VETERINÁRNÍ A FARMACEUTICKÁ UNIVERZITA BRNO
FAKULTA VETERINÁRNÍ HYGIENY A EKOLOGIE
Ústav výživy zvířat

NutriNET 2019

Konference studentské vědecké činnosti z oboru výživa zvířat

20. června 2019, Brno

FACULTAS HYGIENAE VETERINARIAE ET OECOLOGIAE
HVE

NutriNET

UNIVERSITY OF VETERINARY AND PHARMACEUTICAL SCIENCES BRNO
FACULTY OF VETERINARY HYGIENE AND ECOLOGY

Department of Animal Nutrition

NutriNET 2019

International Animal Nutrition PhD Conference

Hustopeče, June 20, 2019

NutriNET 2019
International Animal Nutrition PhD Conference

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BRNO 2019

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ISBN 978-80-263-1465-3

On June 20, 2019, a conference of students of doctoral study programs NutriNET 2019 took place. The conference was organized by the Institute of Animal Nutrition, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno.

The aim of the conference was to present student's work on animal nutrition. The purpose of the conference was to enable students to present their results and knowledge, to get acquainted with the work of students from Czech and Slovak universities and to establish new cooperation.

The conference was a unique opportunity to get to know each other's scientific topics of interest, which are dealt with at individual workplaces of veterinary and agricultural universities within the Czech and Slovak Republics, as well as to start discussions between doctoral students and experienced teachers and researchers.

Prof. Ing. Eva Straková, Ph.D.

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Content

GARLIC EXTRACT FEED ADDITION AND IT´S INFLUENCE ON CHICKEN´S MEAT SENSORIC TRAITS

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ABSTRACT

The aim of the study was to investigate the effect of addition of garlic oil to the diet of Ross 308 broilers on sensory properties of breast and thigh muscles. Cockerels were divided into 3 groups at the age of 7 days. One group was a control without the garlic extract and the experimental group was given the compound feed containing 5 g (G5) and 10 g (G10) of garlic oil extract per 1 kg. The chickens were kept in balance cages. The experiment was performed from 21st to 49th day of chickens´ life. At the end of experiment (49th day of age), 8 birds were randomly selected from each group, weighed and slaughtered. The sensory properties of the breast (n=8) and thigh (n=8) muscles were evaluated after two month storage. The addition of garlic oil extract had a positive, statistically significant effect ($P < 0.01$) on chewiness and juiciness ($P < 0.05$) of breast meat.

Keywords: garlic extract, sensory properties, breast meat

INTRODUCTION

Garlic *Allium sativum* is one of the oldest crops. We know that as a vegetable, spice but also as a medicinal plant (Křižaň a kol., 2011). Garlic can be used either as a whole plant or its crushed or finely chopped cloves, also used as dried, garlic salt, garlic paste, garlic butter and oil just like in our study (Oberbeil and Lentzová, 2003).

Garlic has a positive effect on digestive tract, antibacterial and antimicrobial effect, it reduces inflammatory processes in the intestine (Váňa, 2004).

Important ingredient in garlic is alliin, its trivial name is S-2-propenyl-L-cysteine sulfoxide and it is contained in garlic in the amount 0.2-2%.

It is a thermally stable and pharmaceutically inactive substance (SINGH, 2008), but it metabolizes into the bacterially active allicin, which is a very effective antibiotic that destroys most of the microorganisms (Oberbeil and Lentzová, 2003). Allicin can prevent atherosclerosis, storing fat in blood vessels and can act an antioxidant (Lucier, 2000). Garlic also contains garlicin and allistatin and a variety of enzymes, amino acids, trace elements, and more (Malý et al., 1998).
The large use of antibiotics in livestock farming has caused the emergence of new epidemic resistance strains, causing a number of diseases. The vehicle of the disease is animal products, where eggs and pork and poultry meat are among the most common vehicles. Significant reservoirs of multiresistant strains of S. typhimurium include pigs, broilers, laying hens and turkeys (Šišák, 2001).
The European Parliament has introduced limited use of antibiotics in animal feed in the European Union since January 2006, scientists are looking for alternative components that could be added to feed and positively affect animal health and productivity (Kamruzzaman et al., 2011). For example garlic extracts affect antimicrobially on gram negative and gram positive bacteria of the genera *Escherichia, Salmonella, Stafylococcus, Streptococcus, Proteus, Bacillus* and also on pathogenic bacteria *Mycobacterium tuberculosis* (Kavalcová et al., 2013).

MATERIAL AND METHODS

Animals and diet

Seventy two Ross 308 cockerels were divided into 3 groups at the age of 7 days. There were 2 replicates per pen. Chickens were kept in balance cages. Trial was performed from 21st day of chickens´ life until the 49th day. The first group obtained feed mixture with 5 g (G5) and the second group obtained 10 g (G10) of liquid garlic extract per 1 kg of feed. The control group (C) of cockerels was given feed mixture without garlic extract. Garlic extract in the experimental feed replaced the appropriate proportion of sunflower oil. Nutrient and chemical content of the feed is presented in Table 1 and Table 2, respectively. Body weight of chickens was measured every week and feed consumption was recorded daily. The health status of animals was evaluated daily. The chickens were fed *ad libitum*. At the end of experiment (49th day of age), 6 birds were randomly selected from each group, weighed and slaughtered. Feathers were removed, the chickens were eviscerated, and the carcass yield was calculated. The breast and

thigh muscles without skin were separated from the carcasses after cooling. All visible external fat was removed from the sample muscles. The breast and thigh meat was weighed and their percentage of live body weight were calculated.

Sensory analysis

The sensory properties of the breast (n=8) and thigh (n=8) meat were evaluated by 10 panellists in the sensory laboratory (Department of Food Technology, Mendel University) using previously published methods (ISO 8589 1993). Each sample (breast and thigh) was packed into a plastic case and stored at -20 °C. After two weeks, the samples were thawed at 4 °C and were cooked in a convection oven at 200 °C with 60% humidity for 1 hour. Professional evaluation groups that consisted of trained panellists were used for the sensory analysis (ISO 8586-1 2015). A graphic non-structured scale (100 mm, 0 = the worst, 100 = the best) was used to compare the experimental groups for odour, colour, fibreness, chewiness, juiciness with the control group.

Statistical analysis

The data were analysed using one-way ANOVA with the StatSoft Statistica version 12.0 (Tulsa, Oklahoma, USA). To ensure evidential differences, a Scheffe´s test was applied and $P < 0.05$ was regarded as a statistically significant difference.

Table 1 Composition of the diet used in our experiment

COMPONENT	*(g/kg)*
Maize meal	452
Soybean meal	287
Wheat meal	219
Sunflower oil	30
Monocalcium phosphate	5
Premix*	3
Limestone	3
DL-Methionin	1

*Premix added to 1 kg of feed: lysine 0.6 g; methionine 0.75 g; threonine 0.34 g; calcium 2 g; phosphorus 0.65 g; sodium 0.42 g; copper 5 mg; iron 25 mg; zinc 34 mg; manganese 40 mg; cobalt 0.07 mg; iodine 0.3 mg; selenium 0.06 mg; tocopherol 4,500 mg; calciferol 1,667 IU (international units); phylloquinone 0.50 mg; thiamine 1.4 mg; riboflavin 2.3 mg; cobalamin 10 mg; biotin 0.07 mg; niaciamide 12 mg; folic acid 0.57 mg, calcium pantothenate 4.5 mg; choline chloride 60 mg; salinomycin sodium 23.33 mg.

Table 2 Chemical analysis of diets in dry matter

%	Crude protein	Crude fat	Crude fibre	Ash
C	21.05	8.66	3.68	6.54
G10	20.68	8.56	3.56	6.44
G15	20.38	8.65	3.60	6.36

RESULTS AND DISCUSSION

Schleicher *et al*. (1998) describe that positive effects of herbal supplements on broiler performance, carcass quality and quality traits of meat have been demonstrated. In this study, there were no statistically significant differences (P> 0.05) in odour, colour, fibreness of the breast meat, as shown in Table 3. Odour was evaluated more positively in the control group compared to the experimental groups but not statistically significantly. The Table 3 shows that no foreign odour was observed in the breast meat by all evaluators in all 3 groups. This is a good finding for our experiment, as the evaluators did not detect any garlic smell and odour and according to their evaluation the experimental group (G5,G10) was evaluated slightly better than the control group. Onibi *et al*. (2009) state in their study that meat samples were not influenced by garlic aroma by muscle type and interaction of garlic supplementation and muscle type.

The data in the Table 3 show that the colour of the breast muscle was evaluated by the evaluators in all 3 groups with minimal statistically inconclusive differences, when the control achieved better results than the control group. The evaluators evaluated the color of the breast muscle well and its color did not differ significantly from the typical color for the breast muscle.

Fibreness also did not differ from group to group and no statistically significant differences were found (P> 0.05). The Control and the Garlic Group (G5) got the incredibly best rating.

Table 3 Sensory analysis of chickens´ breast meat

Group	C	G5	G10
	Mean ± standard error		
Sensory trait n	80	80	80
Odour	89.14 ± 0.66	87.64 ± 0.96	87.52 ± 0.91
Colour	90.59 ± 0.66	90.29 ± 0.78	90.35 ± 0.35
Fibreness	79.83 ± 1.02	78.51 ± 1.20	79.26 ± 1.29
Chewiness	50.57 ± 1.73^{a}	54.28 ± 1.67^{ab}	57.98 ± 1.51^{b}
Juiciness	31.59 ± 1.60^{a}	35.94 ± 1.82^{ab}	38.81 ± 1.78^{b}

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

Table 3 shows that the values of chewiness between group G10 and the Control group were statistically significant (P <0.01). However this was not the case between experimental Garlic group (G5) and the Control group (P> 0.05). Furthermore, the juiciness of breast muscle in experimental group G10 was found to be statistically significant (P <0.05) in comparsion to the Control group. Garlic group (G5), when compared against the Control, was also more highly evaulated, but this finding was not statistically significant (P <0.05). It can then be assumed that the addition of garlic oil extract could have a positive effect on the juiceness of the breast muscle. We can see in Table 3 that when garlic extract is added at higher concentrations, the values indicate a more positive affect on the breast muscle´s juiciness. Schleicheret *et al*. (1996) in describing the results of their experiment, concluded that, the meat of the birds which fed on a garlic supplemented diet achieved the highest sensory score. Table 4 showed that were no statistically significant differences (P> 0.05) in the odour, colour, fibreness and chewiness in broilers thigh meat.

Table 4 Sensory analysis of broilers´ thigh meat

Group		C	G5	G10
		Mean ± standard error		
Sensory trait	n	80	80	80
Odour		84.25 ± 0.76	85.22 ± 1.01	86.06 ± 0.85
Colour		82.76 ± 0.82	84.57 ± 0.82	85.11 ± 0.87
Fibreness		85.51 ± 0.80	85.61 ± 0.73	86.49 ± 0.81
Chewiness		71.05 ± 1.59	70.53 ± 1.87	74.49 ± 1.51
Juiciness		62.04 ± 1.42[a]	63.40 ± 1.71[ab]	68.48 ± 1.44[b]

[a,b] – different letters in one line - statistically significant differences ($P < 0.05$).

Table 4 shows that the color of the thigh meat was not statistically significant among all 3 groups (P> 0.05). However, the experimental groups were evaluated better than the control group.
The results of evaluation show that the adding of garlic oil extract has a positive effect on the colour of the thigh muscle. Similarly, no statistically significant differences were found among the evaluated groups of sensory analyses of thigh fibreness and chewiness (P> 0.05). The group of garlic (G10) achieved the best results. From the results, it can be assumed that the higher amount of garlic oil added to the feed, results in more favorable evaulation of the thigh muscle fibreness. Table 4 showes that between the control group and experimental group (G10) there is a statisticaly significant difference (P <0.01) in the juiciness. Onibi *et al.* (2009) describe the results of their study while the diets enhanced with an amount of garlic palatability scores, this was without statisticaly significant differences (P>0.05) between groups.

CONCLUSION

The inclusion of garlic extract in the Ross 308 broiler feed has a positive effect, with statistically significant differences between control group and experimental groups on chewiness and juiciness of breast meat and on juiciness of broilers thight meat.

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EFFECT OF GENOTYPE OF THE MECKLENBURGER CHECKED RABBIT BREED ON LIVE WEIGHT DURING GROWTH

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ABSTRACT

The aim of the present study was to evaluate the effect of the particular genotypes of young Mecklenburger Checked (MC) rabbits on their live weight (LW) up to the 15th week of age. A total of 24 rabbits were used in the present study. The rabbits belonged to the MC breed with three colour genotypes: Standard marked, Chaplins and Self. A statistically significant effect of the genotype on their LW was found at the age of 77, 91 and 105 days. It can be concluded that the Self (kk) genotype of the MC breed reached the highest live weight values as compared to the Standard marked (Kk) and Chaplins (KK) genotypes in course of the entire monitored period. These results can be used for optimization of the pure breeding programme and also for crossbreeding in order to improve meat production.

Keywords: rabbit, Mecklenburger Checked breed, genotype, growth

INTRODUCTION

Almost 70 rabbit breeds are recognized by the Czech Association of Breeders (Zadina, 2003). Recently, medium-sized breeds with their production potential have gained most popularity (approx. 63%). Furthermore, a present trend of many Czech rabbit breeders is to rear some specific breeds with unconventional top colour (Šimek et al., 2017).

The Mecklenburger Checked (MC) breed is a coloured medium-sized breed with white markings. Desirable live weight of adult MC rabbits

ranges from 4 to 5 kg. The so called broken (blanket) pattern is a typical breed trait when the most of the body, including head, is evenly pigmented. Standard marked broken rabbits have the white head underpart, chest, belly and forelimbs and hindlimbs and also a white spot on the forehead (Zadina, 2003; Whitman, 2004). The present increasing interest of the breeders for MC breed is linked to their favourable meat performance and interesting top colour (Šimek, 2014).
When rearing the MC breed, it is necessary to take into consideration certain specific breeding aspects. Based on genetics of the rabbit colouring, the standard marked MC are heterozygous in the English spotting locus (alleles Kk). When mating standard marked parents, 3 genotypes normally occur in the litter – a standard marked heterozygous rabbit (Kk), a less marked dominantly homozygous rabbit called Chaplin (KK) and a self-coloured recessively homozygous rabbit without white patterns (kk). In general, the Chaplins (KK) have been often described as naturally subvital rabbits with predisposition to some diseases, especially concerning their intestinal physiology (Bödeker et al., 1995; Fingerland, 1998). However, there is a lack of studies dealing with growth intensity of the three above mentioned genotypes of MC rabbits.
Thus, the aim of the present study was to evaluate the effect of the particular genotypes of young MC rabbits on their live weight until 15th week of age.

MATERIAL AND METHODS

Animals and husbandry condition

A total of 24 rabbits were used in the present study. The rabbits belonged to the MC breed where three colour genotypes: heterozygous Standard marked (ST, genotype Kk, *n=8*), dominantly homozygous spotted Chaplins (CH, genotype KK, *n=8*) and recessively homozygous non-spotted Self (SE, genotype kk, *n=8*). All the rabbits came from a common hobby stock which realizes breeding and showing activities according to guidelines of the Czech Association of Breeders. The rabbits were housed in outside hutches that were sheltered against unfavourable climatic condition. The rabbits of the three genotypes were housed, fed and handled under the same rules of husbandry and hygiene. All rabbits received the same pelleted diet (16.0% nitrogenous substances, 21.5% crude fibre, 3.0% crude fat, 8.0% ash), while the same feeding technique was performed. The young rabbits were raised up to their 8th week together with their does. The rabbits were

vaccinated at the age of 8 weeks against myxomatosis and rabbit haemorrhagic disease types 1 and 2.

Data collection

The young rabbits were individually weighted at the age of 21 days and subsequently their live weights (LW) were recorded at the age of 35, 49, 63, 77, 91 and 105 days. When collecting data, the rabbits showed no clinical signs of disease.

Statistical analysis

The obtained data were statistically analysed using software Statistica CZ, version 9 (StatSoft Inc., 2011). The values of arithmetic mean and standard error of the mean were determined. A Shapiro-Wilk test was used to test normal distribution of the LW within evaluated genotypes. A normality was found within all 3 genotypes. One-way variance analysis (Anova) was used to determinate differences in the evaluated genotypes. When Anova showed significant differences among the groups, a Tuckey test was used.

RESULTS AND DISCUSSION

The average LWs of the particular genotypes of the MC breed are presented in Table 1.

Table 1 The effect of genotype of the Mecklenburger Checked breed on live weight of the young kits (g)

Age (days)	Genotype						*P*
	ST (*n* = 8)		**SE** (*n* = 8)		**CH** (*n* = 8)		
	mean	SD	mean	SD	mean	SD	
21	288.1	33.77	320.0	24.66	305.68	26.91	NS
35	598.8	210.34	731.9	202.85	667.1	48.27	NS
49	951.3	216.11	1030.0	221.05	937.63	53.17	NS
63	1278.8	187.15	1503.8	221.53	1414.8	190.49	NS
77	1588.8^{a}	220.68	1863.0^{b}	170.92	$1781.0^{a,b}$	169.55	*
91	$1851.2^{A,a}$	279.36	2272.5^{B}	123.61	2116.0^{b}	111.80	**
105	2210.0^{a}	292.42	2559.4^{b}	231.77	$2453.3^{a,b}$	169.03	*

ST = Standard marked; SE = Self; CH = Chaplin; SD = standard deviation;
$*^{,A,B}$ = means within a row with different superscript letters differ (P < 0.01);
$**^{,a,b}$ = means within a row with different superscript letters differ (P < 0.05);
NS = not significant.

In the course of the monitored period, the SE rabbits showed the highest values of their LW among the evaluated genotypes. A statistically significant effect of the genotype on their LW was found at the age of 77, 91 and 105 days. A significantly higher ($P < 0.05$) LW was found in 77-day-old SE rabbits as compared to the ST rabbits (+274.2g). At the age of 91 days, the SE rabbits showed a higher ($P < 0.01$) LW in comparison with the ST rabbits (+421.3g). At the end of the evaluated period (105th day of the rabbits´ age), a significantly higher LW of the SE genotype was found when compared to the ST rabbits (+349.4g). Our findings of the Selves´ growth intensity are consistent with a general statement of Fingerland (1998) who states that only the SE genotypes show better growth characteristics.

Based on the previous studies (Bödeker et al., 1995; Fontanesi et al., 2014), the CH genotypes are generally considered as weak individuals because of the *megacolon* syndrome. Therefore, we expected that the CH genotypes in our trial will show the lowest growth intensity among the evaluated breed genotypes. However, during the entire evaluated period we only found a higher LW in the CH genotypes as compared to the ST genotypes while the significant difference in LW was found in 91-day-old rabbits (+264.8g; $P < 0.05$). It is also important to point out that no signs of digestive disorders were recorded in our trial.

The white markings of the MC breed are genetically based by the same English spotting locus as in the other spotted breeds. However, phenotypic expression of the MC breed is different as compared to the other spotted breeds due to the long-term specific selective breeding (Fingerland, 1998). Thus, it can be assumed the CH genotypes of the MC breed seem to be less sensitive to the generally described *megacolon* syndrome and to growth retardation as compared to the CH genotype of other spotted breeds.

CONCLUSION

Based on our results, it can be concluded that the Self (kk) genotype of the MC breed reached the highest live weight values as compared to the Standard marked (Kk) and Chaplins (KK) genotypes in /course of the entire monitored period. These results can be used for optimization of the pure breeding programme and also for crossbreeding in order to improve meat production. It will be necessary to perform further studies focusing on the health state and adaptability to various husbandry conditions of the spotted genotypes.

ACKNOWLEDGEMENT

The study was financially supported by project 202/2019/FVHE from Internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences Brno.

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ZINC NANOPARTICLES EFFECT ON RAT MICROBIOTA

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ABSTRACT

Zinc nanoparticles (Zn NPs) represent a promising alternative to antibiotics due to their antimicrobial effect. NPs may have a potential to lower the use of antibiotics and consequently spread of antibiotic resistance traits among bacteria, including pathogens in pig production. The experiment was performed on rats. To investigate Zn NPs effect on host-colonizing bacteria, the populations of total aerobic bacteria and coliforms were analyzed in rat feces.

Keywords: nano zinc, antimicrobial activity, animals, nanoparticles, antibiotic replacement

INTRODUCITON

Zinc-based nanomaterials have been applied in several fields including agriculture, chemistry, textile and food industry, electronics, and medicine (Dapkekar et al., 2018 and Kaviyarasu et al., 2017). Due to their antibacterial activity, the Zn nanoparticles (NPs), particularly ZnO-based, have been designed and tested for utilization in veterinary and human medicine. Their bactericidal ability has been demonstrated against diverse bacterial species, including pathogens *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, *Salmonella enterica* (Shankar et al., 2017 and Xie et al., 2011) and antibiotic-resistant strains including methicillin resistant *S. aureus* (MRSA) and extended

spectrum beta-lactamases producing *E. coli* and *Klebsiella pneumoniae* (Alves et al, 2017, and Hameed et al., 2016). Considering their substantial bactericidal potential, Zn nanoparticles represent promising alternatives to antibiotics or an enhancement of antibiotics against drug resistant bacteria (Chen et al., 2014).

MATERIAL AND METHODS

The experiment was performed at the experimental laboratory of Mendel university in Brno, with the approval of the Ethics Commission at the Faculty of AgriSciences, Mendel University in Brno, Czech Republic in accordance with Act No. 246/1992 Coll. for the protection of animals against cruelty. Throughout the experiment, microclimatic conditions were maintained at 23 ± 1 °C, 60% humidity, and the light regime (12 h L, 12 h D) with an illumination of maximum 200 lx. As model animal, male laboratory rats of the outbred strain Wistar albino were used. Animals were divided into seven groups of ten rats each. Rat average initial weight was 144 ± 2 g. Four groups of rats were fed with phosphate-based zinc nanoparticles (ZnA, ZnB, ZnC, ZnD) in the dose of 2,000 mg Zn/kg diet. Fifth group was fed by commercial zinc nanoparticles (ZnO-N) in the dose 2,000 mg Zn/kg diet. Sixth group was fed by ZnO in the dose of 2,000 mg Zn/kg diet. The last (control) group had no addition of Zn in their feed (C). Animals were weighed at regular intervals (day 0, 7, 14, 21 and 28), and feces samples were colected. All groups of rats were fed on mono diet (wheat) with 2.7 mg/kg of Zn. The experiment lasted for 28 d. The animals had access to feed and drinking water *ad libitum*.

Analysis of the total aerobic bacteria and coliforms in feces

The fecal samples were homogenized in sterile phosphate buffer solution (PBS) on ice (1:9 *w*/*v*) and the homogenate was serially diluted in PBS. Subsequently, 1.0 mL of diluted suspension was mixed with sterile molten Plate Count Agar (PCA) and MacConkey Agar (Sigma-Aldrich) in duplicates. The total colony counts from PCA and counts of coliforms from MacConkey Agar were enumerated after 24 h at 37 °C.

The data were processed statistically using STATISTICA.CZ, version 12.0 (Czech Republic). The results were expressed as the mean ± standard deviation (SD). Statistical significance was determined using ANOVA and Scheffé's test (one-way analysis). The analysis of total counts and coliforms in feces was performed using one-way

ANOVA with *post-hoc* Dunnett's C test specialized for unequal variances and unequal sample sizes (IBM SPSS Statistics 21, Version 21.0. Armonk, NY, USA).

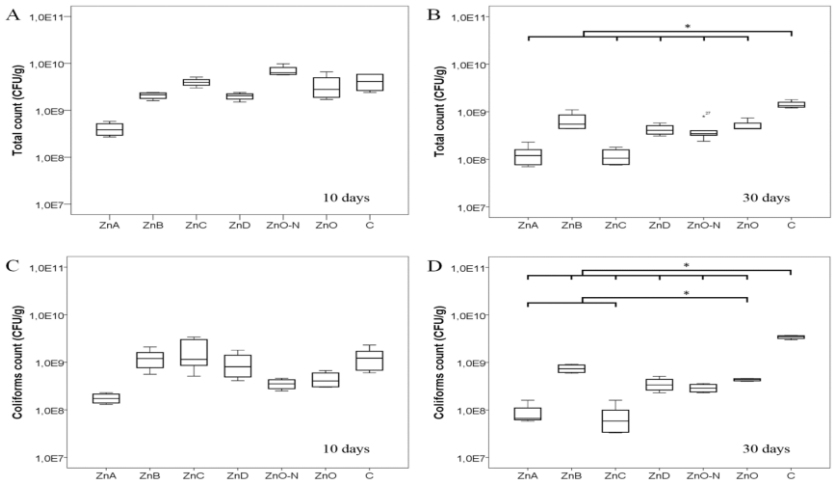
RESULTS AND DISSCUSION

Experimental rats were regularly weighted (0, 7, 14, 21, 28 d). The initial weight of all groups was in the range 130.0–155.0 g. The weight results were comparable in all groups both at the beginning and at the end of the experiment (see Additional file 1: Table S1). Only, the group ZnB showed an accelerated weight gain in comparison to that of all other groups at the end of the experiment.

Figure 1 Rat weight during the experiment (g)

	21.9.	27.9.	6.10.	15.10.	20.10.
Zn-A	138.2	149.4	165.6	190.7	193.7
Zn-B	137.0	162.8	194.4	208.0	218.2
Zn-C	144.0	156.2	187.4	194.6	187.4
Zn-D	130.4	157.2	185.4	201.0	200.6
ZnNPs	155.2	159.6	178.2	190.2	198.8
ZnO	160.2	164.2	185.0	190.8	202.8
KO	144.0	149.6	172.6	182.0	199.6

Figure 2 Effects of Zn NPs and ZnO on bacteria in feces



1.Total bacterial counts after 10 days- A and 30 d- B counts of coliform bacteria after 10 d- C and D-30 d of treatment (mean ± SD, n = 4). *Mean values were significantly different ($P < 0.05$).

The results show lower total count of colonies forming unit (CFU) for variant ZnA after 10 days and ZnA and ZnC after 30 days compared to the control (Fig. 1 A, B). ZnA and ZnO-N showed increased effect against coliform bacteria after 10 days of treatment. Moreover, after 30 days (20 days after treatment) there is obvious strong effect of ZnA, ZnC, ZnO, Zn-N and ZnO agains coliform bacteria.

To investigate Zn NPs effect on host-colonizing bacteria, the population of total aerobic bacteria and coliforms in rat feces was analyzed. Our study demonstrated that dietary supplementation of rats with phosphate-based Zn NPs altered the bacterial population in feces as well. Due to inconsistent results in the control group the bacterial count decline was not significant at day 10; however, over time, the bacterial count was clearly reduced. This phenomenon is in agreement with the work by Feng et al., suggesting that the ileal bacterial community richness decreased in response to higher dose of ZnO NPs (100 mg/kg), and that *Lactobacillus* genus was particularly reduced (Feng et al., 2017). On the contrary, Li et al. pointed out, that ZnO NPs could act anti-inflammatory in a dose-dependent manner. This may be associated with reduction of infection-causing bacteria and, vice versa, gain of probiotics (*Lactobacillus* and *Bifidobacterium*) in colon (Li et al., 2017).

CONCLUSION

The study shows that the effect our Zn formulations on the rat microbiome was similar to that caused by ZnO. In fact, ZnA and ZnC nanoparticles caused even greater inhibition of coliform bacteria than ZnO. Therefore, these nanoparticles have a potential to be used as new antibacterial agents, especially for reduction of coliform bacteria. Further studies, primarily focused on Zn NPs applications in livestock productions, are warranted.

ACKNOWLEDGEMENTS

The study was financially supported by Ministry of Agriculture of the Czech Republic (QK1720349 "Nanoparticles zinc as an alternative to antibiotics in pigs").

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IN VITRO ORGANIC MATTER DIGESTIBILITY OF DRIED GRAPE POMACES FROM SLOVAKIA

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ABSTRACT

The aim of this research was to determine *in vitro* digestibility of organic matter (IVOMD) of grape pomace of four different cultivars of *Vitis vinifera sp.* – Zweigelt (ZW), Green Veltliner (GV), Pinot Blanc (PB) and Pinot Gris (PG) from Slovakia. IVOMD was estimated by two-stage pepsin-cellulase method in Daisy Incubator II (Ankom Technology, U.S.A.). Significant differences in the digestibility of OM of analyzed samples were found ($P<0.05$). Results have shown a significant impact of the grape variety on the digestibility of the grape pomace. The results further showed that digestibility analysis by *in vitro* conditions can be beneficial for animal nutrition research.

Keywords: *Vitis vinifera sp.*, grape pomace, organic matter, *in vitro* digestibility

INTRODUCTION

The grape is the world's largest fruit crop, with an annual production of more than 75 million tonnes (OIV, 2017). It is estimated that around 20-25% of the total weight of grapes used for wine is made up of grape pomace, the solid residue left over after the juice is extracted from the grapes (Laufenberg et al., 2003, Burg, 2004). With respect to animal feed, the nutritional value and the digestibility of these by-products is, due to high fiber content, generally low (Brenes et al., 2016, Gálik et al., 2018). However, grape pomace can be marketed as substantial source of fibre and bioactive substances in animal nutrition (Viveros et

al., 2011, Nistor et al., 2014, Teixeira et al., 2014). In this experiment, we hypothesize that the OM digestibility of grape pomace is closely related to the grape variety. The objective of this study was to determine IVOMD of grape pomaces of four different cultivars of *Vitis vinifera sp.* (Zweigelt, Green Veltliner, Pinot Blanc and Pinot Gris) from Slovakia.

MATERIAL AND METHODS

In the experiment IVOMD of grape pomaces from Slovakia was determined. In total, 12 samples from 4 varieties (Zweigelt, Green Veltliner, Pinot Blanc and Pinot Gris) were analysed. Laboratory samples were processed in the Laboratory of Quality and Nutritive Value of Feeds at the Department of Animal Nutrition at the Slovak Agricultural University by standard laboratory methods and procedures (EC No 152/2009). IVOMD was estimated by two-stage pepsin-cellulase method (PEPCEL) in Daisy Incubator II (Ankom Technology, U.S.A.). An amount of 0.5 g of samples were weighed in triplicate in polypropylene synthetic tissue filter bags (F57, Ankom Technology, U.S.A.) with a pore size of 25 μm. In the first stage samples were incubated in solution of pepsin (activity 10 000 $U.g^{-1}$) and 0.1 M hydrochloric acid at 39°C for 24 hours. After this time samples were placed in a hot air dryer at 80°C for 30 minutes and flushed with hot distilled water three times. The second step of incubation was carried out in solution of cellulase (*Trichoderma viridea*, activity 10 000 $U.g^{-1}$) and acetate buffer with pH 4.8 at 39°C for another 24 hours. Acetate buffer was prepared by dissolving 1.36 g of sodium acetate ($CH_3COONa + H_2O$) in 500 ml of distilled water, then 0.6 ml of acetic acid was added and the solution diluted to 1 litre. The pH was adjusted to 4.8 by addition of sodium hydroxide as necessary. After incubation was complete, the samples were flushed with hot distilled water three times again and finally they were flushed with acetone. The residue was dried at 103±2°C for 12 hours and weighed. The residue was then burned at 530±20°C for 5-6 hours, cooled and reweighed, so the percentage of indigestible organic matter could be determined. The percentage of IVOMD was calculated according to the formula:

$$\text{IVOMD (\%)} = \frac{\text{(OM before incubation – OM after incubation)}}{\text{(OM before incubation)}} \times 100$$

To calculate basic statistic characteristics, to determine significance of differences and to compare results one-way ANOVA and t-test were performed at $P < 0.05$ level. The SAS statistical package was used (SAS Inc., New York City, USA).

RESULTS AND DISCUSSION

Results obtained for DM, OM and IVOMD in analysed grape pomaces are shown in Table 1. The lowest DM content was observed for PG and the highest for ZW. Differences in DM content between all analyzed samples were significant (P<0.05). The OM content in analyzed grape pomaces was similar except for ZW, which had significantly (P<0.05) the lowest OM content. According to several studies, the DM and OM content in dried grape pomace can vary from 901 to 930 $g.kg^{-1}$ and 916 to 967 $g.kg^{-1}$ of DM respectively (Zalikarenab et al., 2007; Winkler et al., 2015; Chikwanha et al., 2018). The composition of grape pomace 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). The highest IVOMD was detected for GV and the lowest for ZW. Significant differences (P<0.05) in IVOMD of analyzed grape pomace samples were found. These results indicate a significant impact of the grape variety on the digestibility of the grape pomace. According to Gálik et al. (2018) the average digestibility coefficients of OM of dried grape pomace is only 18-25%. Winkler et al. (2015) state, that grape pomace from red cultivars is characterized by higher digestibility coefficients of OM (39%) in comparition with pomace from white varieties (34%), what is consistent with our results. On the contrary, Zalikarenab et al. (2007) reported exactly the opposite results, namely that the pomace from white cultivars have a higher digestibility coefficients of OM (38.97%) than those of red varieties (31.64%). These discrepancies in OM digestibility may in general result of different proportion of seeds and stalks with higher fiber content in the analyzed grape pomaces and due to genetic variations among the cultivars. Some studies has shown, that OM digestibility of grape pomace of both varieties can be significantly increased by ensiling (Winkler et al., 2015). In line with this, an addition of polyethylene glycol also increases the estimated OM digestibility of grape pomace (Alipour and Rouzbehan 2005).

Table 1 Organic matter content and digestibility coefficients of analyzed grape pomaces

	DM	OM	IVOMD
	Mean±SD		
Zweigelt	943.43±0.21[a]	939.55±1.64[a]	33.61±0.22[a]
Pinot Blanc	941.77±0.15[b]	957.71±0.24[b]	45.88±0.93[b]
Green Veltliner	928.97±0.42[c]	950.20±1.89[c]	59.56±0.94[c]
Pinot Gris	927.50±0.14[d]	958.81±0.01[d]	53.63±0.72[d]

DM – dry matter ($g.kg^{-1}$); OM – organic matter ($g.kg^{-1}$ of DM); IVOMD – *in vitro* organic matter digestibility (%); SD – standard deviation. Values followed by different letters within a column are significant at the level 0.05.

CONCLUSION

The objective of this study was to determine IVOMD of grape pomace of four different cultivars of *Vitis vinifera sp.* (Zweigelt, Green Veltliner, Pinot Blanc and Pinot Gris) from Slovakia. Significant differences in DM and OM content of the analyzed samples, as well as in the digestibility of their OM were found. These differences in OM digestibility can be related to different agro-climatic conditions of grape varieties from which the pomaces came from. Further research in the future is recommended in this regard, and *in vitro* digestibility analysis could be useful for detection of the nutritional quality of grape pomace.

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ACKNOWLEDGEMENT

The study was supported by the Slovak Research and Development Agency under the contract no. APVV-16-0170 (*By-products from grape processing as a bioactive substances source in animal nutrition*).

IS IT POSSIBLE TO AFFECT QUALITY OF EJACULATE BY ADDITION OF SELENIUM, ZINC, VIT. E AND C INTO DUROC BOAR FEED MIXTURE?

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ABSTRACT

Selenium, zinc, vitamin E and C are the major compounds of antioxidant defence system in organism. All together are fighting against the negative impact of oxidative stress, which is the main cause of male infertility. In our study 12 Duroc boars have been divided into the control group (n = 6) fed only by basic diet and the experimental group (n = 6) fed by basic diet with the supplementation of 0.5 mg selenium (seleno-methionine), 70 mg zinc (zinc-methionine), 70 mg vit. E (alpha-tocopherol) and 350 mg vit. C (L-ascorbic acid) per kilogram of basic diet. Monitored parameters of ejaculate was volume of ejaculate (ml), concentration of sperm (10^6/ml), total rate of sperm (10^9), motility of sperm (%) and amount of morphologically abnormal sperm (%).

Keywords: antioxidant, boar, ejaculate

INTRODUCTION

In seminal plasma, several antioxidants, such as vitamin E, vitamin C, superoxide dismutase, glutathione and thioredoxin can be found, which neutralize free radicals and protect sperm cells from ROS damage (Tremellen 2008). Selenium is the component of glutathione peroxidase as a marker of oxidative stress. It restricts functions of peroxide radicals and protects body tissues from damage (Horký et al. 2012, Pavlata et al., 2011). Moreover, selenium plays an important role in spermiogenesis and maturation of sperm (Blair 2007). Zinc is a part of enzyme superoxide dismutase, an important enzyme in antioxidant chain. Zinc is also important in development of reproductive system

and its activity (Jelínek et al. 2003). Vitamin E, especially α-tocopherol, is important in cell protection during the oxidative stress. Formation of very toxic lipoperoxides is suppressed by tocopherols (Racek 2003). Vitamin C, the L-ascorbic acid, together with α-tocopherol and selenium, protects sperm cell membranes from peroxidation (Horký et al. 2016). All these antioxidants together could have positive effect on sperm quality (Svoboda 2011).

MATERIAL AND METHODS

The experiment was performed at insemination station in Velké Meziříčí (AgroMeřín, Czech Republic) and it was divided into 3 periods by 45 days, depending on boars spermiogenesis. 12 duroc boars were divided into 2 groups: the control group (n = 6) fed by basic feed mixture and the experimental group (n = 6) fed by basic diet with the addition of 0.5 mg Se, 70 mg Zn, 70 mg vit. E and 350 mg vit. C per kilogram of basic feed mixture. Doses of antioxidant were calculated based on Zeman et al. (2006). After the analysis of basic feed mixture, there was measured antioxidants per kg is dose: 0.02 mg selenium, 21.5 mg zinc, 9.9 mg vit. E and 16.0 mg vit. C.
Boars´ ejaculate was taken by hand-gloved technique once a week. Analysis of ejaculate was performed according to Lovercamp et al. (2013). Volume of ejaculate was measured by weighing, with 1 g to 1 ml conversion. Concentration of sperm was determined by using self-calibrating spectrophotometer Spekol 11 (SpermaCueTM, Minitube of America, Verona, WI) at wavelength 340–850 nm. The sample for spectrophotometer measuring was prepared by mixing 9 ml of 1M HCl and 0.25 ml of ejaculate. Total sperm account was calculated by: volume of ejaculate x concentration of sperm. For sperm motility determination, the ejaculate sample (500 µl) was diluted with 500 µl of Androhep diluent and incubated in 37 °C for 30 min. After incubation, the sample was monitored in contrast microscope with digital camera (Olympus microscope IX 71 S8F-3; Tokio, Japan) and Sperm Vision™ software (Minitube of America, Verona, WI). For morphology determination, 50 µl of each ejaculate was fixed by 5 µl 10% buffered formalin, then 5 µl of this sample was dropped on slide and incubated for 30 min (in 25 °C and 100% humidity to immobilize the sperms). Coloration of samples was saturated in water solution of congo-red and then in 0.5% aqueous solution of crystal violet. Sperm morphology was evaluated using a phase contrast microscope (Zeiss, Germany) with an oil immersion lens at a magnification of 1500×. Subjective assessment was performed by a single qualified person.

The statistical analysis was done using STATISTICA.CZ version 10.0 (Czech Republic). The results were stated as the mean ± standard variance. Statistical significance was observed between the groups (the first sampling was taken as a control one) using ANOVA and Scheffe's test – the two-factor analysis (the first factor was the animal group, the second one - the sampling factor) for parameters of ejaculate volume, sperm concentration, motility and percentage of morphologically abnormal sperm. The difference ($P < 0.05$) was considered as significant.

RESULTS AND DISCUSSION

Based on the results it can be considered that supplementation of vitamin-mineral premix has no significant influence on quality of ejaculate. Hadwan et al. (2000) states that the addition of zinc in diet can improve sperm motility and morphology. Moreover, Marin-Guzman et al. (2000) in their experiment show higher amount of sperm cells in the group of boars with supplementation of selenium compared to group without supplementation. We have not confirmed these hypotheses in our experiment.

Although the results were not statistically significant, it can be stated that the addition of antioxidants to the feed ration could stabilize the values of ejaculate quality for ID preparation. Motility in the control group, without the antioxidant supplementation, decreased below the level 70 %, that is required as the lowest level of motility for ID preparation. Also, in morphologically abnormal sperm, percentage of abnormal sperm in control group increased above the required level 20 %. In experimental group, all observed parameters of ejaculate were more or less stable during the whole experiment.

Similar results to ours reached Horky et al. (2016).

Values of qualitative assessment of the ejaculate for the production of insemination doses taken from the standard ČSN 46 7114 ‘’Boars sperm’’.

Table I Average values of analysed parameters in each period

Period	1		2		3	
Group	Control	Experiment	Control	Experiment	Control	Experiment
Volume of ejaculate	**187.33** ±38.73	**217.31** ±104.22	**173.23** ±31.36	**234.46** ±97.87	**200.83** ±49.53	**271.31** ±115.95
Concentration of sperm	**463.67** ±146.20	**465.72** ±161.16	**541.93** ±142.86	**473.76** ±187.60	**498.67** ±187.21	**458.50** ±184.19
Total rate of sperm	**85.79** ±32.97	**93.61** ±39.29	**89.44** ±15.28	**97.69** ±26.39	**92.66** ±25.47	**109.98** ±35.33
Motility of sperm	**71.58** ±3.47	**73.75** ±3.06	**67.33** ±4.31	**74.06** ±4.00	**70.33** ±9.98	**74.08** ±4.01
Morphologically abnormal sperm	**10.00** ±7.35	**8.22** ±4.45	**14.37** ±8.48	**15.86** ±8.13	**23.85** ±11.73	**17.40** ±8.97

CONCLUSION

Although we have not achieved the desire results, we can state that supplementation of antioxidant complex can improve, or at least can stabilize the quality of ejaculate. From the results we can confirm, that addition of vitamin-mineral premix stabilizes the value of sperm motility as well as percentage of morphologically abnormal sperm in comparison with control group of boars without supplementation.

ACKNOWLEDGEMENT

This project was funded from grants and IGA TP 7/2017: Analysis of performance and behaviour of farm animals in relation to ambient temperature variability and possibilities of elimination of its impact.

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THE EFFECT OF UREA ADDITION ON NUTRITIVE VALUE OF GRAPE POMACE SILAGES

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ABSTRACT

The aim of the experiment was to determine the influence of added urea on nutritive value of grape pomace silages after 6 weeks of storage in mini silo bags. The experiment consisted of two variants: variant C (control-without additive) and variant A with the addition of urea. In variant A additional urea was applied on fresh grape pomace (*Vitis vinifera* L.) variety *Pinot Gris* at rate of 2 $kg.t^{-1}$. The silage matter was stored in mini silo bags and hermetic sealed. After 6 weeks of storage, average samples of grape pomace silages were taken for determination of nutrient's content. Application of urea influenced the nutritional quality of the grape pomace silages with statistically higher dry matter, crude protein (by 12.5%), starch and organic matter content, however lower total sugars content. The results confirmed, that the urea addition had positive effect on the protein value (PDIN by 12.5% and PDIE by 10.6%), but not influenced energy value of grape pomace silages.

Keywords: grape pomace; silage; urea; nutritive value

INTRODUCTION

Grapes are one of the most extensively cultivated crops in the world with almost 63 million tons produced worldwide, and the vast majority of the total grape production (75%) is used to produce wine (FAOSTAT, 2013). Approximately 20% of the grapes (by weight) constitute the main winemaking by-product, the grape or wine pomace (Laufenberg et al, 2003). The large amounts of wine pomace obtained from the winemaking process and their potential market has led food researchers to look for new alternatives that exploit this by-product (García-Lomillo and González-SanJosé, 2017). A topic of interest in recent years in feed lots has been the search for strategies that optimise

nutrient synchrony between N and carbohydrate compounds in the rumen in order to promote better nutrient utilisation and energy efficiency, and as a strategy for reducing the risk of environmental pollution (Hristov et al., 2011). The N retention in the rumen is mainly mediated by the rate of degradation of N compounds and carbohydrates and by the energy available for the process of protein synthesis (Tedeschi et al., 2002). The N retention in the rumen is mainly mediated by the rate of degradation of N compounds and carbohydrates and by the energy available for the process of protein synthesis (Milton et al., 1997; Zinn et al., 2003). Urea $CO(NH_2)_2$ is a non-protein nitrogen compound that can only be fed to older ruminants. For its risk-free feeding, it must be sufficient to have enough energy in the feed, especially in the form of easily digestible carbohydrates, and the feed rate must be compensated for the content of minerals and vitamins that are important for the development of ruminal microflora using ammonia released by hydrolysis of urea (Bíro et al., 2016). Although ensiling is an inexpensive conservation technique which can be applied also for fresh grape pomace having a limited storage ability, information on its effect on crude nutrient content, polyphenol stability and nutrient digestibility of grape pomace is scarce (Baumgärtel et al. 2017).

MATERIAL AND METHODS

Fresh grape pomace (*Vitis vinifera* L.) of variety *Pinot Gris* with dry matter content 45% in laboratory conditions was ensilaged. Grape pomace was from University Experimental Farm in Kolinany. Grape pomace was ensilaged in 2 variants: as a control without additive (C) and with as an experimental with urea addition applied at a rate 2 $kg.t^{-1}$ (A). Grape pomace from both variants (n=3) was rammed into mini silo bags and hermetic sealed. The mini silo bags for 6 weeks in climatized laboratory conditions (t 22±1 °C) were stored. Subsequently, average samples of grape pomace silages were analysed in the Laboratory of quality and nutritional value of feed at the Department of Animal Nutrition at the Slovak University of Agriculture in Nitra. Nutrients were analysed by standard analysed methods and procedures (MARDSR no. 2145-2004-100): dry matter (DM)- by drying at 103±2 °C, crude protein (CP)- by the Kjeldahl method, fat (F)- by Soxhlett-Henkel extraction method, Ash (A)- by complete combustion in a muffle furnace, crude fiber (CF): by Hennenberg-Stohmann method, nitrogen free extract (NFE)- by calculation NFE = DM – (CP + F + CF + A), total sugars- by a Luff-Schoorl method, starch- by polarimetric

method, organic matter (OM)- by calculation OM = DM – A. Energy (net energy of lactation- NEL, net energy of gain- NEG) and protein values (PDIN, PDIE) were calculated by regression equations (Regulation of the Government of Slovak Republic no. 439/2006, appendix no.7, part G Nutritive value of feeds). Statistical parameters using SPSS Statistics 20.0 (IBM) (ANOVA-Tukey test) were evaluated.

RESULTS AND DISCUSSION

After the addition of urea, significantly ($P<0.05$) higher content of dry matter in grape pomace silages in comparison with control was determined (Table 1). The crude protein content was higher by 12.5% in silages with urea ($P<0.05$). This findings are similar with Dinić et al. (2015) where the chemical additive with the presence of urea was used. Winkler et al. (2015) found lower crude protein content (109 $g.kg^{-1}$ of dry matter) in grape pomace silages without additive and higher content in silages with chemical additive (130 $g.kg^{-1}$ of dry matter). There were no significant differences in fat and crude fiber content. Statistically nonsignificant differences in mentioned nutrients in the research of Dinić et al. (2015) were observed. The content of nitrogen free extract and total sugars was significantly ($P<0.05$) higher in silages without additive. Different results in sugar content were reported by Winkler et al. (2015) after the addition of a chemical additive. The addition of 2% urea at the time of ensiling has proven beneficial to reduce starch losses during the fermentation process, what resulted in significantly ($P<0.05$) higher starch content in grape pomace silages. The content of organic matter in silages with urea in comparison with silages without additive was significantly higher ($P<0.05$). The value of net energy of lactation and net energy of gain by the application of urea were not influenced (Table 2). Grape pomace silages had very low energy value. However Yaghoubi et al. (2014) found that urea addition (1%) can improved energy content of grape pomace silages. Ensiled grape pomace, which has a low energy content comparable with straw of wheat and oat, might replace other low-quality roughages for ruminants (Winkler et al., 2015). Grape pomace silages with urea had significantly ($P<0.05$) higher content of PDIN (by 12.5%) and PDIE (by 10.6%) in comparison with control.

Table 1 Nutrient's content of grape pomace silages

Nutrients ($g.kg^{-1}$ of dry matter)	Parameter	Variant	
		C	A
Dry matter $g.kg^{-1}$	$\bar{x}$	366.60*	386.88*
	S.D.	7.90	10.20
Crude protein	$\bar{x}$	131.64*	148.03*
	S.D.	3.53	2.51
Fat	$\bar{x}$	108.96	113.15
	S.D.	6.33	6.40
Crude fiber	$\bar{x}$	206.00	200.51
	S.D.	11.47	6.55
Ash	$\bar{x}$	50.13*	47.04*
	S.D.	0.97	0.33
Nitrogen free extract	$\bar{x}$	503.27*	491.29*
	S.D.	4.60	10.05
Starch	$\bar{x}$	51.97*	57.31*
	S.D.	2.33	3.29
Total sugars	$\bar{x}$	15.59*	13.85*
	S.D.	0.87	1.02
Organic matter	$\bar{x}$	949.87*	952.96*
	S.D.	0.97	0.33

*values with the same index in the line are statistically significant at $P < 0.05$, C: grape pomace silage without additive (control), A: grape pomace silage with urea (additive)

Table 2 Nutritive value of grape pomace silages

Nutritive value (in kg^{-1} of dry matter)	Parameter	Variant	
		C	A
PDIN g	$\bar{x}$	72.53*	81.58*
	S.D.	1.96	1.39
PDIE g	$\bar{x}$	43.77*	48.40*
	S.D.	1.01	0.59
Net energy of lactation MJ	$\bar{x}$	3.27	3.27
	S.D.	0.01	0.01
Net energy of gain MJ	$\bar{x}$	2.51	2.51
	S.D.	0.01	0.01

*values with the same index in the line are statistically significant at P <0.05, C: grape pomace silage without additive (control), A: grape pomace silage with urea (additive)

CONCLUSION

Application of urea positively influenced the nutritional quality of the grape pomace silages after 6 weeks of storage. Statistically higher dry matter, crude protein, starch and organic matter content, but lower total sugars content in silage with the addition of urea was found. Furthermore, results confirmed, that the urea addition had positive effect on the protein value (PDIN and PDIE), but did not influence the energy value of grape pomace silages.

ACKNOWLEDGEMENTS

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-16-0170.

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CHEMICAL COMPOSITION OF MUSCLES FROM FATTENED CHICKENS AND DUCKS FED BY DIETS BASED ON LUPIN SEED MEAL

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ABSTRACT

We can extrapolate from the analytical results of breasts and femoral muscles of chickens and ducks that replacing soybean meal with lupin seed meal does not have a negative influence on muscle contents of their slaughter body. From a dietetic point of view we can evaluate positively that feeding diets based on lupin seed meal resulted in fat reduction in muscles and also in their energy reduction.

Keywords: Feeds; lupin; muscle; crude protein; fat; ash; crude energy

INTRODUCTION

Nowadays, there is a long-term focus in agricultural production on planting and production of domestic protein commodities and their use in feed for farm animals. From our point of view, seeds from cultivated plants genus *Lupinus* have good prospects because of their high crude protein contents. Lupin seed meal is a potential component for future feeds. That is why our idea was to test possibilities of using lupin seed meal as a replacement for soybean meal in feeds for fattening broilers and ducks and to test whether this replacement has not negative influence on nutritional value of their muscle. Suchy et al. (2017) dealt with nutritional value of lupin seed and Geigerova et al. (2017) or Karel et al. (2017) dealt with using lupin seed for fattening of broilers.

MATERIAL AND METHODS

The main objective of this experiment was to test the influence of replacing soybean meal with lupin seed meal used for fattening of broilers and ducks. There were two experimental groups and a control group. 50% of soybean meal crude protein was replaced with lupin seed meal in diets in the experimental group E 50% and 100% of soybean meal crude protein was replaced with lupin seed meal in the experimental group E 100%. Diets in the control group K 0% contained the same components as diets in the experimental groups, only the presented replacement was done. The experiments were performed at the accredited farm in Department of Animal Nutrition (University of Veterinary and Pharmaceutical Science Brno) with smart controlled light mode and heat mode. The experiments were done in compliance with technological manuals for fattening of the respective hybrids. Hybrid ROSS 308 broilers and Charry Valley ducks were used. Fattening of broilers took 35 days and fattening of ducks 40 days. At the end of the fattening, 20 members with average weight from each group were selected, 10 of them were males and 10 females. Samples from their femoral muscles and breasts were taken for chemical tests after slaughtering. The samples were dried at temperature 105°C into constant weight. After that the samples were homogenized and then they were ready for the chemical analysis. Crude protein in g/kg (by Kjeldahl, N x 6,25), fat (by analytical machine ANKOM^{XT10}), ash (incinerated at temperature 550°C into constant weight) and crude energy in MJ/kg (by analytical machine AC 500) were determined. Results of analysis were computerized with mathematics-statistic methods (by software Unistat 5.6) using Tukey-HSD test. The files were characterised using arithmetic mean (x) and standard deviation (± SD).

RESULTS AND DISCUSSION

Broilers

It is obvious from Table 1 that diets prepared on basis of the lupin seed meal did not affect significantly the chemical contents of breasts. We can evaluate positively the fat reduction in muscles of the experimental groups. We also observed statistically significant decreasing of energy value of breasts.

Table 1 Average values of basic chemical contents of broilers´ breast muscles (PS) without sex differentiation (FM) in g/kg or MJ/kg, x – arithmetic mean, SD – standard deviation, P ≤ 0,05 ab.

Dry matter	**x**	SD	**Crude protein**	**x**	SD
PS FM K 0%	**252.87**	11.145	PS FM K 0%	**854.14**	28.532
PS FM E 50%	**253.32**	5.674	PS FM E50%	**855.62**	29.900
PS FM E 100%	**250.68**	6.771	PS FM E 100%	**863.85**	27.927
Ash	**x**	SD	**Crude energy**	**x**	SD
PS FM K 0%	**45.51**	2.099	PS FM K 0%	**24.13**[a]	0.363
PS FM E 50%	**46.58**	1.583	PS FM E 50%	**23.82**[b]	0.411
PS FM E 100%	**47.21**	2.109	PS FM E 100%	**23.58**[b]	0.419
Fat	**x**	SD			
PS FM K 0%	**121.93**[a]	24.693			
PS FM E 50%	**109.33**	18.691			
PS FM E 100%	**99.36**[b]	23.916			

We can see in Table 2 that there is statistically significant difference between average values of monitored indicators of control group and experimental groups in femoral muscles.

Table 2 Average values of basic chemical contents of broilers´ femoral muscles (SS) without sex differentiation (FM) in g/kg or MJ/kg, x – arithmetic mean, SD – standard deviation

Dry matter	**x**	SD	**Crude protein**	**x**	SD
SS FM K 0%	**285.85**	9.680	SS FM K 0%	**682.61**	30.829
SS FM E 50%	**281.71**	10.712	SS FM E 50%	**706.23**	25.747
SS FM E 100%	**285.50**	10.033	SS FM E 100%	**687.86**	50.164
Ash	**x**	SD	**Crude energy**	**X**	SD
SS FM K 0%	**37.35**	2.331	SS FM K 0%	**27.29**	0.518
SS FM E 50%	**38.40**	2.004	SS FM E 50%	**27.11**	0.491
SS FM E 100%	**37.72**	1.771	SS FM E 100%	**27.13**	0.487
Fat	**x**	SD			
SS FM K 0%	**312.60**	28.820			
SS FM E 50%	**297.04**	28.798			
SS FM E 100%	**311.81**	25.355			

Ducks

There were not statistically significant differences between average values of monitored indicators of the control group and experimental groups in breast muscles. The values are presented in Table 3.

There was higher ash content and lower energy content in femoral muscles in experimental groups after fattening with lupin seed meal diets. The values are presented in Table 4.

Table 3 Average values of basic chemical contents of ducks´ breast muscles (PS) without sex differentiation (FM) in g/kg or MJ/kg, x – arithmetic mean, SD – standard deviation

Dry matter	**x**	SD	**Crude protein**	**x**	SD
PS FM K 0%	**229.17**	5.764	PS FM K 0%	**849.34**	33.450
PS FM E 50%	**227.65**	7.188	PS FM E 50%	**858.06**	17.865
PS FM E 100%	**224.97**	5.678	PS FM E 100%	**850.40**	25.295
Ash	**x**	SD	**Crude energy**	**x**	SD
PS FM K 0%	**55.73**	2.225	PS FM K 0%	**23.26**	0.201
PS FM E 50%	**54.94**	1.206	PS FM E 50%	**23.20**	0.339
PS FM E 100%	**55.76**	1.955	PS FM E 100%	**23.22**	0.291
Fat	**x**	SD			
PS FM K 0%	**82.17**	10.156			
PS FM E 50%	**80.12**	13.651			
PS FM E 100%	**87.11**	17.060			

Table 4 Average values of basic chemical contents of broilers femoral muscles (SS) without sex differentiation (FM) in g/kg or MJ/kg, x – arithmetic mean, SD – standard deviation, $P \leq 0,05$ ab.

Dry matter	**x**	SD	**Crude protein**	**x**	SD
SS FM K 0%	**261.54**^{a}	7.090	SS FM K 0%	**777.19**	29.306
SS FM E 50%	**254.57**^{b}	7.443	SS FM E 50%	**776.88**	16.732
SS FM E 100%	**255.02**^{b}	6.249	SS FM E 100%	**762.84**	14.829
Ash	**x**	SD	**Crude energy**	**x**	SD
SS FM K 0%	**41.80**^{b}	1.534	SS FM K 0%	**25.44**^{a}	0.369
SS FM E 50%	**43.27**^{a}	1.168	SS FM E 50%	**25.38**	0.418
SS FM E 100%	**43.76**^{a}	1.513	SS FM E 100%	**25.11**^{b}	0.408
Fat	**x**	SD			
SS FM K 0%	**204.01**	21.053			
SS FM E 50%	**195.54**	21.774			
SS FM E 100%	**190.67**	17.204			

CONCLUSION

We can extrapolate from the analytical results of breasts and femoral muscles of chickens and ducks that replacing soybean meal with lupin seed meal does not have negative influence on muscle contents of their slaughter body. We can evaluate positively from a dietetic point of view that feeding diets based on lupin seed meal resulted in fat reduction in muscles and also in energy reduction.

ACKNOWLEDGEMENT

Trial was financially supported by grant project QJ1510136 Optimization of monogastric animal protein nutrition based on lupin white seeds variety (*Lupinus Albus L.*).

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THE IMPACT OF THE PRODUCT WITH HUMIC ACIDS ON THE PRODUCTION PARAMETERS OF BROILER CHICKENS

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ABSTRACT

The effect of humic acids on health and production parameters (body weight and feed conversion ratio) was monitored in the experiment in broiler hybrids (Cobb-500) (n = 60). Chickens of tested group were fed diets supplemented with natural humic compounds in commercial product HUMAC® Natur AFM Monogastric in dose 5 g per 1 kg of feed mixture. In the course of the experiment, one broiler chicken in the control group died and one chicken from each group was eliminated due to the significant growth delay. The average body weight (B.W.) of broilers on day 35 achieved in the control group was 2291 g and in the test group 2282 g. There was no statistically significant difference between the two groups. The use of humic acid supplement did not affect feed conversion ratio during the experiment, which was 1.51 respectively 1.53 kg/kg in the control and in the test group.

Key words: broiler, humic acids, health, production

INTRODUCTION

Humic substances are characterized as soil components which have been created by the decomposition of biological matter containing plant and animal residues and by the activity of microorganisms found in the soil. They occur in natural materials such as sediment, turf, brown coal, lignite and other materials. Due to their chemical structure formed by the aromatic nucleus and the functional group, they are efficient to bind the polar and non-polar compounds. Therefore, they are having the ability to influence the availability of various nutrients in the animal organism (Tichá et al., 2009). Humic substances are used to influence

soil fertility as well as in the animal husbandry to reduce environmental load (nitrogen, phosphorus) and the odour in housing objects (Greene and Cool, 2000, Rashid et al., 2018). Their application is in human and veterinary medicine for prevention, stimulation of the immune system and in the supportive treatment of some diseases, too. The substances are characteristic with the detoxifying effect when they are binding various toxic substances and ensure their removal through excrements. They are able to activate metabolism, promote health, to reduce mortality, to improve the growing intensity and the feed conversion as well as the fattening efficiency index (Veselá et al., 2005; Vaško, 2011; Vaško et al., 2012; Mudroňová et al., 2018).
We observed the effect of humic substances on the health and production parameters in the feed experiment in broiler chickens.

MATERIAL AND METHODS

There were used one-day 60 broiler chickens COBB 500 with the average individual body weight of 50g ± 1 in the experiment. The chicks were divided into two groups of 30 each. The control group (C) was fed with a commercial complete feed mixture (CFM). CFM BR1 was used for first 10 days. CFM BR2 was fed for another 14 days and CFM BR3 was applied till the slaughter. The test group (E) of broiler chickens was fed the same way but HUMAC® Natur AFM Monogastric in dose 5 g per 1 kg of feed mixture was added. Technical parameters and properties of product: humic acid in dry matter min. 57%, fulvic acid min. 5%, formates 3.24 %, particle size up to 100 μm and humidity max. 15%. Chickens were given feed and water ad libitum during fattening period. The mortality was monitored in the course of the experiment. The chicks were weighted at weekly intervals. The feed consumption was monitored weekly and from feed consumption and daily gain the feed conversion ratio was calculated in both groups in weekly frequency and at the end of experiment (on day 35). The comparison of the observed production parameters between groups was done using the t-test using Prism Free Trial software (GraphPad Software, USA).

RESULTS AND DISCUSSION

The effect of humic acids addition on health and production parameters, body weight and feed conversion ratio was monitored in the experiment in broiler hybrids (Cobb-500). The animals were housed in the experimental conditions of the breeding establishment, providing

the conditions for the fattening of broiler chickens. Initially, the temperature was 30.9 °C with a gradual decrease to 21 °C on day 23 in the experimental pens. This temperature was maintained until the end of the experiment. The average relative humidity was 60.93%. The mortality of 1 chicken was registered in the control group in the second week. In the third week, one chicken was eliminated from both groups because of more pronounced growth delay. Results, which are described in Table 1 and 2, were achieved from the individual measurements of the broiler body weight and the consumption of the feed mixture carried out at weekly intervals. The average body weight in one-day chicken was 50g ± 1 in both groups. At day 35, the average body weight was 2291.7 g in the control group and 2281.9 g in the test group. The average body weight of chickens was more or less balanced between groups up to 3 weeks of age. The average body weight of chicken was higher in the control group by 48 g in week 4 and by 10g in week 5. There was no statistically significant difference between the two groups.

Table 1 Average B.W. in the control C and test group E (g/chicken)

Week / group	C	E
1	191.7	192.3
2	514.8	521.5
3	985.5	989.3
4	1633.0	1585.2
5	2291.7	2281.9

The consumption of feed mixture in the control and test groups is shown in Table 2. There were fed 98.37 kg in total in the control group and 104.06 kg of CFM in the control group. The difference between groups is caused due to the final unequal number of chicks in the control n = 28 and in the test group n = 29.

The average consumption of CFM of one chicken per one day increased in the control group from 27.9 g in the first week, to 161.2 g in the fifth week. While in the test group from 29.1 g in the first week to 177.8 g in the last week of the trial.
The feed conversion ratio was calculated from the total consumption of CFM and the daily gain of chicks. The average feed conversion ratio for whole experiment was 1.51 kg/kg in the control and 1.53 kg in the test group. In spite of the fact that in the last week of the experiment the parameter of feed conversion ratio was higher by 0.22 kg (C - 1.71 kg;

E - 1.93 kg) in the test group, it can be stated that the use of humic acid supplement in our experiment did not affect the average feed conversion in the observed groups. There was not observed statistically significant difference.
Demeterová and Šamudovská (2011) reported no significant differences in body weight between group using sodium humate and control group in their broiler experiment. The average body weight of chicks in the control group was 2476.6 g and in the experimental group 2481.5 g on day 37. In their study, the feed consumption was 3.58 kg/chicken in the experimental group and in the control group, it was by 0.07 kg/chicken lower. Similarly, in our experiment we observed the same, with the calculated feed consumption 3.59 kg/chicken respectively 3.51 kg/chicken in the test and in the control group.

Table 2 Quantity of the used feed mixture in the control C and the test E group during fattening period

Week	Used feed mixture (in kg)	
	C	E
1	5.45	5.68
2	12.92	12.68
3	20.80	21.20
4	27.60	28.40
5	31.60	36.10
Total	**98.37**	**104.06**

Marcinčáková et al. (2018) found no positive effect of the addition of humic acids on the feed consumption (control group 3883.3 g, experimental group 3882.7 g) and the feed conversion ratio (control group 1.64, experimental group 1.63) in the experiment with addition of humic acids (0.8%) in broiler chicken.

In our study, the positive effect of the addition of humic acids was observed in the reduction of mortality in the test group, where no individual chickens died compared to the control group with one case of mortality. Therefore, we agree with Vaško et al. (2010), who reported the beneficial effects of the addition of humic acids on the reduction of livestock mortality.

CONCLUSION

Feed additives are used in nutrition of farm animals for improving of the nutritional value of feeds, the nutrient utilization and the production parameters. The possible preparations of this character are humic acids. The achievement of positive results at their application is influenced by several factors. We did not observe any statistically significant differences in production parameters in our experiment in broiler chickens after the addition of humic acid supplement in dose 5 g per kilo of feed mixture. The preliminary results assign a positive effect on some product quality parameters, blood indicators and the immune system stimulation.

ACKNOWLEDGEMENT

The project was supported by the Slovak National Scientific Grant Agency VEGA Grant No. 1/0408/17

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NutriNET 2019
International Animal Nutrition PhD Conference
Proceedings of the conference NutriNET 2019

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Ing. Lucie Všetičková, Ph.D.

Published and printed by: Tribun EU s.r.o.
Cejl 892/32 602 00 Brno

Number of printed copies: 50
Number of pages: 52

This publication did not pass through stylistic revision

Tribun EU, first edition
Brno 2019

ISBN 978-80-263-1465-3

www.knihovnicka.cz