the most important products for human nutrition. Of the tested diets, a diet in which 50% soybean meal was replaced with lupin seemed to be optimal.

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