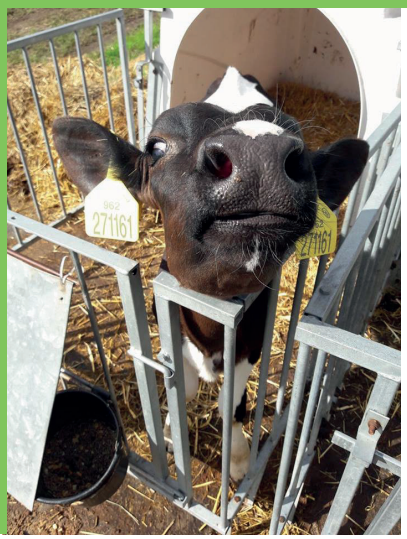
Mendel University in Brno

NutriNET 2018

Faculty of AgriSciences



Proceeding of Reviewed Scientific Papers

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Mendel University in Brno

Faculty of AgriSciences

Department of Animal Nutrition and Forage Production

NutriNET 2018

Proceedings of reviewed scientific papers

The conference is organised in the framework of the project number QJ1510206

May 24–25, 2018

Brno

NutriNET 2018

Proceedings of reviewed scientific papers

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prof. Ing. Jiří Zelenka, CSc

prof. Ing. Ladislav Zeman, CSc, dr. h. c.

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The NutriNET is a conference of PhD students whose research theme corresponds with animal nutrition.

Historically, the first conference, entitled NutriNET held in Brno 21–22 November 2012 under the auspices of Mendel University (Czech Republic).

Furthermore, tradition of PhD students conference addicted to animal nutrition began in 1998 in Prague, Czech University of Live Sciences Prague, where prof. Ing. Zdenek Mudřík, CSc. and his colleagues organized the first doctoral student's conference. Since this year began a string annual conference PhD student dealing with animal nutrition, feedstuffs etc. The conference took place in the annual, sometimes least every two years, but each time at a different university, but always at the institute or department of animal nutrition.

Contributions of these authors were placed in the first three places in year 2018:

1st: Ing. Klára Laloučková

2nd: Ing. Barbora Znoj Novotná

3rd: Ing. Andrea Roztočilová

Traditional conference organizers NutriNET these universities:

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University of South Bohemia in České Budějovice

University of Veterinary and Pharmaceutical Sciences Brno

Mendel University in Brno

Slovak University of Agriculture in Nitra

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EFFECT OF DIFFERENT FORMS OF ZINC ON MICROBIAL INDICATORS OF LABORATORY RATS FECES

DARIA BAHOLET, PAVEL HORKÝ, JIŘÍ SKLÁDANKA, LENKA URBÁNKOVÁ

Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: dariabaholet@gmail.com

ABSTRACT

This project was carried out in the experimental part of the Institute of Animal Nutrition and Forage Production at Mendel university in Brno. In recent years, it has been used as a tool for reducing the use of antibiotics and to prevent diarrheal diseases in weaned piglets. The aim of this methodology was to create a coherent process of zinc nanocomplexes synthesis using phosphates. The microbiological characteristics of the individual zinc nanocomplexes are part of the methodology.

Keywords: zinc nanoparticles; microbial indicator; nutrition

INTRODUCTION

Zinc is a trace element essential for animals and serves as a component of many metalloenzymes, including DNA and RNA synthetises, and plays important role in metabolism and intestinal nutrient absorption. Rapid developments in nanotechnology provide new dimensions for researches possibly used as substitutes of antibiotics and high dietary ZnO.

MATERIAL AND METHODS

As a model animal for this experiment, male rats of the Wistar albino strain were selected; animals were divided into 7 groups of 5 cells. The average weight of the rats at the start of the experiment was 144 grams. Throughout the experiment, microclimatic conditions were observed in the laboratory. They were mainly limited by the temperature measured

by "DATALOGER S 3120" and maintained at 23 °C ± 1 °C. Also, air humidity was measured and maintained at 60 % by the same system. The photoperiod was manually controlled for 12 h day and 12 h night, scheduled with maximum illumination of 200 lx. Mono diet comprised wheatgrass with 2000 mg Zn per kg of the mixture. The feeding experiment lasted for 30 days. Zinc particles and complexes were studied for their antibacterial activity *in vitro*, especially in bacterial cultures of *Staphylococcus aureus*, MRSA and *Escherichia coli*. Antimicrobial activity was evaluated by measuring inhibition zones and growth curves. Using microbiological methods, the most effective complexes were tested on their antibacterial properties against a wide range of microorganisms - bacteria, fungi and yeasts. Findings of new information on nanoparticles, microparticles and zinc complexes will serve as a practical output. The outcome of this phase of the project will be certified methodology.
Feed and excrement samples were distributed on the MW Ethos ONE, where the samples were mineralized in 4 ml concentrated HNO_3 Suprapure, 1 ml H_2O_2, and 5 ml H_2O. Mineralization took place for 35 minutes at 210 °C. Analysis of zinc content was performed on a 240FS Agilent Technologies using flame atomization in an acetylene or air flame. Samples were stored at 5 °C prior to zinc determination. IN. Wavelength 213.86 Nm.

Zn-A

$$3\ Zn(NO_3)_2 \cdot 6H_2O + 2\ (NH_4)_2HPO_4 \rightarrow Zn_3(PO_4)_2$$

The mixed nitrate (4.46 g) was dissolved in water (50 ml) and heated to 60 °C. A solution of ammonium hydrogen phosphate (1.32 g in 20 ml of water) was added, stirring constantly. A white precipitate immediately formed. The suspension was stirred for 2 hours and the volume was refilled to 100 ml.

Zn-B

$$3\ Zn(NO_3)_2 \cdot 6H_2O + 2\ Na_2HPO_4 \cdot 7H_2O \rightarrow Zn_3(PO_4)_2$$

Zinc nitrate (4.46 g) was dissolved in water (50 ml) and heated to 60 °C. A solution of sodium hydrogen phosphate (2.68 g in 20 ml of water) was added, stirring constantly. A white precipitate immediately formed. The suspension was stirred for 2 h and the volume was refilled to 100 ml.

Zn-C

$$2\ Zn(NO_3)_2 \cdot 6H_2O + Na_4P_2O_7 \rightarrow Zn_2(P_2O_7)$$

Zinc nitrate (3 g) was dissolved in water (50 ml) and heated to 60 °C. A solution of sodium pyrophosphate (1.33 g in 20 ml of water) was added, stirring constantly. A white precipitate immediately formed. The suspension was stirred for 2 h and the volume was refilled to 100 ml.

Zn-D

$$5\ Zn(NO_3)_2 \cdot 6H_2O + 2\ Na_5P_3O_{10} \rightarrow Zn_5(P_3O_{10})_2$$

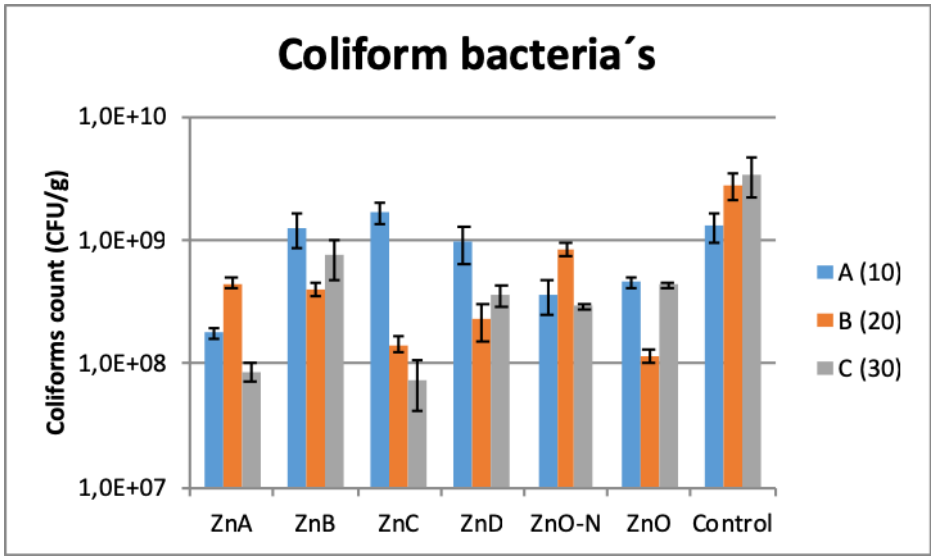
Zinc nitrate (1.49 g) was dissolved in water (50 ml). With stirring, a solution of sodium triphosphate (0.74 g in 20 ml of water) was added. A white precipitate immediately formed. The suspension was stirred for 2h and the volume was made up to 100 ml.

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ).

RESULTS AND DISCUSSION

The first group (Control) served as control with zinc dose not increased. The second group was fed zinc oxide in a dose of 2000 mg

Figure 1. Count of the Coliform´s bacteria in feces



Zn per kg of feed mixture, the third group contained pure zinc nanoparticles (2000 mg Zn per kg of feed mixture) and the remaining four groups included addition of various modifications of zinc nanoparticles (2000 mg Zn per kg of feed mixture). The results are proving that nanoparticles of zinc cause the reduction of coliform bacteria. Figure 1 shows count of the Coliform´s bacteria in feces**.**
Similarly, it has been reported earlier by (Lin, 2008) that nanoparticles exhibit higher bioavailability, and enhance drug absorption. Early reports also verified that dietary supplementation of 20 and 60 mg/kg nano-ZnOs had greater weight gains and better feed conversion ratios than 60 mg/kg ZnO in broilers.

CONCLUSION

Nanotechnology is the emerging technology of present century operating in all fields of science. Zinc oxide nanoparticles stand out as one of the most versatile materials, due to their diverse properties, functionalities, and applications. ZnO NPs have tremendous physical and optical properties. They also possess antimicrobial actions against some bacteria and fungi. As far as synthesis of zinc oxide nanoparticles is concerned they can be synthesized by chemical methods but in recent times due to evolution of green chemistry, biogenic synthesis of ZnO NPs is also possible by using different plant extracts. As far as their usage is concerned nanoparticles play a significant role in agriculture, where colloidal solution of ZnO NPs is used in nanofertilizers. Application of these nanoparticles to crops increases their growth and yield. As food demand is increasing day by day the yield of staple food crops is much low. So it is need of the hour to commercialize metal nanoparticles for sustainable agriculture.

ACKNOWLEDGEMENT

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THE USE OF FERMENTED FEED IN BROILER DIET

MARTIN BARTKOVSKÝ[1], DANA MARCINČÁKOVÁ[2], IVETA MASKAĽOVÁ[3], PETRA TIMKOVIČOVÁ-LACKOVÁ[3], JÁN MAČANGA[1], MILAN ČERTÍK[4], LADISLAV MOLNÁR[5], SLAVOMÍR MARCINČÁK[1]

[1]Department of Food Hygiene, UVLF Košice
[2]Department of Pharmacology and Toxicology, UVLF Košice
[3]Institute of Nutrition, Dietetics and Feed Production, UVLF Košice
[4]Institute of Biotechnology, FCHPT STU Bratislava
[5]Clinic for Birds and Exotic Animals, UVLF Košice

Corresponding email address: bartko.mato@gmail.com

ABSTRACT

The aim of this work was to study the effect of the addition of fermented product on production parameters and carcass yield of COBB 500 broiler chickens. Employment of fermented cereals enriched with polyunsaturated fatty acids (PUFAs) into commercial feeds is therefore promising way of increasing the content of these essential fatty acid within the animals such as broiler chicken. We added 10% of fermented feed into commercial diet while the commercial feed was reduced to a given amount. Our results suggest that addition of fermented feed significantly influenced ($P > 0.05$) carcass weight and weight of body parts.

Keywords: poultry; meat; fermented feed; PUFA

INTRODUCTION

Food production by fermentation is one of the oldest in food technology. Many of these foods have been produced for their unique flavor, aroma and texture that have been and are highly valued by consumers. Several rows of oil-bearing lower fibrous fungi, in particular *Mortierella, Cunninghamella, Mucor, Thamnidium, Pythium* and *Thraustochytrium*, are a good source of PUFAs (Čertík et al., 2013). In addition, fibrous fungi simultaneously reduce the content of antinutrients, partially hydrolyze the biopolymer substrate, and the resulting preferred organic product can be used as an inexpensive food or feed supplement (Slugeň et al., 1994). Solid-state fermentation (SSF) is defined as a solid fermentation in the absence of water (or almost

without) where the substrate must provide sufficient moisture to support the growth and metabolism of microorganisms (Pandey, 2003). Selection of the correct substrate is a key aspect of the SSF. Substrate acts as a physical support and source of nutrients. The solid material may be a naturally occurring solid substrate such as agricultural crops, agro-industrial residues or an inert carrier (Pandey et al., 2000). Advantage of fermented feed is the elimination of antinutritive components and partial hydrolysis of the biopolymers. This may affect the production parameters of poultry fattening. One of the ways to increase the content of PUFAs in cereal diets is to add these important substances to the diet. Great attention is therefore focused on the development of bioprocesses suitable for the production of "organic" cereals enriched with PUFAs. At present, biotechnological processes for the production of γ-linolenic acid using *Mortierella* and *Mucor* are successfully applied on an industrial scale in Japan. Further productivity gains are gained thanks to the still new mutants (Čertík et al., 2013). Growth of lower – filamentous fungi on carbohydrate-containing substrates led to a constant yield of lipids with a desired fatty acid profile. Solid-State fermentation utilizing strain trunks shows considerable potential for the application and utilization of bioproducts enriched with PUFAs from cereals in the veterinary and feed industry (Emelyanova, 1996). The composition of poultry fatty acids can be significantly affected by feeding (Zelenka et al., 2008). SSF presents many advantages for the production of enzymes and chemical products. The advantage of using SSF over today process is higher production of required compounds, better air circulation, production on cheaper media, lower energy and technology costs, and imitation of natural microorganism conditions, thereby improving microorganism production performance. Against the production of pure fermented feed, there is also a way to obtain enzymes. Addition of the enzymes thus produced can increase the digestibility of feed in poultry (Couto and Sanroman, 2006). Growth of the molds on carbohydrate-containing substrates led, after optimization of the culture conditions, to a constant yield of lipids with a desired fatty acid profile. The basic physiology of microbial lipid overproduction is that molds are cultivated with sufficient carbon sources and with a limited amount of other important nutrients such as nitrogen. Grain substrates such as rice bran, wheat bran, oat flakes, crust or barley provide a suitable source of nutrients for mushroom growth and the production of high starch-containing lipids and corresponding levels of organic nitrogen (Čertík et al., 2013).

MATERIAL AND METHODS

In the experiment, 80 COBB 500 hybrids of day-old sexed male broilers were used. The chickens were divided into a control and experimental group (40 pcs), which received a feed supplemented with PUFAs. In this experiment low filamentous fungi (*Mortierella alpina*) were used as a producer of arachidonic (ARA) and eicosapentaenoic (EPA) fatty acid. For the preparation of the spore suspension, which was inoculated with the SSF substrate, *M. alpina* CCF 2861 was grown for 10 days on rice. After 10 days, the spores were washed with distilled water with 0.05% Tween 80 and filtered through a gauze to remove the solid substrate. The spore suspension thus prepared was diluted to a concentration of $2x10^5$ spores / mL. As a substrate we used wheat bran. The control group was fed with standard feed mixtures throughout the feed-off period (42 days). The experimental group (Fermentation Feed) was fed with BR1, BR2, BR3 and from the 10th day of fattening, a 10% bio feed was supplemented, the standard feed were reduced. During calving, chickens had access to water *ad libitum* and feed was given twice a day (morning/evening). During the trial, their health status and feed consumption were monitored. The weight of live broilers was determined by weighing all pieces in each group on the first day of fattening and then during fattening at weekly intervals (14, 21, 28, 35 and 42 days). After the end of the fattening, the average weight of the chickens and the average daily increment of the chickens were calculated. The average daily increment is the weight calculated on the basis of the weight difference of two consecutive weightings over a given period. Feed consumption was recorded daily for each group throughout the fattening period. The average feed consumption for each group was calculated over a certain period (1^{st}-7^{th}, 7^{th}-14^{th}, 14^{th}-21^{th}, 21^{th}-28^{th}, 28^{th}-35^{th}, 35^{th}-42^{nd} day). Subsequently, the average daily feed consumption expressed per one head (ADFI) was calculated. Conversion of feed was calculated as a proportion of the weight of feed consumed during fattening and the increase in the weight of chickens reached at the end of fattening. 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 chickens after slaughtering and weighing before slaughter. To determine the percentage of breast muscle and thigh to the total weight of the carcass, these parts were separated from the body and then weighted. The percentage of breast muscle and thigh in the carcass was calculated as

the weight of the individual parts and the body weight after the slaughter.

RESULTS AND DISCUSSION

Chickens were fed up to the 10th day of age with starter diet, from 10th to the 30th day with grower and from day 31 until slaughter with finisher diet. From the 10th day experimental group were fed with fermented feed in 10% concentration, while the commercial feed was reduced by an equal amount. Table no.1 expresses the nutrient content of the feed, namely the analyzed fermented feed, Starter, Grover and Finisher. Composition of feed has important role in broiler nutrition. Amounts of nutrient affect the production parameters of broiler chicken. By adding fermented feed, we see an increase in nutrients. Because we used wheat bran as a substrate for fermented feed production the amounts of fiber are increased in experimental group.

Table 1. Nutrient composition of commercial and fermented feed

		Grower		Final diet	
	FF	C	E (10%)	C	E (10%)
Dry matter g/kg	964.4	889.9	895.7	884.6	892.9
Crude protein g/kg	184.60	173.60	182.00	166.60	169.20
Crude fat g/kg	36.10	43.00	39.70	41.80	37.60
Crude fibre g/kg	137.40	33.40	46.00	38.90	47.80
NDF g/kg	190.50	123.60	137.20	106.80	127.10
ADF g/kg	150.2	54	60.4	61.90	68.9
Starch g/kg	134.80	416.30	407.60	444.60	438.00
Ash g/kg	62.50	44.00	38.80	34.60	36.70
Ca g/kg	1.70	8.43	8.33	7.96	7.55
Mg g/kg	4.00	1.05	1.17	1.62	1.18
Na g/kg	0.20	1.57	1.43	1.30	1.47

K g/kg	13.00	7.76	8.20	6.10	7.30
P g/kg	10.40	5.60	4.70	5.13	5.43
Cu mg/kg	13.50	13.70	16.30	14.76	18.00
Zn mg/kg	83.00	103.80	93.40	82.30	78.10
Mn mg/kg	109.10	97.50	95.70	80.50	80.50

NDF = neutral detergent fiber; ADF = acid detergent fiber; FF – fermented feed

C – control group; E – experimental group

Table 2. Production parameters

	Daily intake		Daily intake/1 bird		Gains		Conversion	
week	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**
1	173.5	173.5	4.33	4.33	-	-	1.59	1.65
2	372.6	374.4	9.32	9.36	343.8	331.6	1.53	1.64
3	650.0	641.8	16.25	16.05	424	391.7	1.72	1.73
4	945.0	946.5	23.63	23.66	550.7	545.7	1.67	1.82
5	1204.0	1202.9	30.10	30.07	722.2	660.8	1.66	1.85

C – control group; E – experimental group

In **table 2** we see production parameters of broiler chickens. The addition of fermented feed negatively affects the gains and conversion. Despite the fact we are able to enrich the produced meat of chickens by polyunsaturated fatty acids (PUFAs) feeding of fermented feed negatively influenced growth and conversion of the feed. Based on these results, we can see that using the *Mortierella alpina* strain and wheat bran as a substrate we did not achieve the desired improvement over the control group. In the past, strains tested such as *Umbellopsis isabellinum* gave us better results, and production parameters were comparable or better according to control than in this experiment.

Table 3. Weights of carcass and body parts

	Control group	Experimental group
live weight (g)	$2464.1^{a} \pm 226.1$	$2326.1^{b} \pm 172.5$
carcass weight (g)	$1868.6^{a} \pm 185.6$	$1727.4^{b} \pm 134.5$
carcass yield (%)	75.8 ± 10.1	74.3 ± 7.2
breast (g)	$480.5^{a} \pm 56.5$	$426.4^{b} \pm 56.3$
thighs (g)	517.9 ± 46.5	514.6 ± 45.8
wings (g)	178.8 ± 12.6	181.0 ± 16.1
hull (g)	641.1 ± 51.2	609.7 ± 59.8
fat (g)	$35.7^{a} \pm 10.8$	$29.5^{b} \pm 6.8$

^{a,b} – values are statistically significant ($P < 0.05$)

Table 3 show the differences in the carcass and body parts of broilers. Feeding of fermented feed has positively influenced only the weight of the wings. However, in experiment, the experimental group had worse results in weights than control group.

Full-fledged feed additive was created using SSF and the appropriate type of mold, and only with the use of waste materials of agricultural production. Although the bioproduct is essentially only a ballast substance, as shown by a higher fiber content, the fermentation process has significantly affected not only the fatty acid composition but also the composition of the nutrients and consequently the digestibility. The digestibility of the fermented feed is enhanced by mold-produced enzymes that are needed to hydrolyze sources bound in biopolymers of the substrate. The plums of the mold during fermentation penetrate rapidly into the substrate (wheat bran), resulting in an efficient conversion of the substrate into a fermented feed. Such a preferred organic product, along with standard feed mixtures, has had a significant impact on poultry production parameters. In the work, after the application of the organic product, the consumption of the feed was recorded, with the final weight of the chicken being increased, the better gains and the better conversion of the feed. The other most commonly used additives in poultry feed are antimicrobials and antioxidants. Studies show that some additives, especially plants and their extracts, have properties stimulating irritation, antibacterial effects or antioxidant properties (Jang et al., 2008). These substances are referred to as phytogenetics. The mode of action of plant extracts may vary considerably. Possible mechanisms of action are stimulation of endogenous enzymes, regulation of gut microflora and chemical effects (lowering of pH in GIT), which can lead to animal health improvement (Langhout, 2000). There is a discussion that phytogenic additives are

added to a set of non-antibiotic growth stimulators such as organic acids and probiotics that are now well established in animal nutrition. Poultry synthesizes and deposits other lipids only if the energy density or the calorie / feed protein ratio increases, irrespective of the energy source. Therefore, the increased amount of fat in the feed does not affect the fatty tissue of chickens when the calorie / protein ratio remains balanced (Doreau and Chilliard, 1997). A fermented feed produced by Solid-State fermentation, has been favorably influenced by standard feed mixtures by increasing crude protein and crude fiber content. Results in the table 3 shown that the fermented feed affected the feed intake of the experimental group. Despite the increased intake, the test group carcass parameters were worse. The plums of the mold during fermentation penetrate rapidly into the substrate (wheat bran), resulting in an efficient conversion of the substrate into a fermented feed. Such a preferred organic product, along with standard feed mixtures, has not significant impact on poultry production parameters.

CONCLUSION

In the present work the fermented cereal bio-product was enriched with ARA and EPA as a feed additive for poultry. The fermented feed was prepared by Solid-State fermentation using agricultural waste. Subsequent fermentation with lower fibrous fungi resulted in the transformation of bran in a fermented feed enriched with polyunsaturated fatty acids. In addition, the fermentation has reduced the antinutrients contained in the bran, thereby increasing the nutritional value of the fermented feed. Feeding of the fermented feed resulted in a reduction in feed consumption, but not improved feed growth and conversion resulted in increased chicken final weight and lower yield.

ACKNOWLEDGEMENT

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EVALUATION OF PARAMETERS OF EXCRETION DEPENDING ON ZEOLITE ADDITION IN FEEDS FOR GROWING PIGS

LUKÁŠ BUJŇÁK[1], ĽUBOR BINDAS[1], ALENA HREŠKO ŠAMUDOVSKÁ[1], PAVEL NAĎ[1], MAREK HUDÁK[2]

[1]Department of Nutrition, Dietetics and Animal Breeding, University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, Slovak Republic
[2]District Veterinary and Food Administration, Stöcklova 34, 085 01 Bardejov, Slovak Republic

Corresponding email address *lukas.bujnak@uvlf.sk*

ABSTRACT

The aim of this work was to investigate the effect of the six-week addition of zeolite on selected parameters in faeces. Twelve fattening pigs were divided into two groups, the control (n = 6) and the test (n = 6), with an average initial body weight of 30.2 kg ± 2.4 respectively 31.4 kg ± 2.3. In test feed mixture was applied after mixing the addition of natural zeolite in an amount of 20 g/1 kg. Statistical analysis of the results confirmed higher average dry matter content in faeces samples of test group ($P < 0.01$), higher values of crude protein and ammonia in fresh sample of faeces ($P < 0.01$; $P < 0.001$) from the group given zeolite. After determination of blood serum urea of pigs, we recorded a lower level in the test group compared to the control group. However, the difference was not significant ($P > 0.05$).

Keywords: ammonia, excretion, faeces, pigs, zeolite

INTRODUCTION

The primary zeolite structure is characterized by a framework of linked tetrahedrals composed of oxygen atoms surrounding a central cation (Coombs et al., 1997). Zeolites are minerals that have intriguing properties such as water absorption, ion adsorption and cation exchange capacity (Schneider et al., 2017). There are many species of zeolites which differ in chemical formula, void volume, pore size, thermal stability, and ion exchange (Bernardi et al., 2008). Due to their

chemical characteristics, zeolites have a relatively great potential for use in animal production, especially in pig farms, as feed additives with direct and indirect effects on performance, yield and reduction of environmental pollution. The use of zeolites in swine farming is mainly related to animal nutrition. Earlier studies were focused to assess the effect of zeolites on growth performance and other production parameters (Shurson et al., 1984; Lichvar, 1984). The properties of zeolites allow the retention of nitrogen and confer the ability to improve the efficiency in the digestion of proteins (Shurson et al., 1984; Mumpton, 1999; Leung et al., 2007). Newer studies also related zeolites to the preservation of the environment due to their capacity to retain pollutants of animal production, especially ammonia (Islam et al., 2014; Bujňák et al., 2015).
This study was conducted to evaluate the parameters of excretion depending on zeolite addition in feeds for growing pigs.

MATERIAL AND METHODS

Twelve fattening pigs (meat hybrid) were divided in two groups (six in control group and six animals in test group). The experiment was conducted during 42 days, with initial mean body weight (BW) 30.2 ± 2.4 kg in control group and 31.4 ± 2.3 kg in test group. The test group diet was supplemented with natural zeolite in an amount of 2g/100g (fraction 0−0.3 mm). Composition of test and control diet and the analysis of crude protein and energy from the diets are shown in Table 1. After 6 weeks, the faeces were collected in plastic buckets. In the laboratory, they were analysed nitrogen substances (CP) and ammonia (NH_3) from fresh sample and the level of dry matter (DM). For these determinations, the procedures of AOAC (2000) were used. Blood serum was analysed to determine urea using biochemical analyzer. (Chemistry Analyzer Ellipse, Italy). The differences between means were determined, according to the unpaired t-test using GraphPad Prism 6 software.

Table 1. Composition and analysis of the diets

Feeds	**control (-) and test (+ 20 g Zeolite/1 kg feed) diet in %**	
corn	27.5	
wheat	24	
barley	24.1	
soybean meal	21	
premix VM	3	
AA	0.4	
	Analysis in diet	
DM, g/kg	890.2	887.6
CP, g/kg	172.3	171.7
ME, MJ/kg	12.83	12.81

VM – vitamins and minerals, AA – amino acids, DM – dry matter, CP – crude protein, ME – metabolizable energy

RESULTS AND DISCUSSION

In the assessment of parameters of the diets used in the experiment, the test and control diets were almost identical in crude protein content 171.7 vs. 172.3 g/kg and metabolizable energy 12.81 vs. 12.83 MJ/kg. Zeolites have three essential properties: ion adsorption, water absorption and ion exchange capacity (Schneider et al., 2017). In preservation of the environment zeolites have been tested continuously. In the test group there was analysed a higher dry matter content of faeces than in the control group ($P < 0.01$), with individual values within the range 28.5−33.9% vs. 24.5−28.5%. The humidity reduction in the faeces can be explained by the capacity of the zeolites of absorbing more than 60% of their weight in water (Wilson and Mumpton, 1984).

The level of nitrogen excretion parameters in the fresh faeces and serum urea levels in both groups are shown in Table 2. By analyzing the CP content in fresh faeces samples in the test group, we recorded higher mean values of CP compared to the control group ($P < 0.01$). Similarly, when assessing NH_3 excretion in a fresh sample of faeces, we found higher NH_3 levels in the test group, with individual values within the range 981−1376 vs. 615−896 mg/kg. When comparing serum urea concentration, lower mean values were observed in animals in the test group, with mean values of 4.42 ± 0.97 mmol/l compared to the control group, with mean values at 4.86 ± 1.03 mmol/l. Although

this difference was not statistically significant (P > 0.05). In pigs, most of the nitrogen is excreted in the urine, to a lower extent in faeces. The total amount of nitrogen excreted by these two pathways changes only slightly, but the shift of urine excretion to the fecal nitrogen excretion has an impact on ammonia emissions. Ammonia is the final product of organic nitrogen compounds from excreta (urine, faeces) of pigs. Its source is mainly urea present in urine. The ability of zeolite to bind $NH4^+$ was recorded in pigs repeatedly by many authors (Papaioannou et al., 2005; Leung et al., 2007).

Table 2. Parameters of excretion in the faeces and the level of serum urea (Mean ± SD)

		Control group
Faeces	**DM, %**	26.90 ± 1.54
	CP, %	5.53 ± 0.28
	NH_3, mg/kg	750.96 ± 114.28
Blood	**U mmol/l**	4.86 ± 1.03
		Test group
Faeces	**DM, %**	30.63 ± 1.79**
	CP, %	6.25 ± 0.45**
	NH_3, mg/kg	1182.26 ± 155.14 ***
Blood	**U mmol/l**	4.42 ± 0.97

SD – standard deviation; CP – crude protein; NH_3 – ammonia; DM – dry matter; U – urea; significant differences: **P < 0.01, ***P < 0.001.

Data obtained by Ly et al. (1996) showed in growing pigs, higher faecal concentration of ammonia, as well as the amount of its daily output in the treatments with zeolite. Zeolite and clinoptilolite were effective in reducing ammonia produced by the deamination of proteins in the gastrointestinal tract during digestion, preventing their absorption and resulting in increased fecal nitrogen, while the total nitrogen content in urine decreased (Shurson et al., 1984). Islam et al. (2014) reported that the use of 0.5% artificial zeolite in the diet of pigs led to a significant reduction in the emission of ammonia.

Results regarding production performance is variable and can be related to the different doses and kinds of zeolites. The zeolites have potential for the mitigation of pollution and waste control processes produced by the pig industries.

CONCLUSION

The addition of zeolites showed a higher content of crude protein and ammonia in the faeces from the group given zeolite, decreasing their volatilization to the environment. This, along with lower serum urea values in the animals in the test group, indicates a tendency to shift urine nitrogen excretion to the fecal excretion of nitrogen.

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CONTENT OF MYCOTOXINS IN SILAGES ACCORDING TO PLANT SPECIES AND USING OF SILAGE INOCULANTS

HANA DOČKALOVÁ, IVANA KOLÁČKOVÁ, PAVEL HORKÝ, IVA ČÁSLAVOVÁ, LADISLAV ZEMAN

Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: hana.dockalova@mendelu.cz

ABSTRACT

Feed doses for domestic herbivores comprise mostly forage grasses. The quality of forage is directly dependent on the quality of plant species and technological processes, including the use of silage inoculants. Feeds contaminated with mycotoxins can cause health disorders and lower productivity. Mycotoxins are toxic secondary metabolites of microscopic fungi. Ergosterol (ERG) is considered the first indicator of moulds presence in fresh forage and silages. The monitored species were *Lolium perenne*, × *Festulolium pabulare*, × *Festulolium braunii*. Silages were produced in three variants: untreated, treated with chemical ingredient and treated with biological-enzymatic inoculant. Fresh forage and silage (60 days old) were analysed for ergosterol, deoxynivalenol (DON), zearalenon (ZEN) and T2 toxin (T2) content. Content of ergosterol in fresh forage was ranging between 1.4 and 8.2 mg/kg. Content of ergosterol in silages was ranging between 4.8 and 12.9 mg/kg. The highest observed contents of mycotoxins were: in case of DON 215.3 (ppb), ZEN 90.8 (ppb), T2 toxin 57.1 (ppb). Determined values did not exceed the recommended limits for livestock feed.

Keywords: mycotoxins; ergosterol; silage aditives; forage; silage

INTRODUCTION

Phyllosphere of grasses provides an ideal environment for the growth of highly-adaptable microorganisms (Lindow a Brandl 2003). Presence of microbial species and mycotoxins is geographically specific (Chen

et al. 2016; Fröhlich-Nowoisky et al. 2012). Climate, fertilization regime, intensification of production and genotype of a microorganism have effect on production of mycotoxins (Mateo et al. 2013; Karlsson et al. 2017; Aldars-García et al. 2018).
Many od these microbial species produce secondary metabolites characterized by their high toxicity causing growth retardation, decrease in egg and milk production, tumor formation and changes in immune system of farm animals (Gamliel et al. 2017; Bianchini et Bullerman 2014). There is more than 350 currently known mycotoxins that endanger food safety and the number is increasing with new emerging laboratory methods for their detection (Gamliel et al. 2017). European Union Commission Recomendation states that Member States should analyse the presence, co-occurrence and concentration of DON, ZEN, OTA, FB1, FB2, T2 and HT2 in cereal products (Kyprianou 2006).
Lolium perenne L. is highly valuable forage grass with high soluble carbohydrate content. Its disadvantage is proneness to fungal disease. By contrast, × *Festulolium pabulare* has higher resistance to fungal diseases. × *Festulolium braunii* (K. Richt.) A. Camus is also higher quality forage with good endurance, but it is prone to fungal diseases, however proneness is lower then in case of *Lolium perenne* (Skladanka et al. 2014).
Agronomical practices should be focused on prevention and inhibition of *Fusarium graminearum* on field and in silage processing. Tillage and spring sowing are positively correlated with decrease of T2 in plant material. On the contrary, repeated sowing of graminoids on the same location increases T2 levels (Schöneberg et al. 2018).
In an effort to diminish feed degradation in future processing is this experiment following influence of grass species and different aditives on silage quality.

MATERIAL AND METHODS

The experiment (small-plot) was located in the Research Station of Fodder Crops in Vatín (mean annual precipitation of 617 mm, mean annual temperature of 6.9 °C, the soil type Cambisol). The experimental plots were fertilized with 50 kg/ha in March 2008. The monitored species were *Lolium perenne* cv. Kentaur, × *Festulolium pabulare* cv. Felina, × *Festulolium braunii* cv. Perseus. Times of cutting were the beginning of June and the end of July 2008. The experiment was carried out in triplicate and split-plot design of 1.25 x 8 m was used. Fresh forage from the first cut was ensiled

in containers. Experimental containers provided the necessary anaerobic conditions for natural fermentation process. Silages were produced in three variants: untreated, treated with chemical ingredient (43% formic acid, 10% propionic acid, 30% ammonium formate, 2% benzoic acid) and treated with biological-enzymatic inoculant (containing *Enterococcus faecium, Lactobacillus plantarum, Pediococcus acidilacti* and *Lactobacillus salivarius,* cellulase, hemicellulase and amylase, with 1×10^{11} CFU/g). The amount of chemical ingredient added was 4 L/t of ensiled material and that of biological additive was 10 g/t. Silages were sampled 60 days after closing the containers. These samples were frozen and analysed for content of ergosterol (HPLC method) and mycotoxins, namely deoxynivalenol, zearalenon and T2 toxin (ELISA method).

RESULTS AND DISCUSSION

The highest quality forage material was discovered in the first cut. Risk of feed degradation increases with cultivation length (in case of *L. perenne*, there was the increasing more than fivefold in second cut). The highest increase of ergosterol and mycotoxines was observed in the second cut. It is possible to conclude, that the quantity of fungi was influenced by relative convenient climatic conditions.

Although samples were positive for ZEN of × *F. braunii* only in second cut, concurrence of DON and T2 toxin can lead to accumulation in feed components and this accumulation of mycotoxins has negative effects on animal health (Zhang et al. 2018).

Ergosterol was used for fungal detection and quantification. Increasing of ergosterol and mycotoxin contents in second cut of *Lolium perenne* and × *Festulolium braunii* was recorded. In regard to these findings we can assume that fungal abundance increased due to favorable climatic conditions. Highest contamination was measured in *Lolium,* the lowest in × *F. braunii*, which can be caused by higher stress resistance of intergeneric hybrids.

Table 1. Evaluation of mycotoxins DON, ZEN, T2 (ppb) and ERG (mg/kg) in fresh forage

1st cut				
Species	**DON**	**ZEN**	**T2**	**ERG**
Lolium perenne	31.1	0	< LOQ	1.4
× *Festulolium pabulare*	25.8	0	29.9	4.8
× *Festulolium braunii*	31.2	0	28.9	1.6
2nd cut				
Lolium perenne	105.7	> LOQ	54.1	8.2
× *Festulolium pabulare*	84.2	< LOQ	35.9	2.9
× *Festulolium braunii*	44.4	36.4	39	3.7

Table 2. Content of mycotoxins DON, ZEN, T2 (ppb) and ERG (mg/kg) in silages from the first cut

Untreated silages				
Species	**DON**	**ZEN**	**T2**	**ERG**
Lolium perenne	131.0	70.2	< LOQ	6.8
× *Festulolium pabulare*	179.6	53.3	0	7.7
× *Festulolium braunii*	125.1	52.8	> LOQ	5.1
Silages treated with chemical ingredient				
Lolium perenne	115.3	43.0	< LOQ	5.6
× *Festulolium pabulare*	215.3	< LOQ	< LOQ	11.8
× *Festulolium braunii*	133.6	35.0	< LOQ	5.0
Silages treated with biological-enzymatic inoculants				
Lolium perenne	145.1	53.8	27.6	6.1
× *Festulolium pabulare*	204.1	90.8	52.7	12.9
× *Festulolium braunii*	131.0	60.7	57.1	4.8

Increase of mycotoxin levels by ensilaging was documented by Zachariasova et al. (2014). Although concurrence of DON and ZEN is frequent, it was not confirmed in fresh forage, but it was apparent in most of the silage samples (Błajet-Kosicka et al. 2014; Kosicki et al. 2016; Manizan et al. 2018).

Silage with biological additive was less contaminated by mycotoxins than chemical variant of treatment, which was presumably caused by antifungal activity of lactic acid bacteria (Juodeikiene et al. 2018). Although T2 did not occur in biological and untreated variants, its levels rapidly increased with chemical additive. Biological additive depleted DON, ZEN and T2 content in *L. perenne*. However,

in × *Festulolium* spp. ZEN levels decreased and DON levels were elevated.

CONCLUSION

Recommended levels of ZEN (0.5 mg/kg) and DON (10 mg/kg) in feed were not exceeded in any treatment variant.
Due to many influencing climatic factors and mycotoxin levels' dependencies on stress response of filamentous fungi will be this experiment repeated in following years. This will ensure values verification.

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THE EFFECT OF PHYTOGENIC ADDITIVES ON HEALTH PARAMETERS IN RABBITS

SOŇA HALOUZKOVÁ, EVA MRKVICOVÁ, LEOŠ PAVLATA, BARBORA UMLÁŠKOVÁ, ONDŘEJ ŠŤASTNÍK

Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: es.ha@atlas.cz

ABSTRACT

The aim of this study was to prove the harmlessness or positive effects of phytogenic additives on health parameters in rabbits. Experimental feed mixture contained 2.5% of the phytogenic additives premix. The phytogenic additives premix was composed of dehydrated herbs such as dandelion leaves (*Taraxacum officinale*), nettle leaves (*Urtica diotica*), dandelion and daisy blooms (*Bellis perennis*), yarrow leaves (*Achillea millefolium L.*), plantain leaves (*Plantago lanceolata L.*), sage leaves (*Salvia officinalis*), wild strawberry leaves (*Fragaria vesca*) and mint leaves (*Mentha aquatica*). The experiment was performed with five crossbreeds of Californian and New Zealand rabbit weighting about 3.5 kilograms at 4 months of age. The rabbits were being fed with experimental feeding mixture for three weeks. Blood samples were taken at the beginning and at the end of the experiment, where liver and kidney biochemical profile were evaluated and nitrogen and carbohydrate metabolism as well. Control and experimental blood samples were compared. In addition, the results of both control and experimental blood samples were compared with reference range of blood parameters in rabbits. A significant difference was not proved in the most of results between control and experimental samples ($P > 0.05$). However, decreased enzyme activity of gamma glutamyl transferase was demonstrated ($P < 0.05$). According to this result we can say that the phytogenic additives could have a positive effect on the liver profile. The influence on the kidney function, nitrogen or carbohydrate metabolisms was not noticed.

Keywords: rabbit nutrition; biochemical profile of blood; liver profile; feeding additives

INTRODUCTION

The fear of using antibiotics in animal nutrition has been leading to increased requests on using natural and safe feeding additives with better production ensuring at the same time (Frankič et al., 2009). The prohibition of feeding antibiotics, except of medical purposes, led to digestive disturbances and deaths in fattening rabbits (Zotte et al., 2016). According to Wenk (2003) plant extracts have increased the ration palatability, stimulate digestion and physiological functions, increase feed intake and weight gains or antimicrobial and coccidiostatic effects have been noticed.

The antioxidant and anti-inflammatory effects have been proven in plantain (*Plantago lanceolata*) by Khatun (2014) and by Dai et al. (2015) in wild strawberry leaves *(Fragaria vesca)* and mint (*Mentha aquatica*). Besides wild strawberry leaves are rich in phenolic acids and anthocyanins and many other positive effects, such as immunomodulatory, anti-diarrhea, cardioprotective or neuroprotective, have been noticed (Ferlemi and Lamari, 2016).

The experiments of Al-Snafi (2015) with daisy extracts (*Bellis perennis*) led to decreased triglycerides level in rabbit's blood and increased secretion of digestive juices. The support of secretion of digestive juices has been noticed in a yarrow (*Achillea millefolium L.*) as well. In addition, liver-protective and antibacterial effects of the yarrow have been proven (Siewertová, 2015).

A sage (*Salvia officinalis*) is known for its anti-inflammatory and disinfecting effects. It also helps in liver disease, diarrhea and enteritis occurrence (Siewertová, 2015). A rich source of fatty acids and essential amino acids is a nettle (*Urtica dioica*). The nettle improves digestion, decreases a glucose level in blood and has a positive effect on cardiovascular system (Rutto et al., 2013). A dandelion (*Taraxacum officinale*) improves digestion as well, besides it decreases cholesterol and glucose level in blood and dandelion root can be used as a probiotic in rabbit diets (Yarnell and Abascal, 2009).

The aim of this study was to prove the effects of phytogenic additives on health parameters in rabbits.

MATERIAL AND METHODS

The experiment was performed with five crossbreeds of Californian and New Zealand rabbit at 4 months of age. There were 3 castrated males among 2 females weighting about 3.5 kilograms. The rabbits were stabled in a group in a cage with straw bedding in a building.

Blood samples were taken from rabbits ears. The control blood samples had been taken before the experiment started. The rabbits were being fed with feeding mixture (Table 1) contained granulated feed mixture (Biostan), barley and 2.5% of the phytogenic additives premix (Table 2) for three weeks. The feed mixture was served *ad libitum* twice per day. Meadow hay was given daily as well.

Table 1. Experimental feed mixture composition

Feed	Amount (%)
Biostan (complete granulated feed)	49
Barley	48.5
Phytogenic additives premix	2.5

Table 2. Phytogenic additives premix composition

Phytogenic additive	Amount (%)
Dehydrated dandelion leaves *(Taraxacum officinale)*	20
Dehydrated nettle leaves *(Urtica dioica)*	11
Dehydrated dandelion and daisy blooms *(Taraxacum officinale, Bellis perennis)*	12.8
Dehydrated yarrow *(Achillea millefolium L.)*	12.8
Dehydrated plantain (*Plantago lanceolata L.*)	11.8
Dehydrated sage *(Salvia officinalis)*	11.8
Dehydrated wild strawberry leaves *(Fragaria vesca)*	11.8
Dehydrated mint leaves *(Mentha aquatica)*	8

Blood samples were taken again the third week at the end of the experiment (experimental blood samples). Blood parameters were chosen according to liver and kidney profile assessment and nitrogen and carbohydrate metabolisms evaluating. The biochemical profile of blood plasma was analysed with the use of Ellipse (AMS Spa, Italy) analyser. The individual parameters were analysed using individual tests produced by Erba Lachema (Brno, CZ): albumin (Alb 500); total protein (TP 500); AST - aspartate aminotransferase (AST/GOT 500); GGT - gamma-glutamyl transferase (GGT 250); ALP - alkaline phosphatase (ALP AMP 500); ALT - alanine aminotransferase (ALT/GPT 500); LD - lactate dehydrogenase (LDH-L 100); bilirubin (BIL T JG 350); cholesterol (CHOL 250); creatinine (CREA 500), glucose and Randox UK: Urea (Urea, cat. No. UR 107).

Data were processed by Statistica version 12.0 (CZ) with one-way analysis at variance (ANOVA) and by Microsoft Excel. The differences were considered significant at $P < 0.05$. Results were statistically compared between control and experimental blood samples. Both control and experimental blood samples were compared with reference values of rabbit's blood.

RESULTS AND DISCUSSION

The results of biochemical analyses and standard rabbit blood values are summarized in Table 3.

Table 3. Control and experimental blood samples as compared to standard blood values in rabbits

Parameter	Unit	Standard values in rabbits	n	Contr.	SD	Exper.	SD
AST	µkat/l	0.16-1.63	5	0.31[a]	0.03	0.54[b]	0.26
GMT	µkat/l	0.04-0.24	5	0.27[b]	0.06	0.10[a]	0.02
ALP	µkat/l	0.16-1.60	5	3.21	0.40	2.23	0.34
ALT	µkat/l	0.91-4.33	5	1.05	0.26	1.13	0.11
LD	µkat/l	2.20-4.20	5	2.00	0.56	5.09	2.99
Bilirubin	µmol/l	do 20	5	1.43[a]	0.28	6.60[b]	4.14
Glucose	µmol/l	4.20-8.90	5	6.72	0.83	6.47	0.39
Cholesterol	µmol/l	0.10-2.00	5	1.30	0.24	1.43	0.10
Urea	µmol/l	9.10-25.50	5	5.20	1.59	5.18	0.40
Creatinine	µmol/l	53-124	5	100.38	9.16	114.53	24.83
TP	g/l	50-75	5	57.85	4.28	61.18	7.98
Albumin	g/l	25.40	5	38.48	1.37	40.40	4.92

[a,b] – different letters in one line mean statistically significant differences ($P<0.05$)
AST (aspartate aminotransferase), GMT (gamma glutamyl transferase), ALP (alkaline phosphatise), ALT (aspartate alanine aminotransferase), LD (lactate dehydrogenase), TP (total protein)
SD – standard deviation; Contr. – control samples; Exper. – experimental samples
Standard blood values in rabbits has been taken from MediRabbit (2017)

Most of the results corresponded with average blood values in rabbits. A significant difference was not proved in the most of following parameters between control and experimental samples ($P > 0.05$). The enzyme activity of AST and bilirubin concentration were significantly

increased ($P < 0.05$), but those values were in the reference range. On the other hand, the activity of GMT was decreased ($P < 0.05$). The activity of GMT in control samples was higher than the reference range, however in experimental samples was normal. The higher bilirubin concentration could be caused by hemolysis in some experimental blood samples.

GMT is a very important liver enzyme contained in cellular membranes. If a liver damage is occurred, GMT is released from the cells and its activity is increased in blood. Because of decreased enzyme activity of GMT in our experiment, we have assumed phytogenic additives had a positive effect on the liver profile. The positive effect on the liver has been also mentioned by Siewertová (2015) in a yarrow *(Achillea millefolium L.)* and by Treben (2014) in a sage *(Salvia officinalis)* and a plantain (*Plantago lanceolata L.*). We have supposed the different activity of GMT was not caused only by these three additives, however the whole complex of phytogenic additives in premix had an influence on the liver profile. The complex probably supported the liver function and decreased a liver burden indirectly. The authors Neugebauerová and Žďárská (2015) have mentioned the positive effect of daisy leaves and nettle leaves on blood cleaning and detoxication. According to Siewertová (2015) a dandelion leaves make non-degradable complexes with harmful substances in a digestive tract, which are excreted from the body then.

CONCLUSION

The decreased enzyme activity of GMT was found out and could indicate the positive effect of phytogenic additives on the liver profile. The influence on the kidney function, nitrogen and carbohydrate metabolisms was not noticed. The negative effect of addition of 2.5% phytogenic additives premix into rabbit diets was not proved.

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RELATIONSHIP BETWEEN THE CRUDE PROTEIN AND ITS INDIVIDUAL FRACTIONS IN DIFFERENT WHEAT VARIETIES WITH AND WITHOUT RYE TRANSLOCATION 1B / 1R FOR NITROGENOUS SUBSTANCES DIGESTIBILITY, INTAKE, CONVERSION AND PRODUCTION EFFICIENCY OF THE FEED MIXTURE

DANA HOMOLKOVÁ[1], VLADIMÍR PLACHÝ[1], ZDENĚK MUDŘÍK[1], FRANTIŠEK VACEK[1], BORIS HUČKO[1], VÁCLAV DVOŘÁČEK[2], ALOIS KODEŠ[1]

[1]Department of Microbiology, Nutrition and Dietetic, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Praha 6 - Suchdol, Czech Republic
[2]Crop Research Institute, Drnovská 507/73, 161 06 Praha 6 - Ruzyně, Czech Republic

Corresponding email address: homolkovad@af.czu.cz

ABSTRACT

In feed and balance experiments on chickens the effect of nitrogenous substances (NS) content and its fractions (albumin + globulin, glutenin and gliadin) on NS digestibility, feed consumption, conversion and production efficiency (PER) of used feed mixtures in wheat with (WRT) and without rye translocation (WTRT) were monitored.
The experiments were performed in balance cages of experimental stables - the Department of Microbiology, Nutrition and Dietetics Czech University of Live Sciences in Prague.
The ROSS 308 chicken broiler cockerels at the age of 35 days, and the initial mean weight of 2.45 ± 0.20 kg, were included in experiments. The cockerels were housed in balanced cages of 2 pieces. Total 22 cockerels were used. Samples of the monitored wheat, including the NS fraction, were provided by the Research Institute of Plant Production Prague - Ruzyne. The NS content was determined by Kjeldahl method on a Kjeltec 2400™.
The hypothesis that wheat WRT have a potentially higher nutritional value and would be better as a component of compound feed used for

poultry and for monogastries in general, has not been confirmed ($P < 0.05$).

Keywords: wheat; crude protein; rye translocation; nitrogenous substances digestibility

INTRODUCTION

According to the statistics of the Ministry of Agriculture, the share of wheat grains fed to animals in the Czech Republic in 2012 was 54.4% (Roubalová, 2013). The fact that nearly two-thirds of wheat production is used for animal feed may suggest that wheat varieties of high nutritional quality should be specially grown. Until now, however, there are no clear breeders' criteria, suitable wheat varieties and cultivation methods specifically designed for these wheats. Wheat which is not suitable for bakery use or for processing into pasta or other food use, is mainly used as a feeding wheat. Evaluating the systematic effect of varieties for specific nutritional values is complicated because of a large number of external and internal (genetic) factors. In addition to highly significant influences of the year or eventually chemical-technological properties of the grain (Svihus et al., 2002, Wiseman 2006), there are also problems of proper nutritional evaluation of wheat grain as feed (Carré et al., 2007).

Studies of the wheat grain's genetic properties in relation to nutritional value often include the effect of the 1B/1R translocation, unfortunately with highly variable results (Rose 2003, Wiseman 2006). From the current assortment of 70 registered wheat varieties in the Czech Republic we have 10 varieties of translocations 1B/1R. Wheat grain as a component of feed mixtures is primarily a source of energy. In used feed mixtures, wheat accounts approximately 70% of the metabolisable energy (ME) content. In case of digestible protein, it is only 40%. The PER of compound feeds is mainly due to the presence of gross protein and feed energy.

We have chosen to monitor the relation between the representation of proteins and their qualitative components in spite of the fact that the dose of wheat in the feed mixture for chicken broilers is actually only about 40% of their actual needs. Wheat protein is generally considered to be less valuable due to lower representation of essential amino acids (Henry and Kettlewell, 1996; Bedford et al., 2003; Oury et al., 2006). Svihus et al. (2002) confirmed the significant negative effect of protein content and metabolizable energy on the other hand, but also found that

the increasing protein content was positive in relation to the gain in chickens. It can be assumed that the structure of protein fractions of wheat (albumin, globulins, gliadins and glutenins) with different proportions of nutritionally essential amino acids will also be an important factor affecting the wheat grain, as confirmed by Wiseman (2007).
The aim of our monitoring was to determine the relation of NS and their individual fractions (albumin + globulin, gliadin, glutelin) to NS digestibility, feed intake, conversion of feed mixture and PER of used compound feed in studied wheat hybrids with rye translocation (WRT) 1B/1R and without this translocation.

MATERIAL AND METHODS

The balancing attempts were included on the ROSS 308 chicken broiler cockerels at the age of 35 days, and the initial mean weight of 2.45 ± 0.2 kg. The cockerels were housed in balanced cages of 2 pieces. Total 22 cockerels were divided into two groups. In the first group were 10 cockerels feeded with wheat WRT. In the second group were 12 cockerels feeded with wheat without rye translocation (WTRT). During the preparatory period of 3 days, the animals became accustomed to the new environment and feed. As the only feed was wheat WRT and WTRT 1B/1R, the chromium oxide (in addition 2 %) was used as an external digestibility indicator. Cockerels were given *ad libitum* access to food and water. After 7 days of the balance experiment, chickens were slaughtered and the ileum was removed. A mixed sample of both animals from the cage was created. The ileum contents were lyophilized, the dry matter (DM) were determined at 103 °C, and nitrogen content according Kjeldahl method (ČSN 56 0512-12) and chromium oxide iodometric to sodium thiosulfate were determined.

The digestibility of the NS was calculated from the observed values converted to 100 % DM according to the formula:

$$Digestible\ NS\ (\%) = 100 - \frac{(\text{ifeed} * \text{nfeaces})}{(\text{ifeaces} * \text{nfeed})}$$

ifeed – indicator in the feed; nfeaces – nutrients in the feaces;
ifeaces - indicator in the feaces; nfeed – nutrients in the feed

PER was calculated from the observed values converted to the formula:

$$PER = \frac{weight\ gain}{intake\ of\ NS}$$

Chemical analyzes of feed and feaces were done according to the recommended fodder analytical methods (Kacerovský, 1990).

RESULTS AND DISCUSSION

The results in Table 1 show the specific values recorded in the experimental observations. When we look closely to the individual values found and reported, it is quite obvious that none of the detected values significantly differ. The intention of the experiment was to determine the possible relationship of the 1B/1R rye translocation to the improvement of the nutritional value of the grain, and possibly the improvement of its dietary value. We considered, based on the literature, that the individual NS fractions would also be different, which could lead to improved nutrient utilization and to a higher PER of the feed used. Examined samples of wheat WRT showed a CP value of 14.51%, while wheat without this translocation showed CP 13.54%. This difference is not significant but suggests that wheat WRT could potentially have higher nutritional and production value. When looking more in detail on NS quality from the point of individual fractions view, some differences can be seen mainly in the content of albumin and globulin and in glutamine and no difference in the presence of gliadin, as seen in the Table 1. If the differences in the representation of NS, albumin + globulin and glutanin fractions would be significant, we could show the value of the digestibility the CP feed mixtures with the representation of the comparable wheat types. However, CP's digestibility values showed the opposite trend. Wheat WRT showed lower values (65.98%) than WTRT wheat (68.07%). These results already tell us that the rye translocation did not show a positive effect on the nutritional value, which was 3.07 % lower. Similar results were also obtained when evaluating the PER of the monitored wheat. There was no statisticaly significant difference ($P < 0.05$) between feed intake, conversion of feed and PER of both wheat varieties.

Observed values in experiment were different, but the differences are not statistically significant ($P < 0.05$). Several authors confirmed that the nutritional value of wheat for poultry may vary considerably (Mollah et al., 1983; McCracken and Quintin, 2000; Ball et al., 2013).

Table 1. Results of monitoring differences in nutritional values

Type of wheat	WRT	WTRT
	mean ± SD	
CP (%)	14.51 ± 0.23	13.54 ± 0.35
Albumine + globuline from CP (%)	26.25 ± 0.27	24.65 ± 0.12
Gliadine from CP (%)	30.89 ± 0.28	30.80 ± 0.38
Glutanine from CP (%)	42.86 ± 0.16	44.60 ± 0.47
Digestibility of CP (%)	65.98 ± 0.64	68.07 ± 0.19
Intake of feed (g)	133.9 ± 1.29	133.7 ± 1.35
Conversion of feed (%)	1.81 ± 0.04	1.84 ± 0.02
PER	2.70 ± 0.04	2.68 ± 0.06

WRT – 10 animals; WTRT – 12 animals; CP – crude protein; PER – protein efficiency ratio; $P > 0.05$

CONCLUSION

The experiment showed that the presence of rye translocation 1B / 1R in wheat was not a decisive indicator of wheat nutritional value or wheat production efficiency. The reason might be the influence of various external and internal factors to the value of wheat that would determine it to be more favorable for feeding poultry. Therefore, it will be necessary to focus primarily on the genetic influences of wheat that would characterize its feed value. From experience it is known that the wheat which does not meet the food parameters will be classified as feed wheat. This is not right and it is deficient for feeders. Besides studying genetic influences, it must also be a priority to find easily analyzing values that will be proven in practical experiments to determine the nutritional and production value of this widespread and fairly high quality food.

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EFFECT OF SODIUM HUMATE ON SOME PRODUCTION PARAMETERS IN BROILER CHICKENS

ALENA HREŠKO ŠAMUDOVSKÁ[1], MAREK HUDÁK[2], ANDREJ MARCIN[1], LUKÁŠ BUJŇÁK[1], PAVEL NAĎ[1]

[1]Department of Nutrition, Dietetics and Animal Breeding, University of Veterinary Medicine and Pharmacy, Komenského 73, 071 81 Košice, Slovak Republic
[2]District Veterinary and Food Administration in Bardejov, Stöcklova 34, 085 01 Bardejov, Slovak Republic

Corresponding email address: alena.hreskosamudovska@uvlf.sk

ABSTRACT

The objective of the study was to determine the effect of sodium humate added to the diets (in different amounts: 5 g/kg during first two weeks and 7 g/kg from 3th week till 5th week) on growth rate, feed conversion ratio, performance index and flock uniformity in broiler chicks. The body weight of chicks and the flock uniformity on the 35th day of experiment were not significantly influenced. Similarly, the growth rate of chicks during the whole trial period was not significantly influenced, though in the first trial phase the addition of sodium humate led to significant decrease ($P < 0.05$) and in the second phase to non-significant increase of growth rate of chicks. The feed conversion ratio was worse in the trial group. The performance index was not significantly influenced. It was concluded that the usage of sodium humate in diets had no beneficial effects in broilers.

Keywords: humic compounds; growth rate; flock uniformity; performance index; poultry

INTRODUCTION

Humic substances are natural organic compounds, that are generated from the decomposition of organic matter with the aid of bacteria in the soil. Major components of them are humin; humic, fulvic and ulmic acids; and trace minerals (Stevenson 1994). Humic substances have many beneficial effects like antibacterial, antiviral and anti-inflammatory effects. They also improve immune system, reduce odour in faeces and cause a reduction in stress (Islam et al., 2005). Humic

acids and their sodium salts are permitted for oral use in horses, ruminants, swine and poultry for the treatment of diarrhoea, dyspepsia and acute intoxications (EMEA 1999). The use of humic substances in feeds (El-Husseiny et al., 2008; Taklimi et al., 2012; Abdel-Mageed, 2012; Arif et al., 2016) or drinking water (Mirnawati and Marlida, 2013; Lala et al., 2017) can bring a number of advantages for health and productive performance in poultry. Humic substances could be a suitable alternative of feed antibiotics. The mechanism by which humic substances affect poultry performance is largely unknown. They can stabilize gut microflora of animals (Humin Tech, 2004) and can improve intestinal villus height (Taklimi et al., 2012), what results in increased nutrient absorption and improvement of growth performance.
The objective of this experiment was to study the influence of sodium humate on growth performance in broiler chickens.

MATERIAL AND METHODS

In this research, one hundred unsexed one-day-old broiler chicks (Ross 308) with a mean body weight of 41.2 ± 0.33 g per bird were randomly assigned in two equal groups and housed on deep bedding in agreement with the technological instruction for Ross 308 chicks, with controlled light, temperature, animal hygiene and feeding regime. Lighting was continuous throughout the whole experimental period. The birds in both groups were fed with corn-wheat-soybean meal-based complete mixture in the mash form according to the growth phases (phase 1: 1^{st} – 2^{nd} week; phase 2: 3^{rd} – 5^{th} week). No antibiotic growth promoters or anticoccidial drugs were used in the diets. While control birds were fed diets with no additives, treatment birds (HNa group) were fed with diets containing sodium humate in amount: 5 g/kg of diet during 1^{st} phase and 7 g/kg of diet during 2^{nd} phase. Diets and water were provided *ad libitum* over the whole experimental period. Composition of diets used in respective experimental periods are shown in Table 1. Diets were analysed for dry mater, crude protein, ether extract, crude fibre and ash by the AOAC (2001).
Live body weight (BW) and feed consumption were observed weekly. Feed conversion ratio was calculated by dividing total feed intake by total weight gain. Growth rate, flock uniformity and performance index were calculated according to the equations: Growth rate = ((final BW – initial BW) / (0.5 x (initial BW + final BW))) x 100; Flock uniformity (%) = 100 – ((standard deviation / BW mean) x 100); Performance index = (BW / feed conversion ratio) x 100.

Statistical evaluation of the effects of sodium humate on selected production parameters of chicks were determined by unpaired *t*-test. Results were expressed as means ± standard error of the means (SEM).

Table 1. Ingredient composition of the experimental diets

PARAMETERS	Control		HNa	
	1st - 2nd week	3rd - 5th week	1st - 2nd week	3rd - 5th week
Ingredients (%)				
Maize	43.5	50.0	43.5	50.0
Wheat*	12.1	9.0	11.6	8.3
Soybean meal	36.0	33.0	36.0	33.0
Vegetable oil	4.0	4.0	4.0	4.0
Limestone	2.0	1.6	2.0	1.6
Vit-Min. premix	2.0	2.0	2.0	2.0
Lysine	0.4	0.4	0.4	0.4
Sodium humate			0.5	0.7
Chemical analysis (% DM)				
Dry matter**	89.7	90.0	89.8	89.7
Crude protein	25.0	23.1	24.9	23.2
Crude fibre	3.7	4.4	4.0	3.7
Crude ash	8.2	6.7	7.4	6.6
Ether extract	7.0	7.2	7.2	6.9
Calculated analysis				
ME*** (MJ/kg DM)	13.3	13.3	13.3	13.4

*in the experimental group (HNa) appropriate amount of wheat was replaced by sodium humate
**Dry matter
***Metabolizable energy

RESULTS AND DISCUSSION

Evaluating the growth rate of chicks in respective trial phases (Table 2), a significantly lower growth rate of chicks in trial group than in the control group ($P < 0.05$) was found in the first phase (1^{st} – 14^{th} day). The addition of sodium humate to the diet led to increased growth rate of chicks in the second trial phase (15^{th} – 35^{th} day), but the difference was not significant in comparison to the control group (2,51 %). Similarly, the growth rate of chicks was not significantly influenced by the effect of tested additive during the whole monitored period (1^{st} – 35^{th} day). The results of present study are not in agreement with the findings of Abdel-Mageed (2012) who reported that supplementation

diets with humic substances (at a level of 10, 20 or 30 ml/kg diet) significantly increased growth rate in Japanese quail.

Table 2. Effect of sodium humate on growth rate

	Control	HNa
1st – 14th day	156.8 ± 0.75[a]	153.6 ± 0.67[b]
15th – 35th day	139.6 ± 1.21	143.1 ± 0.69
1st – 35th day	191.6 ± 0.07	191.5 ± 0.02

[ab] significant differences ($P < 0.05$)

The addition of natrium humate to the diet didn't significantly influenced the body weight of chicks (Table 3). The average body weight of chicks on the 35th day was found to be lower by 2.59 % in the trial group than in the control group. Some researchers also found that the live body weight in broilers were not affected by sodium humate (Skalická and Koréneková, 2016) and humic acids supplementation (Marcinčáková et al., 2015). Moreover, Rath et al. (2006) found that humic acid treatment significantly decreased body weight of broiler chickens, more pronounced at higher concentration. However, in different studies (Celik et al., 2008; El-Husseiny et al., 2008; Pistová et al., 2016) humic substances supplementation positively influenced live body weight of broiler chicken.

The flock body weight uniformity is one of the most important economical factors in broiler production. Chicks from more uniform flock cause less disruption for the machinery during slaughter and downstream carcass processing (Fasina a Olowo, 2013). In our experiment, the addition of sodium humate had no significant impact on the flock body weight uniformity (Table 3).

Table 3. Effect of sodium humate on body weight (kg/chick), flock uniformity (%) and performance index

	Control	HNa
Body weight (35th day)	1.93 ± 0.06	1.88 ± 0.05
Flock uniformity (35th day)	82.3 ± 1.76	82.7 ± 2.73
Performance index (35th day)	119.2 ± 3.48	111.2 ± 3.24

The value of feed conversion ratio for the period of 35 days was found higher in the trial group (1.70) than in the control group (1.62). Results of present study are in line with the findings of Demeterová (2009) who concluded that supplementation of humic compounds in broiler diet (in

the concentration of 0.7 % throughout the whole experiment) resulted in significant worsening of feed conversion. On the other hand, Ozturk et al. (2012) and Arif et al. (2016) reported that the feed conversion ratio was significantly improved with supplementing varying levels of humic substances in broiler diets. But some other researchers found that the values of feed conversion ratio in broilers were not affected by humate supplementation (Karaoglu et al., 2004; Kaya and Tuncer, 2009; Pistová et al., 2016).

Due to the non-significantly lower body weight of chicks and worse feed conversion ratio a lower value of performance index was found in the trial group (Table 3). The difference between groups, representing 6,71 %, was not statistically significant. However, Abdel-Mageed (2012) reported that dietary humic substances supplementation increased performance index in Japanese quail.

Many studies show that the effect of humic substances may vary and may depend on the composition of humate products and on the amount added to the feed or water.

CONCLUSION

The addition of sodium humate to the diet had no significant impact on the body weight of chicks, flock body weight uniformity, growth rate and production index over the period of 35 days. Though it has led to significantly lower growth rate of chicks in the first trial phase and to increase in feed consumption per kilogram of weight gain over the whole monitored period.

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FIBER CONTENT AND *IN VITRO* DIGESTIBILITY OF OILSEEDS AND THEIR CAKES

RENATA KOLLÁTHOVÁ[1], ĽUBA BALUŠÍKOVÁ[1], PATRÍCIA VAŠEKOVÁ[1], BRANISLAV VARGA[1], MARTIN GAŠPAROVIČ[1], DANIEL BÍRO[1], EVA IVANIŠOVÁ[2], MICHAL ROLINEC[1], BRANISLAV GÁLIK[1]

[1]Department of Animal Nutrition, [2] Department of Plant Storage and Processing, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra

Corresponding email address: xkollathova@uniag.sk

ABSTRACT

The aim of this research was to determine dry matter (DM), organic matter (OM), crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF) content as well as *in vitro* digestibility of organic matter (IVOMD) of four oilseeds (sunflower, soybean, flaxseed and rapessed) and their cakes. Cakes were obtained by using pressing unit FARMER 10 (Farmet, Czech Republic). Fiber and fiber fractions were determined by standard laboratory methods and procedures. IVOMD was estimated by two-stage pepsin-cellulase method in Daisy Incubator II (Ankom Technology, U.S.A.). Significant differences in the composition of analyzed seeds and cakes as well as in the digestibility of their OM were found ($P < 0.05$). Results have shown that *in vitro* digestibility analysis should be beneficial in feeds quality detection. However, nutritional quality of analysed samples was different and affected by many factors.

Keywords: oilseeds; oilseed cakes; fiber; *in vitro* digestibility

INTRODUCTION

Despite their high energy value and high protein content, oilseeds in feed industry are used only marginally. They often contain antinutrients which negatively affect the quality of animal products or animal health (Zeman et al., 2006). Because of high fat content they are mainly used for oil production. In animal nutrition only by-products from the production of vegetable or technical oils, such as extracted meals or cakes, are used with great importance. Oilseeds in their full fat form belong to feeds with the highest energy value. The content of

analytically determined nutrients in oilseeds is highly variable and mostly depends on the presence and proportions of their hulls (Gálik et al., 2011). The objective of this study was to determine DM, OM, CF ADF and NDF content as well as IVOMD of four oilseeds (sunflower, soybean, flaxseed and rapessed) and their cakes.

MATERIAL AND METHODS

In the experiment, DM, OM, CF and fiber fractions (ADF, NDF), as well as IVOMD of oilseeds and their cakes were determined. Four samples of oilseeds (sunflower, soybean, flaxseed and rapeseed) in triplicate were analysed as seeds and cakes. Seeds were processed in the Laboratory of fats and oils (AgroBio Tech Research Centre of the Slovak University of Agriculture in Nitra). Pressing unit FARMER 10 (Farmet, Czech Republic) was used. Laboratory samples were analysed in the Laboratory of Quality and Nutritive Value of Feeds at the Department of Animal Nutrition at the Slovak Agricultural University. CF, ADF and NDF were determined 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.). 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:

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

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, CF, ADF, NDF, OM and IVOMD in analysed oilseeds are shown in Table 1. The lowest DM content was observed for soybean. Ítavo et al. (2015) reported higher value for DM in soybean seed (981.2 g/kg). The highest DM content was observed in rapeseed. In comparison with Rymer and Short (2003) 21.8 g/kg higher content was found. Differences in DM content between soybean and rapeseed were significant ($P < 0.05$). The maximum amount of CF was detected for sunflower seed and the minimum for soybean seed. These differences were significant ($P < 0.05$). According to Nadeem et al. (2010) detected results for sunflower seed were lower. Ciabotti et al. (2016) reported lower value for CF in soybean seed (78 g/kg of DM). The highest ADF content was found in rapeseed and the least ADF was observed in soybean seed. The observed difference in ADF content between rapeseed and soybean seed was significant ($P < 0.05$). According to Rymer and Short (2003) our result for ADF content in rapeseed was 263.50 g/kg of DM higher. This huge difference could be caused by using whole rapeseeds in the experiment. Ítavo et al. (2015) reported higher value for ADF in soybean seed (161.6 g/kg of DM). The highest NDF content was found in sunflower seed and the lowest amount of NDF was detected for soybean seed. Ítavo et al. (2015) reported 120.85 g/kg of DM higher NDF content in soybean seed and lower NDF content for sunflower seed (300.8 g/kg of DM). Differences in NDF content between sunflower and soybean seeds were significant ($P < 0.05$). The OM content in analyzed seeds was similar, except soybean seed. Soybean had the lowest OM content. El-Shemy (2011) reported higher value for OM in soybean seed (951.4 g/kg of DM). The highest OM content was observed in rapeseed. This value was practically identical to sunflower seed and higher than reported by Rymer and Short (2003). The observed difference in OM content between sunflower seed and soybean seed was significant ($P < 0.05$). The highest IVOMD was detected for soybean seed. Probably, this result was affected by the fiber content, in soybean was found the

lowest from oilseeds. Significant differences ($P < 0.05$) in IVOMD of analyzed seed samples were detected, exept between flaxseed and rapeseed. In seeds, higher digestibility coefficients were found in comparison with results of Petrikovič et al. (2000), mainly in rapeseed.

Table 1. Dry matter, crude fiber and fiber fractions, organic matter content and digestibility coefficients of analyzed oilseeds

	Sunflower	**Soybean**	**Flaxseed**	**Rapeseed**
	Mean±SD			
DM	947.25±0.35^{a}	943.20±0.10^{b}	948.25±0.15^{c}	958.80±0.01^{d}
CF	162.45±2.15^{a}	88.40±0.80^{b}	112.70±0.20^{c}	140.35±0.95^{d}
ADF	251.65±0.85^{a}	140.50±2.20^{b}	297.9±2.30^{c}	362.50±1.70^{d}
NDF	410.70±2.50^{a}	142.15±1.15^{b}	302.95±1.25^{c}	277.45±0.75^{d}
OM	963.8±0.2^{a}	946.6±1.2^{b}	960.2±0.2^{c}	963.9±0.2^{a}
IVOMD	76.45±0.51^{a}	94.68±0.30^{b}	85.44±0.31^{c}	85.16±0.41^{c}

DM – dry matter (g/kg); CF – crude fiber (g/kg of DM); ADF – acid detergent fiber (g/kg of DM); NDF – neutral detergent fiber (g/kg of DM); OM – organic matter (g/kg of DM); IVOMD – *in vitro* organic matter digestibility (%); SD – standard deviation. Values followed by different letters within a row are significant at the level 0.05.

Results obtained for DM, CF, ADF, NDF, OM and IVOMD in analyzed cakes are shown in Table 2. The situation with DM content in cakes was exactly the opposite then in seeds. The lowest DM content was determined in rapeseed cake and the highest DM content was observed in soybean cake. These differences were significant ($P < 0.05$). Leming and Lember (2005) found lower DM content in rapeseed cake (917 g/kg). Analysed DM content in soybean cake was higher than reported by Geremew et al. (2015). The content of CF in cakes was considerably variable with the minimum for soybean cake and the maximum for sunflower cake. These differences were also significant ($P < 0.05$). Geremew et al. (2015) reported higher CF content for soybean cake (64.8 g/kg of DM) and Chung et al. (2009) obtained 56.4 g/kg of DM higher value for sunflower cake. Sunflower cake contained the highest amount of ADF, as well. In contrast, ADF content in soybean cake was 225.75 g/kg of DM lower. These differences were significant ($P < 0.05$). Nadeem et al. (2010) found even higher ADF content in sunflower cake (396 g/kg of DM). Analysed results for ADF content in soybean cake were 51.85 g/kg of DM higher than reported by Geremew et al. (2015). The highest NDF content was also detected in sunflower cake, but this result was

49.1 g/kg of DM lower than reported by Chung et al. (2009). Soybean cake contained the lowest amount of NDF. Our results for NDF content in soybean cake were 29.45 g/kg of DM lower than reported by Goes et al. (2010). Differences in NDF content between sunflower and soybean cakes were significant ($P < 0.05$). From cakes rapeseed cake had the lowest OM content and the highest OM content was observed in flaxseed cake. These differences were significant ($P < 0.05$). Leming and Lember (2005) found lower OM content in rapeseed cake (929 g/kg of DM). Our result for flaxseed cake was higher than reported by Latif et al. (2008). The highest IVOMD was observed in soybean cake. This result was probably affected by the fiber content, in soybean cake found the lowest from cakes. The IVOMD of flaxseed, rapeseed and flaxseed cake was similar (around 85 %). The lowest IVOMD was found for sunflower cake and rapeseed cake. These results were consistent with the high fiber content of whole sunflower and rape seeds. Significant differences ($P < 0.05$) in IVOMD of all the analyzed cake samples were observed. In cakes, higher digestibility coefficients were found in comparison with results of Petrikovič et al. (2000), mainly in soybean cake.

Table 2. Dry matter, crude fiber and fiber fractions, organic matter content and digestibility coefficients of analyzed cakes

	Sunflower	**Soybean**	**Flaxseed**	**Rapeseed**
	Mean±SD			
DM	947.60±0.30^a	950.35±0.05^b	946.55±0.25^c	945.00±0.20^d
CF	221.60±1.90^a	53.35±0.15^b	95.85±1.45^c	111.90±0.60^d
ADF	365.60±0.80^a	139.85±1.95^b	272.85±1.45^c	260.30±1.80^d
NDF	325.90±3.00^a	93.55±0.95^b	277.35±1.85^c	177.95±0.55^d
OM	945.9±0.3^a	943.9±0.1^b	949.0±0.1^c	938.1±0.3^d
IVOMD	65.63±0.67^a	94.54±0.71^b	85.34±1.45^c	75.58±0.59^d

DM – dry matter (g/kg); CF – crude fiber (g/kg of DM); ADF – acid detergent fiber (g/kg of DM); NDF – neutral detergent fiber (g/kg of DM); OM – organic matter (g/kg of DM); IVOMD – *in vitro* organic matter digestibility (%); SD – standard deviation. Values followed by different letters within a row are significant at the level 0.05.

CONCLUSION

The aim of this research was to determine DM, OM, CF, ADF and NDF content as well as IVOMD of four oilseeds (sunflower, soybean, flaxseed, rapeseed) and their cakes. Significant differences in the

composition of analyzed seeds and cakes as well as in the digestibility of their OM were found. These differences in OM digestibility are probably related to different fiber content in analysed samples. Digestibility analysis by *in vitro* conditions can be beneficial for animal nutrition research. Compared with other studies of various authors, the fiber content in oilseeds and cakes is related to the variety and environment in which they are grown.

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EVALUATION OF NITROGEN FRACTIONS OF SILAGES IN RUMINANTS NUTRITION

MARIE KOUKOLOVÁ[1, 2], PETR HOMOLKA[1, 2], VERONIKA KOUKOLOVÁ[2]

[1]Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Czech Republic
[2]Institute of Animal Science, Prague-Uhříněves, Czech Republic

Corresponding email address: koukolova.marie@vuzv.cz

ABSTRACT

The objective of this study was to evaluate the nutritional value of typical silages used in ruminant nutrition by Cornell system. This experiment includes samples of five (n = 5) silages (clover, corn, rye grass, grass and alfalfa). Silages were analysed for individual nitrogen fractions (A, B1, B2, B3 a C). The results declared influence of fibre content (CF, NDF, ADF, ADL) on individual nitrogen fractions. The highest values of crude protein (187.8 and 192.8 g/kg DM) and fraction A (612.1 and 594.7 g/kg CP) were found for for alfalfa and rye grass silages, respectively. The same trend was confirmed for fraction C, which is associated with lignification. The highest value of this fraction C was 391.6 g/kg CP for grass silage corresponding with ADL ($r = 0.777$, $P < 0.01$) of grass silage. A strong correlation was found between fraction A and B2 ($P < 0.05$, $r = 0.711$).

Keywords: Cornell system; nitrogen fractions, ruminants, silages.

INTRODUCTION

Cornell system (CNCPS; Cornell Net Carbohydrate and Protein System) is system for evaluation of the fractions of nitrogenous substances (Lanzas et al., 2008) into 5 fractions (A, B1, B2, B3 and C) according their degradability and passage rate in the gastrointestinal tract (Van Soest, 1994). Fraction A represents non-protein nitrogen (NPN) which can be separated using trichloroacetic acid and is very fast degradable in the rumen. Fraction B1 (rapidly degradable protein) is expressed by the estimating of the true soluble protein in borate phosphate buffer at pH 6.7-6.8. Fraction B2 (intermediately degradable protein) is classified as neutral detergent soluble protein. Fraction B3

(slowly degradable protein) is insoluble protein in neutral detergent but soluble in acidic detergent. Fraction C (bound (indigestible) protein) represents protein insoluble in acid detergent. This part of protein is indigestible and may occur in excrement (Van Soest, 1994; Licitra et al., 1996; Bovera et al., 2003; Parashuramalu et al., 2013).

MATERIAL AND METHODS

Silages (n = 5) were analysed for individual nutrients as dry matter (DM), crude protein (CP), ether extract (EE), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and brutto energy (BE). Silages were analyzed for individual nitrogen fraction according to method by Licitra et al. (1996):

a) non-protein nitrogen using trichloroacetic acid (TCA),
b) soluble nitrogen and protein,
c) nitrogen insoluble in acid detergent using the Fibertec apparatus,
d) nitrogen insoluble in neutral detergent using the Fibertec apparatus.

Individual nitrogen fractions (A, B1, B2, B3, C) calculations (g/kg CP) (Cazzato et al., 2012):

fraction A = NPN (g/kg SOLP) × 0,01 × SOLP (g/kg CP),
fraction B1 = SOLP - fraction A (g/kg CP),
fraction B2 = 1000 - A - B1 - B3 - C (g/kg CP),
fraction B3 = NDIP - ADIP (g/kg CP),
fraction C = ADIP (g/kg CP).

Where: A = non-protein nitrogen, ADIP = acid detergent insoluble protein, B1 = rapidly degradable protein, B2 = intermediately degradable protein, B3 = slowly degradable protein, C = bound (indigestible) protein, CP = crude protein, NDIP = neutral detergent insoluble protein, SOLP = soluble protein.

Data were statistically analyzed in the SAS 9.3 GLM (SAS Institute, 2003) procedure (PROC GLM), using the PROC CORR procedure was evaluated by correlation coefficients between the observed variables. Test statistically significant differences were evaluated by Scheffe analysis.

RESULTS AND DISCUSSION

The chemical composition and energy values of silages are presented in Table 1. The chemical compositions of the feeds were affected by

many factors, such the soil type, fertilization, climate and processing to by-product (Van Soest, 1994; Valderrama and Anrique, 2011). The CP of the samples ranged from 69.5 to 192.8 g/kg DM. As expected, the CP was higher for protein silages (clover, rye grass and alfalfa). Values of ADL ranged from 21.1 (rye grass silage) to 103.7 g/kg DM (grass silage). There was declared strong correlation ($r = -0.664$, $P < 0.05$) between OM and NDF.

Table 1. Chemical composition (g/kg DM) and brutto energy (MJ/kg DM) of the estimated samples

	DM	CP	EE	NFE	OM	CF	ADF	NDF	ADL	BE
1	933.5	179.8	17.5	439.4	905.6	268.9	380.3	423.1	52.9	18.3
2	931.3	69.5	28.1	647.4	955.2	210.3	259.9	434.4	28.0	18.2
3	943.5	192.8	26.4	332.9	807.8	255.6	257.8	364.2	21.1	18.7
4	898.7	161.3	24.4	474.5	926.1	265.9	409.7	459.4	103.7	19.3
5	903.4	187.8	17.3	306.6	876.7	365.0	79.9	468.6	94.0	19.3

ADF = acid detergent fibre, ADL = acid detergent lignin, BE = brutto energy, CF = crude fibre, CP = crude protein, DM = dry matter, EE = ether extract, NDF = neutral detergent fibre, NFE = nitrogen free extract, OM = organic matter.
1 = clover silage, 2 = corn silage, 3 = rye grass silage, 4 = grass silage, 5 = alfalfa silage.

The nitrogen fractions of the estimated samples are in Table 2. Fraction A as instantaneously degraded part of the feed in the rumen varied from 327.3 to 612.1 g/kg CP. The highest values of fraction A were found in alfalfa and rye grass silages (612.1 and 594.7 g/kg CP) corresponded with CP of these silages (187.8 and 192.8 g/kg DM, respectively). In the study of Peletkova and Broderick (1996) was fraction A of alfalfa silage lower (422.8 g/kg CP) in comparison to our results. Strong correlations ($P < 0.001$) of fraction A were found for CP ($r = 0.896$) and OM ($r = -0.879$). Other statistically significant correlations ($P<0.05$) of fraction A were found for CF ($r = 0.726$), BE ($r = 0.570$) and fraction B2 ($r = 0.710$). Values of fraction B1 varied from 1.0 to 68.9 g/kg CP. Strong correlation of fraction B1 was found for NDF ($r = -0.829$, $P<0.01$), ADL ($r = -0.597$, $P < 0.05$) and OM ($r = -0.635$, $P < 0.05$). Relationship of NDF and B1 is in Graph 1. Fraction B2 varied from 177.9 to 456.2 g/kg CP. For this fraction was the best correlation ($P < 0.001$) with OM. Other statistically significant correlations ($P<0.05$) of fraction B2 were found for NDF ($r = -0.692$) and ADF ($r = -0.518$). Fraction B3 represent values from 7.0 (grass silage) to 224.4 g/kg CP (clover silage). Bounded fraction C varied

from 73.8 (corn silage) to 391.6 g/kg CP (grass silage). Similar value of fraction C found Purwin et al. (2012) (172 g/kg CP of clover silage and 108 g/kg CP of alfalfa silage. These results correspond with very high values of ADF (409.7 g/kg DM) and ADL (103.7 g/kg DM) of this grass silage (Table 1). Which was declared with correlation of fraction C ($P < 0.01$) with ADL ($r = 0.777$; Graph 2) and NDIP ($r = 0.761$). Relationship ($P < 0.05$) of fraction C was also found with IP ($r = 0.723$).

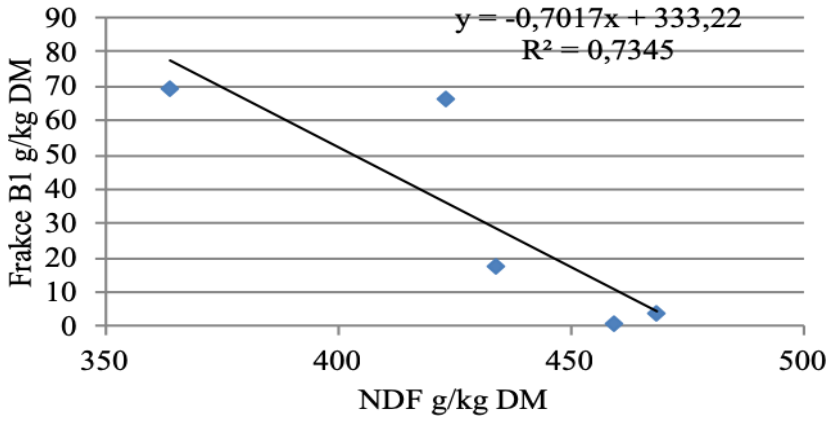
Table 2. Determination of the nitrogen fractions (g/kg CP) of the estimated samples.

Sample	A	B1	B2	B3	C
1	384.9^{b}	66.5^{a}	183.2^{b,c}	224.4^{a}	141.0^{b}
2	327.3^{c}	17.6^{b}	456.2^{b,c}	125.2^{b}	73.8^{c}
3	594.7^{a}	68.9^{a}	215.4^{a}	20.8^{c}	100.2^{c}
4	422.0^{b}	1.0^{b}	177.9^{c}	7.0^{c}	391.6^{a}
5	612.1^{a}	3.6^{b}	192.6^{a,b}	60.4^{b}	131.2^{b}

A = non-protein nitrogen, B1 = rapidly degradable protein, B3 = slowly degradable protein, C = bound (indigestible) protein, CP = crude protein.
1 = clover silage, 2 = corn silage, 3 = rye grass silage, 4 = grass silage, 5 = alfalfa silage.
^{a,b,c} Values with different superscript differ significantly ($P<0.05$)

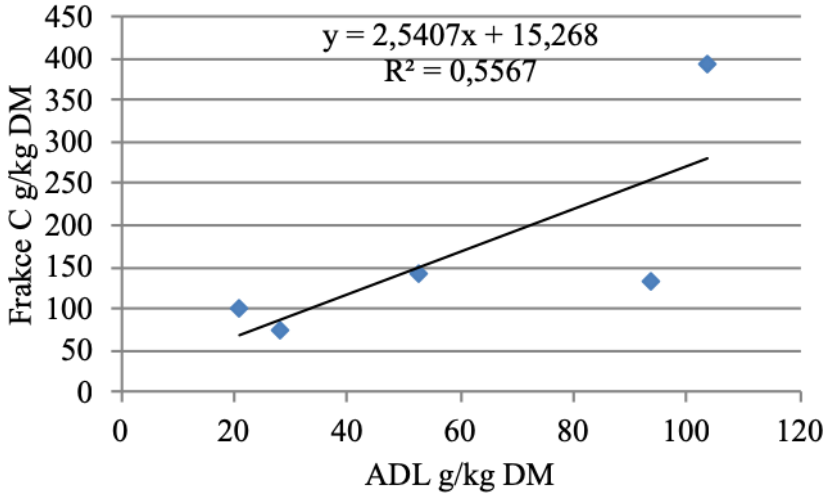
Generally, there were found statistically significant differences ($P<0.05$) among estimated silages (Table 2) in individual nitrogen fractions (A, B1, B2, B3, C).

Graph 1. The trendline of the NDF and fraction B1



NDF = neutral detergent fibre.

Graph 2. The trendline of the ADL and fraction C



ADL = acid detergent lignin.

CONCLUSION

Results of this study show importance of nitrogen fractions, especially influence of higher content of fibre fractions (CF, NDF, ADF, ADL) on individual nitrogen fractions. The fractionation of CP provides a broader view of the digestibility of the received feeds in the ruminant digestive tract. These procedures allow proper balancing of feed nutrients, their ratios and their concentrations for the proper functioning of the rumen.

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STUDY ON ANTIBACTERIAL PROPERTIES OF EDIBLE OILS CONTAINING MEDIUM-CHAIN FATTY ACIDS

KLÁRA LALOUČKOVÁ[1, 2], EVA SKŘIVANOVÁ[1, 2], PETRA HOVORKOVÁ[1]

[1]Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Kamýcká 129, 165 21 Praha 6-Suchdol, Czech Republic

[2]Department of Physiology of Nutrition and Quality of Animal Products, Institute of Animal Science, Přátelství 815, 104 00 Praha-Uhříněves, Czech Republic

Corresponding email address: lalouckova@af.czu.cz

ABSTRACT

The antibacterial properties of eight edible oils containing medium-chain fatty acids (MCFA) were determined by defining their minimum inhibitory concentration (MIC). The tested oils included: babassu (*Attalea speciosa*, syn. *Orbignya speciosa*), coconut (*Cocos nucifera*), *Cuphea* (*C. lanceolata* and *C. ignea*), murumuru (*Astrocaryum murumuru*), palm, palm kernel and red palm (*Elaeis guineensis*), and tucuma (*Astrocaryum vulgare*). Oils' antibacterial effect was examined againstselected bacteria, namely: *Bifidobacterium animalis*, *B. longum*, *Campylobacter jejuni*, *Clostridium perfringens*, *Enterococcus cecorum*, *Escherichia coli*, *Lactobacillus acidophilus*, *L. fermentum*, *Listeria monocytogenes*, *Salmonella enteritidis*, *S. infantis*, *S. typhimurium* and *Staphylococcus aureus*. The profile of fatty acids in tested oils was identified using gas chromatography (GC/FID). MIC was determined by a broth microdilution test. There was no potential of tested oils to inhibit the growth of Gram-negative bacterial strains. Moreover, palm and palm red oil did not exhibit any antibacterial action. MIC of oils that showed antibacterial activity ranged from 0.14 to 4.5 mg/ml. The lowest measured MIC was found in tucuma oil to *C. perfringens* (0.14 mg/ml). Other oils inhibited the growth of *C. perfringens* in concentrations from 0.25 to 4.5 mg/ml. *E. cecorum* strains were inhibited by coconut, babassu, *Cuphea*, palm kernel, murumuru and

tucuma oil in MIC range between 1.12 – 4.5 mg/ml. Only *Cuphea* from all tested oils showed inhibitory properties against *L. monocytogenes* (MIC 1.12 mg/ml). *S. aureus* strain growth was inhibited by palm oil at MIC between 0.56 to 2.25 mg/ml (coconut, babassu, *Cuphea*, palm kernel, murumuru and tucuma oil). Negative susceptibility to all tested oils, evaluated as a positive effect, was detected towards *B. animalis*, *B. longum*, *L. acidophilus* and *L. fermentum* strains. It can be concluded that the edible oils containing medium-chain fatty acids show antibacterial effect towards Gram positive strains of bacteria. Negative influence on beneficial intestinal microbiota can be promising.

Keywords: commensal microbiota; pathogen; inhibition; nutrition; palm; lauric acid

INTRODUCTION

Continuing burden of zoonoses and foodborne illnesses has been reported in developed (Havelaar et al., 2010) and developing (Byarugaba, 2004) countries. It is necessary to consider the possible transmission of pathogens, since animals could serve as potential reservoir of serious human diseases (Levin and Antia, 2001). Hence, an increasing concern about reducing bacterial pathogens in animal husbandry is still valid.

Foodborne pathogens have been controlled by antibiotics since their discover in medicine (Aminov, 2010). Nevertheless, the use of antibiotics in both human and veterinary medicine has currently been viewed with a caution, due to the public health concern (Grave et al., 2010). Consequently, there is a widespread interest in alternative antibacterial compounds research and development. Over the past few decades, organic acids and their derivatives have been used in animal production as well as the food industry because of their antibacterial properties (Davies and Davies, 2010). Medium-chain fatty acids (MCFA) are saturated unbranched monocarboxylic acids with 6 – 12 carbons (Bach and Babayan, 1982) and represent a class of natural compounds with antibacterial properties (Desbois and Smith, 2010). MCFAs are natural components of milk and various feed materials, especially coconut and palm oils (Edem, 2002). The three most valuable MCFAs in these plant oils are lauric acid ($C_{12:0}$), capric acid ($C_{10:0}$) and caprylic acid ($C_{8:0}$) (Oyi et al., 2010). Lauric acid may reach a proportion of up to 50% in coconut oil (Marten et al., 2006; Arlee et al., 2013) and is the MCFA with the highest potential anti-infective effect on harmful microorganisms: it inhibits the growth of algae

(McGrattan, 1976), both Gram negative (Bergsson et al., 1998; Bergsson et al., 1999) and Gram positive bacteria (Kabara et al., 1972; Feldlaufer et al., 1993), fungi (Kabara et al., 1972; Bergsson et al., 2001), and protozoa (Dohme et al., 2001). Therefore, the antibacterial activity of chosen edible oils rich in MCFA was tested *in vitro* towards several foodborne pathogens with the aim to evaluate their prospective as natural antibacterial compounds that can serve as an alternative to in-feed antimicrobials.

MATERIAL AND METHODS

Bacterial strains and culture conditions

Fourteen bacterial strains (Table 1) were used to assess the antibacterial properties of plant oils including eight pathogenic microorganisms and six beneficial intestinal strains. The sources of bacterial strains were as follows: CCM, Czech Collection of Microorganisms (Brno, Czech Republic); ATCC, American Type Culture Collection (Manassas, USA); CNCTC, Czech National Collection of Type Cultures (National Institute of Public Health, Prague, Czech Republic); and CIP, Collections of Pasteur Institute (Paris, France). *C. perfringens* no. 56 was kindly provided by Prof F. Van Immerseel from the Ghent University (Belgium).

Table 1. Strains used for the determination of antibacterial properties of edible oils containing MCFA

Species	Strain
Bifidobacterium animalis	CCM 4988 MA5
Bifidobacterium longum	CCM 4990 TP1
Campylobacter jejuni	CCM 6189 CAMP/VFU 612/21
Clostridium perfringens	CIP 105178 CNCTC 5454 UGent 56
Enterococcus cecorum	CCM 3659 ^{T} CCM 4285
Escherichia coli	ATCC 29522 C6
Lactobacillus acidophilus	CCM 4833
Lactobacillus fermentum	CCM 91
Listeria monocytogenes	ATCC 7644
Salmonella enteritidis	ATCC 13076
Salmonella infantis	K2
Salmonella typhimurium	K3
Staphylococcus aureus	ATCC 25923

All bacterial strains were cultured and maintained in appropriate broth (Oxoid Inc., Basingstoke, Hampshire, UK) at 37 °C for 24 hours (or 48 hours) under aerobic or anaerobic conditions, as appropriate.

Antimicrobial compounds

Edible oils, namely coconut (*Cocos nucifera*) and palm kernel (*Elaeis guineensis*) oil were purchased from Sigma-Aldrich (Czech Republic). Palm, red palm (*Elaeis guineensis*), babassu (*Attalea speciosa*), tucuma (*Astrocaryum vulgare*) oil, and murumuru butter (*Astrocaryum murumuru*) were purchased from Sweet Natural Botanicals (USA). *Cuphea* seeds were purchased from the US Department of Agriculture – Agricultural Research Service (Plant Germplasm Inspection Station, USA), dried and extracted using 80 g/kg methanol for 24 hours. This extract was filtered and dried at 40 °C using a vacuum dryer Rotavapor R-200 (Buchi, Switzerland).

Fatty acids determination in edible oils

The fatty acid (FA) profile was determined by gas chromatography/flame ionisation detection (GC-FID) at the Institute of Animal Science (Prague, Czech Republic). Alkaline trans-methylation of the extracted FAs was carried out as described by Raes et al. (2003). HP 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, USA) with a programmed 60 m DB-23 capillary column (J&W Scientific, Folsom, USA) was used for GC analysis of methyl esters. FAs were identified based on retention times by comparison with the retention times of FAME Mix 37 standards (Sigma-Aldrich).

Preparation of plant oils for microdilution tests

The oils were weighed and diluted in the same amount of dimethylsulfoxide (DMSO; Lach-Ner, Czech Republic), followed by an addition of emulsifier (Tween 80; Sigma-Aldrich) to form an emulsion, that was consecutively diluted in Wilkins-Chalgren medium containing a lipase from porcine pancreas (Sigma-Aldrich), resulting in a final oil concentration of 4.5 mg/mL. The final concentrations of DMSO and Tween 80 did not exceed 1% and 0.1%, respectively.
Antibacterial tests both with unhydrolysed and hydrolysed oils were performed to examine antimicrobial properties of the oils. FA moieties esterified to the glycerol backbone of triacylglycerol had to be firstly hydrolysed into their free forms (free FAs). The hydrolysis was catalysed by lipase. The minimum required amount of lipase was calculated based on the molecular weight of the predominant FA (i.e. lauric or capric acid), as well as on the enzymatic activity specified by the manufacturer. The resulting emulsions of medium, lipase, and oil was warmed to 37 °C and shaken for 1 h.

Determination of antibacterial effects of MCFA, their derivatives and plant oils in vitro

Antibacterial activity of the tested compounds was evaluated in vitro by broth microdilution method using 96-well microtiter plates, modified according to recently proposed recommendations for an effective assessment of anti-infective potential of natural products (Cos et al., 2006). Seven two-fold dilutions were prepared, starting with an initial concentration of 4.5 mg/mL (volume-adjusted depending on the density of compound). Dilutions of each compound was prepared in Wilkins-Chalgren broth, or a corresponding medium recommended for

cultivation of certain bacteria (Oxoid, UK). The bacterial inoculum was standardized to density 1 x 10^7 CFU/mL using the McFarland scale, and inoculated into wells (10 µL/well). Microplates were incubated at 37 °C for 24 hours (or 48 hours) under aerobic conditions. The growth of microorganisms was assessed by culture turbidity, determined by Infinite® 200 PRO Microplate Reader (Tecan, Switzerland) at 405 nm. Minimum inhibitory concentrations (MIC 80) were expressed as the lowest compound concentrations that resulted in 80% growth reduction compared with the growth of extract-free control. The susceptibility of all microorganisms to penicillin G was evaluated as a control of compound effectiveness and bacterial viability. Positive controls containing 10 µL of bacterial suspension and 90 µL Wilkins-Chalgren broth, negative controls containing 100 µL Wilkins-Chalgren broth were added as well. All samples were tested in three independent experiments, each carried out in triplicate. The results were statistically evaluated using MS Excell programme. The final MIC values represent modes of the obtained MICs measured in the individual independent experiments.

RESULTS AND DISCUSSION

Lauric acid was the most prevalent MCFA in coconut, palm kernel, babassu, tucuma, and murumuru oil, that is generally in agreement with previous findings (Dubois et al. 2007). This FA is considered the most effective MCFA against pathogenic bacteria (Galbraith et al. 1971). *Cuphea* oil consisted mainly from capric acid ($C_{10:0}$), but the measured amount is lower compared to results of Zentek et al. (2011) (i.e. ± 85%). Palm and red palm oil did not show any significant presence of MCFA (Graph 1), that is a consistent result when compared to known facts (Edem, 2002).

MIC of active tested oils ranged between 0.14–4.5 mg/ml (Table 2). The lowest MIC has been detected in tucuma oil against *C. perfringens* CIP 105178 - 0.14 mg/ml. The most susceptible strains of bacteria according to the number of oils (6) that inhibited their growth were *E. cecorum* CCM 3659, *E. cecorum* CCM 4285, and *S. aureus* ATCC 25923. The highest antibacterial activity has been proven in *Cuphea* oil that inhibited growth of 7 bacterial strain (35% success). The beneficial antibacterial effect of whole *Cuphea* seeds, when combined with exogenic lipase, was already observed by Dierick et al. (2003). The performed *in vitro* experiment showed, that there is a negative susceptibility of beneficial microbiota towards edible oils containing MCFA. The resistance of *Bifidobacterium* to MCFAs can be caused by

the lack of ferredoxin system responsible for the reduction of the nitro group in metronidazole, resulting in higher resistance to metronidazole, an antibiotic effective against most obligatory anaerobes (Pelissier et al. 2010). The negative antibacterial effect observed against *Lactobacillus acidophilus* and *L. fermentum* corresponds to the findings of a previous study by Kodicek and Worden (1945).

Graph 1. Fatty acid composition of tested oils (mg/kg)

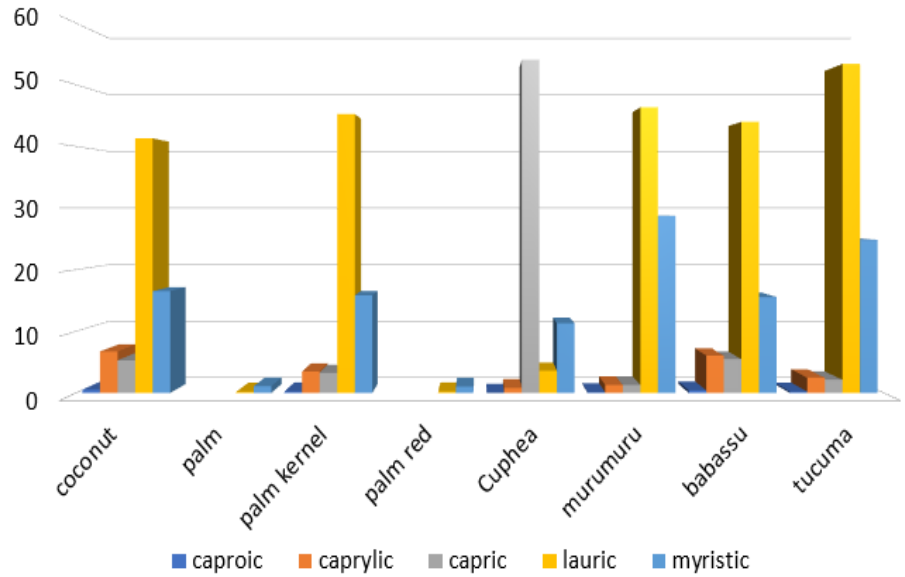

Table 2. Summary - MIC of tested oil (mg/ml)

	BA		BL		CJ		CP			ECe	
OIL	**CCM 4988**	**MA5**	**CCM 4990**	**TP1**	**CAMP/ VFU 642/04**	**CCM 6189**	**CNCTC 3659**	**UGent 56**	**CIP 105178**	**CCM 3659**	**CCM 4285**
Coconut											
Palm											
Red palm											
Palm Kernel											
Cuphea											

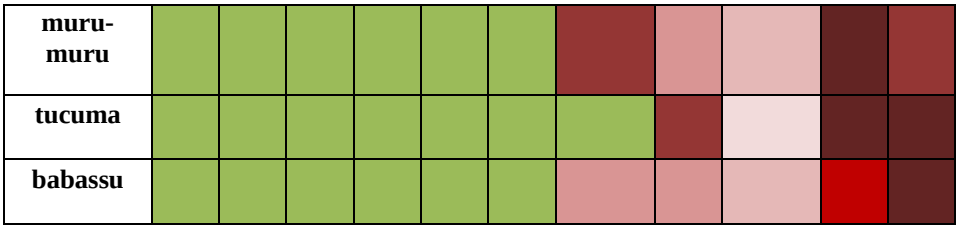

Table 2. (continuation) Summary - MIC of tested oil (mg/ml)

	ECo		LA	LF	LM	SE	SI	ST	SA
OIL	**ATCC 29522**	**C6**	**CCM 4833**	**CCM 91**	**ATCC 7644**	**ATCC 13076**	**K2**	**K3**	**ATCC 25923**
Coconut									
Palm									
Red palm									
Palm Kernel									
Cuphea									
muru-muru									
tucuma									
babassu									

BA – *B. animalis*, **BL** – *B. longum*, **CJ** – *C. jejuni*, **CP** – *C. perfringens*, **ECe** – *E. cecorum*, **ECo** – *E. coli*, **LA** – *L. acidophilus*, **LF** – *L. fermentum*, **LM** – *L. monocytogenes*, **SE** – *S. enteritidis*, **SI** – *S. infantis*, **ST** – *S. typhimurium*, **SA** – *S. aureus*

MIC (mg/ml)

0.14	0.28	0.56	1.12	2.25	4.5	> 4.5

CONCLUSION

Edible oils rich in MCFA show antibacterial activity against Gram positive bacteria after cleavage by pancreatic lipase and negative susceptibility to *Lactobacillus* spp. and *Bifidobacterium* spp. That is a promising effect of oils containing MCFA. Addition of oils rich in MCFA into the diet can be an alternative to the conventional antibiotics without side-effects (antibiotic resistance).

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COMPARISON OF NUTRIENT COMPOSITION OF SORGHUM VARIETIES DEPENDING ON DIFFERENT SOIL TYPES

KATEŘINA MRVOVÁ[1], LEOŠ PAVLATA[1], VLADIMÍR SMUTNÝ[2], BARBORA UMLÁŠKOVÁ[1], PETR DOLEŽAL[1]

[1]Department of Animal Nutrition and Forage Production, [2]Department of Agrosystems and Bioclimatology, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: kacka.mrvova@seznam.cz

ABSTRACT

The aim of this study was compare the nutrient composition of 10 selected varieties of sorghum at two different habbitats, it is soil types (clay loam soil – fluvisol and light sandy soil) in South Moravia region. Sampling of sorghum varieties at two different locations was realized 12 weeks after sowing, followed by analysis of nutrient composition as dry matter, ash, fat, N-substances, fibre, ADF, NDF and organic matter digestibility. Based on the data found it was concluded that it depends more on sorghum variety and its form than on a soil types.

Keywords: grain sorghum, silage sorghum, nutrition, C4-plants, ruminant nutrition, chemical composition

INTRODUCTION

Sorghum is one of the most cultivated crops in the world, especially in countries with warm climate and dry areas. Sorghum can be located in regions where corn does not do well (Wang et al. 2016) and where may be more useful and does high yield, because sorghum needs 1/3 less water than corn. But we must know that sorghum has higher temperature requirements than corn.

This crop can be used as a human food, even for animal nutrition to substitute only part of corn in diets for most livestock (Wang et al. 2016). Sorghum is useful for silage production, especially in conditions of rainfall deficit and even in the growing period when maize usually gives poor yields (Szempliński et al. 2014).

Sorghum is known to be relatively more tolerant to soil salinity and acidity than other comparable crops such as maize (Reddy et al. 2004), but it depends on varieties or hybrids of sorghum. It has also low nutrient requirements including nitrogen and is less suffering from diseases and pests (Podrábský 2017). The soil claims for sorghum are smaller compared to maize. The roots of the sorghum reach to a depth of up to 150 cm, even deeper in the permeable soils (Hermuth et al. 2012). Sorghum is grown on very diverse soils, from sandy loam soil to heavy clay soil. For the successful cultivation of sorghum the most suitable medium is loam soil (Hermuth et al. 2012). Unsuitable for cultivation of this crop are only cold and wet soils (Usťak 2014).
The aim of this study was to compare the nutrient composition of selected varieties of sorghum at two different locations, it is different soil types in South Moravia region.

MATERIAL AND METHODS

Characteristic of field experimental station in Žabčice

Field experiment was processed in Žabčice located in maize production area in the South Moravian region. This territory belongs among the warmest regions in the Czech Republic. The drought is increased by the winds that cause a large evaporation of soil moisture.

Soil characteristic
Sorghum was sown at two locations. The first location named Obora has clay loam soil and the soil type is fluvisol. Obora has good availability of groundwater (Svratka River), which fluctuates 0.8 – 2.5 m below the soil surface during the year. The second location Písky has light sandy soil and it is drier then Obora.
Part of the seeds was provided by KWS company and SEED SERVICE company.

Characteristic of selected varieties

Some varieties are of grain-sorghum type: Express, Arsenio, KHS5G07, Sweet Susana and Buffalo Grain BMR. Some are hybrids with Sudanese grass (*Sorghum sudanense*) – Latte, KWS Sole. And the other variants are pure *Sorghum bicolor* (KWS Zerberus) or hybrid *Sorghum bicolour x bicolour* (KWS Kalisto) and hybrid of *Sorghum saccharatum* and *Sorghum sudanense* (Big Kahuna BMR).
Sampling of sorghums (10 on Obora and the same 10 varieties on Písky locations) was realized in July 14th (12 weeks after sowing). From each

variety about 2 kg of fresh matter was sampled, than the samples were processed on a chopped forage.

Analyzed parameters

The nutrient compositions of sorghums were analysed in laboratory at Mendel University in Brno. The basic analysis of sample is represented by Weende analysis, which includes: dry matter, ash, nitrogenous substances, fat, and fibre. Other parameters such as ADF (Acid Detergent Fibre), NDF (Neutral Detergent Fibre) were determined using ANKOM machine. OMD (Organic Matter Digestibility) was determined by pepsin-cellulase method.

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

RESULTS AND DISCUSSION

The important indicator of sorghum quality for silage preparation is dry matter content, which should be between 28% and 35% in harvest period. From table 1 can be read that dry matter values of sorghums are very different, ranging from 16% to 28%. The lower dry matter value has Big Kahuna BMR variety at both locations; conversely the highest value has KHS5G07 variety at Písky location. Dry matter value is related with earliness of varieties. From table 1 it is obvious that some varieties are more earlier, for example KHS5G07, Arsenio both at Písky location, because of higher dry matter value.

Přikryl (2010) compared changes in nutritive value of sorghum depending on the date of sampling. He found that the optimal time for the preservation of sorghum was when the dry matter content was about 16%. He suggested that for the preservation of sorghum it is necessary to choose a two-phase harvest with respect to the low content of dry matter. But intensive withering is necessary to adjust the dry matter content to at least 28%. So, he decided to choose phase with low dry matter, and his experiment was based on other parameters, especially N-substances. At this time N-substances value was 17.1% which is optimal.

Rajčáková (2005) compared sorghum at four different locations in south Slovakia. She determined N-substances in range of 13.1–18.6% also as optimum values.

N-substances are the highest quality indicator of sorghum. Range of N-substances determined in sorghum is from 13% to 18% (Doležal 2014). The results in table 1 show that N-substances are relatively low, especially at Obora locations, even though urea fertilization. Second habitat Písky, which should be less productive, was not fertilized by nitrogen and had highest content of N-substances. This may correspond with the fact that the soil is well supplied with nitrogen. However, low values do not mean a problem, because we can increase N-substances in crop by higher fertilization.

Another important parameter is NDF. Scientific literature states that if the NDF content is too high, it causes increase of feed volume and reduce the potential intake of dry matter by animals. The range of NDF values by Rajčáková (2005) was from 54% to 55.2%. In our research there were the values of this parameter from 47.50% to 60.08%, this is very large dispersion which relates to the content of fibre.

The range of OMD – organic matter digestibility in this study is from 70.68% to 85.59%, therefore relatively high. It is caused by the fact that it is a relatively young growth. Přikryl (2010) measured values of OMD from 40.9% to 71.5% - the later sampling, the lower OMD.

These results demonstrate large divergence among selected varieties. Differences between habitats are not too high, but it depends on variety.

Table 1. Analyzed parameters of selected varieties from second sampling in July 14th

	%	%	%	%	%	%	%	%
	Dry matter	**Ash**	**N-substances**	**Fat**	**Fibre**	**ADF**	**NDF**	**OMD**
Express (G)								
Obora	25.00	7.14	9.91	2.82	24.52	28.04	49.11	79.49
Písky	25.00	7.63	14.49	2.63	23.20	26.94	49.94	84.83
Sweet Susana (G)								
Obora	27.00	6.53	9.03	2.14	25.94	29.74	51.47	78.01
Písky	25.00	8.03	12.72	2.84	25.60	29.34	52.69	79.61

Buffalo Grain BMR (G)								
Obora	19.00	7.14	9.93	3.36	26.38	27.48	49.58	85.59
Písky	19.00	9.25	15.94	3.74	23.12	25.76	49.36	79.26
Big Kahuna BMR								
Obora	16.00	8.42	11.24	3.18	28.53	29.62	51,56	75.40
Písky	18.00	10.55	16.48	3.62	22.54	23.80	47.50	87.29
Latte								
Obora	20.00	7.00	7.84	3.40	30.09	32.75	54,16	77.76
Písky	18.00	10.28	12.87	3.67	27.30	29.00	52.63	81.36
KWS Sole								
Obora	26.00	6,31	8.55	2.06	33.33	38.13	60.08	70.68
Písky	25.00	7.94	10.70	2.66	31.75	35.82	58.02	72.41
KWS Zerberus								
Obora	22.00	5.95	8.73	2.84	32.24	34.40	56.83	77.06
Písky	16.00	9.82	12.34	2.90	28.44	31.75	52.79	76.35
KWS Kallisto								
Obora	23.00	6.52	8.23	2.51	29.40	31.18	52.12	76.76
Písky	20.00	7.91	9.73	2.16	30.00	33.41	55.66	73.56
Arsenio (G)								
Obora	25.00	6.04	9.46	2.13	23.48	26.36	45.06	78.19
Písky	27.00	7.03	11.89	3.28	20.86	23.36	41.28	82.58

KHS5G07 (G)								
Obora	26.00	5.99	8.96	2.68	28.13	29.20	51.86	77.35
Písky	28.00	7.48	12.41	3.42	22.52	24.38	46.97	81.45

(G) – grain form of sorghum

According to the results presented in table 2 and 3 the statistically significant difference was only for two parameters – Nitrogenous substances and ash. Other parameters are statistically insignificant.
Average values in table 2 and 3 are with minimal differences. Average value of dry matter is very similar; the difference is only 0.80%. The "biggest" divergent average value in tab. 2 is for nitrogenous substances, where the difference is about 3.32%. It is related with table 1, where large differences in nitrogen values between the habitats are demostrated, as already mentioned.
Significant differences are in ash value (6.70 ± 0.75; 8.59 ± 1.26%), the difference between the locations is about 1.89%, which can be caused by different mineral content in the soil.

Table 2. Nutrient parameters (mean ± standard deviation) in sorghum depending on soil type

	Dry matter [%]	Ash [%]	N-substances [%]	Fat [%]
Obora	22.90 ± 3.60^{a}	6.70 ± 0.75^{a}	9.18 ± 0.98^{a}	2.71 ± 0.50^{a}
Písky	22.10 ± 4.33^{a}	8.59 ± 1.26^{b}	12.5 ± 2.13^{b}	3.09 ± 0.53^{a}

Table 3. Nutrient parameters (mean ± standard deviation) in sorghum depending on soil type – acid detergent fibre (ADF), neutral detergent fibre (NDF) and organic matter digestibility (OMD)

	Fibre [%]	ADF [%]	NDF [%]	OMD [%]
Obora	28.20 ± 3.20^{a}	30.69 ± 3.55^{a}	52.18 ± 4.16^{a}	77.63 ± 3.68^{a}
Písky	25.53 ± 3.67^{a}	28.36 ± 4.27^{a}	50.68 ± 4.78^{a}	79.87 ± 4.71^{a}

As can be seen from tables 4 and 5, the differences between grain and non-grain varieties are statistically significant in dry matter, N-substances (11.47 ± 2.42; 10.67 ± 2.68%), ADF (27.06 ± 2.14; 31.98 ± 3.99%), NDF (48.73 ± 3.46; 54.14 ± 3.63%) and also OMD (80.64 ± 2.88; 76.83 ± 4.73%). Difference between average dry matter contents is 4.20%, between average fibre content 4.98% and between average NDF contents even 5.41%. These big divergences among these parameters show that it really depends on the variety and especially on form of sorghum.

Table 4. Nutrient parameters (mean ± standard deviation) in different sorghum form

Variety	Dry matter [%]	Ash [%]	N-substances [%]	Fat [%]
Grain	24.60 ± 3.13^{a}	7.22 ± 0.97^{a}	11.47 ± 2.42^{a}	2.90 ± 0.54^{a}
Non-grain	20.40 ± 3.53^{b}	8.07 ± 1.68^{a}	10.67 ± 2.68^{b}	2.90 ± 0.57^{a}

Table 5. Nutrient parameters (mean ± standard deviation) in different sorghum form – acid detergent fibre (ADF), neutral detergent fibre (NDF) and organic matter digestibility (OMD)

Variety	Fibre [%]	ADF [%]	NDF [%]	OMD [%]
Grain	24.38 ± 2.15^{a}	27.06 ± 2.14^{a}	48.73 ± 3.46^{a}	80.64 ± 2.88^{a}
Non-grain	29.36 ± 3.04^{b}	31.98 ± 3.99^{b}	54.14 ± 3.63^{b}	76.83 ± 4.73^{b}

The correlation among nutrient parameters is recorded in table 6. The table shows relationships among analyzed nutrient parameters. OMD parameter has positive correlation with N-substances, fat, fibre and negative correlation with ADF and NDF. Strong relationship has also N-substances with fat, fibre, ADF and OMD.

Table 6. Correlation (r) in all analyzed samples

	Dry matter	Ash	N-sub.	Fat	Fibre	ADF	NDF	OMD
Dry matter		-0.67**	-0.28	-0.47*	-0.18	-0.05	-0.14	-0.08
Ash			0.79**	0.60**	-0.24	-0.26	-0.08	-0.31
N-sub.				0.58**	-0.63**	-0.60**	-0.40	0.57**
Fat					-0.39	-0.52*	-0.36	0.59**
Fibre						0.96**	0.92**	-0.77**
ADF							0.94**	-0.82**
NDF								-0.70**
OMD								

* P < 0.05; ** P < 0.01

CONCLUSION

The results of this research suggest that differences of selected nutritive parameters between compared locations are not too high. And this is very interesting finding, because we can state that both locations are appropriate for sorghum growth - dried and less fertile Písky as well as more fertile Obora. So, dry conditions of South Moravia region are suitable for sorghum growing.

It can not be state that the dry matter is demonstrably higher at one or at the other habitat; it depends mainly on the variety of sorghum. Only N-substances and ash are significantly influenced by habitat conditions, but these parameters can be affected by fertilization.

But if we talk about differences between grain and non-grain forms of sorghum regardless of habitat, we can state that these divergents are very big. So, the form of sorghum is also very important if we have to choose the ideal sorghum variety for animal nutrition.

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THE INFLUNECE OF L-CARNITINE SUPPLEMENTATION ON QUALITATIVE AND QUANTITATIVE PARAMETERS OF DUROC BOAR EJACULATE

MAGDALENA PŘIBILOVÁ[1], PAVEL HORKÝ[1], LENKA URBÁNKOVÁ[1], MILAN VEČEŘA[2], PAVEL NEVRKLA[2], JIŘÍ SKLÁDANKA[1]

[1]Department of Animal Nutrition and Forage Production, [2]Department of Animal Breeding, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: magdalena.pribilova@mendelu.cz

ABSTRACT

The aim of this study was to monitore qualitative and quantitative changes in duroc boar ejaculate after a supplementation of L-carnitine into the basic feed ration. The monitored ejaculate parameters included volume of ejaculate, sperm concentration, total rate of sperm, motility and percentage of morphologically abnormal sperm. Amount of L-carnitine in ejaculate was monitored as well. For the experiment were selected 12 duroc boars and were divided into two groups. Control group (n = 6) was fed by basic feed mixture only. Experimental group (n = 6) was fed by basic feed mixture with the addition of 500 mg of L-carnitine/kg of the basic mixture. The experiment ran from July to August (60 days).

Based on the results we confirmed the hypothesis, that L-carnitine has a positive effect on quality of ejaculate. In sperm motility there was insignificant increase in experimental group, but there was statistically significant difference between groups ($P < 0.05$). In the amount of morphologically abnormal sperm, there was statistically significant increase in control group ($P < 0.05$) and as well statistically significant difference between groups ($P < 0.05$).

Keywords: L-carnitine, boar, ejaculate, sperm

INTRODUCTION

L-carnitine is vitaminous amino-acid-like compound, synthetized from lysine and methionine in brain, liver and kindey (Vaz and Wanders,

2002; Cibulka, 2005). L-carnitine improves qualitative parameters of ejaculate, especially an increase of concentration of sperm and motility (Vitali et al. 1995). L-carnitine plays a very important role in lipid metabolism. It brings long-chain fatty acids into the mitochondria for beta-oxidation, thus producing the energy (ATP) necessary for proper sperm motility (Hoppel, 2003; Horký et al. 2012). It is also very important for detoxification of the organism, because it eliminates acetyl-CoA from mitochondria, excess of which has a toxic effect (Arrigoni-Martelli and Caso, 2001) and protects the cell membranes from the oxidative damage caused by peroxidation of polyunsaturated fatty acids, being the integral part of the membrane phospholipids (Kalaiselvi and Panneerselvam, 1998; Horký, 2014).

MATERIAL AND METHODS

An experiment was performed at station of insemination in Velké Meziříčí (N 49° 23.46667 ', E 15°52.70135') and lasted 60 days (July – August), which were divided into 3 periods (Period 1 = day 0, Period 2 = day 1 – 30, Period 3 = day 31 – 60). For the experiment was chosen 12 duroc boars of average weight 255 ± 20 kg and age 2 ± 0.3 years. Boars were housed individually in pens (2.5 × 2.5 m). The boars were divided into two groups, where the control group (n = 6) was fed by the basic feed mixture only and the experimental group (n = 6) was fed by the basic feed mixture with addition of 500 mg of L-carnitine per kg of the feed ration.

In **Table 1** there is a composition of basic feed mixture. The energy value of feed was 12.6 MJ/kg and was fed in dose 3.5 kg/boar/day. Water was accessible ad libitum.

Table 1. Composition of feed mixture

Component	% of feed mixture
Barley grain	36.00
Wheat grain	20.36
Oat grain	20.00
Soybean meal (SBM)	14.50
EKPO T (biscuit meal)	3.00
BergaFat (palm oil)	2.10
Calcium carbonate	1.50
Monocalciumphosphate	1.20
Mineral vitamin premix for boars 0.5%	0.50
Sodium chloride	0.40
Magnesium oxide	0.15
L-Lysine HCl	0.14
L- Threonine	0.09
Methionine DL	0.06

Ejaculate was taken once a week with hand gloved technique. Methodology of ejaculate analysis was determined by Lovercamp et al. (2013). Biochemical analysis were performed according to the methodology by Cerovsky et al. (2009).

Data has been processed by Statistica version 10.0 (CZ). 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 L-carnitine ejaculate volume, sperm concentration and motility, percentage of morphologically abnormal sperm. The difference ($P < 0.05$) was considered as significant.

RESULTS AND DISCUSSION

Table 2 shows the effect of L-carnitine supplementation on qualitative ejaculate parameters of each group and in each period of the experiment. From the results it's obvious, that addition of 500 mg of L-carnitine had insignificant effect on volume of ejaculate, concentration of sperm and total rate of sperm. On the contrary, the control group reached better values of these parameters than the experimental group,

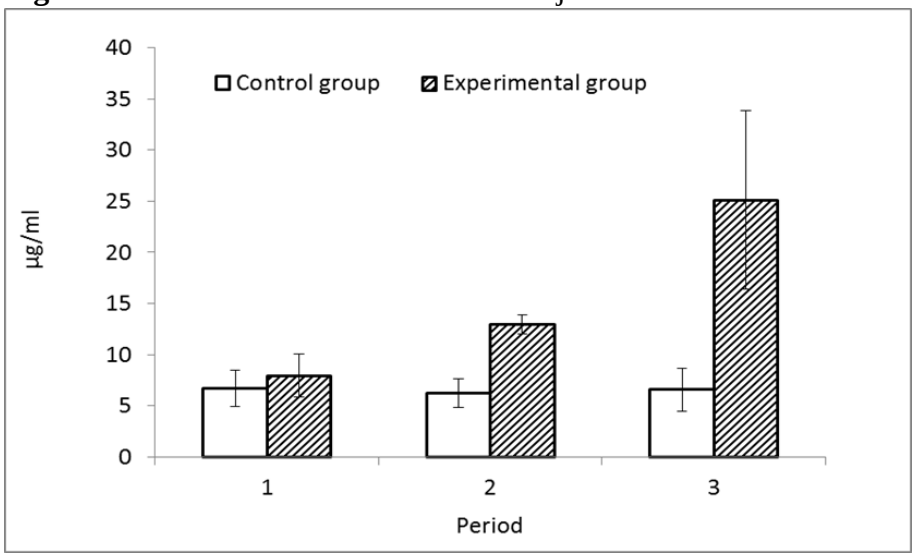
but results were not statisticaly significant. Statistically significant effect of supplementation of L-carnitine has been proven in motility and the amount of morphologically abnormal sperm. Already after 30 days of supplementation there was statistically significant difference between groups in motility of sperm (about 10.62 %), ($P < 0.05$). In the 3rd period there were significant differences in motility (11.35 %) and the amount of morphologically abnormal sperm (about 5.62 %), ($P < 0.05$).

Table 2. Average values of analysed parametres in each period (means ± standard deviation)

Period	1		2		3	
	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group
Volume of ejaculate	**158.33** ±35.59	**150.00** ±41.44	**178.95** ±36.91	**173.06** ±35.07	**158.12** ±36.47	**175.42** ±37.72
Concentration of sperm	**518.33** ±156.25	**607.5** ±117.01	**462.5** ±126.85	**527.50** ±89.12	**572.91** ±130.14	**556.87** ±20.59
Total rate of sperm	**80.70** ±27.85	**76.77** ±12.43	**85.32** ±39.38	**81.86** ±18.74	**87.43** ±16.74	**84.04** ±16.82
Motility	**71.66** ±7.52	**77.50** ±5.00	**66.04*** ±6.02	**76.66*** ±4.71	**65.62*** ±5.58	**76.97*** ±4.13
Morphologically abnormal sperm	**9.83** ±6.58	**6.50** ±7.04	**11.41** ±6.34	**8.25** ±6.39	**14.87*** ±10.58	**9.25*** ±2.31

After biochemical analysis, it can be observed statistically significant increase of L-carnitine concentration in experimental group. was occurred already after 30 days (about 5.02 μg/ml) and after 60 days (about 12.12 μg/ml) of supplementation ($P < 0.05$). Statistically significant differences between groups were occurred after 30 days (about 6.78 μg/ml) and 60 days (about 18.55 μg/ml) of supplementation ($P < 0.05$).

Figure 1. Concentration of L-carnitine in ejaculate



CONCLUSION

We confirmed the hypothesis, that L-carnitine has a positive effect on quality of ejaculate, in observed parameters: motility of sperm, the amount of morphologically abnormal sperm and concentration of L-carnitine in ejaculate. In volume of ejaculate, concentration of sperm and total rate of sperm there weren't noticed any positive changes during the experiment.

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EFFECT OF PURPLE WHEAT RU 687-12 ON PERFORMANCE PARAMETERS OF LAYING HENS AT THE END OF THE LAY

ANDREA ROZTOČILOVÁ, ONDŘEJ ŠŤASTNÍK, EVA MRKVICOVÁ, LEOŠ PAVLATA

Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: xroztoc1@node.mendelu.cz

ABSTRACT

The aim of this study was to determine the effect of wheat with colored grain RU 687-12 in a feed ration for laying hens after 69 weeks of age. The influence on performance parameters and qualitative parameters of the eggs were evaluated. The experimental diets contained 60 % of wheat with purple grain pigmentation RU 687-12. The control diet contained 60 % of common wheat. Differences in yolk weight between groups in qualitative parameters were statistically significant ($P < 0.05$). Hens fed with purple wheat grain achieved higher total number of eggs with higher yolk weight in this trial.

Keywords: colored wheat, purple wheat, weight of yolk, poultry nutrition

INTRODUCTION

Different colours of wheat grains are caused by pigments including anthocyanins, flavonoids, carotenoids and another else. Wheat with coloured grain contain more anthocyanins than common wheat. Anthocyanins are secondary metabolites of flavonoids emerging in flowers. This substances screen flowers before stress factors and mycotoxic infections (Chalker-Scott, 1999). One of the most commonly occurring anthocyanins is cyanidin followed by delphinidin, peonidin, pelargonidin, malvidin (Oomah and Mazza, 1999). The anthocyanins have a positive effect on health status of animals and humans. For example, anthocyanins have anti-bacterial, anti-carcinogenic and antioxidation effects, act as a prevention against heart diseases (Martinek and Vyhnánek, 2014), prevention of liver damage (Mazza, 2000) and it can bind free radicals (Li et al., 2005). The RU

687-12 is wheat cultivar with a purple pericarp (Martinek et al. 2010). Purple colour is caused mainly by cyanidin 3-O-glucoside and peonidin 3-O-glucoside (Abdel-Aal and Hucl, 2003). The aim of this study was to determine the effect of purple wheat cultivar RU 687-12 on quantitative and qualitative parameters of laying hens eggs after 69 weeks of age.

MATERIAL AND METHODS

A total of 30 laying hens were divided into two groups at the end of the laying periods in 69 weeks. Experimental group (n=15) was fed with feed mixture which contained 60 % purple wheat RU 687-12 with higher content of anthocyanins (36.66 mg/kg). Control group was fed with feed mixture where purple wheat was substituted by common wheat variety Bohemia (4.88 mg/kg). Feed mixtures were prepared to be isonitrogenous and isoenergetic. The compositions of feed mixtures are shown in Table 1 and Table 2. Hens were fed *ad libitum*.

Table 1. Composition of feed mixture (g/kg)

Component	RU 687-12	Control
Wheat	600	600
Soybean meal	187.8	200
Maize	71.05	54.1
Rapeseed oil	31.7	31.7
Limestone milled	74	74
Monocalciumphosphate (24.5% P)	5	5
Methionin (DL-methionin)	0.45	0.5
Wheat gluten	0	4.7
Premix*	30	30

*Premix supplied to 1 kg of feed mixture: lysine 1.35 g; methionine 75 g; calcium 8.85 g; phosphorus 65 g; sodium 42 g; copper 500 mg; iron 2.500 mg; zinc 3.400 mg; manganese 4.000 mg; cobalt 7 mg; iodine 30 mg; selenium 6 mg; tocopherol 450.000 mg; calciferol 166.700 IU; phylochinon 350 mg; thiamine 140 mg; B2 230 mg; B6 200 mg; cobalamine 1.000 mg; biotin 7 mg; niacinamid 1.200 mg; folic acid 57 mg, calcium pantothenate 450 mg; choline chloride 6000 mg; salinomycin sodium 2.333 mg.

Table 2. Chemical composition of rations (g/kg of dry matter basis)

Group	RU 687-12	Control
Crude ash	155.4	157.2
Crude protein (N*6.25)	198.3	200.4
Ether extract	53.1	57.6
Crude fibre	42.9	51.1

Room temperature and humidity was controlled every day. Lighting system was set to 16 hours light and 8 hours dark. Laid eggs and feed consumption was monitored every day. Eggs qualitative analysis was performed every 14th days. During the eggs qualitative analysis egg weight, eggshell weight, eggshell thickness, eggshell strength, eggshell weight ratio, Haugh units, weight of yolk and colour of yolk was monitored. The egg weight, yolk weight and shell weight were determined using a laboratory scale. Egg shell strength was determined by machine Egg Force Reader. The color of the yolk was assessed by the color fan DSM. The eggshell thickness was monitored in 3 places: in the center of the eggshell and on the blunt and sharp of the peak shell. The arithmetic mean was calculated from measured data. The experiment lasted to 78 weeks of age of laying hens. The laying hens were slaughtered by decapitation by the end of the trial.
Data has been processed by Microsoft Excel (USA) and Statistica version 10.0. We used one-way analysis (ANOVA). Differences between groups were evaluated using the Sheffe´s test. Statistically significant differences were evaluated with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Number of laid eggs during the trial and feed consumption are presented in Table 3. Experimental group RU 687-12 laid more eggs than control group (770 pcs vs 675 pcs). The mean weight of one egg was higher in control group (64.62 g) than RU 687-12 group (63.43 g). Zelenka (2014) write that egg size may be partially influenced by the content of polyunsaturated fatty acids. Weight of eggs increases with the age of the hen; it can be reason of why we found higher eggs weight in our trial.

Table 3. Mean feed consumption and mean eggs production per trial

	n	total number of eggs per period	number of eggs per hen and day	mean weight 1 egg (g)	feed consumption per egg
Control	14	675	0.747	64.62	0.152
RU 687-12	15	770	0.802	63.43	0.144

Feed intake is influenced by the need of energy and its concentration in the feed mixture. From the start of laying to its peak, feed consumption will increase by as much as 40%. Growth nutrients requirements at the beginning of the laying are more than the increase in feed intake. This is a reason, why mixture must be as concentrated as possible (Zelenka, 2014). Suto et al. (1994) suggest that the hens reared on litter have higher feed consumption than hens in caged systems. Hendrix Genetics (2018) indicates the average feed consumption of the Bovans Brown hybrid 114 g per day, so the observed consumption is slightly lower than the stated value. After calculating total daily egg production better production results were found in the experimental group. In experimental group egg production was 50.97 g compared to control group where egg production was only 48.29 g. The laying curve is shown in Figure 1 and results of eggs qualitative analyses are shown in Table 4.

Figure 1. Laying curve per trial

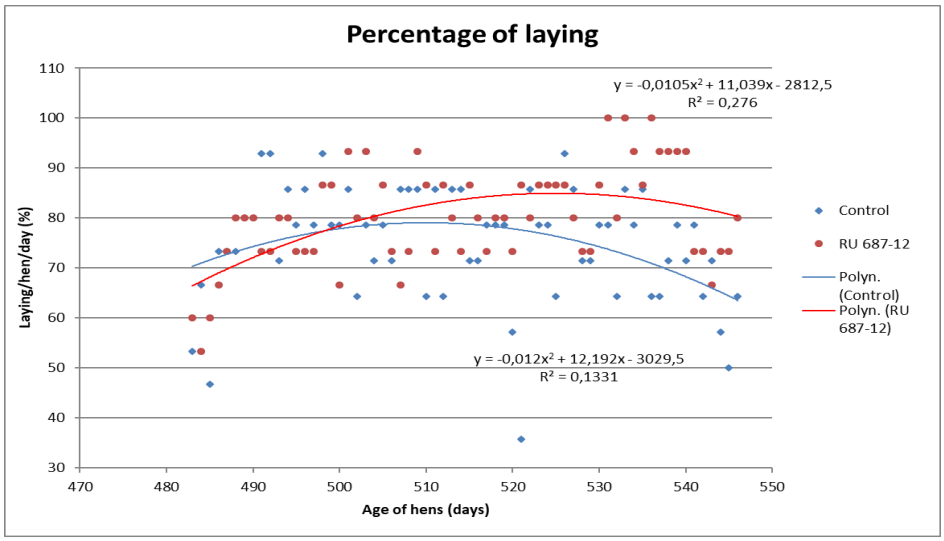

Table 4. Results of eggs qualitative analyses

Group	Control			RU 687-12		
n	130			130		
	mean	±	SE	mean	±	SE
egg weight	64.17	±	0.4704 [a]	63.61	±	0.4720 [a]
egshell strength	34.17	±	0.6044 [a]	34.51	±	0.5449 [a]
Haugh units	85.01	±	1.0190 [a]	86.21	±	0.8438 [a]
yolk weight	15.99	±	0.1421 [a]	16.54	±	0.1112 [b]
percentage eggshell ratio	9.270	±	0.1104 [a]	9.411	±	0.0859 [a]
coulur yolk	4.123	±	0.0907 [a]	4.192	±	0.0796 [a]
eggshell weight	5.907	±	0.0511 [a]	5.962	±	0.0521 [a]
egshell thickness	39.28	±	0.3965 [a]	39.83	±	0.3827 [a]

$P < 0.05$, SE = standard error

The table 4 shows that the feeding of purple wheat RU 687-12 didn´t have a statistically significant effect on the thickness and strength of the shell. Yolk weight was found statistically significant ($P < 0.05$) in RU 687-12 group than control group.

CONCLUSION

Hens fed with purple wheat grain achieve higher total number of eggs with higher yolk weight with better feed conversion ratio at the end of the lay.

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THE DIFFERENT TYPES OF BINDER IN PELLETED PET RABBIT DIETS AND ITS EFFECTS ON GROWTH OF THE YOUNG DWARF LOP RABBITS

VLASTIMIL ŠIMEK[1], DAVID ZAPLETAL[1], LENKA KUDĚLKOVÁ[1], PETRA JAKEŠOVÁ[1], EVA STRAKOVÁ[2], MIROSLAV MACHÁČEK[1], PAVEL SUCHÝ[1]

[1]Department of Animal Husbandry and Animal Hygiene, [2]Department of Animal Nutrition, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Palackého tř. 1946/1, 612 42 Brno, Czech Republic

Corresponding email address: simekv@vfu.cz

ABSTRACT

The aim of this study was to evaluate growth of the young rabbit kits of the Dwarf Lop breed in association with the different diet composition. A total of 4 types of the rabbit pelleted diets were used in the present study. The does with their young kits were divided into 2 groups (control and experimental group). The rabbits in the control group (*n=20*) received a foreign commercial diet for the dwarf rabbits in course of the entire monitored period (up to the 15th week of the rabbit´s age). The rabbits in the experimental group (*n=38*) received the pre-weaning diet containing a noticeable share of the white lupin (*Lupinus albus*) seeds var. Amiga. After weaning, the young rabbits of the experimental group were divided into 2 post-weaning dietary experimental groups (group M, *n=15*; group G, *n =22*) and received these diets up to the 15th week of age. Both of the experimental post-weaning diets showed the same ingredient composition, except of the binder (diet M, sugarbeet molasses; diet G, vegetable crude glycerol from rapeseed oil). The 21-day-old young rabbits of the experimental group showed higher ($P < 0.05$) live weight as compared to the control group (+32.57 g). Concerning the post-weaning period, no significant differences in the live weight were found between the experimental groups M and G. Based on our results, it can be concluded that the crude glycerol at the rate of 3.0% in the diet can be used as the altenative binder in the diets for growing dwarf rabbits.

Keywords: dwarf rabbit; diet; mollasses; crude glycerol; live weight

INTRODUCTION

Under the recent conditions of the Czech Republic as well as other central and western European countries, the pet rabbits represent favourite companion animals where selected dwarf rabbit breeds show the highest popularity among the rabbit breeds (González-Redondo and Contreraz-Chacón, 2012; Šimek et al., 2017). The appropriate nutrition plays a key role in pet rabbit husbandry. Good health state and long livespan are preferred aspects of the pet rabbit rearing. The mollasses is used as traditional binder in pelleted rabbit diets where improves palatability of the diet (Proença and Mayer, 2014). The crude glycerol, a waste by-product of bio diesel production, has been recently used as the successful alternative binder in nutrition of some farmed animals whereas the dietary use of glycerol improved both the production performance of these animals and the technological and hygienic quality of the pellets (Schröder and Südekum, 1999; Donkin et al., 2009; Kroupa et al., 2011). Generally, in rabbit feeding, the use of the crude glycerol is rare so far. There is a lack of studies deals with the crude glycerol as alternative binder in rabbit diets. In meat-type rabbits, Iñigo et al. (2011) studied dietary effects of the crude glycerol on performance of meat-type rabbits. The use of the crude glycerol in the diets for the dwarf rabbits has not been studied yet.
The aim of the present study was to evaluate the dietary effect of the different ingredient composition on the live weight of young growing rabbits of the Dwarf Lop breed.

MATERIAL AND METHODS

Animals and housing. The study was conducted on a total of 58 rabbits. These rabbits belonged to the Dwarf Lop breed and came from the common hobby stock which realizes breeding and show activities in according to guidelines of the Czech Small Animal Breeders Association. The rabbits were housed in outside hutch sheltered againt unfavourable climatic condition. All of the raising young kits were housed and cared under the same husbandry and hygiene rules.
Experimental design and nutrition. A total of 4 types of the rabbit pelleted diets were used in the present study (Table 1).

Table 1. Ingredient composition (g/kg of the diet as fed) of the used pelleted diets for the dwarf rabbits.

Ingredient	Control diet	Pre-weaning experimental diet	Post-weaning experimental diets	
			M	G
Alfalfa meal	417.0	290.0	270.0	270.0
Barley	85.0	100.0	30.0	30.0
Wheat bran	226.0	100.0	175.0	175.0
Oat	0	100.0	140.0	140.0
Oat bran	60.0	0	100.0	100.0
WLS	0	250.0	200.0	200.0
Chicory root	0	48.0	0	0
Sugarbeet pulp	59.0	0	20.0	20.0
Malt sprouts	151.0	50.0	0	0
Melglyko	0	30.0	0	0
Mollasses	19.0	0	30.0	0
Glycerol	0	0	0	30.0
VMP	0	10.0	5.0	5.0
MCP	1.0	7.0	15.0	15.0
$CaCO_3$	8.5	10.0	10.0	10.0
NaCl	3.5	5.0	5.0	5.0

M, post-weaning experimental diet M (with mollasses); G, post-weaning experimental diet G (with crude glycerol); WLS, white lupin seeds var. Amiga; VMP, vitamin and mineral premix; MCP, monocalciumphosphate; $CaCO_3$, calcium carbonate; NaCl, sodium chloride.

The does with the young kits were divided into 2 groups (control group, experimental group). The rabbit kits in the control group (*n=20*) received a foreign complete commercial pelleted diet designed for the dwarf rabbits (Berkel-Futter Light 6008, Coesfeld, Germany) in course of the entire monitored period (up to the 15th week of the rabbit´s age). The experimental pre-weaning diet contained a noticeable share of the white lupin seeds var. Amiga (250 g/kg of the diet). The weaning in both control and experimental groups was performed at the age of 8 weeks. The young rabbits of the experimental group (*n=38*) were after weaning subsequently divided into 2 post-weaning experimental groups (group M and group G) while they received the specific diets up to the 15th week of age. In the experimental post-weaning groups M (*n=15*) and G (*n=22*), one-week gradual adaptation to the post-weaning diets

was performed according to the recommendation of Lowe (2010). The ingredient composition of the both of the post-weaning diets M and G was the same, except for the the binder type (diet M contained sugarbeet mollasses; diet G contained crude glycerol from rapeseed oil). Moreover, the both of diet M and G contained white lupin seeds at the same rate (200 g/kg of the diet). The rabbits in all the monitored dietary groups were fed once a day (30 g/kg of live weight the diet/day) and they were unlimited access to drinking water. The meadow hay was offered three-times a week. Live weights (LW) of the rabbit kits were recorded for the first time at the age of 3 weeks and subsequently in two-week period up to the 15th week of age. The individual LW of each rabbit was recorded.

Statistical analysis. Obtained results were statistically analyzed using software Statistica CZ, version 10 (StatSoft Inc., 2011). One-way Anova was used to deteminate differences among the evaluated LW of the groups. When Anova showed significant differences among the groups, HSD test was used. The differences were considered significant at $P < 0.05$ (*).

RESULTS AND DISCUSSION

The average values of the LW of the young Dwarf Lop kits from 21st to 49th day of age in the control and experimental groups are presented in Table 2.

Table 2. Live weight of Dwarf Lop rabbits at the age of 21- 49 days depending on dietary group.

Age	Units	Dietary group				*P*
		Control		Experimental		
		x	SEM	x	SEM	
21 days	g	192.75	12.90	225.32	6.15	*
35 days	g	342.00	13.04	381.05	11.01	ns
49 day	g	519.50	23.20	530.55	17.37	ns

*: $P < 0,05$; ns, not significant; x, Aritmetic Mean; SEM, Standard Error of the Mean.

On the 21st day of age, the kits of the pre-weaning exprerimental group showed significantly higher ($P < 0.05$) LW as compared to the control group (+32.57 g). Weight evaluation of the rabbit kits at the age of 21 days can be used as an indirect non-invasive method of milk production evaluation of the does. Furthemore, the lactation peak of the rabbit does

was found at the age of 21 days after the parturition (Zerrouki et al., 2005). A higher milk production of the does in the experimental group in the present study could be associated with higher ether extract content in diet due to inclusion of the white lupin seeds (Volek et al., 2014). The chemical analysis of the lupin seeds recently revealed their high content of the exter extract, while differences among the particular lupin varieties were found (Straková et al., 2006). Live weight of the Dwarf Lop kits at the age 35 and 49 days showed no significant differences ($P > 0.05$) between both dietary groups.
The average values of the LW of the young Dwarf Lop kits from 63rd to 105th day of age in the control and experimental groups are presented in Table 3.

Table 3. Live weight of the Dwarf Lop rabbits at the age of 63 - 105 days depending on dietary group.

Age	Units	Dietary group						*P*
		Control		M		G		
		x	SEM	x	SEM	x	SEM	
63 days	g	714.00	27.54	781.73	26.92	706.14	20.79	ns
77 days	g	857.94	39.91	957.00	41.56	902.14	21.65	ns
91 days	g	959.41	44.10	1075.00	43.39	1048.81	22.33	ns
105 days	g	1061.76^{a}	41.84	1207.14^{b}	52.37	1178.57^{a,b}	24.50	*

*,^{a,b}: Means within a row with different superscript letters differ ($P < 0.05$); ns, not significant; x, Aritmetic Mean; SEM, Standard Error of the Mean. M, post-weaning experimental diet M (with mollasses); G, post-weaning experimental diet G (with crude glycerol).

Within the entire post-weaning period, the rabbits of the experimental group M showed the highest LW among the evaluated groups. On the 105 days of age, young rabbits of group M showed significantly higher ($P < 0.05$) LW as compared to the rabbits of the control group (+145.38 g). No significant differences ($P > 0.05$) in LW were found between the experimental groups M and G during the entire monitored period in our experiment. Beside that, Iñigo et al. (2011) found no significance difference in LW of growing of the meat-type rabbit kits fed diets with added crude glycerol; the above mentioned authors recommend inclusion of the crude glycerol at rate 2.5 and also 5.0% per kg of the rabbit diets. Furthermore, Retore et al. (2012) evaluated different sources and dietary rates of the glycerol in the feed mixtures used for the meat-type rabbits. Based on results of the growth performance of

rabbits, they concluded that vegetable crude glycerol can be added in the diets for growing New Zealand White rabbits under condition of production system. In their study, the best LW values gained rabbits fed diet with inclusion of the vegetable crude glycerol at the level of 6%.

CONCLUSION

Results of the present study show that that different diet composition had the significant effect on the live weight of the growing Dwarf Lop kits. The 21-day-old young rabbits of the pre-weaning experimental group showed higher live weight as compared to the control group. Concerning the post-weaning period, no significant differences in live weight were found between the experimental groups M and G.
Based on our results, it can be concluded that the crude glycerol at the rate of 3% in kg the diet can be used as the altenative binder in the diets for the growing dwarf rabbits. Regarding effect of the dietary glycerol on the internal environment of dwarf rabbits, there is need to perform further studies to evaluate its specific health effects.

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THE RELATIONSHIP BETWEEN UREA CONCENTRATION IN MILK, PRODUCTION AND MILK COMPONENTS IN NUTRITIONAL AND SEASONAL DEPENDENCE OF DAIRY COWS

PETRA TIMKOVIČOVÁ LACKOVÁ, IVETA MASKAĽOVÁ, VLADIMÍR VAJDA

Department of Nutrition, Dietetics and Animal Breeding, University of Veterinary medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic

Corresponding email address: pl.lackova@gmail.com

ABSTRACT

The aim of this work was to evaluate the influence of nutrition on the level of production, components and metabolites in milk in seasonal dependence in dairy cows during the first lactation phase. In the field conditions, at the level of the production group, by evaluating of the nutrition according to analyzed nutrients content in the feed ration and the feed ration intake in relationship to the level of milk production and milk components at monthly intervals was confirmed the seasonal dependence variation of the urea in milk with increased values in the summer months ($P < 0.001$) compared to the autumn and winter months. In the total number of dairy cows (1972) in the first lactation phase evaluated at monthly intervals, of the yearly average 38 % of the animals showed an over-limit content of urea in milk. In seasonal dependence the highest level of urea in milk (31.1 ± 5.1 mg/100 ml) was confirmed in the summer months with increased values individually in 56 % of dairy cows.

The dynamics of urea content in milk was in indirect dependence ($P < 0.001$) with protein content and in direct dependence ($P < 0.001$) with the quantity of milk. Analysis of urea and protein content in milk in critical summer months through increased urea content in milk and decrease protein content was determined decreased transformation of nitrogen from feed to milk and increased nitrogen excretion to the environment, which is the ecological pollution of environment in compairing of cold months without the influence of heat stress.

Keywords: nutrients; milk components; urea in milk; milk protein;

INTRODUCTION

The content of lactose in milk and the quantity of milk are affected by the composition of the feed ration throught the starch content in total mix ration (TMR) and its rumen fermentation to produce propionic acid, which in gluconeogenesis in the liver affects blood glucose levels (Tripathi, 2014). Precursors for milk protein synthesis in the mammary gland are metabolizable amino acids that are transported from the small intestine into the mammary gland and amino acids mobilized from the body reserves (Mass et al., 1997). Synthesis of fat in the mammary gland is supported by two sources. The first source is the synthesis of short and medium chain fatty acids *de novo* in the mammary gland of acetic acid and beta hydroxybutyric acid after the microbial fermentation of carbohydrates in rumen. A second source are long chain fatty acids circulating in the blood (Bauman, 2003). In view of the relationship among the protein nutrition of dairy cows, ammonia content in the rumen and urea content in blood and milk, evaluating of urea in milk is an important tool for assessing the protein-energy ratio of nutrients in TMR. Analysis of urea in milk is a direct method of determining the extent of nitrogen transformation, evaluating of protein nutrition and estimating nitrogen excretion in urine (Hopkins et al., 2001).

MATERIAL AND METHODS

In feeding experiment on Holstein dairy cows in field conditions of farm the cows were fed by a TMR in the first lactation phase. The analysis of the effect of the feed ration on the level of milk production, milk components and metabolites was monitored in the group of dairy cows in first lactation phase in seasonal dependence. Samples of TMR were analyzed for dry mater (DM), crude protein (CP), fat, acid and neutral detergent fibre (ADF, NDF), starch, ash and minerals content according to conventional methods (Commission Regulation EC No. 152/2009 of 27.1.2009). Non-fibre carbohydrates (NFC) and net energy for lactation (NEL) were calculated by the equation based on the analyzed nutrient content in the feeds. The dairy cows were milked twice a day and the milk production, milk components and metabolites were recorded once per month by Plemenárska služba SR.

RESULTS AND DISCUSSION

The chemical composition of the diets, milk yield, components and metabolites in milk are showed in Table 1. The feed rations in dairy cows in the first lactation phase were formed on the based of Corn silage 20.0 – 34.0 kg, Alfalfa silage 6.0 – 16.0 kg, Grass silage 2.0 – 5.0 kg, Grass hay 0.8 – 1.0 kg, Alfalfa hay 1.0 kg, Wheat straw 0.5 – 0.8 kg, Gluten 4.0 kg, Cereals meal 1.0 – 4.5 kg, Rapeseed meal 2.0 – 4.0 kg, Rye 8.0 kg, Grain mixture 1.0 – 4.5 kg.

Table 1. Chemical composition of TMR, milk yield and milk components of dairy cows in first lactation phase

	SPRING March, April, May	**SUMMER** June, July, August	**AUTUMN** September, October, November	**WINTER** December, January, February	**x ± SD**
Number of cows	497	479	499	497	1972
Chemical composition of TMR					
DM g/kg	455.7±0.6	453.6±10.8	465.4±12.8	429.1±23.9	450.9±12.0
CP g/kg DM	156.0±1.7	148.3±11.1	153.7±2.3	153.4±8.0	152.9±5.8
Starch g/kg DM	257.3±0.6	245.1±29.5	255.4±20.0	270.9±23.5	257.2±18.4
Fat g/kg DM	43.0	44.3±5.5	44.0±1.7	43.2±1.3	43.6±2.3
NDF g/kg DM	349.0±3.5	356.3±17.2	338.1±16.1	349.2±4.9	348.2±10.4
ADF g/kg DM	215.0±1.7	225.1±24.3	206.2±14.9	217.3±4.2	215.9±7.8
NFC g/kg DM	379.7±2.3	385.7±6.7	391.0±1.2	387.0±1.1	385.9±2.8
NEL MJ/kg DM	65.5±0.8	66.0±0.1	66.9±0.4	66.4±0.9	66.2±0.6
Ash g/kg DM	72.7±0.6	67.5±1,8	73.7±2.3	68.4±7.9	70.6±3.2
Milk Yield and Milk Composition					
Milk kg/day	46.8±7.4	41.9±6.9	41.3±6.9	42.1±7.3	43.0±7.5
Urea mg/100 ml	30.7±7.5	31.1±5.1[a,e]	24.5±4.2[e]	26.3±7.3[a]	28.1±6.8
Urea 15-30 mg/100 ml	43.4 % 24.1±3.7	43.8 % 26.9±2.5	89.4 % 24.0±2.9	60.4 % 24.1±3.2	59.3 % 24.8±3.1
Urea> 30 mg/100 ml	55.0 % 35.1±3.6	56.2 % 34.4±3.8	8.8 % 32.1±1.9	33.0 % 33.2±2.4	38.3 % 33.7±2.9
Proteins %	3.0±0.3	2.9±0.3[b,f]	3.1±0.3[f]	3.2±0.3[b]	3.1±0.3
Proteins kg	1.4±0.1	1.2±0.1[d,g]	1.3±0.1[g]	1.3±0.1[d]	1.3±0.1
Fat %	3.3±0.7	3.2±0.7[c]	3.4±0.6	3.5±0.7[c]	3.3±0.7
Fat kg	1.6±0.2	1.3±0.3	1.4±0.2	1.5±0.3	1.5±0,1
Fat/Proteins	1.1±0.3	1.1±0.2	1.1±0.2	1.1±0.2	1.1±0.2
Lactose %	4.8±0.2	4.8±0,2	4.9±0.2	4.9±0.1	4.9±0.2
Lactose kg	2.2±0.1	2.0±0.1	2.0±0.1	2.1±0.04	2.1±0.2
SC 1000/1ml	462.4±1361.4	516.6±1277.9	521.9±1171.7	272.6±743.6	442.8±1165.7
Excretion N in urine g/day	243.4 41.8 %	246.7 43.3 %	194.4 32.7 %	209.6 37.8 %	223.1 38.9 %
Excretion N in milk g/day	220.0 37.8 %	190.5 33.3 %	200.7 33.8 %	211.2 38.1 %	205.6 35.8 %
Intake N in TMR	581.8	571.1	594.3	554.0	575.3

g/day					
ETN %	32.2	31.2	38.2	32.1	32.7

DM – dry matter; CP –crude protein; NDF – neutral detergent fiber; ADF – acid detergent fiber; NFC – non- fibre carbohydrates; NEL – net energy for lactation;SC – Somatic cells; TMR – total mix ration; N - nitrogen; ETN -– Efficiency transformation nitrogen in milk; a,b,c,e,f: P< 0,001; d,g : P< 0,05; Content of minerals in feed ration: Ca 7.2 – 10.2 g/kg DM; Mg 3.6 – 4.1 g/kg DM; Na 2.8 – 3.7 g/kg DM; K 11.2 - 12.0 g/kg DM; P 3.6 – 4.1 g/kg DM; Cu 27.0 – 61.8 mg/kg DM; Zn 64.0 – 96.4 mg/kg DM; Mn 61 - 176.5 mg/kg DM

Evaluating of milk production in dairy cows in the first lactation phase, at yearly average of daily milk yield was 43.0 ± 7.5 kg. The highest level of production in seasonal dependence was confirmed in the spring months with a daily milk yield of 46.8 ± 7.4 kg accompanied by a seasonally highest level of protein production of 1.4 ± 0.1 kg and fat of 1.6 ± 0.2 kg per day. The production parameters in the other evaluated periods were balanced on average at a level of 41.0 - 42.0 kg of daily milk yield in daily average protein production of 1.2 – 1.3 kg and fat of 1.3 – 1.5 kg per day.

The urea content in milk at yearly average was 28.1 ± 6.8 mg /100 ml and individual increased values above 30 mg/100 ml were confirmed in a quarter of the examined milk samples. Seasonally the highest values were confirmed in the spring and summer months an average 30.7 ± 7.5 or 31.1 ± 5.1 mg/100 ml. In this period individually at 55 % or 56 % of dairy cows were increased content of urea in milk with an average value of 35.1 ± 3.6 or 34.4 ± 3.8 mg/100 ml.

The content of milk components at yearly average shows protein values of 3.10 ± 0.3 % and fat of 3.3 ± 0.7 % with the lowest values in summer months.

Seasonally increased content of urea in milk during the summer months compared to autumn and winter months were significantly influenced ($P < 0.001$) together with a decreased of % protein in milk ($P < 0.001$), decreased protein production in milk ($P < 0.05$) with the highest amount of nitrogen excretion to the environment through urine and the lowest transformation of nitrogen in milk at reduced dry matter intake due to heat stress in dairy cows.

Heat stress with stressful effects in dairy cows is reflected by an increase in the temperature of the environment, when the animal uses more energy to regulate body temperature and release it from the body by skin and breathing. In heat stress as a defensive mechanism a reduced dry matter intake to prevent further production of heat followed by a reduced nutrients supply to the mammary gland for milk synthesis (West, 2003; Rhoads et al., 2009).

From the nutritional view in the summer period in TMR is the highest proportion of NDF and the lowest content of CP and starch, which at reduces dry matter intake due to heat stress metabolically stimulates lipomobilization and proteolysis in dairy cows.

Evaluation of the relationship between urea content in milk and % protein in milk was within regression dependencies. The regression relation between the urea content in milk and % protein in milk (Fig. 1) was confirmed at $P < 0.001$. Evaluating of the relationship between the urea content in milk and milk yield (Fig. 2) was confirmed the dependence at $P < 0.001$.

Figure 1. Dependence between urea in milk and % proteins in milk

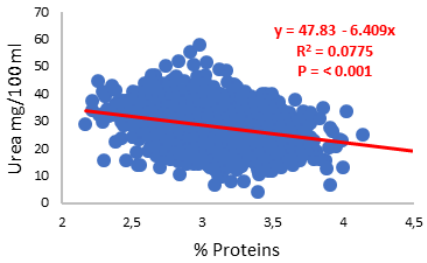
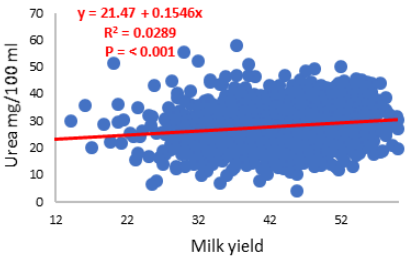


Figure 2. Dependence between urea in milk and milk yield



The evaluation of urea in milk in dairy cows is an important diagnostic tool for assessing the protein - energy ratio of nutrients in feed ration and is also an efficient marker for evaluating ammonia level in rumen (Hopkins et al., 2001). The optimal level of urea in milk is 15 - 30 mg/100 ml of milk (Říha et al., 2000). Increased urea content in milk occurs with unbalanced protein and energy intake in feed ration (Stallings et al., 2009). In the insuffficient of energy in a feed ration, i.e. relative excess of crude protein, when there is insufficient growth of rumen microflora and insufficient ammonia uptake in rumen or more often with absolute excess of crude protein and adequate energy intake. Low urea content in milk occurs in deficiency protein intake in a feed ration, where low ammonia production in the rumen for rumen microflora growth occurs with simultaneous excessive intake of energy in the feed ration (Hopkins et al., 2001).

The content of urea and protein in milk in the continuous monthly evaluation in the graphical representation (Fig. 3, Fig. 4) is used for evaluating the relationship between protein and energy intaken in the

feed ration. In the most critical summer months (Fig.3, Tab. 2) the feed ration was formed to production 40 liters of milk.

Figure 3. Relationship of protein and urea in milk in Summer

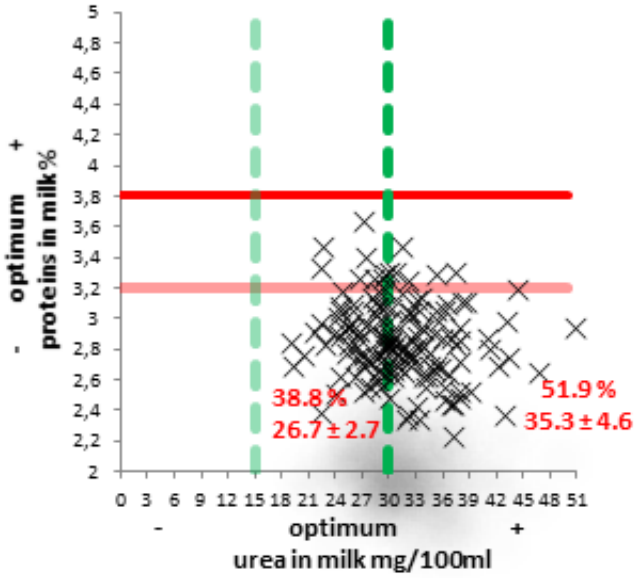


Table 2. Evaluation of milk in Summer

	1st group	**2nd group**
	Urea 15-30mg/100ml Proteins <3,2%	**Urea >30mg/100ml Proteins < 3,2 %**
Urea in milk mg/100 ml	38.8 % 26.7 ± 2.7	51.9 % 35.3 ± 4.6
Milk kg/day	40.3 ± 5.0	39.6±6.4
Proteins %	2.8 ± 0.2	2.8±0.2
Fat %	3.2 ± 0.5	3.4±0.7
SC 1000/1 ml	460.5 ± 1168.8	504.2 ± 1551.9
Fat/Proteins	1.1 ± 0.2	1.2 ± 0.3
Excretion N in urine g/day	211.3 35.8 %	280.5 47.5 %
Excretion N in milk g/day	176.9 30.0 %	173.8 29.4 %
Intake N in TMR g/day	590.4	590.4
ETN %	31.7	30.5

Due to the heat stress during this period was the reduced dry mater intake with increased portion of left overs on the level of 10 – 12 %. The production indicators showed that the average milk yield was maintained at an estimated level of 40 liters but a significant decrease

in milk components with protein content averaged 2.8 ± 0.2 % with an individual decrease of protein content in 90.7 % of dairy cows. At this level of production, an increased urea content in milk was confirmed at 35.1 ± 4.5 mg/100 ml, while in 51.9 % of dairy cows the milk urea level was 35.3 ± 4.6 mg/100 ml of milk. In 38.8 % of dairy cows the content of urea in milk fluctuated in the reference range at 26.7 ± 2.7 mg/100 ml of milk. In the evaluated sections the amount of excreted nitrogen in the urine was calculated by regression equations by Kauffman and St-Pierre (2001) and efficiency transformation N in to milk (ETN) by Huhtanen et al., (2015).

In the 1st group of animals with an optimal urea value in milk at an average level of 26.7 ± 2.7 mg/100 ml was the daily excretion of nitrogen in urine at the level of 211.3 g/day, what representing 35.8 % of the nitrogen intake in TMR. The nitrogen excreted in the milk was 176.9 g/day, what representing 30.0 % of the nitrogen intake in TMR.

In the 2nd group of animals with increased urea level in milk an average 35.3 ± 4.6 mg/100 ml and calculated daily excretion of nitrogen in urine was 280.5 g/day (47.5%) and the amount of nitrogen excreted in milk at 173.8 g/day (29.4%) for each dairy cow. The confirmed difference excretion nitrogen in urine within the comparison groups of 69.2 g/day represents the amount of CP adequate in 1 kg of soybean meal. The observed values show a expressive ecological pollution of nitrogen through the urine and its low transformation in to milk.

In the colder autumn months (Fig.4, Tab. 3) without a heat stress, the feed ration was formulated to production of 37 liters of milk.

Figure 4. Relationship of protein and urea in milk in Autumn

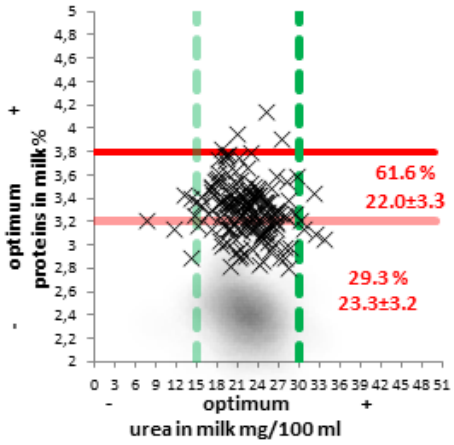


Table 3. Evaluation of milk in Autumn

	1st group	2nd group
	Urea 15-30mg/100 ml Proteins 3.2-3.8 %	**Urea15-30mg/100 ml Proteins< 3.2 %**
Urea in milk mg/100 ml	61.6 % 22.0 ± 3.3	29.3 % 23.3±3.2
Milk kg/day	39.3 ± 6.2	43.9±6.7
Proteins %	3.4±0.1	3.0±0.1
Fat %	3.6±0.6	3.1±0.6
SC 1000/1 ml	603.6±1299.8	309.1±730.6
Fat/Proteins	1.1±0.2	1.0±0.2
Excretion N in urine g/day	174.7 29.5 %	184.2 31.1 %
Excretion N in milk g/day	209.4 35.3 %	206.4 34.8 %
Intake N in TMR g/day	592.8	592.8
ETN %	32.1	32.8

At this level of production an optimal urea level in milk of 22.0 ± 3.3 mg/100 ml was confirmed in 93.3 % of dairy cows with optimal protein content in milk at 3.4 ± 0.1 % in 61.6 % of dairy cows. In the evaluated sections in the 1st group of animals with containing urea and protein in milk at the reference levels the nitrogen excreted in the urine was 174.7 g/day, i.e. 29.5 % of the intake N in TMR. The amount of nitrogen excreted in the milk was 209.4 g / day, i.e. 35.3 % of the intake N in TMR for each dairy cow.

In the 2nd group of animals with optimal urea level in milk but with a decrease in protein content in milk at 3.0 ± 0.1 % the calculated daily nitrogen excreted in urine was 184.2 g/day, i.e. 31.1 % of the intake N in TMR. The amount of nitrogen excreted in milk was 206.4 g/day, i.e. 34.8 % of the intake N in TMR for each dairy cow. Which is the higher value than calculated by ETN by Huhtanen et al., (2015). The observed values indicate lower nitrogen excretion in urine and its higher transformation in milk.

CONCLUSION

The evaluating of the level of nutrition, production, components and metabolites in milk in relationship to the analysis of the urea content in milk is efficient method for determining the extent and efficiency of nitrogen transformation and the evaluating of the protein nutrition of dairy cows in the productive phase as well as influence ecological

pollution of excreted nitrogen to the enviroment in the seasonal dependence.

ACKNOWLEDGEMENT

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VARIATION OF BIOCHEMICAL PARAMETERS OF ENERGY AND LIVER METABOLISM IN PERIPARTURIENT DAIRY COWS

BARBORA UMLÁŠKOVÁ[1], KATEŘINA MRVOVÁ[1], ONDŘEJ ŠŤASTNÍK[1], LEOŠ PAVLATA[1]

[1]Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: barbora.umlaskova@seznam.cz

ABSTRACT

The goal of the study was to monitor biochemical parameters characterizing disorder of energy metabolism in dairy cows. In consequence of that find out if there is any correlation between those and liver failure. The trial was conducted on 12 healthy dairy cows, pluriparous, no heifers. Blood samples were taken three times. The first one before parturition (14 – 30 days before partition), second one in early peripartal period (4^{th} to 10^{th} day after parturition) and the last one 2 months after parturition. In the blood samples selected biochemical parameters were analysed (triglycerides – TGs; cholesterol – CHOL; non-esterified fatty acids – NEFA; beta-hydroxybutyrate – BHB; total bilirubine – TBIL; aspartate aminotransferase – AST; gamma-glutamyl transferase – GGT; creatine kinase – CK; UREA; total protein – TP; albumin - ALB). Increased concentration of BHB (1.15 ± 0.39 mmol/l) showed development of negative energy balance after parturition. Moreover body mass loss was verified by raised CK in serum. The liver failure is confirmed by detection of increased liver enzymes (AST, GGT). The results of the correlation analysis showed a significant correlation between the development of ketosis (BHB), loss of weight (NEFA, CK, UREA) and liver stress in cow's blood plasma.

Keywords: ketosis; negative energy balance; body condition score; peripartum period; beta-hydroxybutyrate

INTRODUCTION

Ketosis is one of the most spread metabolic disease in dairy cows frequently observed during early lactation period. It can cause huge economic losses in milk production and occurrence of peripartal disease as displaced abomasa, metritis and retained placenta (Ospina 2010). The origin of appearance of ketosis includes biochemical and hormonal changes and the presence of predisposing factors (Zhang 2012). Typical signs of the disease are increased levels of ketone bodies in the blood, milk and urine (Tehrani-Sahrif et al. 2012). The transition period in cows takes from 3 weeks before to 3 weeks after parturition. This period is the most important period for observing possible incidence of subclinical or clinical ketosis (Drackley 1999). Ketone bodies comprise beta-hydroxybutyrate (BHB), acetoacetate and aceton. These are the products of masive lipolysis and following ketogenesis after calving and onset of lactation. Cows with higher body condition score are more predicted for this metabolic dissease. With deepening negative energy balance (NEB) liver stress increases. Some degree of NEB relating to upcoming energy demands and inadequated energy intake is identified by an increase in circulating concentrations of non esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) (Ospina 2010). Diagnosis of hepatopaties is based on blood biochemical examination (Pavlata 2014). Increased values of liver enzyme in blood (aspartate aminotransferase – AST, gama-glutamyl transferase – GGT) confirm liver damage. The aim of this study is to monitor the biochemical parameters of energy metabolism in dairy cows in periparturient period and evaluating their correlation with biochemical parameters of liver function.

MATERIAL AND METHODS

Animals

The study was conducted on private dairy farm located in South Moravia in Czech Republic. Experimental animals were breed of Holstein Friesians, only dairy cows. Heifers were expelled from the trial. The animals were kept on free stall housing with no bedding. All the animals were healthy with no signs of illness, fed with TMR (total mixture ration) according the reproductive period. Composition of TMR in different part of lactation period is detailed in table 1. During the trial, randomly selected dairy cows (pluriparous, n = 12) were observed and chosen for taking blood free times during tree months.

Table 1. Composition of TMR (Total mixed ration) in different part of lactation period (kg)

	dry period	calving	peak of lactation
Maize silage		19.00	29.50
Alfalfa silage	15.00		4.00
Barley			2.40
Sugar beet pulp	6.60	4.40	4.20
Straw	4.30	1.60	0.40
Amygold (Mayze malt)		1.20	3.20
Brewer´s malt			4.40
Coarse meal (mix)			4.40
Mineral supplement (DOVP VIII 15 s4)			4.40
Rape meal solvent extract	0.60	2.60	
Mollases			0.50
Mineral supplement (Premin Sucho)	0.30		
Mineral supplement (MP iont mínus)		0.5	
Urea			0.04
Lipids (C16)			0.16

Clinical biochemistry

Blood samples were taken from each of these animals (n = 12). The first taking of blood was done before parturition in all animals at the same time (14–30 days before parturition). The second one just in early peripartal period in interval from 2 to 10 days after parturition. And the last blood sample was taken at the end of the trial, that means 2 months after parturition. The blood sample was taken from *coccygeal vein* to non heparinized tube. Samples were centrifuged at 3000 revolutions per minute for 10 minutes following day. That is the reason why glycaemia was not observed. The biochemical parametres determining the energy and liver metabolism (triglycerides – TGs; cholesterol – CHOL; non-esterified fatty acids – NEFA; beta-hydroxybutyrate – BHB; total bilirubine – TBIL; aspartate aminotransferase – AST; gamma-glutamyl transferase – GGT; creatine kinase – CK; UREA; total protein – TP; albumin – ALB) were established in biochemical laboratory.

Statistical analysis

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). One-way analysis (ANOVA) was used for data evaluation. To ensure evidential differences Scheffe´s test was applied and $P < 0.05$ was regarded as statistically significant difference. The relationship of the set parameters was tested by correlation analysis. For the relationship of values, the correlation coefficient (r) was calculated.

RESULTS AND DISCUSSION

The results of biochemical parameters of cow´s plasma are presented in table 2, table 4 and figure 1. The relationship between individual biochemical values can be seen in table 3.

Table 2. Biochemical profile (mean ± standard deviation) in dairy cows (n = 12) during peripartum period - triglycerides (TGs), non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB)

Blood taking No.	BHB [mmol/l]	NEFA [mmol/l]	TAG [mmol/l]
I	0.58 ± 0.11 [a]	0.20 ± 0.05 [a]	0.31 ± 0.19 [a]
II	1.15 ± 0.39 [b]	0.65 ± 0.28 [b]	0.26 ± 0.22 [a]
III	0.77 ± 0.18 [a]	0.42 ± 0.09 [c]	0.20 ± 0.03 [a]

[a, b] - different letter in column means statistically significant difference $P \leq 0.05$
I – 14 – 30 days till the parturition
II – 4 – 10 days after parturition
III – 2 months after parturiton

According the results in table 2, the value of serum beta-hydroxy-butyrate (BHB), with critical thresholds 0.8 mmol/l, does not pass this reference in the average value in the first blood taking (before parturition). On the other hand, the second sampling that was done after calving (from 4th to 10th post parturition day) shows increased values (1.15 ± 0.39 mmol/l). This increase was established in almost all experimental animals (82 %). Increased BHB concentration in blood indicates stimulation of lipolysis by reason of NEB after calving and rising lactation (Zhang 2012). Cows with BHB concentration ≥ 1.2 mmol/l are considered to be in subclinical ketosis (Kaufman 2018). According the results from 2014, 89% cows fed with high-energy food suffered from subclinical ketosis (Schulz 2014). As can be seen in table 2, two months after parturition, ketone bodies, concretely BHB declined to the normal references.

Hand in hand with rising BHB, non-esterified fatty acids (NEFA) go. Dairy cows mobilise lipids that are necessary for energy in early lactation from body mass (Tamminga 1997). Lipids in triglyceride form are split into glycerol and free fatty acids in a process called lipolysis (Hofirek 2009). NEFA are products of lipid degradation. That explains their increase during early periparturition period, from 0.20 ± 0.05 mmol/l to 0.65 ± 0.28 mmo/l, with gradual decrease in the next 2 months. It had been proved that NEFA value over 0.7 mmol/l for longer period than one week after calving indicate several negative energy balance or serious health problems (Krempaský 2014). The correlation between biochemical parameters is recorded in table 3. As can be seen there is NEFA and BHB significant correlation. With increased values of NEFA, amount of ketones rises too.

Table 3. Correlation (r) in all blood samples

	AST	GGT	CK	BHB	NEFA	UREA	TP	ALB	TBIL	CHOL	TGs
AST		0.98**	0.61**	0.14	0.41*	-0.05	0.26	0.04	0.07	0.47**	-0.08
GGT			-0.11	0.00	0.23	0.04	0.19	0.19	0.18	0.31	-0.02
CK				-0.25	-0.25	-0.07	0.24	-0.18	-0.08	0.16	-0.13
BHB					0.76**	-0.40*	-0.25	-0.13	0.59**	-0.17	0.09
NEFA						-0.49**	-0.11	-0.21	0.74**	-0.11	-0.09
UREA							0.18	0.45*	-0.37*	0.39*	-0.07
TP								0.50**	-0.34	0.61**	-0.16
ALB									-0.41*	0.65**	-0.13
TBIL										-0.49**	0.07
CHOL											-0.24
TGs											

*P < 0.05; **P < 0.01

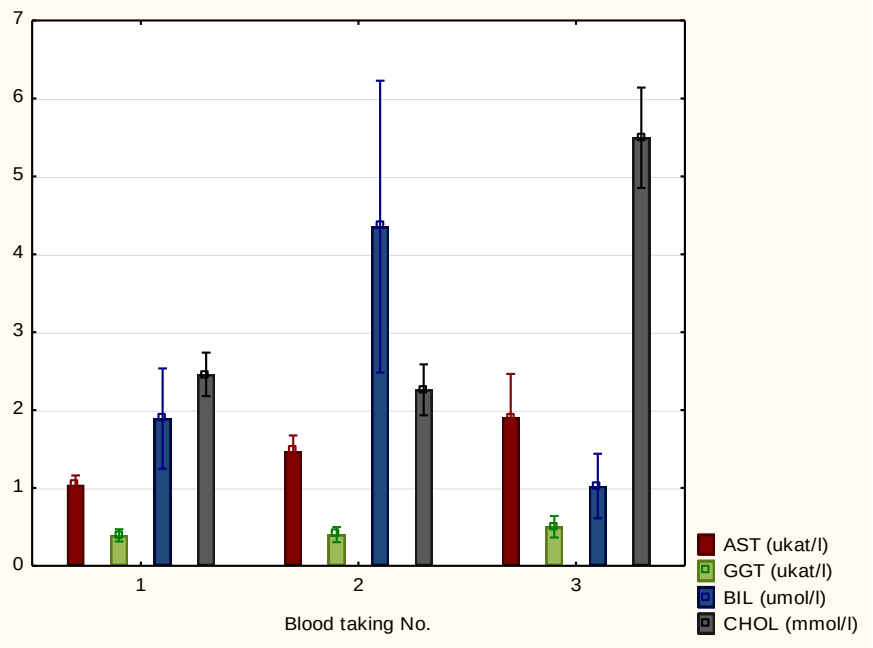
During the trial statistically significant difference was not proved in blood triacylglycerol level (0.31 ± 0.19 mmol/l, 0.26 ± 0.22 mmol/l and 0.20 ± 0.03 mmol/l).

The level of cholesterol was in the reference range in the first taking of samples. There was observed mild decrease in some indiduals in the first week after calving, but not in average value. The significant difference was proved in the last blood taking. There are noticed increased values of cholesterol that is probably caused by increased intake of energy in TMR (total mixture ration) after parturition.

Aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) are the enzymes, while are raised, predicate liver failure. The critical thresholds for these are 1.5 μkat/l and 0.5 μkat/l,

respectively (Hofírek 2009). In the figure 1, the values of AST and GGT are represented. According the reference range above-mentioned only 3 samples in sampling No. 2. were increased. However almost all of the values of AST were observed increasing in sampling taken 2 months after parturition. But in confrontation with the other sources, the values over 1 µkat/l are pathological already (Doubek 2007, Laboklin 2014). In consideration of that all of the average values of AST (1.04 ± 0.19 µkat/l, 1.47 ± 0.30 µkat/l, 1.91 ± 0.67 µkat/l) were over 1 µkat/l, there can be supposed some level of liver stress.

Figure 1. Biochemical profile (mean ± standard deviation) in dairy cows (n = 12) during peripartum period – AST, GGT, total bilirubine (TBIL), cholesterol (CHOL)



Bilirubin belongs to another biochemical parameters of the liver damage. Hyperbilirubinemia (excess bilirubin in the blood) is noticed during starving, ketosis, liver insufficiency etc (Doubek 2007). No statistical differences were observed in the bilirubin levels in serum in the period before parturition and in the early peripartum period (1.89 ± 1.01 µmol/l and 4.35 ± 2.79 µmol/l). Whereas the statistical difference was detected between those samples and the last one that was taken two months after calving (1.03 ± 0.49 µmol/l) but still in

reference range (0.17 – 8.5 µmol/l). The bilirubin decline at the end of the experimental is illustrated in figure 1.
High creatin kinase (CK) in blood is an indicator for destruction of skeletal muscle. In cows it can be caused, among other things, as a consequence of syndrom recumbency in cows in peripartal period. The reference values for CK are not supposed to get over 6 µkat/l. Increased level in early peripartal period can be explained by parturition itself. The average value in sampling No. 3 is caused by the two individual values, that are 3 to 6 times increased than references. In table of correlation (table 3) can be seen some affinity between liver damage (AST) and rhabdomyolysis. That is predictable effect of calving and subsequent starving, when goes to body mass loss and hepatic burden. Urea is the final product of protein metabolism in mammals that is produsedin liver and excreated by kidney. The increased concentration of urea is observed in blood just in course of liver failure or redundancy of protein from muscle breakdown. High urea concentration after calving is caused by body mass lost and proteolysis. The high intake of protein diet can be possible.

Table 4. Biochemical profile (mean ± standard deviation) in dairy cows (n = 12) during peripartum period –creatine kinase (CK), UREA, total protein (TP), albumin (ALB)

Blood taking No.	CK [µkat/l]	UREA [mmol/l]	TP [g/l]	ALB [g/l]
I	4.01 ± 5.24 [a]	6.34 ± 1.55 [a]	70.17 ± 3.72 [a]	32.77 ± 1.81 [a]
II	5.45 ± 5.98 [a]	4.50 ± 0.71 [b]	66.55 ± 5.67 [a]	31.36 ± 2.06 [a]
III	8.81 ± 12.18 [a]	6.40 ± 0.81 [b]	78.11 ± 4.56 [b]	35.16 ± 1.92 [b]

[a, b] - different letter in column means statistically significant difference $P \leq 0.05$
I – 14 – 30 days till the parturition
II – 4 – 10 days after parturition
III – 2 month after parturiton

The values of total protein (TP) and albumin (ALB) are closely related. Hypoalbuminemia occurs when liver insufficiency happens. Concentrations were not alterated in average values (TP [g/l] – 70.17 ± 3.72 g/l , 66.55 ± 5.67, 78.11 ± 4.56; ALB [g/l] – 32.77 ± 1.81, 31.36 ± 2.06, 35.16 ± 1.92). Only some individuals were lower than refference values (TP: 60 – 85 g/l, ALB: 30 – 42g/l).

CONCLUSION

The results of the experiment showed and confirmed changes induced by negative energy balance. During the trial body mass loss was observed in all experimental animals. As a consequence of that

alteration of ketone bodies such as beta-hydroxybutyrate was determined. Moreover, ketosis is accompanied by liver damage characterized by increased values of liver enzymes and total bilirubin. However, levels of bilirubine were not outside the reference interval. It was proved correlation between biochemical parameters of energy metabolism and parameters characterising liver function.

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THE INFLUENCE OF SELENIUM NANOPARTICLES ON GLUTATHIONE CONCENTRATION IN ANIMAL ORGANISM

LENKA URBÁNKOVÁ, MAGDALENA PŘIBILOVÁ, PAVEL HORKÝ, JIŘÍ SKLÁDANKA

Department of Animal Nutrition and Forage Production, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Corresponding email address: lenka.urbankova@mendelu.cz

ABSTRACT

The aim of the experiment was to determine the influence of different forms of selenium (sodium selenite, selenium nanoparticles) on the antioxidant status of laboratory rats. The male of Wistar albino rats strain were sorted into 4 groups. The first group served as control with no selenium (Se) administration. The second group was fed with mixture containing sodium selenite (Na_2SeO_3). The third and the fourth group were fed with different forms of selenium nanoparticles. Selenium nanoparticles were modified by polyvinylalcohol (PVA 49 kDa, PVA 100 kDa). After 30 days of experiment, the rats were slaughtered and the total content of selenium in liver and blood tissue and also changes in concentration of reduced (GSH) and oxidized (GSSG) form of glutathione were measured. The results showed that selenium addition to the feed dose would increase the total amount of selenium in the liver and blood. Concentration of oxidized and reduced glutathione in the organism has also been affected.

Keywords: rats, nanoparticles, liver, blood, antioxidant status

INTRODUCTION

The antioxidant status of animals can be positively affected by addition of antioxidants, including vitamin E and antioxidant enzyme cofactors, such as selenium, which is an important element in selenoproteins, of which at least 16 have an antioxidant role. Interaction between Se and Vitamin E may increase the production of glutathione peroxidase, which is an important part of the antioxidant system (Arruda et al. 2015; Horky et al. 2016b; Chen et al. 2016a; Skalickova et al. 2017;

Tran & Webster 2011; Wang et al. 2007; Zhang et al. 2001). The selenium content in soils in Europe is generally low therefore, it should be added to livestock feed (Horky et al. 2012; Kursa et al. 2010). The two most widely used inorganic selenium forms are selenate and selenite. Both can be converted into less toxic insoluble elemental selenium forms. Howeover, the biological nature of this reaction is not known yet (Chen et al. 2016b). At higher doses, selenium may be toxic (FernandezLlamosas et al. 2016; Horky 2014). Thus, alternative nanotechnological solutions are searched instead of conventional alternatives as nanoparticles show new promising properties, which could supress toxicity with maintaining the positive effects of selenium on an organism (Arruda et al. 2015; Fernandez-Llamosas et al. 2016; Mohapatra et al. 2014; Skalickova et al. 2017). The synthesis and application of selenium nanoparticles (SeNPs) attracted increased attention due to several benefits, such as low toxicity, biocompatibility and chemical stability (Zhang et al. 2001). The aim of our study was to compare two different forms of dietary nanoselenium with sodium selenite to show whether selenium nanoparticles can increase the antioxidant status of rat metabolism and serve as an alternative source of nutrition supplements for an animal organism.

MATERIAL AND METHODS

Animals

The experiment was carried out in the experimental facility of the Department of Animal Nutrition and Forage Production of Mendel University in Brno, in accordance with the act on the protection of animals against cruelty No. 246/1992 Coll. Throughout the whole experiment, microclimatic conditions were measured and controlled at 23 ± 1 °C at constant humidity of 60 %. The light regime was maintained at 12 h of light and 12 h of dark with a maximum illumination of 200 lx.

Laboratory rats of the outbreed strain Wistar albino were selected as model animals in number of 32 pieces with an average initial weight of 150 ± 5 g. The rats were divided into 4 groups of 8 pieces. The first group was a control with no addition of selenium in their feed. The second group was supplemented with selenium in the form of Na_2SeO_3 at a dose of 1.2 mg/kg/diet. The third and fourth group were fed with selenium in form of Se-49 and Se-100 nanoparticles at a dose of 1.2 mg Se/kg/diet, respectively. The groups 2, 3 and 4 were fed with monodietus containing 0.03 mg Se/kg/diet. The experiment duration was 30 days. The animals had an access to feed and drinking water ad

libitum. At the end of the experiment, the animals were sacrificed and samples of blood and liver were collected and subjected to chemical analyses.

Preparation of selenium nanoparticles

The average particle size distribution was determined by quasi-elastic laser light scattering with a Malvern Zetasizer (NANO-ZS, Malvern Instruments Ltd., Worcestershire, United Kingdom). Solutions of nanoparticles were measured according to experimental conditions stated in (Dostalova et al. 2016). The structures of nanoparticles were observed using scanning electron microscopy (FE Tescan Mira II LMU, Brno, Czech Republic) under the conditions showed in (Dostalova et al. 2016; Chudobova et al. 2014).

Se-49: PVA 49 kDa (0.19 g) was added to a solution of 1.88 mL $Na_2SeO_3 \cdot 5H2O$ (2.63 g/50 mL) in water (80 mL). Cysteine (9 mg/mL) was added with mixing and left for 2 h. Then, the colour turned to light orange and water was added to final 100 mL volume.

Se-100: The preparation was the same as in previous case with one exception of using PVA 100 kDa instead of PVA 49 kDa. Undissolved PVA was filtered off. After addition of cysteine, the colour turned to orange and water was added to final 100 mL volume.

Preparation of samples for GSH and GSSG detection

Liver: Two grams of samples from each variant were homogenized in a fritted bowl with the addition of liquid nitrogen and 1.5 mL of water. After homogenization, each sample was sonicated using an ultrasound needle for 2 min, shaken for 10 min, and centrifuged for 20 min at 25,000 g and at 4 °C. 100 µL of supernatant was taken from each sample and mixed with 100 µL of 10% TFA and centrifuged again for 20 min at 25,000 g and 4 °C. After the centrifugation, the supernatant was taken and analysed by HPLC-ED.

Blood: Sample processing was performed by pipetting 200 µL of sample from each variant, placing it into liquid nitrogen for 2 min and adding 500 µL of water. Each sample was sonicated with an ultrasound needle for 2 min, shaken for 1 min, and centrifuged for 20 min at 25,000 g and at 4 °C. 200 µL of supernatant was taken from each sample and mixed with 200 µL of 10% TFA. The samples were again centrifuged for 20 min at 25,000 g and 4 °C. After centrifugation, the supernatant was analysed by HPLC-ED.

Preparation of samples for selenium detection
Samples of liver weighting 0.3 g and samples of blood weighting 0.5 g were disintegrated by dry method in a muffle furnace (LAC, Czech Republic) and mineralized in 2.5 mL concentrated nitric acid (Horky et al. 2016a).

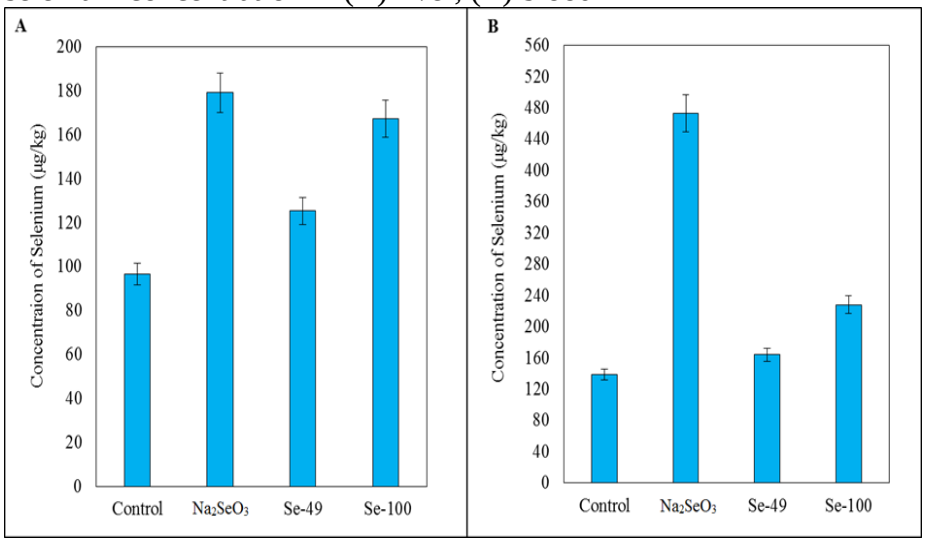
Determination of reduced and oxidized glutathione, and selenium
Reduced and oxidized glutathiones were determined using high performance liquid chromatography with electrochemical detection (HPLC-ED). Experimental conditions were adopted from (Zitka et al. 2012). Selenium was determined on 280Z Agilent Technologies atomic absorption spectrometer (Agilent, USA) with electrothermal atomization under the conditions stated in (Horky et al. 2016a).

Statistics
The data were processed statistically using STATISTICA.CZ, version 10.0 (Czech Republic), number of measurements were 3, $P < 0.05$ were considered significant using ANOVA and Scheffe's test for the parameters GSH; GSSG; Se.

RESULTS AND DISCUSSION

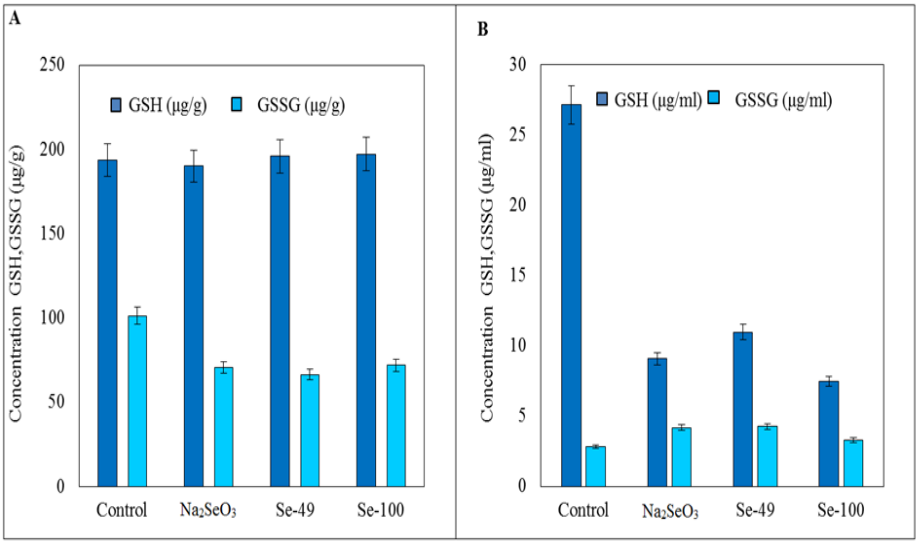
In the experiment the effect of two forms of selenium (Na_2SeO_3, Se NPs), included in the feed ration for rats on the antioxidant status of organism was monitoring. In blood and liver samples the total selenium level was determined. Another measured value was concentration of glutathione as one of the markers of oxidative stress in the body.
The level of selenium in liver was statistically significant increased in all experimental groups compared with control (Na_2SeO_3 by 85%, Se-49 by 30% and Se-100 by 73%) (Figure 1A). In the blood samples increasing amount of selenium was measured, especially in the Na_2SeO_3 group, where the level was nearly twice as high, and in the Se-100 group, which increased by 64% ($P < 0.05$) (Figure 1B). These results are in agreement with results of Horky et al. (2016) and show that the addition of selenium to the feed dose has an effect on increasing the amount of selenium in the liver and blood tissues.

Figure 1. Influence of sodium selenite and selenium nanoparticles on selenium concentration in (A) liver, (B) blood



The level of GSH and GSSG is a parameter which has a direct correlation with selenium and indicates antioxidant potential of organism. In the Figure 2A are shown a concentrations of GSH and GSSG in liver. The GSH level was almost equal for all groups and the GSSG showed a statistically significant decrease in groups Na_2SeO_3 by 30%, Se-49 by 39% and Se-100 by 29%. The concentration of GSH and GSSG in blood is shown in the Figure 2B. The significant decrease of GSH was observed in all experimental groups (Na_2SeO_3 by 72%, Se-49 by 59%, Se-100 by 67%, $P < 0.05$). On the other hand the increase of GSSG in all experimental groups was measured. Kominkova et al. (2015) states that the optimum ratio of GSH and GSSG is 90 : 10. The results of our experiment were in contradiction with the results of Horky et al. (2016), which was probably caused due to the intentional use of higher selenium doses and stress.

Figure 2. Influence of sodium selenite and selenium nanoparticles on GSH and GSSG concentration in (A) liver, (B) blood



CONCLUSION

The results are consistent with the assumed hypothesis, it has been found that the addition of selenium nanoparticles increases the amount of this element in both the liver and blood. However, sodium selenite was more effective compared to selenium nanoparticles (especially in blood). The results can serve as a basis for further research. Alterations in reduced and oxidized glutathiones revealed marked changes in the antioxidant status based selenium treatment, however, we confirmed that nano-form of selenium has less negative effects than standard one. This leads us to support an idea to use nanoSe as an alterative source of selenium. It would be appropriate to test these selenium sources even at lower concentrations in order to avoid potential toxicity.

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MIDDLE, LONG AND VERY LONG CHAIN FATTY ACID COMPOSITION OF MAIZE SILAGES FROM STAY GREEN AND GRAIN HYBRID

PATRÍCIA VAŠEKOVÁ[1], MIROSLAV JURÁČEK[1], DANIEL BÍRO[1], MILAN ŠIMKO[1], BRANISLAV GÁLIK[1], MICHAL ROLINEC[1], ONDREJ HANUŠOVSKÝ[1], ĽUBA BALUŠÍKOVÁ[1], RENATA KOLLÁTHOVÁ[1], ONDREJ PASTIERIK[2]

[1]Slovak University of Agriculture in Nitra, Nitra, Slovak Republic
[2]AGRORAMA Šaľa, Slovak Republic

Corresponding email address: xvasekova@is.uniag.sk

ABSTRACT

The aim of this research was to determine content of selected the fatty acid (with middle, long and very long chain) in maize silages of different hybrids with FAO 340 (stay green hybrid) and FAO 420 (grain hybrid). Tested hybrids were grown under identical agro climatic conditions. Maize hybrids for silage were harvested on 1171 growing degree days (Hybrid A) and on 1277 (Hybrid B), with dry matter content 33% (Hybrid A) and 32 % (Hybrid B). Whole plants were cut to average 10 mm length of cut and ensiled in plastic containers with volume 50 dm^3. After 2 months samples were taken for determination of fatty acid content by gas chromatography. The results confirmed differences in fatty acid composition of maize silages made from stay green and grain hybrid. In silages of stay green hybrid there was detected significantly ($P < 0.05$) higher content of α-linoleic acid, behenic acid and cis-11-eicosenoic acid and significantly lower content of linoleic acid. Statistically significant ($P < 0.05$) higher polyunsaturated fatty acids content was found in silage from stay green hybrid in comparison to silage from grain hybrid.

Keywords: fatty acid content; maize; stay green hybrid; grain hybrid; silage

INTRODUCTION

Maize (*Zea mays L.*) is the crop with highest production at world level and it occupies the third place (after wheat and rice) in the top of cultivated surfaces. The surface cultivated with maize is increasing

yearly due to multiple uses of this crop: as food for humans and animals, pharmaceutical industry, composite materials but especially due to increasing demand as bio-fuel (Iordan et al., 2015). Every year around the world, hundreds of new hybrid combinations are being developed to better adapt to the conditions in individual agricultural areas of the world. Maize silage is made out of whole ensiled maize plants. It is one of the most valuable forages for ruminant livestock and it is used wherever maize can grow, from temperate regions to the tropics. The popularity of maize silage is due to several factors. It is a consistent source of palatable and high-energy forage for all classes of ruminants, including dairy cattle, beef cattle, sheep and goats (Roth et al., 2001). It is one of the most high-yielding forage crops, requires less labour (since it is harvested in a single operation) and is generally less costly (per t DM) to produce than other forage crops (NASS, 2015; Arvalis, 2011; Roth et al., 2001). Though relatively easy to produce, maize silage requires good crop and harvest management as well as careful ensiling practices (Arvalis, 2011). Forages contain relatively low amounts of lipids, but they can often be the major source of fatty acids in the diet (Vanhatalo et al., 2007). The concentration of lipids in forages varies cosiderably, depending on species and hybrid and in response to environmental factors, N fertilizer application rates, stage of maturity and conservation method (Boufaïed, 2003; Vanhatalo et al., 2007; Juráček et al., 2012; Lehel et al., 2013). Maize silage is a carbohydrate feed, is a major energy component in feed rates (Doležal et al. 2012; Bíro et al. 2014). Maize represents the main feed in rations for ruminants due to the high yield potential and excellent feeding value (Loučka et al., 2013). Maize silage is a suitable component of feed rates not only for ruminants, but also for non-ruminants (Gálik et al., 2013; Kubelková et al., 2013).

MATERIAL AND METHODS

Maize matter of different hybrids without additive was ensilaged. Hybrid A (FAO 340) was stay-green hybrid and Hybrid B (FAO 420) was grain hybrid with dent type of grain. Hybrids were planted in Mojmírovce (+48° 10' 38.6394" +18° 4' 18.4794" west part of Slovakia), on a soil Anthrosolic Chernozems, with population density 78, 000. The row spacing was 76 cm. Soil has been standard pre-treated, fertilized by 160 kg N, 23 kg P and 23 kg K per hectare. Whole plants of maize were harvested on 1171 growing degree days (GDD, hybrid A) and on 1277 GDD (hybrid B). All plants were cut above the first internode. Hybrid A contained 33% of dry matter and hybrid B

32% of dry matter. Chop length 10 mm of whole plant maize was acquired by harvester with kernel processor (CLAAS ltd. USA). Plastic containers (50 dm^3) were filled by chopped matter of each hybrid separately, sealed and stored for 2 months. Three average samples (n=3) of silage from each hybrid (3 replicates) were taken for further analysis. For fat and fatty acid content in maize silage standard laboratory methods and procedures were used. Crude fat was determined with petroleum ether (Soxhlet extraction). After fat extraction, lipid fraction was hydrolysed on glycerol and free fatty acids. Subsequently fatty acids were esterified on fatty acid methyl esters. The fatty acid methyl esters were quantified by gas chromatography (Agilent 6890A GC, Agilent Technologies, USA) equipped with a flame-ionization detector (FID). Quantified fatty acids are expressed in a gram from the 100 grams of the total fatty acids content present in the sample.
The results were statistically analysed by a one-way ANOVA, the differences in average means of fatty acids between different maize silages were tested with T-test (SAS system 9.2, SAS Institute Inc. USA).

RESULTS AND DISCUSSION

The highest content from all fatty acids was determined in linoleic acid, the second highest content was in oleic acid and the third highest content was in palmitic acid in maize silage of both hybrids. In silage of Hybrid B was statistically significant ($P < 0.05$) higher content of linoleic acid. Differences in oleic acid, palmitic acid content were not statistically significant. In agreement with our results Balušíková et al. (2017) found the highest content in linoleic acid followed oleic and palmitic acid in maize silage. Silage of Hybrid A had content of linoleic acid 46.26 g, silage of Hybrid B 49.06 $g.100g^{-1}$, while Ferlay et al. (2006) determined content of its acid in maize silage 48.6 g and AbuGhazaleh et al. (2007) 48.5 $g.100g^{-1}$. In silage of Hybrid A was identified composition of α-linoleic acid 10.18 $g.100g^{-1}$ and in silage of Hybrid B 8.30 $g.100\ g^{-1}$, while the differences were statistically significant ($P < 0.05$). Alves et al. (2011) found similar content α-linoleic acid in maize silage (10.64 g and 10.5 $g.100g^{-1}$).
Dierking et al. (2010) reported a higher value of α-linoleic acid in alfalfa (24.79 $g.100g^{-1}$), in *Dactylis glomerata* (26.70 $g.100g^{-1}$). Statistically significant ($P < 0.05$) higher content of behenic acid was in silage of Hybrid A. Lower content of cis-11-eicosenoic acid was found in silage of Hybrid B in comparison to silage of hybrid A. Differences

in cis-11-eicosenoic acid content were statistically significant (P<0.05). In maize silage was found content of polyunsaturated fatty acids 56.81 g (Hybrid A) 54.49 g (Hybrid B), monounsaturated fatty acids 21.68 g (Hybrid A), 21.85 g (Hybrid B) and saturated fatty acids 17.81 g (Hybrid A) and 18.29 g.100g^{-1} (Hybrid B). The differences were statistically significant (P<0.05) only in polyunsaturated fatty acids content. Alezones et al. (2010) detected lower portion of polyunsaturated fatty acids (46.1-51.2 g.100g^{-1}) in 12 different maize hybrids. The ratio n-6/n-3 of fatty acids was in silage of Hybrid A 4.4:1 and in silage of Hybrid B 5.36:1.

Table 1. Fatty acid and crude fat content in maize silages of different hybrids

Parameter		Hybrid A		Hybrid B	
		Mean	S.D.	Mean	S.D.
Crude fat g/kg dry matter		26.93	1.290	31.33	2.150
Fatty acid (g.100 g^{-1} total fatty acids)					
C12:0	lauric acid	0.24	0.020	0.20	0.049
C14:0	myristic acid	0.26	0.026	0.24	0.053
C16:0	palmitic acid	13.45	0.263	14.24	0.835
C16:1	palmitoleic acid	0.35	0.036	0.26	0.041
C18:0	stearic acid	2.14	0.042	2.21	0.099
C18:1	oleic acid	21.05	0.281	21.30	0.430
C18:2	linoleic acid	46.26[a]	1.239	49.06[a]	0.456
C18:3	α-linolenic acid	10.18[a]	0.630	8.30[a]	0.009
C20:0	arachidic acid	0.68	0.010	0.60	0.051
C20:1	cis-11-eicosenoic acid	0.28[a]	0.010	0.24[a]	0.006
C22:0	behenic acid	0.47[a]	0.010	0.34[a]	0.029
C24:0	lignoceric acid	0.54	0.008	0.50	0.031
PUFA		56.81[a]	0.615	54.49[a]	0.265
MUFA		21.68	0.255	21.85	0.391
SFA		17.81	0.352	18.29	0.895
n6:n3		4.4:1	/	5.36:1	/

PUFA: polyunsaturated fatty acids, MUFA: monounsaturated fatty acids; SFA: saturated fatty acids; *values with the same index in row are significant at P < 0.05

CONCLUSIONS

The aim of this research was to determine selected the fatty acid content (with middle, long and very long chain) in maize silages of

different hybrids with FAO 340 (stay green hybrid) and FAO 420 (grain hybrid). Both maize silage of two hybrids had the highest linoleic acid, followed by oleic acid and third highest content of palmitic acid. The results confirmed differences in fatty acid content in maize silages made from stay green and grain hybrid. In silages of stay green hybrid was detected significantly higher content of α-linoleic acid, behenic acid and cis-11-eicosenoic acid and significantly lower content of linoleic acid. Statistically significant higher polyunsaturated fatty acids content was found in silage from stay green hybrid in comparison to silage from grain hybrid.

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PREDICTION OF NDF DIGESTIBILITY USING THREE METHODS

BARBORA ZNOJ NOVOTNÁ[1], FILIP JANČÍK[2], FRANTIŠEK LÁD[1], PETRA KUBELKOVÁ[2], LENKA HANUSOVÁ[1], LUBOŠ ZÁBRANSKÝ[1], PAVLÍNA VAZDOVÁ[1], ALENA KYRYANOVÁ[1]

[1]Department of Animal Husbandry Sciences, Faculty of Agriculture, University of South Bohemia in České Budějovice, Studentská 1668, 370 05 České Budějovice, Czech Republic

[2]Institute of Animal Science, Přátelství 815, 104 00 Praha Uhříněves, Czech Republic

Corresponding email address: novotb00@zf.jcu.cz

ABSTRACT

This experiment was aimed at the prediction of neutral detergent fiber digestibility (NDFD) by various methods. Thirty two samples of ryegrass were analysed for the content of ash, ether extract, crude protein, neutral detergent fiber, acid detergent fiber and non-structural carbohydrates. Prediction of NDFD was executed by three different methods: *in sacco* incubation in rumen (IS), *in vitro* incubation in rumen fluid (IVR) and *in vitro* incubation in pepsin and cellulase solutions (IVE). Samples showed various nutrients and digestibility values because of various growing season. IVE method proved lower values than other methods, the mean of NDFD by IVE was 63.5 %. The mean of NDFD by IVR was 81.1% and by IS was 81.3%. Statistically high significant correlations ($p < 0.001$) were found among all methods. IVR = 9.3115 + 0.88341 × IS ($r = 0.915$), IVE = - 42.69 + 1.3064 × IS ($r = 0.894$), IVE = - 51.14 + 1.4133 × IVR ($r = 0.934$). Although IVE method is not usually used for NDFD determination, based on these preliminary results it seems to be suitable method.

Keywords: digestibility; neutral detergent fiber; in vitro; in sacco

INTRODUCTION

Carbohydrates are the main energy source for ruminant. They usually constitute 60-70% of dairy cows total diet. Carbohydrates provide energy for rumen microbes and ensure correct function of digestive tract. They are divided into two groups. The first group is non-structural carbohydrates (NSC). For this group, very high digestibility is typical. The second group consists of structural carbohydrates, i.e. fiber. Neutral detergent fiber (NDF) is the best expression of fiber currently available, but recommendations are also given for acid detergent fiber (ADF) because of its widespread use (National Research Council, 2001). Adequate fiber content in total diet is necessary for digestive tract function and it is able to influence milk fat percentage in the positive way. Not only fiber content, but also particle size is important (Mertens, 1995). However, NDF is in the negative correlation with dry matter intake and digestibility (Albrecht and Broderick, 1990; Jung and Allen, 1995). The significant characteristic of fiber is rumen degradibility and digestibility. Oba and Allen (1999) stated that 1% increase of NDF degradability give rise to increase of dry matter intake by 0.17 kg and daily milk production by 0.25 kg.

Nutrients and digestibility determination are essential for correct composition of diet (Beever and Mould, 2000). A lot of ways of digestibility determination is known: *in vivo* and *in vitro* methods or calculation using prediction equations (Horrock and Vallentine, 1999; Undersander and Moore, 2002) but these estimations are not exact enough. *In vivo* methods are generally considered as the most accurate, but have many disadvantages like high costs, laboriousness and necessity of live animals (Huhtanen et al., 2006). Robinson et al. (2004) casted doubt on using *in vivo* methods as reference methods. Very often used *in vitro* method was formed by Tilley and Terry (1963). The principle of this method is the incubation of samples in rumen fluid and in pepsin - hydrochloric acid solution. Another method using rumen fluid was presented by Ankom Technology Corp. (Macedor, NY). Popular method without the necessity of using live animals is pepsin-cellulase method. While older sources did not recommend this method for the determination of organic matter digestibility of individual crops, but rather for the comparison among the plants (Nocek, 1988), new researches showed high correlation between *in vitro* and *in vivo* methods and recommended pepsin-cellulase method as reliable method

for the determination of digestibility (Nousiainen et al., 2003; Forejtova et al., 2005; Jancik, 2007). Barchiesi-Ferrari et al. (2011) reported that the discovery of equations for making this method more accurate is important, because not only crop species but also vegetation stage should be taken into consideration. Near infrared spectroscopy (NIRS) also can be used for digestibility determination, but high number of calibration samples and reliable reference methods for results verify is necessary (Rinne et al., 2006). Prediction of NDF digestibility is possible according to relation between acid detergent lignin (ADL) and NDF: true digestibility NDF (tdNDF) = 0.75 × (NDF - ADL) × (1 - (ADL / NDF) × 0.667), but *in vitro* methods provides more exact results (Trinacty et al., 2013).

The aim of this study was to compare *in sacco* and two *in vitro* methods for prediction of NDF digestibility of ryegrass forages.

MATERIAL AND METHODS

The experiment was conducted on the 32 samples of Italian ryegrass (*Lolium multiflorum*) from Plant Breeding Station Vetrov (Oseva UNI, a. s. Chocen). Analyses were done in the laboratory of Faculty of Agriculture, University of South Bohemia in Ceske Budejovice and in the Institute of Animal Science, Prague Uhrineves.

Sampling, samples treatment, the determination of dry matter (DM), crude protein (CP) ash and ether extract (EE) was done in agreement with Commission Regulation No 152/2009 (European Commission, 2009). DM was determined in laboratory drier Memmert BE 600 (Memmert GmbH + Co. KG), ash in muffle furnace 003 LP (Elektricke pece Svoboda), CP in distillation unit UDK 159 (VELP Scientifica) and EE in Det-Gras N Soxhlet Extractor (J. P. Selecta S. A).

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed in instrument Ankom Fiber Analyzer (Ankom Technology Corp., Macedon, NY) according to official Ankom methods. Non-structural carbohydrates (NSC) were calculated using the formula 100 - (NDF + CP + EE + ASH) (National Research Council, 2001).

The principle of *in sacco* method (IS) for the determination of NDF digestibility is the incubation of samples in rumen. For the experiment, two Holstein cows in dry period with rumen cannulas were used. Cows were feed by total mix ratio (TMR) constained from lucerne silage 10,5 kg, corn silage 10 kg, wheat straw 2 kg, meadow hay 1 kg, mineral and vitamin supplements 0,3 kg per day. Samples were weighted into bags R1020 (Ankom Technology Corp., Macedon, NY) with a 50 ± 10-micron porosity. The size of bags was adjusted to parameter

15–20 mg sample / cm^2 active surface of bag (Michalet-Doreau and Ould-Bah, 1992), that means bag size 10 × 10 cm and free surface 10 × 8 cm. Samples in bags on special carrier were put into the rumen. Three replicates from each sample were put into each cow. Because of comparison with in vitro incubation in rumen fluid (the same duration), 48 h incubation time was chosen. Afterwards remainders of samples were quantitatively transferred from bags to cups and residual DM, ash and NDF were determined.

In vitro true digestibility of NDF was done by *in vitro* method using rumen fluid (IVR) from manufacturer Ankom in filter bags F57 in Daisy Incubator II (Ankom Technology Corp., Macedon, NY). Three replicates from each sample were exposed to the solution of combined buffer and rumen fluid, for 48 hours at 39 °C. The NDF analyse was done after incubation.

The determination of enzymatically soluble NDF was carried by modified pepsin-cellulase method (IVE) according to Mika et al. (2009). Three replicates from each sample were exposed to the pepsin - HCl solution for 24 hours at 40 °C. Thereafter, the samples were exposed to cellulase solution in acetate buffer for 24 hours at 40 °C. The incubation of samples was done in filter bags F57 in Daisy Incubator II. The samples were washed, dried to constant weight at 105 °C and weighed. The NDF analysis was done, bags were weighed and incinerated at 550 °C.

Results were evaluated using t-test and correlation analysis by software Statistica 12.

RESULTS AND DISCUSSION

Table 1. Nutrients content (g/kg DM), NDF digestibility (% NDF)

							NDF digestibility		
Sample	**Ash**	**EE**	**CP**	**NDF**	**ADF**	**NSC**	**IS**	**IVR**	**IVE**
1	132.5	43.0	274.7	465.1	301.0	84.7	93.9	92.4	86.9
2	111.9	36.7	203.6	413.3	282.6	234.5	91.9	89.0	77.8
3	115.8	33.0	211.5	377.0	278.2	262.7	92.1	90.9	80.7
4	103.7	36.3	186.0	450.8	278.6	223.2	92.1	90.8	81.1
5	115.8	36.1	190.5	472.0	286.4	185.6	90.0	89.6	76.9
6	121.7	34.8	198.5	525.9	336.9	119.1	92.1	90.6	74.8
7	116.3	30.7	172.4	534.1	296.3	146.5	85.6	86.3	60.8
8	110.4	31.0	147.7	525.5	313.5	185.4	87.8	88.2	70.5
9	113.04	29.5	213.2	467.3	313.4	176.6	83.8	85.8	72.0
10	101.0	25.6	174.6	475.0	304.7	223.8	77.8	81.6	62.9
11	82.7	24.1	129.7	469.7	298.5	293.8	78.9	79.7	56.4
12	66.0	18.5	107.9	417.4	284.4	390.2	86.9	70.0	39.7
13	57.1	20.9	120.0	478.4	302.3	323.6	75.4	75.3	58.8
14	75.2	21.5	126.1	519.0	301.6	258.2	68.6	71.4	49.0
15	65.7	21.2	121.7	599.6	338.8	191.8	73.4	70.7	52.4
16	59.1	13.5	100.5	614.1	374.6	212.9	59.4	57.5	35.9
17	127.3	43.4	266.1	429.9	327.1	133.3	91.5	91.9	77.5
18	111.1	33.7	211.0	442.4	269.4	201.8	94.9	92.6	85.9
19	110.3	31.4	192.4	400.8	277.0	265.1	89.7	90.9	79.0
20	107.1	36.0	198.9	417.4	293.0	240.6	86.7	89.6	76.5
21	110.1	34.0	185.2	434.5	284.1	236.2	90.0	87.0	72.0
22	100.7	30.4	143.2	481.1	309.1	244.6	89.9	88.7	70.2
23	96.5	30.1	134.8	542.0	294.4	196.6	82.6	85.1	69.4
24	100.4	27.7	143.6	552.3	335.6	176.0	81.6	83.4	64.6
25	125.4	36.4	248.2	441.6	321.9	148.4	84.0	75.3	73.3
26	101.9	29.6	171.0	440.6	275.4	256.8	77.4	83.4	64.7
27	91.0	22.5	143.7	473.9	334.6	268.9	75.5	83.9	56.8
28	73.8	23.7	130.2	475.2	280.0	297.2	75.2	75.6	50.3
29	78.0	18.1	121.5	535.0	358.8	247.4	68.4	68.6	39.1
30	71.9	18.8	120.5	541.2	332.5	247.6	64.5	67.7	41.8
31	54.5	18.5	118.3	553.6	316.3	255.0	61.0	64.7	41.7
32	61.8	14.8	92.8	574.1	330.1	256.5	59.4	58.4	33.9

EE = ether extract, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, NSC = non- structural carbohydrates IS = in sacco, IVR = in vitro using rumen fluid, IVE = in vitro enzymatic using pepsin and cellulase solutions

Table 1. shows nutrients content and NDF digestibility values determined by three different methods. Differences in values were caused by unequal growth phases of ryegrass samples. This variability corresponds to studies of Coblentz et al. (1998) and Cone et al. (1999) that deal with higher NDF content and lower content of CP and NSC and lower digestibility in older vegetation.

Table 2. Basic descriptive statistics of NDF digestibility (%)

	IS	**IVR**	**IVE**
Min.	59.3	57.5	33.9
Max.	94.9	92.6	86.9
Mean	81.3	81.1	63.5
Std. Dev.	10.7	10.3	15.6

IS = in sacco, IVR = in vitro using rumen fluid, IVE = in vitro enzymatic using pepsin and cellulase solutions

In sacco (IS) method and *in vitro* method using rumen fluid (IVR) gave very similar values of NDF digestibility, t-test did not reveal significant difference between these methods ($p = 0.8277$). It is not in concordance with the findings of Altman and Bland (1983) and Ceballos et al. (2008), which reported that method using Daisy incubator underestimated DM and NDF disappearances compared to IS. However, *in vitro* enzymatic method using pepsin and cellulase solutions (IVE) proved considerably and statistically significant ($p < 0.001$) lower results than IS and IVE methods.

The IS and IVR methods showed very high values of NDF digestibility. Trinacty et al. (2013) brought out that IS 48 h incubation time is criticised because the retention time of chyme staying in rumen is lower than 30 h. The 48 h incubation time overvalues NDF digestibility value. However, problem of shorter incubation is the decrease of the results reproducibility. This is a reason why the 48 h incubation time is set like standard and is used for the determination of reference values for near infrared spectroscopy (NIRS). According to Varvikko and Vanhatalo, (1990) part of sample is washed out through the fabric of bag and so NDF digestibility is overvalued and processes for the correction of wash out sample loss were found (Weisbjerg et al., 1990). This finding is not in agreement with results of this experiment, because of samples incubated by IVR method proved the same high results although bags F57 have lower porosity (25 microns). Moreover, IVE method used the same bags and the same time of incubation and results were distinctively lower. Tas et al. (2005) published results of ryegrass

NDF digestibility using analysis of forage and cows feaces. Average NDF digestibility during the heading season was 86.9%. The NDF digestibility in the same growing season was by IS 93.1%, by IVR 97.3% and by IVE 82.0%.
Statistically significant ($p < 0.001$) correlations were proved among used methods although IVE method provided significantly lower values. Following equations were produced by correlation analyse:

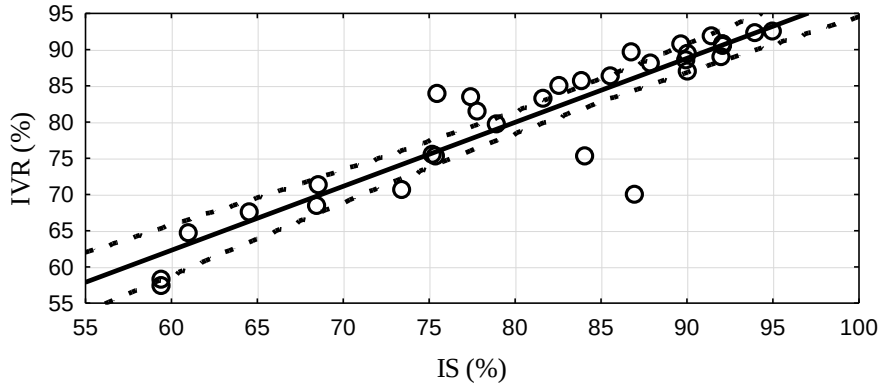
$$IVR = 9.3115 + 0.88341 \times IS \qquad (r = 0.91456)$$

$$IVE = -42.69 + 1.3064 \times IS \qquad (r = 0.89394)$$

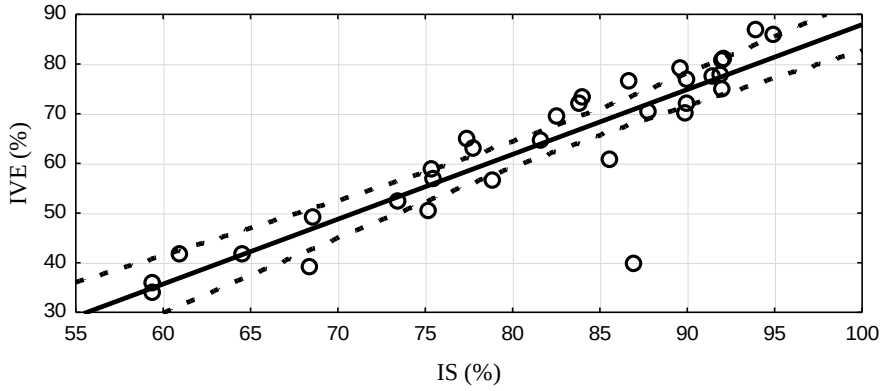
$$IVE = -51.14 + 1.4133 \times IVR \qquad (r = 0.93410)$$

A graphical representation of relationship between IVR and IS is depicted in Graph 1., between IVE and IS in Graph 2.,and Graph 3. shows relation between IVR and IVE.

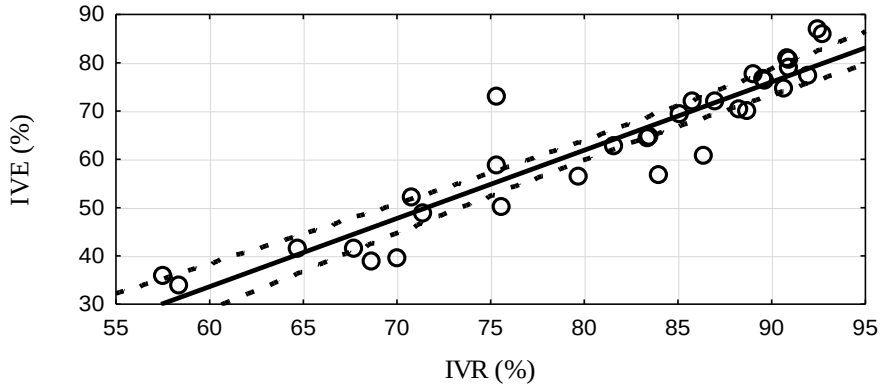
Graph 1. NDF digestibility (%) – correlation of IVR and IS



Graph 2. NDF digestibility (%) – correlation of IVE and IS



Graph 3. NDF digestibility (%) – correlation of IVE and IVR



IS = in sacco, IVR = in vitro using rumen fluid, IVE = in vitro enzymatic using pepsin and cellulase solutions

CONCLUSION

Statistically significant correlations were found among all using methods for NDF digestibility prediction. Pepsin-cellulase method proved distinctively lower values than *in sacco* method and *in vitro* method using rumen fluid. Partial results from this experiment give preliminary information about meaningfulness of another researching and developing of this method, because it seems to be very clean, undemanding and affordable alternative to methods that require live donors of rumen fluid.

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