UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE

DEPARTMENT OF ANIMAL NUTRITION AND HUSBANDRY

NutriNET 2021

KOŠICE, 9. september 2021

UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE

DEPARTMENT OF ANIMAL NUTRITION AND HUSBANDRY

NutriNET 2021

Proceedings of reviewed scientific papers

Editors: MVDr. Lukáš Bujňák, PhD.
doc. MVDr. Pavel Naď, PhD.

Reviewers: doc. MVDr. Iveta Maskaľová, PhD.
doc. MVDr. František Zigo, PhD.
MVDr. Bc. Andrej Marcin, CSc.
MVDr. Tomáš Mihok, PhD.

Approved by rector of University of Veterinary Medicine and Pharmacy in Košice as a proceeding of reviewed scientific papers.

KOŠICE 2021

ISBN 978-80-8077-713-5

CONTENT

THE EFFECT OF HIGH-ENERGY DIET ON PROGRESSION OF DIABETES MELLITUS IN SPONTANEOUS DIABETIC RATS

RUDOLF DUPAK, ANNA KALAFOVA, MONIKA SCHNEIDGENOVA, JANA HRNKOVA, MARCELA CAPCAROVA

Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovak Republic

Corresponding email address: xdupak@uniag.sk

ABSTRACT

The influence of high-energy diet on Zucker diabetic fatty rats was evaluated. Zucker diabetic fatty rats were divided into three groups – non-diabetic (lean), diabetic with normal diet and diabetic with high-energy diet. A high-energy diet was composed of 30 % saturated fatty acid, 5% starch and 15% disaccharides (20 $MJ.kg^{-1}$). Our results suggest that addition of high-energy diet in ZDF rats increased and significantly influenced body weight, water intake and blood glucose levels ($P < 0.001$) against non-diabetic ZDF rats. Supplementation of high-energy diet had inclination to *Diabetes mellitus* and leads to obesity, hyperglycaemia, and other diabetic complications. Our study confirmed that high-energy diet shorted prediabetic state and accelerated the onset symptoms of *Diabetes mellitus* in animal models.

Keywords: diabetes; high-energy diet; hyperglycaemia; ZDF rats

INTRODUCTION

Diabetes mellitus type 2 (DMT2) is most prevalent type of diabetes and is significantly increasing (Henning, 2018). DMT2 is a heterogenous group of metabolic disorders described by insulin resistance and impaired insulin secretion and it is characterized by elevated fasting blood glucose (Stride and Hattersley, 2002). Diabetic research is mainly focused on exploring new substances that would serve in the regulation or prevention of diabetes. Animal models have a long history in this field

of research and play an important role (Rees and Alcolado, 2005; Dupak et al., 2020). These models reflect glucose intolerance, obesity, dyslipidaemia, hypertension, and other diabetic complications in human DMT2. Several genetic and environmental factors contribute to the causes and progression of diabetes. High-energy diet is one of the most important environmental factors, which contributes to the prevalence of obesity (Schrauwen and Westerterp, 2000; Capcarova et al., 2018). The acceleration of DM symptoms is interesting as it can be effective in use of animal models to shorten the duration of the experiment (King, 2012). The aim of this study was to determine the effect of high-energy diet on onset and progression of DMT2 in Zucker diabetic fatty (ZDF) rats, which allows a better understanding of effects of high-energy diet on shorten of prediabetic state in research of DM in spontaneous diabetic rats.

MATERIAL AND METHODS

Animals and experimental design

A total of 30 male ZDF rats were used in the experiment. ZDF rats were divided to three groups as follows: L (lean, non-diabetic control, n = 10), ND (diabetic with normal diet, n = 10) and HD (diabetic with high-energy diet, n = 10). Animals were obtained from Breeding Facility of the Institute of Experimental Pharmacology and Toxicology (Dobra Voda, Slovak Republic, SK CH 24016) in the age of 12 weeks. ZDF rats were housed in number of two rats per plastic cage (800 cm^2) with a 12 hours light-dark cycle at 23 °C. All animals were provided with water and diet on *ad libitum* base and the feed, water consumption and body weight were monitored during the 8 weeks of the experiment. The composition of feed mixture is presented in Table 1. The HD group received modified diet where the number of calories increased substantially (enriched KKZ-P/M, 30 % saturated fatty acid, 5 % starch and 15 % disaccharides, 20 MJ/kg).

Table 1. The composition of feed mixture

Analytical compounds (%)	
Nitrogenous compounds	19.10
Dietary fibre	3.60
Oils and fats	5.10
Ash	5.85
Humidity	9.10
Additives	
Vitamins	E672 Vitamin A – 20,000 u.m., E671 Vitamin D3 – 2000 u.m., Vitamin E – 70 mg
Amino acids	DL-Methionine 1.2 g, L-Lysine 0.8 g

u.m. – unit of measure

Glucose analysis

Blood glucose was determined in 1st, 4th and 8th week of the experiment. It was measured after overnight fasting by a FreeStyle Optium Neo Glucose system (Abbottt Diabetes care Ltd., UK) using test strips. Few drops of blood were taken from the tail vein of rat. DMT2 was diagnosed if the blood glucose concentration exceeded 15 mmol/L.

Statistical analysis

The data are expressed as means ± standard error of the mean (SEM). Means of the results from the treatments were compared by one-way ANOVA test followed by Bonferroni post-tests to compare replicate means by row. The differences were considered significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$.

RESULTS AND DISCUSSION

Diabetic animal models affected by high-energy diets that mimic different physiological conditions in humans are commonly used in research (Heydemann, 2016). Accelerating the onset of diabetic complications in animal models can be beneficial primarily in terms of time as well as economics. *Diabetes mellitus* begins in ZDF rats at approximately 10 weeks of age on a normal diet (Yokoi et al., 2013). In our experiment a high-energy diet caused a significant increase ($P < 0.001$) of body weight against lean non-diabetic control from the first week till the end of the experiment. ZDF rats on a normal diet reached the highest weight at week 8 and there was a significant increase ($P < 0.001$) compared to the lean group during whole experiment and a significant increase ($P < 0.01$) against high-energy diet in 8th week (Figure 1.). A weight gain is associated with insulin resistance and lack of β-cells compensation leads to impaired glucose tolerance (King, 2012).

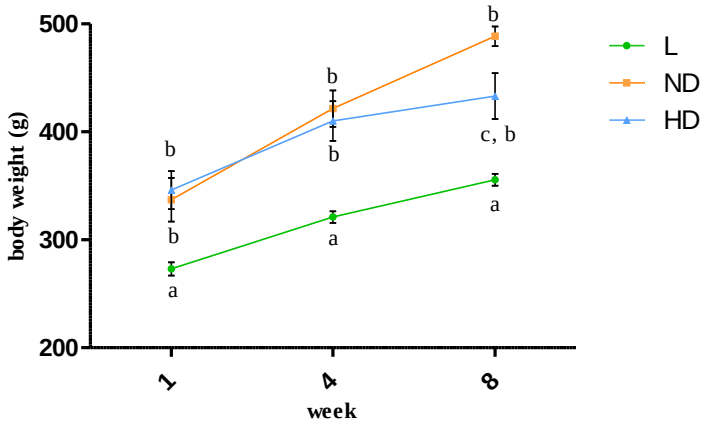


Figure 1. The effect of high-energy diet on body weight in ZDF rats
L – control non-diabetic group, ND – diabetic with normal diet group, HD – diabetic with high-energy diet group. Values are mean ± SEM, a-b in particular week means significant differences ($P < 0.001$), a-c and b-c in particular week means significant differences ($P < 0.01$).

The high-energy diet caused significant increase ($P < 0.01$) of feed consumption when compared to the lean in the end of experiment. Diabetic group with normal diet showed significant increase ($P < 0.001$) against the lean and also HD group throughout the experiment (Figure 2.). When observing water intake, we found significant increase ($P < 0.001$) in high-energy group from the 4th week to 8th week when compared to the lean (Figure 3.). A significant increase of feed and water consumption in the diabetic rats observed also Oyedemi et al. (2011). These increases are known markers of DMT2 in diabetic rats as well as in humans.

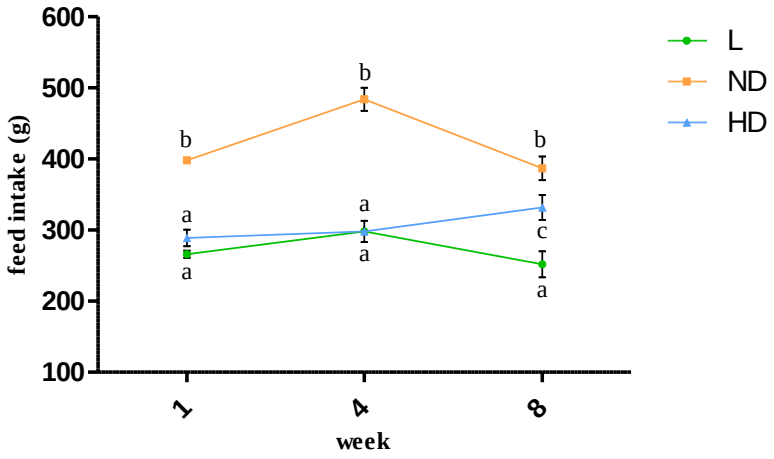


Figure 2. The effect of high-energy diet on feed intake in ZDF rats
L – control non-diabetic group, ND – diabetic with normal diet group, HD – diabetic with high-energy diet group. Values are mean ± SEM, a-b in particular week means significant differences ($P < 0.001$), a-c in particular week means significant differences ($P < 0.001$), b-c in particular week means significant differences ($P < 0.05$).

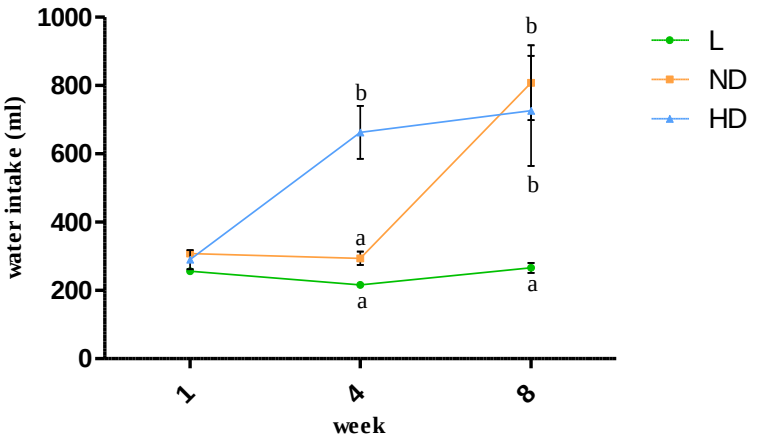


Figure 3. The effect of high-energy diet on water intake level in ZDF rats
L – control non-diabetic group, ND – diabetic with normal diet group, HD – diabetic with high-energy diet group. Values are mean ± SEM, a-b in particular week means significant differences ($P < 0.001$).

Fasting hyperglycaemia is one of the key features of DMT2 and is well suited for ZDF rat model (Clark et al., 1983). After high-energy diet, blood glucose of ZDF rats started raising significantly ($P < 0.001$) compared to the lean group at all weeks and the ND group at week 1 and 4 and $P < 0.01$ in week 8. Similarly, Magalhaes et al. (2019) found after high-fat diet in streptozotocin male Wistar rats raised glucose values (27.7 mmol.l^{-1}). In our study in the end of the experiment levels of blood glucose in HD group were 16.55 mmol.l^{-1}.

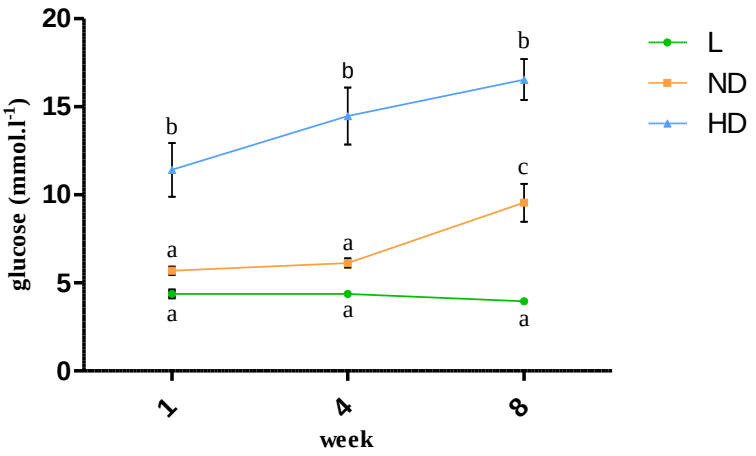


Figure 4. The effect of high-energy diet on glucose level in ZDF rats
L – control non-diabetic group, ND – diabetic with normal diet group, HD – diabetic with high-energy diet group. Values are mean ± SEM, a-b in particular week means significant differences ($P < 0.001$), a-c and b-c in particular week means significant differences ($P < 0.001$).

CONCLUSION

Our results confirm that application of high-energy diet caused the earlier onset of diabetic complications in ZDF rats. We conclude that high-energy diet in ZDF rats was effective in generating a DMT2 in shortening the prediabetic state in order to better understanding of potential effect of therapeutic agents in diabetic research, with metabolic characteristics similar to those of human DMT2.

ACKNOWLEDGEMENT

The project was supported by the VEGA 1/0144/19, KEGA 034SPU-4/2019 and the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund.

REFERENCES

Capcarova, M., Kalafova, A., Schwarzova, M., Prnova, M. S., Svik, K., Schneidgenova, M., Slovak, L., Bovdisova, I., Toman, R., Lory, V., Zorad, S. (2018): The high-energy diet affecting development of diabetes symptoms in Zucker diabetic fatty rats. Biologia 73, 659-671.

Clark, J. B., Palmer, C. J., Shaw, W. N. (1983): The diabetic Zucker fatty rat. Proceedings of the Society for Experimental Biology and Medicine 173, 68-75.

Dupak, R., Jaszcza, K., Kalafova, A., Schneidgenova, M., Ivanisova, E., Tokarova, K., Brindza, J., Capcarova, M. (2020): Characterization of compounds in Cornelian cherry (*Cornus mas* L.) and its effect on interior milieu in ZDF. Emirates Journal of Food and Agriculture 32, 368-375.

Henning, J. R. (2018): Type-2 diabetes mellitus and cardiovascular disease. Future Cardiology 14, 491-509.

Heydemann, A. (2016): An overview of murine high fat diet as a model for type 2 diabetes mellitus. Journal of Diabetes Research 2016.

King, A. J. F. (2012): The use of animal models in diabetes research. The British Journal of Pharmacology 166, 877-894.

Magalhaes, D. A., Kume, W. T., Correia, F. S., Queiroz, T. S., Neto, E. W. A., Santos, M. P., Kawashita, N. H., Franca, S. A. (2019): High-fat diet and streptozotocin in the induction of type 2 diabetes mellitus: a new proposal. Anais da Academia Brasileira de Ciencias 91, 1-14.

Oyedemi, S. O., Adewusi, E. A., Aiyegoro, O. A., Akinpelu, D. A. (2011): Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia Africana* (Smith) on streptozotocin-induced diabetic Wistar rats. Asian Pacific Journal of Tropical Biomedicine 1, 353-358.

Rees, D. A., Alcolado, J. C. (2005): Animal models of diabetes mellitus. Diabetic Medicine: a Journal of the British Diabetic Assoc. 22, 359-370.

Stride, A., Hattersley, A. T. (2002): Different genes, different diabetes. Lessons from maturity-onset diabetes of the young. Annals of Medicine 34, 207-216.

Yokoi, N., Hoshino, M., Hidaka, S., Yoshida, E., Beppu, M., Hoshikawa, R., Sudo, K., Kawada, A., Takagi, S., Seino, S. (2013): A novel rat model of type 2 diabetes: the Zucker fatty diabetes mellitus ZDFM rat. Journal of diabetes research 2013, 1-9.

THE ROLE OF NUTRITION IN DEVELOPMENT OF CATARACTS, DIABETIC RETINOPATHY AND OTHER EYE-RELATED DISEASES

JANA HRNKOVÁ, RUDOLF DUPÁK, MARCELA CAPCAROVÁ

Faculty of Biotechnology and Food sciences

Slovak University of Agriculture in Nitra

Corresponding email address: jankahrnkova1@gmail.com

ABSTRACT

In recent years, there has been a fundamental change among the causes of blindness. The number of chronic, age-related diseases is increasing and the number of infectious diseases is decreasing. In this review, we provide basic information about the pathophysiology of the four major ocular age-related diseases - cataracts, diabetic retinopathy, glaucoma, and age-related macular degeneration. We focus on the specific role of nutrition in preventing or slowing the progression of eye diseases with an emphasis on antioxidant research.

Keywords: cataracts; diabetic retinopathy; glaucoma; age-related macular degeneration; nutrition; antioxidants

INTRODUCTION

Epidemiological data from the WHO show that more than one billion people worldwide suffer from a visual impairment of a different degree. The most common causes of visual impairment with subsequent blindness include cataracts, diabetic retinopathy, glaucoma, and age-related macular degeneration (Kuchynka, 2016; WHO, 2019; Heruye, 2020). The goal of many research projects is to understand the mechanisms of development of these diseases and to find a way to prevent their occurrence or slow down progression. One such factor with a significant impact on the development of ocular pathology is nutrition, especially nutrients with antioxidant and anti-inflammatory effects, such as carotenoids, bioflavonoids, antioxidant vitamins, omega-3 fatty acids, selected minerals and microelements (Heruye, 2020; Xu, 2020;

Francisco, 2020). The influence of individual components of nutrition is monitored either experimentally on animal models, but it is also studied in numerous, variously designed clinical studies. Despite the mixed results, supplementation with nutrients with antioxidant properties has proven to be a very promising alternative for the treatment of many chronic diseases, including eye diseases (Capcarova, 2018; Hrnková, 2021).

CATARACTS

Cataracts are still the main cause of blindness also in the 21st century, affecting around 94 million people (Kuchynka, 2016; WHO, 2019; Heruye, 2020). Only the inhabitants of the most developed countries in the world have unrestricted access to surgical treatment, but to most people on earth it still remains inaccessible. The effort to find a compound that could prevent or slow the progression of the disease without the need for surgery, which would mean a huge benefit, especially for regions with limited possibilities of microsurgery treatment comes from these factors. The main causes of cataractogenesis are considered to be the age, genetic predisposition, congenital mutations of proteins in lens, physical environmental influences and overproduction of sugar alcohols due to metabolic diseases, especially *diabetes mellitus* (Kanski, 2008; Kuchynka, 2016; Heruye, 2020). The final result of all these processes is excessive oxidative stress, which leads to exhaustion or failure of the antioxidant system of the lens with subsequent development of cataracts, the lens loses its transparency, vision deteriorates and without treatment decreases to the level of blindness (Dukuran, 2006; Kuchynka, 2016; Heruye, 2020)

The results of experimental works focused on the research of the anticataractogenic effect of various substances show that substances with antioxidant potential are of fundamental importance. Experiments are usually performed on animal models (most commonly Wistar rats, mice, rabbits, dogs, etc.) in which cataract development is induced by oxidative stress, metabolic disease (eg. streptozocin-induced diabetes), UV radiation, or steroid administration (Yamakoshi, 2002; Dukuran, 2006; Heruye, 2020, Hrnková, 2021). The most frequently investigated substances include the antioxidants vitamin C, vitamin E, glutathione,

carotenoids, the flavonoid quercetin and the polyphenol resveratrol. The anti-cataractogenic effect is very likely to inhibit the oxidation of lipids, proteins, nucleic acids and peroxide formation (Doganay, 2006; Shetty, 2010; Dubey, 2016; Singh, 2019, Lim, 2020).

Among the most important clinical trials for monitoring the effect of micronutrients on the development of cataracts are the Age-related Eye Disease Study (AREDS) and the Roche European American Cataract Trial (REACT). In AREDS, a positive effect of selected micronutrients on slowing the progression of age-related macular degeneration (AMD) was demonstrated, but there was demonstrated no effect on cataract development. The REACT results support the statement that increased intake of vitamin E, C and beta-carotene in the early stages of cataracts may have a positive effect on slowing disease progression (Schalch, 2003; Chiu, 2007; Lim, 2020). The Blue Mountains Eye Study confirmed the positive effect of a healthy diet and normal body mass index (BMI) on reducing the risk of formation of cataracts in the Australian population (Tan, 2019). The effect of increased intake of vitamins A, B, C, E of zinc, copper, carotenoids, lutein and zeaxanthin has been studied in many other studies (Glaser, 2015; Mathew, 2012; Braakhuis, 2019; Heruye, 2020; Francisco, 2020). Unlike experimental works, the results are still relatively contradictory and ambiguous.

DIABETIC RETINOPATHY

Diabetic retinopathy (DR) is the most common microvascular complication of *diabetes mellitus* (DM) and a major cause of blindness in the adult population in developed countries. The prevalence of DR in 2019 was around 27%, which means that out of 463 million diabetics, at about 125 million of them was developed some form of diabetic retinopathy (Matos, 2020). Risk factors for the development of diabetic retinopathy include the duration of the underlying disease, insufficient compensation of *diabetes*, type 1 *diabetes*, arterial hypertension and dyslipidemia. After 20 years of *diabetes*, diabetic retinopathy occurs at practically all patients with type 1 *diabetes* and at almost 60% of patients with type 2 *diabetes* (Kuchynka, 2016; Matos, 2020). The basic pathomechanism of the development of diabetic retinopathy is the pathological effect of chronic hyperglycemia on vascular endothelium.

At the cellular level, it is a chronic inflammation that leads to an increase in oxidative stress with consequent damage to the retinal microcirculation. The final consequence is pathological changes that can result in irreversible visual impairment (Kuchynka, 2016; Kanski, 2008, Matos, 2020).

A number of experimental works as well as clinical studies focus on the research of substances with anti-inflammatory and antioxidant effect with possible potential for use in the prevention or treatment of diabetic retinopathy. Such substances undoubtedly include bioflavonoids, especially their subgroup anthocyanins, as well as antioxidant vitamins A, E, C, green tea extracts and many others (Lee, 2010; Wu, 2014; Wong, 2018; Matos, 2020; Dupák, 2021). In animal models, most often in rats with streptozocin-induced type I diabetes, their positive effect on slowing the development of complicated stages of diabetic retinopathy was proved, mainly due to their high antioxidant potential, effect on sugar and lipid metabolism and positive effect on insulin resistance (Matos, 2020).

Antioxidant supplementation has also been studied in clinical trials on patients with *diabetes mellitus* as well as on healthy population at risk of developing diabetes. The effect of quercetin and myricetin on the development of diabetic complications with a proven slowing of disease progression was studied on more than 10,000 participants (Matos, 2020). Mahoney et al. studied the effect of high levels of flavonoids in the diet on a sample of 381 diabetics. They found that such an enriched diet reduced the risk of disease progression by 30%. Positive effect of anthocyanins have been demonstrated on the reduction of inflammatory markers in the blood of diabetics, the improvement of glycated hemoglobin levels and glycaemia (Matos, 2020). Great attention is focusing on the so-called pycnogenol, a substance derived from maritime pine (Pinus maritima). Several clinical studies have confirmed that with a sufficiently long period of antioxidant supplementation with representation of pycnogenol slows the progression of diabetic retinopathy, improves retinal morphology, and stabilizes visual function (Matos, 2020). Despite these encouraging results of some studies, it is not yet possible to draw generally valid conclusions. The individual

studies are designed in a very diverse manner and therefore the results cannot be clearly interpreted.

AGE-RELATED MACULAR DEGENERATION

Age-related macular degeneration (AMD) is a degenerative, progressive disease of the central part of the retina. The exact cause is unknown, the main proven risk factors include age, heredity, race and smoking. It occurs in two forms, most cases have so-called dry form, only in about 20% of cases an aggressive so-called wet form is developed, which is the cause of almost 90% of blindness in developed countries (Kanski, 2008; Kuchynka 2016; Bagheriová, 2020). Therapy does not exist yet, the wet form can be put into a stabilized inactive state by injecting biological treatment directly into the vitreous of the eye. To prevent the progression of the dry form, compouds with a precisely defined antioxidant composition, which were developed based on the AREDS / AREDS2 study (see table1), are given.

The AREDS study is currently considered as the basic study of the effect of nutrients on eye diseases, according to which other similar clinical studies are being designed. It was performed on 4,757 patients aged 55-80 years with a diagnosis of AMD or cataracts, or both, and lasted 5 years. Various combinations of vitamin C, E, beta-carotene, zinc and copper were tested. In 2006, the AREDS2 study began and included 4,203 subjects aged 50-85 years, exclusively with a diagnosis of AMD. Lutein and zeaxanthin were added to the substances studied in the first study and beta-carotene was excluded. The main findings of the AREDS/AREDS2 study are that increased intake of selected micronutrients reduces the risk of advanced AMD progression by up to 25%, but has no effect on cataract development and its progression. At the same time, the study recommends to smokers to avoid increased beta-carotene intake, due to the higher risk of developing lung cancer, and prefer formulas with the composition tested in AREDS2 (Schalch, 2003; Chew, 2012; Glaser, 2015; Francisco, 2020). Both studies result in commercially available preparations with AREDS/AREDS2 compositions which are a common part of the treatment of patients with dry form of AMD.

Table 1. Compounds according to AREDS/AREDS2 study

Nutrient	**AREDS**	**AREDS2**
Vitamin C	500mg	500mg
Vitamin E	400 IU	400IU
Beta-carotene	15mg	-
Copper	2mg	2mg
Lutein	-	10mg
Zeaxanthin	-	2mg
Zinc	80mg	80mg

GLAUCOMA

Glaucoma is defined as progressive optic nerve neuropathy that leads to changes in the visual field, retinal ganglion cells die, what leads to gradual irreversible loss of vision (Kanski, 2008; Kuchynka 2016; Bagheriová, 2020). According to the WHO, there are currently more than 70 million people with this diagnosis, of which about 11 million are blind (WHO, 2019). The disease is multifactorial, the exact pathomechanism of the development of glaucoma changes has not yet been clearly described. The main risk factor and at the same time the only one that we can therapeutically influence is high intraocular pressure. Chronic elevation of intraocular pressure damages the optic nerve and retinal cells mechanically and by ischemia due to hypoperfusion. Treatment is aimed at reducing the pressure with medication or by surgery, despite that, there is a group of patients with continued progression of changes, even with good compensation of the ocular pressure values. Normotensive glaucoma is a separate unit where the glaucoma neuropathy develops and intraocular pressure is within normal limits (Kanski, 2008; Kuchynka 2016; Bagheriová, 2020). The influence of other mechanisms, such as toxic, immunopathological reactions and the influence of oxidative stress are expected. It was proved that oxidative stress damages individual structures of the eye and leads to very early morphological changes in the preclinical stage of glaucoma. In this context, antioxidant

supplementation appears to be a possible way of treatment, especially in the early stages, unless irreversible changes have occurred yet (Garcia-Medina, 2020; Francisco, 2020).

Experimental studies are most often performed on rodents, rabbits, but also dogs (beagles). In rats, the positive effect of vitamin A and vitamin E on the reduction of ganglion cell apoptosis by the mechanism of their action against lipid peroxidation was demonstrated. Vitamins of group B in experiments on mice have shown a protective effect on mitochondria and slowed down the process of reducing the number of nerve fibers from the optic nerve. Coenzyme Q was administered to rats in the vitreous. It has been researched, that it has a protective effect on ischemia-damaged retinal ganglion cells. Gingko biloba extracts have a significant anti-inflammatory, antithrombotic and vasoprotective effect. In experiments in rats, the protective effect of mitochondrias in particular has been demonstrated. Resveratrol was injected into the anterior chamber to rats. Its protective effect on ganglion cells is most likely based on a reduction in the level of reactive oxygen species in the ischemic retina (Garcia-Medina, 2020; Ramdas, 2018). Although many of these experiments have shown very encouraging results, we cannot simply extrapolate them to human medicine.

Antioxidant supplementation has also been studied in clinical trials on patients with glaucoma. A positive effect of increased intake of omega-3-unsaturated fatty acids on improved results of findings in visual field at glaucoma patients has been demonstrated. Gingko biloba, given especially to patients with normotensive glaucoma, improves visual field findings, has a neuroprotective effect and has a positive effect on the rheological properties of the blood, all leading to improved retinal and optic hemodynamics (Garcia-Medina, 2020). Anthocyanins, substances highly present in fruit berries, are flavonoids with significant antioxidant potential. They have an antiplatelet effect, normalize endothelin level and thus improve ocular microcirculation. A study of blackcurrant anthocyanins found a significant decrease in intraocular pressure and improvement in the visual field (Oghuro, 2012; Oghuro, 2013). Some studies have shown a positive effect of some flavonoids, glutathione and nitrates contained mainly in dark green leafy vegetables on slowing the

progression of glaucoma changes, as well as the effect of green tea extracts and many other substances (Kang, 2016).
Due to the fact that this is an incoherent group of studies, it is necessary to evaluate the results with some reservation.

CONCLUSION

Millions of people around the world suffer severe visual impairment and loss of vision due to chronic eye diseases, and their number is growing every year. Vision loss has a significant impact on the quality of life of the affected individual, reduces his/her independence, mobility, increases the risk of injuries, worsens his/her social role, he/she often loses employment, which can lead to mental disorders and social isolation. There are also significant economic consequences for society, both in the form of ever-increasing health care costs and indirect losses resulting from reduced productivity, the inability to improve qualifications, the need to pay early or disability pensions. Although modern medicine provides a wide range of diagnostic and therapeutic procedures for a large number of eye diseases, they are very difficult to approach or inaccessible to most of the world's population. Therefore, a number of experiments and studies are being made, focusing at researching new methods that could prevent the progression of disease changes. The impact of nutrition and especially the increased intake of antioxidants has been the subject of interest in recent years. Very positive results are brought mainly by experimental work on animal models, to which can be implemented the nutrition of individual nutrients with the expected positive effect in humans, or examine the impact of high and low energy diet on individual parameters of homeostasis and overall condition of animals, all in an environment with stable conditions and strict control of the animal regime. Clinical studies, on the other hand, do not yet bring such convincing results, as real-life conditions differ significantly from the laboratory environment. Further research will be needed to understand the exact mechanism of effect of individual antioxidants on pathological processes, as well as to determine the optimal dose of individual substances while maintaining safety for the organism.

ACKNOWLEDGEMENT

The work was supported by research grants APVV 19/0243 and VEGA 1/0144/19. This publication was supported by the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and inovative food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund.

REFERENCES

Bagheriová N., Wajdová NB.(2020): Willsův oční manuál. Diagnostika a léčba očních chorob v praxi. Triton, Praha, ISBN 978-80-7533-808-6,365p.

Braakhuis AJ., Donaldson CI. et al.(2019): Nutritional Strategies to Prevent Lens Cataract: current Status and Future Strategies. Nutrients, 11(5): 1186.

Capcarova M., Soltesova Prnova M. et al (2018): Rodent animal model for research in diabetes: a mini-review. Slovak J.Anim.Sci., 51 (3): 138-145, ISSN 1337-9984.

Doganay S, Borazan M et al (2006): The effect of resveratrol in experimental cataract model formed by sodium selenite. Curr. Eye.Res. 31:147-153.

Dubey S, Deep P et al (2016): Phytochemical characterization and evaluation of anticataract potential of seabuckthorn leaf extract. Vet Ophthalmol.19(2):144-148.

Dukuran AH, Everekliogluc et al (2006): Ingestion of IH636 grape seed proanthocyanidin extract to prevent selenite- induced oxidative stress in experimental cataract. J Cataract Refract Surg. 32(6):1041-5.

Dupák R., Hrnková J., Capcarová M. (2021): Vplyv environmentálnych faktorov na vznik a vývoj diabetes mellitus. Elektronický zborník vedeckých prác, 24. Košický morfologický deň, 6/2021: 64-68. ISBN 978-80-8077-705-0, 298p.

Francisco SG, Smith KM., AragonésG. et al (2020): Dietarry Patterns, Carbohydrates, and age-related Eye diseases. Nutrients, 12(9): 2862.

Garcia-Medina JJ., Rubio-Velazquez E. et al (2020): Glaucoma et antioxidants: review and update. Antioxidants (Basel). 9(11): 1031.

Glaser TS., Doss LE. et al (2015): The Association of Dietary Lutein/Zeaxantin and B Vitamins with Cataracts in the Age-Related Eye Disease Study AREDS Report No. 37. Ophthalmology, 122(7): 1471-1479.

Heruye SH., Nkenyi LNM. et al (2020): Current Trends in the Pharmacotherapy of Cataracts. Pharmaceuticals (Basel), 13(1): 15.

Hrnková J., Dupák R., Capcarová M. (2021): Rakytník rešetliakový - potenciálny kandidát pre farmakologickú liečbu katarakty? Elektronický zborník vedeckých prác, 24. Košický morfologický deň, 6/2021: 121-125. ISBN 978-80-8077-705-0, 298p.

Chew EY., Clemons T., Sangiovanni JP. Et al (2012): The age-related eye diseases study 2 (AREDS 2): study design and baseline characteristics (AREDS2 report number 1) Ophthalmology. 119:2282-2289.

Chiu CHJ. Taylor A. (2007): Nutritional antioxidants and age-related cataract and maculopathy. Exp Eye Res. 84 (2): 229-45.

Kang JH., Willett WC., Rosner BA. et al (2016): Association of dietary nitrate intake with primary open-angle glaucoma: a prospective analysis from the Nurses´health study and health professionals follow-upstudy.JamaOphtalmol.134:294-303.

Kanski JJ. (2008): Klinische Ophthalmologie, Lehrbuch und Atlas. Elsevier, Munchen, ISBN 978-3-437-23472-9, 974p

Kuchynka P a kol. (2016): Oční lékařství. Grada Praha, ISBN 978-80-247-5079-8, 903p

Lee Ch., Gayton EL. et al (2010): Micronutrients and Diabetic Retinopathy: A systematic Review. Ophthalmology, 117 (1): 71-78.

Lim JC., Arredondo MC. et al (2020): Vitamin C and the Lens: New Insights into Delaying the Onset of Cataract. Nutrients. 14;12(10):3142.

Mathew MC., Ervin AM. et al (2012): Antioxidant vitamin supplementation for preventing and slowing the progression of age-related cataract. Cochrane Database Syst Rev., 6: CD004567.

Matos AL., Bruno DF. et al (2020): The Benefits of Flavonoids in Diabetic retinopathy. Nutrients, 12(10): 3169.

Oghuro H., Oghuro I. et al (2012): Two-year randomized, placebo-controlled study of black currant anthocyanins on visual field in glaucoma. Ophthalmologica. 228:26-35.

Oghuro H., Oghuro I. et al (2013): Effects of black currant anthocyanins on intraocular pressure in healthy volunteers and patients with glaucoma. J.Ocul. Pharmacol. Ther. 29: 61-67.

Ramdas WD., Schouten J., Webers C. (2018): The effect of vitamins on glaucoma: a systematic review and meta-analysis. Nutrients.10:359.

Schalch W., Chylack LT. (2003): Antioxidant micronutrients and cataract. Review and comparison of the AREDS and REACT cataract studies. Opthalmologe. 100(3): 181-9.

Shetty LL., Harikiran H. et al (2010): In vitro prophylactic cataract prevention study on glucose induced cataract by quercetin and alpha tocopherol. Int J. Pharm.Sci. Res. 1:41-45.

Singh APOD., Singh R. et al (2019): Health benefits of resveratrol. Evidence from clinical studies. Med.Res. Rev. 39:1851-1891. Tan AG., Kifley A., Flood VM. et al (2019): The combination of healthy diet and healthy body weight is associated with lower risk of nuclear cataract in the Blue Mountains Eye Study. J.Nutr. 1; 149(9): 1617-1622.

Xu J., Qiuli F. etal (2020). Advances in pharmacotherapy of cataracts: Ann Transl. Med, 8(22):1552.

Yamakoshi J., Saito M. et al (2002): Procyanidin-rich extract from grape seeds prevents cataract formation in hereditary cataractous (ICR/f) rats. J Agric Food Chemic. 14;50(17): 4983-8.

WHO: World report on vision (2019) ISBN: 9789241516570,180p.

Wong MYZ., Man REK. et al (2018): Dietary intake and diabetic retinopathy: A systematic review. PLoS ONE 13(1): e0186582.

Wu Y., Tang L. et al (2014): Oxidative Stress: Implications for the Development of Diabetic Retinopathy and Antioxidant Therapeutic Perspectives. Oxid.MedCellLongev, 10.1155/2014/752387.

MYCOTOXIC CONTAMINATION OF SILAGES: A REVIEW

MÁRIA KALÚZOVÁ, MIROSLAV JURÁČEK, DANIEL BÍRO, MILAN ŠIMKO, BRANISLAV GÁLIK, MICHAL ROLINEC, ONDREJ HANUŠOVSKÝ, EVA MIXTAJOVÁ, RENATA KOLLÁTHOVÁ, PAVOL BREK

Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Trieda A. Hlinku 2, 94976 Nitra, Slovakia

Corresponding email address: xkaluzova@uniag.sk

ABSTRACT

This review aims to summarize the presence of 6 major mycotoxins in maize and alfalfa silage from countries of Europe and focuses on their regulation and prevention in feed and food. Contamination of feed with mycotoxins is constantly one of the most observed topics, and their content should be monitored due to threats to human and animal health. Ruminants are exposed to this risk through silage, so precautionary measures are taken before and after harvesting, as well as proper silage management. Deoxynivalenol had the highest concentration in all samples of maize silage, followed by zearalenone and fumonisins. The most observed mycotoxin in alfalfa silages was deoxynivalenol, and the second one was zearalenone. In maize silage, the permitted limit for zearalenone was exceeded in two studies, while in alfalfa silage, the limit for any of the mycotoxins was not exceeded.

Keywords: mycotoxins; maize silage; alfalfa silage, Europe

INTRODUCTION

Many species of microscopic fungi are able to produce secondary metabolites (mycotoxins) that have harmful effects not only to animals but also to humans, and in addition their presence in feed poses a threat to food safety (Fabiszewska et al., 2019). As Gallo et al. (2021) reported, mycotoxins are a result of the secondary metabolism of microscopic

fungi, also characterized as low molecular weight products, produced primarily by *Aspergillus*, *Penicillium* and *Fusarium* species. Most studies have focused on the occurrence and incidence of mycotoxins in feeds made from cereals. However, the wide range of mycotoxins present in preserved feeds has led to the study of hay and silage (Dagnac et al., 2016). Cogan et al. (2016) are of the opinion, that there is relatively little research focused on the concentration of mycotoxins in silages, as this may be a potential source of infection and a risk to animal health. Likewise, Del Palacio et al. (2016) focused their attention on contaminated feed as one of the main risk factors for ruminant health. According to Jovaišienė et al. (2017), the ingestion of feed contaminated with mycotoxins is most often chronic, accompanied by reduced intake, production, and fertility. Other concomitant symptoms of mycotoxicosis include diarrhea, fever, or itching and bleeding. Mycotoxins may also be present in animal products, posing a risk to human health (Cogan et al., 2016). Therefore, Gallo et al. (2021) and Ogunade et al. (2018) recommend the use of high-quality silage without toxins and undesirable microorganisms to ensure animal health. Ensuring quality and healthy feed is relatively challenging, as the regulation of mycotoxins in the field is difficult (Gallo et al., 2021). There are many factors influencing the formation and development of mycotoxins, such as inappropriate storage and handling conditions (Juan et al., 2020), but also factors beyond human control (climate change, alternating periods of drought and rain, weather during growth, flowering, and harvesting) (Biomin, 2021). Kosicki et al. (2016) point to at least some possibilities for reducing the growth of microscopic fungi and the production of mycotoxins, namely: pre-harvest control, appropriate harvest management and adequate storage conditions.

The aim of this article is to provide information on the occurrence and concentration of mycotoxins in maize and alfalfa silages in some countries of the Europe, and on the prevention of microscopic fungi in silages. The review is focused on the 6 main groups of mycotoxins: aflatoxins, zearalenone, T-2 toxin, ochratoxins, fumonisins and deoxynivalenol.

CONTAMINATION OF MAIZE SILAGES

Several studies point to the fact that contamination of feed and food with mycotoxins is a global problem. Biomin (2021) published the results in an annual report, according to which the incidence of mycotoxins (aflatoxins, zearalenone, T-2 toxin, ochratoxins, fumonisins and deoxynivalenol) in Europe ranges from 47-62%. Mycotoxins can be produced (formed) in maize silage from pre-harvest or post-harvest species of the genus *Fusarium*, *Alternaria, Penicillium* and *Aspergillus* (Dagnac et al., 2016; Panasiuk et al., 2019). Very often, maize is contaminated with fumonisins, with the participation of other mycotoxins (Kosicki et al., 2016). This is also proven by the latest results from Biomin (2021), because in Northern Europe most samples were contaminated with fumonisins, and in Italy all tested samples of maize silage (n=58) were positive for fumonisins. However, deoxynivalenol still poses the greatest threat, contaminating 97% of samples in France, 90% of samples in Germany, 92% of samples in Romania and 68% of samples in Ukraine. Compared to 2019, mycotoxin levels decreased but, conversely, zearalenone increased and for instance in Hungary, up to 71% of samples were positive for zearalenone (Biomin, 2021).

Table 1 Average concentration of mycotoxins in maize silage from some European countries in terms of 12% moisture

Mycotoxin (µg.kg^{-1})						Country	Reference
AFL	ZEA	T-2	OTA	FUM	DON		
10.0[a]	623.0	199.0	-	-	2180.0	Lithuania	Venslovas et al., 2021
-	255.8	-	-	565.1[b]	1316.4	Spain	Dagnac et al., 2016
0.3[a]	61.4	-	-	669.9[c]	297.2	Spain	Rodriguez-Blanco et al., 2019
0.1	61.1	1.7	1.6	19.2	557.2	Poland	Kosicki et al., 2016
-	2065.0	-	-	-	2805.0	Germany	Jensen et al., 2020

-	18.0	-	-	4210.0	160.0	Italy	Biomin, 2021
20.2[a]	-	-	-	-	1949.2	Italy	Gallo et al., 2021

AFL - aflatoxins, ZEA - zearalenone, T-2 - T-2 toxin, OTA - ochratoxins, FUM - fumonisins, DON - deoxynivalenol, [a] - AFB1, [b,c] - FB1 + FB2, - not detected

CONTAMINATION OF ALFALFA SILAGES

Silage is considered a high-value feed in many countries and, in addition to maize, other crops are often ensiled, such as alfalfa (Vaičiulienė et al., 2020; Rodríguez-Blanco et al., 2019). Relatively few authors deal with the occurrence of mycotoxins in alfalfa silage in Europe. Rodriguez-Blanco et al. (2019) found aflatoxins in only one sample of alfalfa silage and the presence of other mycotoxins was ruled out.

Table 2 Average concentration of mycotoxins in alfalfa silage from some European countries in terms of 12% moisture

Alfalfa silage							
Mycotoxin (µg.kg^{-1})						**Country**	**Reference**
AFL	**ZEA**	**T-2**	**OTA**	**FUM**	**DON**		
0.6[a]	237.6	44.0	-	-	484.0	Lithuania	Vaičiulienė et al., 2020
-	67.5	-	-	-	652.7	Czech Republic	Skládanka et al., 2017
-	0.4	-	-	-	114.8	Czech Republic	Hodulíková et al., 2016
2.7[b]	-	-	-	-	-	Spain	Rodríguez-Blanco et al., 2019

AFL - aflatoxins, ZEA - zearalenone, T-2 - T-2 toxin, OTA - ochratoxins, FUM - fumonisins, DON - deoxynivalenol, [a] - AFB1, [b] - AFG1+AFG2, - not detected

In contrast, in an experiment by Hodulíková et al. (2016) deoxynivalenol predominated, and besides it, they also noted the presence of zearalenone. Hodulíková et al. (2016) further state that the preservation

of forage did not affect the content of DON or ZEA, but its wilting may suppress the development of mycotoxins. On the contrary, Skladanka et al. (2019) state that the content of deoxynivalenol was reduced by ensiling green fodder. Also, alfalfa silage in a study by Skladanka et al. (2017) was positive for DON and ZEA, while its concentration was almost 7 times higher than in results of Hodulíková et al. (2016). Vaičiulienė et al. (2020) found the presence of all mycotoxins except OTA and FUM, and according to their research, their concentration can be reduced using herbal extracts. None results of the authors listed in Table 2 exceeded the maximum permitted levels of mycotoxins in complete feedstuffs for adult ruminants.

REGULATION AND PREVENTION

Stoškus et al. (2019) point out that it is necessary to focus on quality and safety, as human and animal health is endangered by feeding low-quality silage to animals. On the surface of each plant there is an epiphytic microflora, which also includes microscopic fungi, which can produce mycotoxins in the pre-harvest and post-harvest stages. (Skladanka et al., 2017; Dell´Orto et al., 2015). According to this knowledge, preventive measures should be implemented before ensiling, and it is appropriate to continue them during ensiling by applying good manufacturing practices. These procedures include ensuring the appropriate dry matter content, maturity stage, correct cut length and particle size of mass, selecting the appropriate covering material and, in addition, correctly assess the size of a silo to the feeding speed of animals. (Dell´Orto et al., 2015). According to Skladanka et al. (2017), the effect of mycotoxins can also be suppressed by using silage additives based on either biological or chemical origin. On the other hand, Vaičiulienė et al. (2020) recommend the use of oregano or thyme extract, or a combination of these herbs with additives. To ensure human and animal health, many countries have established regulations of maximum levels for mycotoxins in feed and food. (Magnoli et al., 2019). These regulations have been implemented in more than 100 countries and therefore differ greatly from one another. The European Union has introduced one of the most extensive and detailed regulations, that has set for all its member countries (Magnoli et al., 2019; Pinotti et al., 2016). Commission

Directive 2003/100/EC, Commission Recommendation 2006/576/EC a Commission Recommendation 2013/165/EU specify maximum acceptable levels of mycotoxins in individual animal feeds. The maximum levels for mycotoxins in complete feedingstuff for adult ruminants are as follows: aflatoxin B1 20 $\mu g.kg^{-1}$, zearalenone 500 $\mu g.kg^{-1}$, T-2 toxin 250 $\mu g.kg^{-1}$, deoxynivalenol 5000 $\mu g.kg^{-1}$, ochratoxin A 100 $\mu g.kg^{-1}$ and fumonisin B1, B2 separately or in combination 50 000 $\mu g.kg^{-1}$ (Commission Recommendation, 2013; European Commission, 2006; European Commission, 2003).

CONCLUSION

The production of high-quality and health-friendly silage is relatively complicated, but with the help of proven procedures it is easier to implement. In addition, mycotoxin contamination has a negative impact on human and animal health, causes economic losses, and is not only a problem in Europe but worldwide. Maize silage was contaminated with fumonisins, with the participation of other mycotoxins (most often DON and ZEA). The authors didn´t find fumonisins in alfalfa silage; on the contrary, the greatest was deoxynivalenol. Due to the presence of mycotoxins, the European Union has set regulation with maximum levels of individual mycotoxins in feed and food. Many authors state that to this issue is not given enough attention, and therefore searching for the best and safest solutions to reduce the development of mycotoxins. These include the use of herbal extracts and silage additives (although their positive effect has not always been demonstrated). It has been shown that the suppression of the development of mycotoxins can be effectively achieved by wilting the forage before ensiling. Firstly however, the attention should be focused on preventive measures before ensiling, and continue during preservation, storage and feeding silage to animals.

ACKNOWLEDGEMENT

This research was funded by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences, project no. 1/0474/19: The application

of additives in animal nutrition for nutrients transformation improve with the accent on quality, safety and sustainability of animal production.

REFERENCES

Biomin (2021). World mycotoxin report impact 2021. Available from https://www.romerlabs.com/en/knowledge-center/knowledge-library/events/news/webinar-world-mycotoxin-report-impact-2021/?utm_campaign=%5BRL%5D%20World%20MTX%20Survey%20Webinar%202021&utm_medium=email&_hsmi=113058909&_hsenc=p2ANqtz-9A9PI7GMCpD_b8mP1kpXRySsBQ6T6pnlSGb5N-UfNUnVcHoP4yiAhtfHuTHb80akwWBN3PCKsei8Bl9RKZbUNNunB8fA&utm_content=113058909&utm_source=hs_email (accessed Aug 10, 2021).

Cogan, T., Hawkey, R., Higgie, E., Lee, M. R. F., Mee, E., Parfitt, D., Raj, J., Roderick, S., Walker, S., Ward, P., Wilkinson, J. M. (2017). Silage and total mixed ration hygienic quality on commercial farms: Implications for animal production. Grass and Forage Science, 72(4), 601-613.

Commission Recommendation No 2013/165/EU of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products. Off. J. L (2013), 91, 12–15.

Dagnac, T., Latorre, A., Fernández Lorenzo, B., Llompart, M. (2016). Validation and application of a liquid chromatography-tandem mass spectrometry based method for the assessment of the co-occurrence of mycotoxins in maize silages from dairy farms in NW Spain. Food Additives & Contaminants: Part A, 33(12), 1850-1863.

Del Palacio, A., Bettucci, L., Pan, D. (2016). Fusarium and Aspergillus mycotoxins contaminating wheat silage for dairy cattle feeding in Uruguay. Brazilian journal of microbiology, 47, 1000-1005.

Dell'Orto, V., Baldi, G., Cheli, F. (2015). Mycotoxins in silage: Checkpoints for effective management and control. World Mycotoxin Journal, 8(5), 603-617.

European Commission. Commission Directive 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. Off. J. L (2003), 285, 33–37.

European Commission. Commission Recommendation 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-

2 and HT-2 and fumonisins in products intended for animal feeding. Off. J. L (2006), 229, 7–9.

Gallo, A., Fancello, F., Ghilardelli, F., Zara, S., Froldi, F., Spanghero, M. (2021). Effects of several lactic acid bacteria inoculants on fermentation and mycotoxins in corn silage. Animal Feed Science and Technology, 277, 114962.

Hodulíková, L., Skládanka, J., Mlejnková, V., Knot, P., Klusoňová, I., Horký, P., Konečná, K., Knotová, D., Nedělník, J., Sláma, P. (2016). Effect of Soil Contamination of Fodder and Wilting on the Occurrence of Fungi and Mycotoxins in Alfalfa Silages. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 64, 169.

Jovaišienė, J., Bakutis, B., Baliukonienė, V., Matusevičius, P., Lipiński, K., Antoszkiewicz, Z., Fijałkowska, M. (2017). Biogenic amines and mycotoxins concentrations in baled silage from organic and conventional farms. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 69, 269-277.

Kosicki, R., Błajet-Kosicka, A., Grajewski, J., Twarużek, M. (2016). Multiannual mycotoxin survey in feed materials and feedingstuffs. Animal Feed Science and Technology, 215, 165-180.

Magnoli, A. P., Poloni, V. L., Cavaglieri, L. (2019). Impact of mycotoxin contamination in the animal feed industry. Current Opinion in Food Science, 29, 99-108.

Panasiuk, L., Jedziniak, P., Pietruszka, K., Piatkowska, M., Bocian, L. (2019). Frequency and levels of regulated and emerging mycotoxins in silage in Poland. Mycotoxin research, 35(1), 17-25.

Pinotti, L., Ottoboni, M., Giromini, C., Dell'Orto, V., Cheli, F. (2016). Mycotoxin contamination in the EU feed supply chain: A focus on cereal byproducts. Toxins, 8(2), 45.

Rodríguez-Blanco, M., Ramos, A. J., Sanchis, V., Marín, S. (2019). Mycotoxins occurrence and fungal populations in different types of silages for dairy cows in Spain. Fungal Biology, 125, 103-114.

Skladanka, J., Adam, V., Zitka, O., Mlejnkova, V., Kalhotka, L., Horky, P., Konecna, K., Hodulikova, L., Knotova, D., Balabanova, M., Slama, P., Skarpa, P. (2017). Comparison of biogenic amines and mycotoxins in alfalfa and red clover fodder depending on additives. International journal of environmental research and public health, 14(4), 418.

Skladanka, J., Kovarova, D., Kolackova, I., Roztocilova, A., Konecna, K., Knotova, D., Horky, P., Skalickova, S., Hodulikova, L., Balabanova, M.,

Slama, P., Knot, P., Čáslavová, I. (2019). Content of mycotoxins in green matter and silage of red clover and alfalfa. In: 18th International Symposium Forage Conservation, Brno, Czech Republic, 60-61.

Vaičiulienė, G., Bakutis, B., Jovaišienė, J., Falkauskas, R., Gerulis, G., Baliukonienė, V. (2020). Origanum vulgare and Thymus vulgaris Extract Usability to Improve Silage Hygienic Quality and Reduce Mycotoxin Concentrations. Journal of Microbiology and Biotechnology, 30 (8), 1149-1155.

Venslovas, E., Merkeviciute-Venslove, L., Mankeviciene, A., Kochiieru, Y., Slepetiene, A., Ceseviciene, J. (2021). The prevalence of mycotoxins and their relation to nutrient composition of maize and grass silage. Zemdirbyste-Agriculture, 108(2).

Fabiszewska, A. U., Zielińska, K. J., Wróbel, B. (2019). Trends in designing microbial silage quality by biotechnological methods using lactic acid bacteria inoculants: a minireview. World Journal of Microbiology and Biotechnology, 35(5), 1-8.

Juan, C., Mannai, A., Salem, H. B., Oueslati, S., Berrada, H., Juan-García, A., Mañes, J. (2020). Mycotoxins presence in pre-and post-fermented silage from Tunisia. Arabian Journal of Chemistry, 13(8), 6753-6761.

Ogunade, I. M., Martinez-Tuppia, C., Queiroz, O. C. M., Jiang, Y., Drouin, P., Wu, F., Vyas, D., Adesogan, A. T. (2018). Silage review: Mycotoxins in silage: Occurrence, effects, prevention, and mitigation. Journal of dairy science, 101(5), 4034-4059.

Jensen, T., De Boevre, M., De Saeger, S., Preußke, N., Sönnichsen, F. D., Kramer, E., Klink, H., Verreet, J.A., Birr, T. (2020). Effect of ensiling duration on the fate of deoxynivalenol, zearalenone and their derivatives in maize silage. Mycotoxin research, 36(2), 127-136.

Stoškus, R., Jatkauskas, J., Vrotniakienė, V., & Juozaitienė, V. (2019). The effect of mixed bacterial inoculant on the microbial population and aerobic stability of lucerne and maize balage. Journal of Animal and Feed Sciences, 28(4), 383-391.

THE EFFECT OF SUBSTITUTING SOY EXTRACT MEAL IN FEED WITH LUPINE SEED MEAL ON THE SLAUGHTER VALUE OF BROILER CHICKENS

MARTIN KUTLVAŠR[1], PAVEL SUCHÝ, EVA STRAKOVÁ[1]

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

Corresponding email address: H20354@vfu.cz

ABSTRACT

The aim of this work was to verify the possibility of substituting soy extract meal with meal from dehulled lupine seeds of the white Zulika variety in feed intended for feeding broiler chickens. The possibility of 50% and 100% substitution was selected for experimental observation in complete broiler chicken feed. Broiler chickens were observed separately according to their sex, and without sex distinction, to study the influence of the substitution on their yield and slaughter value. During our observations, we focused on white lupine as a promising commodity because it can be successfully grown under the climate conditions in the Czech Republic, and given that the content of crude protein in its seeds is comparable with the content of crude protein in soy seeds. White lupine contains high quality protein and quality oil, with a desirable ratio of n-3 and n-6 fatty acids. The benefit of using lupine seed products is that they do not have to be heat treated and can be fed in their natural form. Furthermore, lupine seeds are not a genetically modified organism. Statistically significant ($P \leq 0.05$) differences were captured among the mean values for neck yield in chickens and broiler chickens observed without sex distinction, in heart yield in roosters, and in breast muscle yield in chickens, although we believe that these significances are not in direct relation with lupine in the complete feed in the form of lupine meal. The achieved results confirm that soy extract meal intended for feeding broiler chickens can be substituted with dehulled lupine seeds without reducing the production efficiency of this feed. Based on the

results achieved and based on a number of experiments and operational observations, substituting 50% of soy extract meal with lupine meal can be recommended to breeders, farmers and the feeding industry.

Keywords: soy extract meal; lupine meal; broiler chickens; slaughter value

INTRODUCTION

In European countries, efforts are focused on breeding and increasing the production of protein commodities. For these reasons, it is necessary to especially focus on European culture crops with a high content of crude protein, while also focusing attention on the quality of protein commodities, which is given by the amino acid spectrum. The highest ratio of protein commodities is primarily intended for feeding food animals, where this feed represents an irreplaceable component in the make-up of feed doses and optimizing feed. However, the high price of imported soy commodities and the issue of genetically modified crops, including soy varieties, are exerting pressure to produce other protein feed.

The *Lupinus* genus is part of the legume family of plants (*Fabaceae*). Forty-four genera have been identified in the Czech Republic. It is typical for the majority of species in this group of plants to have tuberous bacteria with nitrogenous bacteria on the roots, which bonds with atmospheric nitrogen, thereby contributing to improving the soil. From the botanical viewpoint, as stated by Pelikán et. al (2012), the legume family has characteristic flowers merged into grape-like flowers, and the pollination of generative organs is provided by insects. One of the most common herbs in our countryside is *Lupinuspolyphyllus*, originally a herb from North America, which is now widespread throughout Europe. Due to its high content of protein in its green matter and especially in the seed, culture varieties of lupines have been developed. It is estimated that there are over 250 individual varieties grown worldwide. It is often labelled as a ‘sweet lupine’. Unlike the wild species, they have a minimal content of alkaloids (Pelikán et. al, 2012). Therefore, they are safe to use in feeding farm animals, although they are also nutritious for humans. The culture species of lupines can be divided into three groups: narrow-leafed, white, and yellow varieties. Lucas et. al (2015) point out that

lupines are Europe's protein crop; the authors go on to claim that it is necessary to create a variety of lupines for sustainable growth by cross-breeding, with a view to obtaining high-quality protein components from lupines and creating products that are marketable. Lupine seeds are very beneficial in diets intended for feeding broiler chickens. Kaczmarek et. al (2016) examined the effect of incorporating white lupine protein meal on the yield and digestibility of nutrients in broiler chickens. The effect of extrusion on the digestibility of nutrients, metabolized energy, and the nutritional value of yellow lupine seeds in broiler chickens was investigated by Rutkowski et. al (2016). The authors concluded that, compared to raw seeds, crude fat was more digestible and nitrogen retention was better after yellow lupine seeds had been extruded. In their experiments, Laudadio and Tufarelli (2011) used peeled micronized lupine (*L. albus*cv. Multitalia) as the main source of protein in the diet of broilers. The aim of this experiment was to find out whether using lupine in the diet affected growth, slaughter value, and the composition of fatty acids in the chicken's meat.

Our interest here was the variety of white lupines, especially the Zulika variety, because its application in the feed industry has been verified by biological tests in feeding ROSS 308 broiler chickens. Our results are only the interim results of the project QJ1510136 "Optimization of the Protein Nutrition of Monogastric Animals on the Basis of White Lupine Seed Varieties (*Lupinusalbus*)", run by the Ministry of Agriculture of the Czech Republic.

MATERIAL AND METHODS

The intermediate goal of this work was to verify the effect of substituting soy extract meal with meal from dehulled lupine seeds of the white Zulika variety by means of biologically monitoring ROSS 308 broiler chickens. The complete feed (control and experimental) had the same component composition, except for the 50% and 100% substitution of soy meal for lupine meal. The component composition of the commercially produced complete feed was based on wheat meal, corn meal, soy extract, lupine meal, soy oils and additives. The preparation of the complete feed was based on Utility Model No. 31533. Table 1 states the nutritional make-up of the feed. Experimental monitoring was done

in an accredited facility of the Faculty of Veterinary Hygiene and Ecology pursuant to technological instructions for feeding ROSS 308 broiler chickens. The experiment on ROSS 308 broiler chickens: Control Group C0% – 80 ♀♂ (40♀ + 40♂), Experimental Group E50% – 80 ♀♂ (40♀ + 40♂), and Experimental Group E100% – 80 ♀♂ (40♀ + 40♂). The broiler chickens were monitored on the 1st–34th day of age, were housed in deep litter, and were fed and given water *ad libitní.* Of the monitored indicators, attention was focused on: live weight (kg), conversion of feed (kg/kg of live weight), mortality of fed broilers (number, %), and the slaughter yield of body and edible muscle and organs. The results were processed by mathematical and statistical methods (Unistat 5.6) using the Tukey-HSD test. The sets were characterized by a mean value ($\bar{x}$) and standard deviation (± SD).

Table 1: Nutritional make-up of feed BR 1, BR 2, BR 3 of the control group and experimental groups

Mixture	**BR 1**				
Nutrient	**C0%**	**E50%**	**Difference (%)**	**E100%**	**Difference (%)**
Crude Protein	247.09	236.70	**-4.21**	243.19	**-1.58**
Fat	54.16	55.56	**2.59**	65.01	**20.04**
Fibre	26.85	23.64	**-11.98**	28.71	**6.91**
NFE	607.69	622.27	**2.40**	606.46	**-0.20**
Starch	428.07	433.96	**1.38**	425.27	**-0.65**
Organic Weight	935.80	938.17	**0.25**	943.36	**0.81**
Ash	64.20	61.83	**-3.68**	56.52	**-11.96**
Net Energy	19.07	18.93	**-0.72**	19.33	**1.35**
Mixture	**BR 2**				
Nutrient	**C0%**	**E50%**	**Difference (%)**	**E100%**	**Difference (%)**
Crude Protein	217.07	210.64	**-2.96**	204.07	**-5.99**
Fat	65.87	73.19	**11.12**	78.29	**18.86**
Fibre	25.83	23.35	**-9.59**	42.59	**64.88**
NFE	630.06	644.09	**2.23**	626.89	**-0.50**
Starch	444.31	468.99	**5.56**	447.73	**0.77**

Organic Weight	938.83	951.28	**1.33**	951.85	**1.39**
Ash	61.06	48.83	**-20.03**	48.15	**-21.14**
Net Energy	19.35	19.56	**1.08**	19.57	**1.17**
Mixture	**BR 3**				
Nutrient	**C0%**	**E50%**	**Difference (%)**	**E100%**	**Difference (%)**
Crude Protein	200.07	183.41	**-8.33**	191.54	**-4.26**
Fat	62.71	79.13	**26.19**	90.06	**43.93**
Fibre	24.35	29.86	**22.63**	22.94	**-5.80**
NFE	665.44	663.04	**-0.36**	651.38	**-2.11**
Starch	492.82	487.26	**-1.13**	479.30	**-2.74**
Organic Weight	952.57	955.33	**0.29**	955.92	**0.35**
Ash	47.43	44.67	**-5.82**	44.08	**-7.07**
Net Energy	19.29	19.53	**1.22**	19.69	**2.05**
NFE - Nitrogen-free extracts					

RESULTS AND DISCUSSION

The presented work is part of a project that has been investigating the possible partial or complete substitution of soy extract meal with lupine seed meal. In the first part we focused on the effectiveness of the feed, which is characterized by the so-called Effectiveness of Feed Index (EFI). The calculation of the Effectiveness of Feed Index is based on the natality rate of chickens (NR), which represents the number of surviving chickens during the whole feeding period, the average weight of the chickens at the end of the feed (EW), the length of feeding (LF), and the conversion of feed (EFI = (NR x AW) : (LF x CF) x 100). Table 2 shows the achieved yield of indicators.

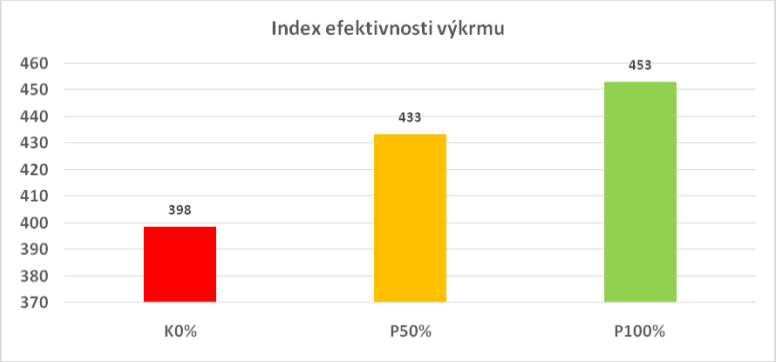
Table 2: Results of the yield indicators for the fed chickens (NR – Natality rate/survival rate, EW – end weight, LF – length of feeding, CF – conversion of feed)

Indicator	Group	
NR (%)	C0%	85.00
	E50%	90.00
	E100%	95.00
AW (kg)	C0%	2.327
	E50%	2.423
	E100%	2.351
LF (days)	C0%	34
	E50%	34
	E100%	34
CF (kg)	C0%	1.46
	E50%	1.48
	E100%	1.45

We calculated the Effectiveness of Feed Index (EFI) based on the results of the yield indicators. The higher the Index, the more effective the feed. The results in Graph 1 clearly show that substituting soy meal with lupine seed significantly increased the effectiveness of the chicken feed. Based on the yield indicators, the natality rate of chickens, which are the surviving chickens housed during the feeding period, significantly affected the increase in the EFI. We conducted several experiments in this project, both at the university and out in the agricultural field. We always observed a lower death rate in chickens when lupine meal was used. This suggests that lupine diets lead to the chicken's improved health.

The results of the work prove that lupine seeds (lupine meal) are a suitable protein component in broiler chicken feed, which is also in line with the findings of Jeroch et. al (2016). Based on their experiments, the authors claim that given the nutritional and dietary values of lupine seeds, they are a suitable feed ingredient for practically all kinds and categories of poultry. This shared conclusion is also consistent with the results of our work, in which we used lupine seed meal as a substitute for soy extract meal in feed intended for broiler chickens.

Graph 1. Results of the EFI for chickens at 50% and 100% soy meal substitute (Effectiveness of Feed Index, C0%, E50%, E100%)



In this interim part of work we were interested in whether substituting soy extract meal with lupine seeds affected the slaughter value of broiler chickens. Ten hens and ten roosters were randomly selected at the end of the experiment (34th feeding day), which was dictated by the mean live slaughter weight of the relevant group. The mean live weight of the selected group of chickens is stated in Table 3.

Table 3: Mean slaughter weight of the selected group, SW – slaughter weight, F – chickens, M – roosters

Indicator	Group	F	M	FM
SW kg	**C0%**	2.28	2.76	2.52
	E50%	2.40	2.85	2.63
	E100%	2.47	2.73	2.60

The weight of the slaughter-processed carcass (WSPC) was calculated from the slaughter value of the broiler chickens after slaughter processing. The calculation of the slaughter yield of edible organs and muscles was based on the weight of the neck, the weight of the heart, the weight of the liver, the weight of the stomach, the weight of the abdominal fat, the weight of breast muscle tissue (both breast muscles), the weight of the thighs (both thighs) and the weight of the thigh muscle tissue after removing the bone. The results of the slaughter analysis are stated in Table 4. The results show that using lupine seed as a substitute for soy meal does not substantially influence the slaughter value of

broiler chickens. Statistically significant ($P \leq 0.05$) differences between the mean values for YN (F, FM), YH (M) and YBM (F) cannot be directly related to the lupine feed.

Table 4: The results of the slaughter analyses of chickens in %, YSPC – yield slaughter of processed chickens, YN – yield of neck, YH – yield of heart, YL – yield of liver, YS – yield of stomach, YAF – yield of abdominal fat, YBM – yield of breast muscle, YT – yield of thighs, YTM – yield of thigh muscle (ab proof $P \leq 0.05$)

Indicator	Group	F	M	FM
YSPC %	C0%	**70.04** ±1.927	**70.59** ±1.559	**70.32** ±1.729
	E50%	**69.98** ±2.038	**70.53** ±1.224	**70.25** ±1.661
	E100%	**70.37** ±1.348	**69.59** ±1.159	**69.98** ±1.286
YN %	C0%	**1.33**[a]±0.189	**1.43**±0.186	**1.38**[a]±0.189
	E50%	**1.14**[b]±0.112	**1.36**±0.213	**1.25**[b]±0.201
	E100%	**1.28**±0.090	**1.35**±0.127	**1.32**±0.112
YH %	C0%	**0.45**±0.038	**0.51**[a]±0.045	**0.48**±0.052
	E50%	**0.46** ±0.050	**0.44**[b]±0.043	**0.45**±0.046
	E100%	**0.45**±0.055	**0.52**[a]±0.067	**0.49**±0.069
YL %	C0%	**1.97**±0.191	**2.03**±0.213	**2.00**±0.199
	E50%	**1.93**±0.213	**2.00**±0.175	**1.96**±0.193
	E100%	**1.93**±0.198	**2.16**±0.169	**2.04** ±0.215
YS %	C0%	**0.84**±0.120	**2.03**±0.213	**0.82**±0.141
	E50%	**0.83**±0.092	**2.00**±0.175	**0.75**±0.123
	E100%	**0.83**±0.099	**2.16**±0.169	**0.78**±0.094
YAF %	C0%	**1.34**±0.373	**1.30**±0.342	**1.32**±0.349
	E50%	**1.47**±0.233	**1.43**±0.311	**1.45**±0.268
	E100%	**1.31**±0.518	**1.25**±0.350	**1.28**±0.431
YBM %	C0%	**22.29**±1.304	**22.51**±1.085	**22.40**±1.173
	E50%	**20.95**[b]±1.506	**22.24**±1.009	**21.59**±1.412
	E100%	**22.57**[a]±1.192	**21.79**±0.993	**22.18**±1.140
YT %	C0%	**17.24**±1.106	**18.41**±1.362	**17.83**±1.348
	E50%	**17.78**±0.989	**18.36**±0.916	**18.07**±0.973
	E100%	**18.26**±0.078	**17.41**±3.835	**17.83**±2.729
YTM %	C0%	**13.83**±1.371	**14.47**±1.262	**14.15**±1.324
	E50%	**14.19** ±0.967	**14.50** ±0.892	**14.34** ±0.920
	E100%	**14.59** ±0.823	**14.85** ±0.850	**14.72** ±0.825

The results we obtained were in accordance with the results of the experimental work conducted by Laudadio and Tufarelli (2011) and

Nalle et. al (2012), who state that including lupine into the diet did not have any negative effect on the slaughter value of broilers. Mikulski et. al (2014) drew similar conclusions regarding the feed of turkeys, and Laudadio and Tufarelli (2011) came to similar conclusions regarding the feed of guinea fowl.

CONCLUSION

Based on the results obtained, it can be stated that substituting soy meal with meal from dehulled lupine seeds was effective (evaluated based on the calculated Effectiveness of Feed Index). This is mainly due to the improved health of the chickens in the experimental group (evaluated based on the significantly lower death rate – higher birth rate).
The fact that administering diets containing lupine meal does not negatively influence the slaughter value of the broiler chickens is seen as a positive outcome (evaluated based on the broiler chicken's yield, edible organs and muscles).

ACKNOWLEDGEMENT

The results of this work were financially supported by the project QJ1510136 "Optimization of Protein Nutrition of Monogastric Animals on the Basis of White Lupine Seed Varieties (*Lupinus albus*)" run by the Ministry of Agriculture of the Czech Republic.

REFERENCES

Jeroch H., Kozłowski K., Schöne F., Zduńczyk Z. (2016): Lupines (Lupinusspp.) as a protein feed stuff for poultry. 1) varieties, composition and nutritional values for poultry. European Poultry Science, 80, 14.

Kaczmarek SA., Hejdysz M., Kubiś M., Rutkowski A. (2016): Influence of graded inclusion of white lupin (Lupinusalbus) meal on performance, nutrient digestibility and intestinal morphology of broiler chickens. British Poultry Science, 57, 364–374.

Laudadio V., Tufarelli V. (2011): Dehulled-micronised lupin (Lupinusalbus L. cv. Multitalia) as the main protein source for broilers: influence on growth performance, carcass traits and meat fatty acid composition. Journal of the Science of Food and Agriculture, 91, 2081–2087.

Lucas MM., Stoddard FL, Annicchiarico P., Frías J., Martínez-Villaluenga C., Sussmann, D., Duranti M., Seger A., Zander PM., Pueyo JJ. (2015): The future of lupin as a protein crop in Europe. Frontiers in Plant Science. 6 (September), art. no. 705, 6.

Mikulski D., Zduńczyk Z., Juśkiewicz J., Rogiewicz A., Jankowski J. (2014): The effect of different blue lupine (L. angustifolius) inclusion levels on gastrointestinal function, growth performance and meat quality in growing-finishing turkeys. Animal Feed Science and Technology, 198, 347–352.

Nalle CL., Ravindran V., Ravindran G. (2012): Nutritional value of white lupins (Lupinusalbus) for broilers: apparent metabolisable energy, apparentileal amino acid digestibility and production performance. Animal, 6, 579–585.

Pelikán J., Hýbl M. (2012): FabaceaeLINDL Plant Families. (Legumes) Czech Republic. Agricultural Research, Ltd. Troubsko. Crop Research Institute, Ltd. Troubsko. Baštan Publishing, 230 s.

RutkowskiA., Kaczmarek SA., Hejdysz M., Jamroz D. (2016): Effect of extrusion on nutrients digestibility, Metabolizable Energy and Nutritional value of yellow lupine seeds for broiler chickens. Annals of Animal Science, 16, 1059–1072.

IN VITRO ANTIBACTERIAL COMBINATORY EFFECT OF GENTAMICIN AND ZINC PYRITHIONE

LUCIE MALÁ[1,2], KLÁRA LALOUČKOVÁ[1,2], EVA SKŘIVANOVÁ[1,2]

[1]Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Praha 6 – Suchdol, Czech Republic

[2]Department of Nutritional Physiology and Animal Product Quality, Institute of Animal Science, Přátelství 815, 104 00 Praha 22 - Uhříněves Czech Republic

Corresponding email address: malalucie@af.czu.cz

ABSTRACT

Streptococcal strains of bacteria belong to the major pathogen groups inducing those causing bovine mastitis. They not only negatively impact economic profit due to milk losses and therapy costs, but they are an important animal health and welfare issue as well. Thus, antimicrobial therapy is the main way of treatment for dairy cows infected with mastitis-causing microorganisms. However, selective pressure on bacteria caused by excessive prescribing and using of antibiotics in veterinary medicine, is one of the main causes of bacterial resistance to antibiotics. In addition to the possibility of using antibacterial alternatives, a reduction in the prevalence of antibiotic-resistant bacteria can be achieved by combining several substances (either pure alternatives or other substances with antibiotics) at significantly lower concentrations than the antibacterial dose alone. The aim of this study was to evaluate *in vitro* combinatory effect of gentamicin and zinc pyrithione against three bovine mastitis strains, especially *Streptococcus agalactiae* (CCM 6187, DSM 6784) and *Streptococcus dysgalactiae* (DSM 20662), by the standardized microdilution checkerboard method in 96-well microtitration plates. The minimum inhibitory concentrations (MICs), that are necessary to evaluate the fractional inhibitory concentrations (FICs), were determined as the lowest concentration

limiting the growth of bacteria in wells compared to a positive control by ≥80%. The combinatory effect of gentamicin and zinc pyrithione was evaluated by values of FICs indices: synergy (FICs ≤0.5); indifference (FICs 0.5≤4); antagonism (FICs >4). Results showed significant synergistic effect against all tested streptococcal strains. The lowest FIC was found in *Str. dysgalactiae* (DSM 20662) (FIC 0.22). However, another tested strains *Str. agalactiae* (CCM 6187, DSM 6784) showed comparable values of FICs. Regarding the synergistic effect, values of FICs ranged from 0.22 to 0.39.

Keywords: gentamicin; zinc pyrithione; bovine mastitis; synergistic effect

INTRODUCTION

Bacteria of the genus *Streptococcus* are a major mastitis-causing pathogens present in dairy products (Forsman et al. 1997) causing also economic losses in animal production and affecting human health (Richards et al. 2014). Streptococcal species, *Streptococcus agalactiae* and *Streptococcus dysgalactiae*, are the main species involved in clinical and subclinical bovine mastitis (Lundberg et al. 2014; Richards et al. 2014), which is defined as "inflammation of the mammary gland" (Erskine et al. 2004). The worldwide estimated economic losses of farms caused by clinical mastitis are between $69 and $110 per cow (Hogeveen et al. 2011). The loss comprises production losses connected with reduced milk production, treatment losses linked to necessary remedies, and loss of animal value due to the fibrotic changes in the udders (Jingar et al. 2017).

The main treatment for dairy cows with mastitis is antimicrobial therapy, but usage of antibiotics is accompanied with various disadvantages, including a low cure rate, increasing occurrence of resistance, and the presence of antibiotic residues in milk (Gomes and Henriques 2016). Commonly used antimicrobial classes for the treatment of streptococcal mastitis are β-lactams, macrolides and lincosamides (Barkema et al. 2006). However, long-term, widespread, and unreasonable use of the antimicrobials have made bacteria more resistant. For example, the main pathogenic *Streptococcus* spp. isolates that cause mastitis are resistant to penicillin, erythromycin and enrofloxacin, or lincomycin (Klimiene et al.

2011). In addition to antibiotic therapy, there are modern approaches of mastitis treatment and prevention including intramammary teat seals (Kromker et al. 2014) and anti-inflammatory drugs (Breen 2017).

Nevertheless, antimicrobial resistance in mastitis-causing streptococci is abundant and slightly increasing. The degree of resistance is strongly dependent on the streptococcal species, antibiotic type, location, and herd (Kabelitz et al. 2021). As more antibiotics are rendered ineffective by drug-resistant bacteria, focus must be shifted towards alternative therapies for treating infections (Ghosh et al. 2019). There is a number of antibacterial and immune compounds used in livestock such as probiotics and prebiotics (Gibson et al. 2004), synbiotics (Bomba et al. 2006), organic acids (Gibson et al. 2004), and clay minerals (Lemke et al. 2001), but also feed enzymes and phytogenic additives may be a way of concept in mastitis prevention (Toghyani et al. 2011). Antimicrobial peptides, immunomodulators, vaccines or bacteriophages can also be used for a prevention or treatment of invasive diseases, and they are often used in relation with bovine mastitis (Bretaudeau et al. 2020).

In addition to the use of antibacterial alternatives, a reduction in the prevalence of antibiotic-resistant bacteria can be achieved by combining several substances (either pure alternatives or other substances with antibiotics) at significantly lower concentrations than the antibacterial dose alone (Soren et al. 2015).

In recent years, frequently discussed compound is the so-called zinc pyrithione, which has strong antibacterial activity, especially against Gram-positive pathogens (Blanchard et al. 2016; Schwartz et al. 2016). Considering the considerable antibacterial effects of zinc pyrithione, it is possible to test the combination of this promising compound together with gentamicin, which has synergistic effects in combination with various substances (Chaves & Tadi 2020). Based on the mechanism of action of these compounds, there is a presumption of an enhanced effect, which could lead to the application of combination gentamicin/zinc pyrithione in practice.

The present research was carried out to evaluate combinatory effect of gentamicin and zinc pyrithione against bovine streptococcal strains, namely *Str. agalactiae* and *Str. dysgalactiae*, by the checkerboard method in broth.

MATERIAL AND METHODS

Bacterial strains and culture media

The bacterial type strains were purchased from the Czech Collection of Microorganisms (CCM; Brno, CZ) and from the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen – DSM; Braunschweig, DE).
The combinatory effect of tested compounds was determined against *Str. agalactiae* (CCM 6187, DSM 6784) and *Str. dysgalactiae* (DSM 20662) that were during the experiment grown and maintained in Tryptone-Soya broth (TSB) (Oxoid; Prague, CZ) for CCM strain and in Tryptone-Soya broth with Yeast Extract (Oxoid; Prague, CZ) for DSM strains. The bacterial cultures were incubated at 37 ºC for 24 h under aerobic conditions.

Evaluation of minimum inhibitory concentrations and combinatory effect

Using guidelines of the Clinical and Laboratory Standards Institute (CLSI 2015), the antibacterial activities of gentamicin and zinc pyrithione were evaluated *in vitro* by the broth microdilution method, modified as per the recommendations of Cos et al. (2006). The antibacterial combinatory effect of gentamicin with zinc pyrithione was tested *in vitro* using the microdilution broth checkerboard methods, as described in the Clinical Microbiology Procedures Handbook (Leber, 2016). The determination of minimal inhibitory concentrations (MICs) of gentamicin and zinc pyrithione, as well as determination of fractional inhibitory concentrations (FICs), was performed in 96-well microtitration plates. For the testing of combinatory effects of gentamicin/zinc pyrithione, eight two-fold serial dilutions of gentamicin were placed in the horizontal rows of the plate and were subsequently cross-diluted vertically by eight twofold serial dilutions of zinc pyrithione, resulting in 64 different combinations of concentrations. The initial concentrations of zinc pyrithione (0.5–4 µg/ml) as well as gentamicin (2-8 µg/ml) were chosen based on the tested strain.
The bacterial inoculum was standardized to achieve a density of 5 x 10^5 CFU/ml using the McFarland scale and inoculated into wells (10 µl). Microplates were incubated at 37 ºC for 24 h under aerobic conditions.

The growth of microorganisms was assessed as the turbidity determined by an Infinite 200® PRO microplate reader (Tecan, Männedorf, Switzerland) at 405 nm. The MICs were calculated as the mode of lowest compound concentrations that resulted in an 80% growth reduction compared to that of the compound-free growth control concentrations. The combinatory effects of gentamicin/zinc pyrithione were evaluated as ΣFIC, derived from the equation:

$$\Sigma FIC = FIC_A + FIC_B$$

FIC_A = MIC_A in the presence of B/MIC_A (alone);

FIC_B = MIC_B in the presence of A/MIC_B (alone)

According to the value of FICs, three different types of interactions can be defined (Odds 2003): synergy (ΣFIC ≤0.5); indifference (ΣFIC 0.5≤4); antagonism (ΣFIC >4).

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

RESULTS AND DISCUSSION

Complete results of combinatory effect testing of gentamicin and zinc pyrithione calculated as mean values are presented in Table 1 and 2.

Results showed significant synergistic effect against all of the tested streptococcal strains. The lowest FIC was found in *Str. dysgalactiae* DSM 20662 (FIC 0.22). However, another tested strains *Str. agalactiae* CCM 6187, and DSM 6784 showed similar values of FICs. Regarding the synergistic effect, values of FICs ranged from 0.22 to 0.39.

In recent years, no comprehensive study of the combination effect of zinc pyrithione with antibiotics has been performed. However, for the successful implementation of combination therapy of substances, the knowledge of their MICs, and the knowledge of the mechanisms of action determining the character of a particular substance, is important (Basri et al. 2014).

Zinc pyrithione is a bactericidal substance, which originates from the naturally occurring antimicrobial substance - aspergillic acid (Woodward 1947). Previous studies confirmed its strong antimicrobial activity of against Gram-positive and Gram-negative bacteria (Blanchard et al. 2016; Schwartz et al. 2016). The available data on the mode of

antimicrobial action suggest that zinc pyrithione is membrane active compound, as indicated by the inhibition of uptake of several unrelated substrates in both bacteria and fungi (Chandler et al. 1978). Further investigation on the action at the membrane suggests that zinc pyrithione forms stable interactions with the bacterial membrane phospholipid phosphatidylethanolamine resulting to disaggregate phospholipids on the outside of the membrane. In addition, current voltage analysis demonstrates that the depolarization of the bacterial membrane is accompanied by a decrease in membrane electrical conductance in a manner consistent with inhibition of the primary proton pump and consistent with a mode of action of zinc pyrithione on plasma membrane ion channels (Dinning et al. 1998a). Therefore, zinc pyrithione inhibits membrane transport via a direct or indirect effect on the primary proton pump that energizes transport, and the site of action of zinc pyrithione is likely to be intracellular rather than extracellular (Dinning et al. 1998b). Other studies on the mode of action of this substance has demonstrated its potent bactericidal activity to be linked to the ability to chelate, for example to form cyclic complexes with the ions of heavy metals (Albert et al. 1956).

Gentamicin is a commonly used antibiotic in veterinary medicine which has been used for the treatment of a wide range of infections caused by Gram-positive and Gram-negative bacteria (Corvec et al. 2013). The antibacterial mechanism of action of gentamicin is to bind to the 30S ribosomal subunit, thereby inducing inhibition of protein synthesis and causing the bacterial cell to die (Yoshizaw 1998).

By binding active hydroxyl or amine groups of gentamicin, complexes with metals can be formed. Therefore, combination of gentamicin and zinc pyrithione may increase the bactericidal activity of both substances. The mechanism leading to this effect is supposed to be related with the production of reactive oxygen species of zinc pyrithione under the influence of gentamicin, which cause oxidative stress inside the cells (Wang et al. 2016). Free radicals can act directly on the polyunsaturated fatty acids present in the membranes of bacterial cells, and thus initiate lipid peroxidation. The primary effect of lipid peroxidation is to alter membrane properties, which can significantly damage the membrane-bound proteins (Humphries & Sweda 1998).

Table 1. The combinatory effect of gentamicin and zinc pyrithione against *Streptococcus agalactiae*.

	MIC of tested compounds (µg/ml):		**GEN + ZnP in concentration (µg/ml):**													
			0,25		0,125		0,0625		0,03125		0,015625		0,0078125		0,00390625	
Strain *Str.A.*	MIC GEN	MIC ZnP	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC
CCM 6187	3,33	0,25	0,06	1,02	0,27	0,58	0,35	**0,36**	0,42	**0,25**	0,67	**0,26**	0,67	**0,23**	1,67	0,52

	MIC of tested compounds (µg/ml):		**GEN + ZnP in concentration (µg/ml):**													
			2		1		0,5		0,25		0,125		0,0625		0,03125	
	MIC GEN	MIC ZnP	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC
DSM 6784	2,11	0,69	0,06	2,91	0,06	1,47	0,06	0,75	0,06	**0,39**	0,17	**0,26**	0,33	**0,25**	0,53	**0,30**

GEN – gentamicin; ZnP – zinc pyrithione; *Str.A.* – *Streptococcus agalactiae*; MIC – minimal inhibitory concentration; FIC – fraction inhibitory concentration.

Table 2. The combinatory effect of gentamicin and zinc pyrithione against *Streptococcus dysgalactiae.*

	MIC of tested compounds (µg/ml):		**GEN + ZnP in concentration (µg/ml):**													
			1		0,5		0,25		0,125		0,0625		0,03125		0,015625	
Strain *Str.D.*	MIC GEN	MIC ZnP	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC	MIC GEN	FIC
DSM 20662	0,94	0,83	0,02	1,22	0,02	0,62	0,08	**0,39**	0,09	**0,24**	0,14	**0,22**	0,70	0,51	0,77	0,58

GEN – gentamicin; ZnP – zinc pyrithione*; Str.D. – Streptococcus dysgalactiae;* MIC – minimal inhibitory concentration; FIC – fraction inhibitory concentration

In summary combination of gentamicin and zinc pyrithione possess significant synergistic effect against all tested streptococcal strains resulting of their strong activity against streptococcal species.

CONCLUSION

The data obtained during the experiment confirmed that combination of GEN and ZnP has significant synergistic effect to bovine mastitis-causing strains of *Str. agalactiae* (CCM 6187, DSM 6784) and *Str. dysgalactiae* (DSM 20662). Results of this work may suggest the use of the combination of these substances in practice. Nevertheless, these assumptions need to be confirmed by *in vivo* studies.

ACKNOWLEDGEMENT

The project was supported by the Centre for the investigation of synthesis and transformation of nutritional substances in the food chain in interaction with potentially harmful substances of anthropogenic origin: comprehensive assessment of soil contamination risks for the quality of agricultural products (No. CZ.02.1.01/0.0/0.0/16_019/0000845), and by the by the project of the Ministry of Agriculture of the Czech Republic No. MZeRO0718.

REFERENCES

Albert A., Rees C.W., Tomlinson A.J.H. (1956): The influence of chemical constitution on anti-bacterial activity. Part VIII. 2-Mercaptopyridine-N-oxide, and some general observations on metal binding agents. British Journal of Experimental Pathology, 37, 500-511.

Barkema H.W., Schukken Y.H., Zadoks R.N. (2006): Invited review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. Journal of Dairy Science, 89, 1877–1895.

Basri D.F., Xian L.W., Abdul Shukor N.I.M, Latip J. (2014): Bacteriostatic antimicrobial combination: Antagonistic interaction between Epsilon-Viniferin and vancomycin against methicillin-resistant *Staphylococcus aureus*. Biomed Research International, 1-8.

Blanchard C, Brooks L, Ebsworth-Mojica K, Didione L, Wucher B, Dewhurst S, Krysan D, Dunman PM, Wozniak RAF, Fey PD. (2016): Zinc Pyrithione Improves the Antibacterial Activity of Silver Sulfadiazine Ointment. MSphere.

Bomba A., Jonecova Z., Koscova J., Nemcova R. (2006): The improvement of probiotics efficacy by synergistically acting components of natural origin: a review. Biologia, 61, 729-734.

Breen J. (2017): The importance of non-steroidal anti-inflammatory drugs (NSAIDs) in mastitis therapeutics. Livestock, 4, 182–185

Bretaudeau L, Tremblais K, Aubrit F, Meichenin M, Arnaud I. (2020): Good manufacturing practice (GMP) compliance for phage therapy medicinal products. Frontiers in Microbiology, 11.

CLSI. (2015): Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard M07-A10 – Tenth Edition. Clinical and Laboratory Standards Institute, Wayne, PA.

Corvec S., Tafin U.F., Betrisey B., Borens O., Trampuz A. (2013): Activities of fosfomycin, tigecycline, colistin, and gentamicin against extended-spectrum-lactamase-producing escherichia coli in a foreign-body infection model. Antimicrobial Agents Chemother, 57, 1421–1427.

Cos P., Vlietinck A.J., Berghe D.V., Maes L. (2006): Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. Journal of Ethnopharmacology, 106, 290-302.

Dinning A.J., Al-Adham I.S.I., Eastwood I.M., Austin P., Collier P.J. (1998a): Pyrithione biocides as inhibitors of bacterial ATP synthesis. Journal of Applied Microbiology, 85, 141-6.

Dinning A.J., AL-Adham I.S., Austin P., Charlton M., Collier P.J. (1998b): Pyrithione biocide interactions with bacterial phospholipid head groups. Journal of Applied Microbiology, 85, 132-140.

Erskine R., Cullor J., Schaellibaum M., Yancey B., Zecconi A. (2004): Bovine mastitis pathogens and trends in resistance to antibacterial drugs. In National Mastitis Council Research Committee Report, Proceedings of the Annual Meeting North Carolina, USA, 1st of January. NMC Research Committee, Minnesota, USA.

Forsman P., Tilsala-Timisjärvi A., Alatossava T. (1997): Identification of staphylococcal and streptococcal causes ofbovine mastitis using 16S-23S rRNA spacer regions.Microbiology, 143, 3491–3500.

Gibson G.R., Probert H.M., Loo J.V., Rastall R.A., Roberfroid M.B. (2004): Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutrition Research Reviews, 17, 259-275.

Gomes F., Henriques M. (2016): Control of bovine mastitis: Old and recent therapeutic approaches. Current Microbiology, 72, 377–382.

Gosh Ch., Sarkar P., Issa R., Haldar J. (2019): Alternatives to conventional antibiotics in the era of antimicrobial resistance. Trends in Microbiology, 27, 323-338.

Hogeveen H., Huijps K., Lam TJ.G.M (2011): Economic aspects of mastitis: new developments. New Zealand Veterinary Journal, 58, 16-23.

Humphries K.M., Szweda L.I. (1998): Selective inactivation of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase: reaction of lipoic acid with 4-hydroxy-2-nonenal. *Biochemistry*, *37*, 15835-15841.

Chandler C.J., Segel I.H. (1978): Mechanism of the antibacterial action of pyrithione: effects on membrane transport, ATP levels and protein synthesis. Antimicrob Agents Chemother, 14, 60-8.

Chaves B.J., Tadi P. (2020): Gentamicin. In: StatPearls. StatPearls Publishing, Treasure Island (FL).

Jingar S.C., Singh M., Roy A.K. (2017): Economic losses due to clinical mastitis in cross-bred cows. International Journal of Livestock Research, 8, 254–263.

Kabelitz T., Aubry E., van Vorst K., Amon T., Fulde M. (2021): Role of *Streptococcus* spp. in bovine mastitis. Microorganisms, **9**, 149.

Klimiene I., Ruzauskas M., Spakauskas V., Matusevicius A., Mockeliunas R., Pereckiene A., Butrimaite Ambrozevicienl C., Virgailis M. (2011): Antimicrobial resistance patterns to beta-lactams of gram-positive cocci isolated from bovine mastitis in Lithuania. Polish Journal of Veterinary Science 14, 467-472.

Kromker V., Grabowski N.T., Friedrich J. (2014): New infection rate of bovine mammary glands after application of an internal teat seal at dry-off. Journal of Dairy Research, 81, 54–58.

Leber A. (2016): Synergism Testing: Broth Microdilution Checkerboard and Broth Macrodilution Methods. Leber A, editor. Clinical Microbiology Procedures Handbook. ASM Press, Washington, DC.

Lemke S.L., Mayura K., Reeves W.R., Wang N., Fickey C., Phillips T.D. (2001): Investigation of organophilic montmorillonite clay inclusion in zearalenonecontaminated diets using the mouse uterine weight bioassay. Journal of Toxicology and Environmental Health, 62, 243-258.

Lundberg Å., Nyman A., Unnerstad H.E., Waller K.P. (2014): Prevalence of bacterial genotypes and outcome ofbovine clinical mastitis due to Streptococcus dysgalactiae and Streptococcus uberis. Acta Veterinary Scandinavia, 56, 80.

Odds FC. (2003): Synergy, antagonism, and what the chequerboard puts between them. Journal of Antimicrobial Chemotherapy, 52, 1.

Richards V.P., Palmer S.R., Bitar P.D.P., Qin, X., Weinstock G.M., Highlander S.K., Town C.D., Burne R.A., Stanhope M.J. (2014): Phylogenomics and the dynamic genome evolution f the genus Streptococcus.Genome Biologx and Evolution, 6, 741–753.

Schwartz J.R. (2016): Zinc Pyrithione: A topical antimicrobial with complex pharmaceutics. Journal of Drugs in Dermatology, 15, 140-144.

Soren O., Brinch K.S., Patel D., Liu Y., Liu A., Coates A., Hu Y. (2015): Antimicrobial peptide novicidin synergizes with rifampin, ceftriaxone, and ceftazidime against antibiotic-resistant Enterobacteriaceae in vitro. Antimicrobial Agents and Chemotherapy, 59, 6233-6240.

Toghyani M, Toghyani M, Gheisari A, Ghalamkari G, Eghbalsaied S. (2011): Evaluation of cinnamon and garlic as antibiotic growth promoter substitutions on performance, immune responses, serum biochemical and haematological parameters in broiler chicks. Livestock Science, 138, 167-173.

Wang Y., Tang H., Wu D., Dong L., Yuanfang L., Aoneng C., Wang H. (2016): Enhanced bactericidal toxicity of silver nanoparticles by the antibiotic *gentamicin. Environmental Science: Nano Journal*, 3, *788–798.*

Woodward C.R. (1947): Production of Aspergillic Acid by Surface Cultures of *Aspergillus flavus*. Journal of Bacteriology, 54, 375-379.

Yoshizawa S., Fourmy D., Puglisi J.D. (1998): Structural origins of gentamicin antibiotic action. The EMBO Journal, 6437-6448.

IN VITRO FERMENTATION PROFILE IN THE CAECUM OF RABBITS WITH USE OF DRIED COW COLOSTRUM

KATARZYNA MIKOŁAJCZYK-LASZECKA[1], EWA PECKA-KIEŁB[2], DOROTA MIŚTA[2], BOŻENA KRÓLICZEWSKA[2], JOLANTA BUJOK[2], FRANTISEK ZIGO[3], ANDRZEJ ZACHWIEJA[1]

[1] Institute of Animal Breeding Wroclaw University of Environmental and Life Sciences, ul. Chelmonskiego 38C, 51-631 Wroclaw, Poland;

[2] Department of Animal Physiology and Biostructure, Wroclaw University of Environmental and Life Sciences, Norwida 31, 50-375 Wroclaw, Poland;

[3]University of Veterinary Medicine and Pharmacy in Košice, Department of Animal Husbandry Košice, Slovakia

Corresponding email address: katarzyna.mikolajczyk@upwr.edu.pl

ABSTRACT

The purpose of this study was to determine the effect of dried bovine colostrum on the *in vitro* fermentation profile in the caecum of rabbits. The material for the study consisted of the caecal contents collected from eight White Giant rabbits. The obtained material was mixed with a buffer in the 1:4 ratio and homogenized. The caecal samples were allocated to three groups: control group (C) - 1 g of mixed concentrate, groups D1 and D2 with 0.1 g and 0.2 g of dried colostrum, respectively; the samples were then subjected to 12-hour fermentation under anaerobic conditions at 39 °C . After fermentation, the pH value was determined, the amount of methane produced was measured, and the level and profile of volatile fatty acids (VFAs) were established using a gas chromatograph. In addition, ammonia levels were determined in the samples using a modified Conway microdiffusion method, and readings were taken using a Lambda XLS spectrophotometer. Bovine colostrum used as a feed substrate was collected from 9 Polish Holstein-Friesian cows of Black-and-White variety, kept in the free-stall system and fed TMR. Before drying, the colostrum was subjected to selection in order to eliminate

samples of low microbiological quality. The results of the study were statistically analysed using one-way analysis of variance (ANOVA). The obtained results show a beneficial effect of dried bovine colostrum on the fermentation profile in the rabbit caecum, resulting in a decrease in the production of methane and ammonia.

Key words: rabbits, *in vitro* fermentation, caecum, dried colostrum, VFA, methane

INTRODUCTION

High-yielding cows produce as much as 25 liters of colostrum in the first day after calving, while a calf consumes about 4-5 liters; colostrum production in sheep is 0.4 - 1.3 liters (Szulc and Zachwieja, 1998, Daels, 2006). Excess colostrum, especially of good quality, can be preserved and then used as a source of immunoglobulins, minerals and biologically active substances not only in calf nutrition but also in other animal species and in human diet (Zachwieja and Knecht 1999, Huguet et al., 2006, Nagaraja et al., 2011, Pandey et al., 2011, Zachwieja et al., 2014, Pecka et al., 2012).

Ruminant colostrum has nutraceutical, immunomodeling, antioxidant, and antihypertensive properties (Huguet et al., 2006, Nagaraja et al., 2011, Mikolajczyk et al., 2019). It is full of antibacterial and bacteriostatic substances, including immunoglobulins, lactoperoxidase, lactenins, lactoferrin, lysozyme, and leukocytes (Gapper et al., 2007, Boudry et al., 2008). It also contains cytokines and growth hormones that enhance intestinal development in newborns (Palm et al., 2013, Li et al., 2013).

Rabbits are medium-sized herbivorous mammals in which microbial fermentation processes occur in the cecum (Kuijper et al., 2004). Volatile fatty acids (VFA), which are produced, among others, in the cecum of rabbits, provide 30 to 40% of the daily requirement of metabolic energy (Engelhardt, 1995). They influence intestinal peristalsis, promote mucosal blood flow and are responsible for the transport of chloride and sodium through the intestinal wall (Vidyasagar and Ramakrishna, 2002). During fermentation of the caecal contents of healthy and adequately fed rabbits, the percentages of acetate to propionate and butyrate should be

(respectively): 73.3-77.2%, 5.5- 7.56%, 15.12-18.90%, and the ammonia content is about 4 mmol/l (Gidenne and Bellier 2000, García et al., 2000, Belenguer et al., 2008).

There is a close relationship between the type and abundance of bacteria in the caecum and the level and proportion of individual volatile fatty acids (Sirotek et al., 2004). The microbiome in the caecum of animals is highly dependent on their diet. The feed additive introduced to rabbit diet should not negatively affect the gastrointestinal bacteria and consequently the fermentation processes in the caecum, whose products include volatile fatty acids (Engelhardt, 1995, García et al., 2000, Sirotek et al., 2004, Guedes et al., 2009).

The level of volatile fatty acids produced in the caecum and their mutual proportions may be treated as one of the exponents of appropriate animal nutrition. Therefore, it seems important to analyze the influence of the addition of dried bovine colostrum to feed on the level of VFAs, methane and ammonia in the caecal content. The activity of liver enzymes in blood is also a source of information about metabolic processes taking place in the animal organism. The use of different feed additives affects the stimulation of protein fraction levels and liver enzyme activity in rabbit serum (Ahamefule et al., 2008, Dkhil and Al-Quraishy, 2010, Ogunsan et al., 2011).

The aim of this study was to determine the effect of addition of dried bovine colostrum on the in vitro fermentation profile in the caecum of rabbits.

MATERIAL AND METHODS

Preparation of dried bovine colostrum

Bovine colostrum for substrate was collected during the first full milking after parturition from 9 Polish Holstein-Friesian cows of Black-and-White variety kept in the free-stall system and fed TMR; the animals were in their second or third lactation. Before drying the colostrum, the number of somatic cells and the total number of microorganisms in the mammary gland secretion were determined with the use of Somacount 150 Bentley Instruments Inc. apparatus from each cow in order to eliminate the samples in which these numbers exceed 400 thousand/ml SCC and 100 thousand/ml TMC.

The colostrum thus selected was homogenized and then dried. Drying process was performed using a B-290 spray dryer made by BUCHII at pressure 7.5 bar and 140˚C inlet 140˚C and 60˚C outlet air temperature.

Animals and Nutrition

Eight White Giant rabbits were maintained until 14 weeks of age (3454±493.27g) in stainless steel cages with *ad libitum* access to drinking water and complete pellet feed according to rabbit feeding standards (Table 1) (Gugolek, 2011).

Table 1. Composition of complete feed used in rabbit diet

Ingredients	
Dried grass[%]	13
Dried alfalfa [%]	15
Wheat bran [%]	17
Wheat grain [%]	10
Barley grain [%]	10
Corn [%]	15
Extraction soybean meal [%]	10
Rape meal [%]	5
Rapeseed oil [%]	1
Vitamin-mineral premix[1] [%]	4

Table 2. Basic composition of substrates used in *in vitro* fermentation

Group	**Dry matter %**	**Ash %**	**Protein %**	**Fat %**	**Energy MJ/kg**
C	98.00	5.50	17.00	3.60	10.95
D1	95.29	4.36	40.82	15.45	20.17
D2	97.84	3.49	64.39	40.71	24.49

***In vitro* fermentation of caecal content**

The experimental material consisted of caecal content samples collected from eight rabbits. The obtained material was mixed with a buffer (Janssen et al., 2009) in the 1:4 ratio and homogenized. The caecal content samples were assigned to three groups: control group (C), where 1 g of mixed concentrated feed of known composition was used as substrate (Table 1 and 2); in groups D1 and D2 the substrate was 0.1 and 0.2 g of dried colostrum (Table 2).

To obtain anaerobic conditions, the bottles were saturated with CO_2 fed from a pressure cylinder. They were then sealed tightly using a capping machine. The samples were subjected to 12 h *in vitro* fermentation in a shaking water bath at 39˚C.

Analysis of selected fermentation products

At the end of incubation in the serum bottles, the overhead pressure generated by the fermentation gases was measured. The gas obtained was analyzed for methane content using a gas chromatograph (Agilent Technologies 7890A GC System) with TCD and FID detectors.

In the obtained suspension, pH value was measured using a CP-401 pH meter (ELMETRON, Zabrze, Poland) with an EPP-3 electrode and a temperature sensor. The prepared solution was centrifuged for 15 min at 13 000 rpm. Formic acid (0.1 ml per 2 ml solution) was added to the resulting sample to inhibit fermentation processes.

Liquid samples were analyzed using a gas chromatograph with an FID detector to determine the total concentration of volatile fatty acids (VFAs) and the percentage of individual acids: acetate, propionate, isobutyrate, butyrate, isovalerate and valerate. Identification of volatile fatty acids and determination of their levels in the samples were performed by comparing retention times and peak area with the Supelco standard using ChemStation software. Additionally, ammonia levels were determined in the obtained samples using a modified Conway microdiffusion method with Nessler's reagent, and the reading was taken using a PerkinElmer Lambda XLS spectrophotometer.

Statistical analysis

The results of the study were statistically processed by one-way ANOVA using the Statistica 10.0 computer program. Differences were considered at a significance level of $P < 0.01$.

RESULTS AND DISCUSSION

The caecum of rabbits is where microorganisms digest carbohydrates and protein. Volatile fatty acids (VFA) are the main end products of the fermentation process in the caecum, providing an important source of energy for the rabbit. The caecum also plays an important role in the etiology of digestive disorders (Guedes et al., 2009).

Volatile fatty acid (VFA) concentration and caecal pH are classic variables characterizing the extent and pattern of the caecal fermentation, providing an indirect estimate of the caecal microbial activity. In the rabbit, changes in these characteristics (an increase in caecal pH or a decrease in caecal VFA, as well as changes in individual VFA) are correlated with the health status of the growing animal, causing damage to gut microbiome (García et al., 2002).

The pH value of the caecal contents was similar in all groups (Table 3). In the study of Guedes et al. (2009), the pH value of the caecal contents of rabbits is around 6.05, which confirms the values obtained in this study. The concentration of VFA increased significantly after the addition of 0.1 g of dried colostrum and amounted to 255.59 mmol/l of content, and a slightly lower level of VFA was recorded in group D2 than in group D1. The high level of VFA in the cecum of rabbits has a positive effect on the wellbeing of the animal, providing, among other things, protection against enteropathogenic *E. coli* infection (Mourão et al., 2006).

In the study by Miśta (2009), along with the application of 10% and 15% of humic-mineral-fat supplement, there was an increase in the total concentration of volatile fatty acids, but up to the 6th hour of fermentation, so in the time the process takes under physiological conditions.

The application of dried colostrum substrate, which contains high levels of protein and fat (Table 2), resulted in a slight decrease in the proportion of acetate. On the other hand, the lowest concentration of butyrate was recorded in group D2 (19.96 mmol/100 mol). The validity of the results obtained in this experiment find confirmation nn the study by Bover et al. (2006), where lower butyrate concentration was recorded after the addition of dried alfalfa containing about 20% protein per dry matter.

The application of colostrum resulted in an increased production of propionate. Along with the increase in the amount of colostrum there was a decrease in the ratio of acetate to propionate (A:P) and the utilization ratio of VFAs expressed as the non-glucogenic to glucogenic ratio (NGR). According to Bover (2008), the highest VFA production and the proportion of acetate and propionate were recorded with the application of *Lolium perenne* ryegrass hay rich in protein and fat.

There was a reduction in ammonia level under the influence of dried colostrum: the ammonia levels in group D1 and D2 were (respectively) 32.59 and 41.48 mmol/l, while in the control group it was 42.42 mmol/l. Liu et al. (2018), reported a reduction in ammonia production using alfalfa as a high-protein feed.

During fermentation, total gas production increased in both groups (D1 and D2) under the influence of the substrate. Zhu et al. (2016) showed no significant differences in the diversity and abundance of methanogenic bacteria populations in the cecum of rabbits that were fed diets with various fiber to starch ratios.

Ruminants and other herbivores, including rabbits, emit methane at different levels. It is probably related to differences in the rumen and caecal microbiome of herbivores (Mi et al., 2018).

Methanobrevibacter and *Methanosphaera* that inhabit the cecal environment are the two dominant H2-using organisms. *Metanobrevibacter* produces one mole of methane per one mole of carbon dioxide, while *Methanosphaera* requires four moles of methanol to produce three moles of CH4. This means that *Metanobrevibacter* has a greater ability to produce methane than *Methanosphaera* (Cavicchioli, 2011). In the study by Liu et al. (2018), it was shown that the proportion of *Methanosphaera* decreased with a higher fineness of alfalfa meal.

There was a decrease in methane concentration in both experimental groups. In group D1 and D2 methane emission was at the level of 25.67 and 22.81 mmol/l, respectively, while in the control group this value was 26.12 mmol/l.

In the available literature, there are no studies investigating the effect of dried bovine colostrum additive to rabbit feed on volatile fatty acid profile.

Table 3. Influence of dried colostrum substrates on VFA profile and pH value in the caecum

Ferementation parameters	C	D1	D2	Mean	S.E.M.	p-value
pH	6.21	6.24	6.23	6.23	0.02	0.799
VFA [a]	211.55	255.59	251.10	239.41	13.34	0.384
Acetate [b]	66.97	64.68	64.99	65.55	1.70	0.861
Propionate [b]	8.35	8.32	11.00	9.22	0.87	0.356
Isobutyrate [b]	0.58	0.54	0.62	0.58	0.05	0.839
Butyrate [b]	20.51	22.81	19.68	21.00	1.34	0.613
Isovalerate [b]	0.79	0.85	1.09	0.91	0.08	0.278
Valerate [b]	1.26	1.46	1.54	1.42	0.09	0.486
Caproate [b]	1.50	1.34	1.07	1.30	0.16	0.578
A:P	10.76	10.02	7.64	9.47	1.18	0.551
P:B	0.47	0.39	0.64	0.50	0.06	0.233
NGR	13.47	12.90	9.80	12.06	1.37	0.518
Ammonia [a]	42.42	32.59	41.48	38.83	6.07	0.788
Gas production [a]	171.78	175.07	176.35	174.40	5.43	0.996
Methane [a]	26.12	25.67	22.81	24.87	2.92	0.494

C - control group; D1- dried colostrum substrates 0.1 g; D2 - dried colostrum substrates 0.2 g; S.E.M. - standard error of the mean; [a] mmol/L; [b] mol/100mol A:P - acetic to propionic acid ratio; P:B - propionic to butyric acid ratio; p-value

CONCLUSION

Summing up the obtained analytical results, it may be concluded that the use of dried bovine colostrum has a beneficial effect on the fermentation profile in the caecum of rabbits, resulting in a decrease in the production of methane and ammonia.

REFERENCES

Ahamefule, F.O., Obua, B.E., Ukweni, I.A., Oguike, M.A., Amaka, R.A. (2008): Haematiological and biochemical profile of weanter rabbits fed raw or processed pigeon pea eed meal based diets. African Journal of Agricultural Research 3(4), 315-319.

Belenguer, A., Fondevila, M., Balcell, J., Abecia, L., Lachica, M., Carro, M.D. (2008): In vivo and in vitro study of caecal fermentation pattern and methanogenesis in rabbits. Nutrition and Digestive Physiology, 535-540.

Borad, S. G., & Singh, A. K. (2018): Colostrum immunoglobulins: Processing, preservation and application aspects. International Dairy Journal 85, 201–210.

Boudry, C., Dehoux, J., Portetelle, D., Buldgen, A. (2008): Bovine colostrum as a natura growth promotor for newly weaned piglets: a review. Biotechnolgy Agronomy, Society and Environment 12 (2), 157-170.

Bovera F., Calabro` S., Cutrignell M I., Infascelli F., Piccolo G., Nizza S., Tudisco R., Nizza A. (2008): Prediction of rabbit caecal fermentation characteristics fromfaeces by in vitro gas production technique: roughages. Journal of Animal Physiology and Animal Nutrition 92(3), 260-71.

Bovera, F., D'Urso, S., Di Meo, C., Piccolo, G., Calabrò, S., Nizza, A. (2006): Comparison of Rabbit Caecal Content and Rabbit Hard Faeces as Source of Inoculum for the In vitro Gas Production Technique. Asian-Australasian Journal of Animal Sciences 19,11, 1649 - 1657.

Cavicchioli, R. (2011): Archaea—timeline of the third domain. Nature Reviews Microbiology 9(1), 51–61.

Daels, Peter F.(2006): Induction of Lactation and Adoption of the Orphan Foal. Annual Resort Symposium, Rome 19-21.

Dkhil, A.M., Al-Quraishy, S. (2010): Effects of extensive consumption of hot red pepper fruit on liver of rabbit. Journal of Medicinal Plants Research 4(23), 2533-2538

Engelhardt, W. (1995): Absorption of short-chain fatty acids from the large intestine. In: Cummings J.H., Rombeau J.L., Sakata T., (Eds.), Physiological and Clinical Aspects of Short- Chain Fatty Acids. Cambridge University Press, Cambridge 149-170.

Gapper, David, L.W., Copestake, D.E.J., Otter, D.E., Indyk, H.E. (2007): Analysis of bovine immunoglobulin G in milk, clostrum and dietary supplements: a revive. Analical and Bioanalytical Chemistry 389:93-109.

García, J., Carbaño, R., Pérez-Alba, L., Blas, J.C. (2000): Effect offiber source on cecal fermentation and nitrogen recycled through cecotrophy in rabbits. Journal of Animal Science 78, 638-646.

García, J., Gidenne, T., Luisa Falcao-e-Cunha, de Blas, C. (2002): Identification of the main factors that influence caecal fermentation traits in growing rabbits. Animal Research 51(2), 165–173.

Gidenne, T., Bellier, R. (2000): Use of digestible fibre in replacement to available carbohydrates. Effect on digestion, rate of the rabbit. Livestock Production Science 63:141-152.

Guedes, C.M., Mourão, J.L., Silva, M.J., Rodrigues, M.A.M., Pinheiro, V. (2009): Effect of age and mannanoligosachaccharides supplementation on production of volatile fatty acids in the caecum of rabbits. Animal Feed Science and Technology 150 (3), 330-336.

Gugołek, A. (2011): Zalecenia żywieniowe i wartość pokarmowa pasz - Zwierzęta futerkowe, Praca Zbiorowa, IFiŻZ Jabłonna.

Huguet, A., Séve, B., Dividich, J.L., Huërou-Luron, I. (2006): Effect of bovine colostrumsuplemented diet on some gut parameters in weaned piglets. Reproduction Nutrition Development 46, 167-178.

Janssen, PWM., Lentle, RG., Hulls, C., Ravindran, V., Amerah, AM. (2009): Spatiotemporal mapping of the motility of the isolated chicken caecum. Journal of Comparative Physiology B 179, 593-604.

Kuijper, D. P. J., van Wieren, S. E., Bakker, J. P. (2004): Digestive strategies in two sympatrically occurring lagomorphs. Journal of Zoology 264(2), 171–178.

Li, Y., Østergaard, M. V., Jiang, P., Chatterton, D. E. W., Thymann, T., Kvistgaard, A. S., Sangild, P. T. (2013): Whey Protein Processing Influences Formula-Induced Gut Maturation in Preterm Pigs. The Journal of Nutrition 143(12), 1934–1942.

Liu, S., Yuan, M., Jin, D., Wang, Z., Zou, H., Wang, L., Peng, Q. (2018): Effects of the particle of ground alfalfa hay on the growth performance, methane production and archaeal populations of rabbits. PLOS ONE 13(9).

Mi, L., Yang, B., Hu, X., Luo, Y., Liu, J., Yu, Z., & Wang, J. (2018): Comparative Analysis of the Microbiota Between Sheep Rumen and Rabbit Cecum Provides New Insight Into Their Differential Methane Production. Frontiers in Microbiology 9.

Mikołajczyk, K., Pecka-Kiełb E., Zachwieja A. (2019): Impact of the volume and the profile of volatile fatty acids in the rumen fermentation on cow productivity and milk composition. Mljekarstvo 69, 4.

Mourão, J. L., Pinheiro, V., Alves, A., Guedes, C. M., Pinto, L., Saavedra, M. J., Kocher, A. (2006): Effect of mannan oligosaccharides on the performance, intestinal morphology and cecal fermentation of fattening rabbits. Animal Feed Science and Technology 126(1-2), 107–120.

Nagaraja, L., Dar, A. A., Pandey, N. N., Mondal, D. B. (2011): Bovine colostrum as immunomodulator for prevention of Escherichia coli diarrhea in weaned rabbits. African Journal of Agricultural Research 6 (22), 5066-5072.

Ogunsan, E.A., Dogo, G.I., Aliero, B.L., Hassan, W.A., Tambuwal, F.M., Ogo, N.I., Jatau J. (2011): The Nutritive Value of Zornia glochidiata as a No-conventional Feedstuff in Rabbit in Sokoto. Pakistan Journal of Nutrition 10 (7), 658-662.

Pandey, N.N., Dar, A.A., Mondal, D.B., Nagaraja, L. (2011): Bovine colostrums: A veterinary nutraceutical. Journal of Veterynary Medicine and Animal Health 3(3), 31-35.

Pecka E., Zachwieja A., Góralska-Kowalska M. (2012): Poziom wybranych makroelementów oraz selenu w siarze krów w zależności od ich wieku oraz liczby komórek somatycznych. Przemysł Chemiczny 91/5, 926-928.

Sirotek, K., Slováková, L., Kopečný, Marounek, M. (2004): Fermentation of pectin and glucose, and activity of pectin-degrading enzymes in the rabbit caecal bacterium Bacteroides caccae, The Society for Applied Microbiology 38, 327-332.

Szulc, T., Zachwieja, A. (1998): Siara eliksir życia osesków. Wrocław 10-15.

Vidyasagar, S., Ramakrishna, B.S. (2002): Effects of butyrate on active sodium and chloride transport in rat and rabbit distal colon. Journal of Physiology 539, 1:163-173.

Zachwieja, A., Knecht, D. (1999): Wykorzystanie siary bydlęcej w odchowie prosiąt dla zwiększenia ich odporności. Medycyna Weterynaryjna 55 (6), 400-402.

Zachwieja, A., Łupińska, A., Pecka, E., Paczyńska, K., Tumanowicz, J. (2014): Zmiany składu siary krów w zależności od metodyki jej konserwacji. (maszynopis).

Zhu, Y., Sun, Y., Wang, C., & Li, F. (2016): Impact of dietary fibre:starch ratio in shaping caecal archaea revealed in rabbits. Journal of Animal Physiology and Animal Nutrition 101(4), 635–640.

THE INFLUENCE OF *QUILLAJA SAPONARIA* ON THE FERMENTATION PROFILE IN THE CAECUM OF GEESE *IN VITRO*

KATARZYNA MIKOŁAJCZYK-LASZECKA[1], EWA PECKA-KIEŁB[2], DOROTA MIŚTA[2], BOŻENA KRÓLICZEWSKA[2], ARTUR KOWALCZYK[1], EWA ŁUKASZEWICZ[1], FRANTISEK ZIGO[3], ANDRZEJ ZACHWIEJA[1]

[1] Institute of Animal Breeding Wroclaw University of Environmental and Life Sciences, ul. Chelmonskiego 38C, 51-631 Wroclaw, Poland;

[2] Department of Animal Physiology and Biostructure, Wroclaw University of Environmental and Life Sciences, Norwida 31, 50-375 Wroclaw, Poland;

[3]University of Veterinary Medicine and Pharmacy in Košice, Department of Animal Husbandry Košice, Slovakia

Corresponding email address: katarzyna.mikolajczyk@upwr.edu.pl

ABSTRACT

The aim of this study was to determine the effect of *Quillaja saponaria* on *in vitro* fermentation profile in the caecum of geese. The material for the study consisted of caecal contents collected from 24 White Koluda geese, aged 17 weeks (12♀ and 12♂). The animals were kept in a semi-intensive system, and geese were given oats for the last two weeks before slaughter. Eight pooled samples were made; each sample was a mixture of caecal contents taken from three birds of the same sex. The samples were homogenized and assigned to three groups: control group (K) - 0.5 g oats; group D1 - 0.5 g oats with 0.15 g saponins; and group D2 - 0.5 g oats with 0.30 g saponins. The caecal samples prepared in this way were mixed with a buffer solution in the ratio of 1:5 and subjected to homogenization. The pH of the obtained suspension was measured using a CP-401 pH-meter. In order to obtain anaerobic conditions the bottles were saturated with CO_2 administered from a pressure bottle. The samples were subjected to 8-hour in vitro fermentation in a shaking water bath at 39 °C.

The samples were then analyzed using a gas chromatograph with an FID detector to determine their total volatile fatty acid (VFA) concentration and VFA profile, as well as their methane content. Additionally, ammonia levels were determined in the obtained samples using a modified Conway microdiffusion method with Nesler reagent. Along with the addition of saponins extracted from *Quillaja saponaria*, a statistical trend was observed in the reduction of acetate production and a decrease in the ratio of acetate to propionate between the control group and the D2 group. The applied additive in the D2 group resulted in an increase ($P<0.05$) of butyrate production in relation to the control group. The concentration of ammonia in the analyzed section of the gastrointestinal tract was the lowest in this group.

Keywords: geese, saponins, *in vitro* fermentation, volatile fatty acids, ammonia

INTRODUCTION

Native to South America, *Quillaja saponaria* belongs to the family Quillajaceae; it is commonly found in Bolivia, Peru and Chile (Luebert, 2013). It is the main source of saponins used for industrial purposes: saponins extracted from Quillaja saponaria are used in cosmetics, pharmacology and as additives to food and drink (Fleck et al., 2019). Extensive research shows that *Quillaja saponaria* used as feed additive contributes to the reduction of methane emissions in the gastrointestinal tract of animals (Jayanegara et al., 2014). Thanks to their extraordinary properties, saponins are also used as feed additives with immunomodulatory properties. They stimulate liver function, improve feed utilization, animal growth rate and slaughter yield (He et al. 2005, Westendarp, 2005).

Geese are herbivorous waterfowl whose meat is considered a healthy food, with high levels of amino acids, low fat content, and high levels of unsaturated fatty acids, which have positive effects on human health (Li et al., 2019).

Geese are unique birds with extremely capacious stomachs and flexible esophagus, thanks to which they can consume large volumes of feed per day. The length of the gastrointestinal tract of geese is determined by the

type of feed consumed and the age of the animal (Duszynska-Stolarska, 2016). The catabolic processes of dietary fiber take place in the caecum and large intestine thanks to the microorganisms living there (Wang et al., 2014). The species richness of microorganisms in the gastrointestinal tract of geese is determined by the amount of protein and minerals supplied in the feed and the level of acidity (Duszynska-Stolarska, 2016). The caecum of geese is an environment for the growth of microorganisms that can symbiotically convert dietary fiber into short-chain fatty acids to provide energy (Chen et al., 2014). The caecum, as opposed to the stomach, does not secrete digestive enzymes; their production is possible thanks to the microorganisms present in them. The fermentation process in caeca of geese is similar to rumen of ruminants, therefore, these animals can consume relatively large amounts of fiber (Ye et al., 2017). Some greenhouse gases emitted by livestock are formed by fermentation occurring in the rumen of ruminants and in the large intestine of monogastric animals. These gases include methane, which accounts for about 18% of the greenhouse gases of the earth's atmosphere (Wei-lian et al., 2005).

Disorders of food digestion and absorption have negative effects on the survival and reproduction of geese. The more developed the stomach, the better the absorption of nutrients from the provided feed. The correct physiology of the goose stomach determines the intestinal motility, which increases the digestion of the food intake, better energy utilization and performance, and can prevent the entry of pathogenic bacteria (Lu et al., 2011).

Goose farming is becoming more and more specialized and common nowadays. An important aspect in breeding geese is their nutrition. Additives to the diet of these animals determine a positive effect on carcass yield and gastrointestinal tract development (Wang et al., 2014). White Koluda geese, which are also called "Polish Oat geese", are very popular in Poland. The name results from the method of feeding the geese with fattening oats in the last 3 weeks of their breeding. Fattening with oats determines health values and taste of meat and fat from geese (Wereńska et al., 2021).

The latest data from Eurostat, the statistical office of the The |European Union show that Poland is the largest poultry meat producer among the

European Union countries. According to the National Poultry Council - Chamber of Commerce, more than 8 million of commodity geese were slaughtered in 2018, while the laying stock amounted to about 201 thousand in 2017.

In order to reduce the production and emission of methane and ammonia, feed additives are sought that reduce the production of gases in the digestive tract while maintaining proper physiological processes in the animal body. Such additives are currently used in cattle and swine, and include saponins, tannins, aluminosilicates, among others. Reducing CH4 emissions in the digestive tract can decrease animal energy losses by 2 to 12% (Kolling et al., 2018).

The aim of the present study was to determine the effect of *Quillaja saponaria* on the formation of the fermentation profile in the caecum of geese in the *in vitro* conditions.

MATERIAL AND METHODS

The material for the study consisted of the caeca collected from 24 White Koluda geese, aged 17 weeks (12♀ and 12♂). The animals were kept in a semi-intensive system, during the last two weeks before slaughtering the geese were given oats, which were analyzed and used as a substrate during fermentation.

Quillaja saponaria extract from Sigma-Aldrich (No S4521) was used as an additive to the incubated caecal contents. Eight pooled trials were performed, each trial was a mixture of cecal contents collected from three birds of the same sex. There were 3 groups in each trial. The samples were homogenized and assigned to three groups: control group (K) - 0.5 g oats; group D1 - 0.5 g oats with 0.15 g saponins; and group D3- 0.5 g oats with 0.30 g saponins.

In vitro fermentation of caecal contents

The obtained material was mixed with a buffer (Adjiri et al.,1992) in the ratio of 1:5 and homogenized. The pH in the obtained suspension was measured using a CP-401 pH meter (ELMETRON, Zabrze, Poland) with an EPP-3 electrode and a temperature sensor. The prepared solution was centrifuged for 15 min at 13 000 rpm. Formic acid (0.1 ml per 2 ml of solution) was then added to inhibit fermentation processes. To obtain

anaerobic conditions, the bottles were saturated with CO_2 fed from a pressure bottle. Then they were sealed tightly with a capsule. The samples were subjected to 8-hour in vitro fermentation in a shaking water bath at 39 ^oC.

Analysis of selected fermentation products

At the end of incubation in serum bottles, the pressure generated by the fermentation gases was measured. The resulting gas was analyzed for methane content using a gas chromatograph (Agilent Technologies 7890A GC System) with a TCD and FID detector. Liquid samples taken both before and after incubation were analyzed using a gas chromatograph with an FID detector to determine the total concentration of volatile fatty acids (VFA) and the percentage of individual acids: acetate, propionate, isobutyrate, butyrate, isovalerate and valerate. The identification and levels of volatile fatty acids in the analyzed samples were carried out by comparing retention times and area under peak with the Supelco standard using the ChemStation software. Additionally, ammonia levels were determined in the obtained samples using a modification of the Conway microdiffusion method with Nesler's reagent, and readings were taken using a PerkinElmer Lambda XLS spectrophotometer.

Feed analysis

Representative samples of the oats fed to the geese and used as substrate were analysed for basic nutrients (Table 1): dry matter (AOAC Official Method 934.01), crude ash (AOAC Official Method 942.05), crude protein (Kjeldahl method, AOAC Official Method 984. 13 on Foss Kjeltec 2300), crude fat (AOAC Official Method 920.39), crude fiber (AOAC Official Method 978.10 on Foss Fibertec 1020), ADF (AOAC Official Method 973.18 on Foss Fibertec 1020), NDF, and energy (JAOAC v. 56, 1352-1356, 1973 on Foss Fibertec 1020).

Statistical analysis

The results of the study were statistically processed by one-way ANOVA analysis of variance using Statistica 13.3 computer program. The differences were analysed at the significance levels of 0.05 and 0.01. Probability values between 0.05 and 0.10 were reported as statistical trends.

Table 1. Composition and nutritional value of oats from goose diets

Diet nutritional value	
Ash [%]	89.41
Crude protein [%]	12.55
Crude fibre [%]	12.91
Crude fat [%]	4.94
NDF [%]	23.71
ADF [%]	12.88
Gross energy [MJ/kg]	16.11

RESULTS AND DISCUSSION

Due to high consumer demand for goose meat, poultry producers strive to increase the rate of muscle tissue growth in these animals. The greatest increase in muscle tissue in the White Koluda goose occurs between 2 and 12 weeks of age (Murawska, 2013). According to Mazanowski (2000), the weight of White Koluda geese at 17 weeks fluctuated around 5700 g. In our study, the average weight of geese was lower and was 4451 g±354.30, while the caecal weight was 63.04 g±21.78. The drying coefficient of the stomach contents was 0.7±0.05 and the proportion of dry matter was 68.58%±11.75.

Volatile fatty acids (VFA) produced in the gastrointestinal tract are an important source of energy for many animals, and can also be substrates or activators of many metabolic processes (Kristensen, 2005). In hens, after absorption into the blood, they satisfy energy needs at the level of 8%, in ostrich as much as 75% (Józefiak, 2004). Literature data do not give the percentage of energy obtained from VFAs produced in the digestive tract of geese. Volatile fatty acids are products of anaerobic bacterial fermentation, mainly of dietary fiber and resistant starch. The energy availability (MJ/kg) from fiber determined by the production of

VFAs in geese is higher than in hens by about 9% (Kirchgessner et al., 1999). The most important task of acid-base management in poultry is to keep the pH value constant, otherwise production rates are reduced. Volatile fatty acids affect the pH of the intestinal contents, act as bioregulators and growth promoters (Mroz et al., 2006).

In fresh caecal contents of geese, the concentration of VFAs is 52.11±19.42 μmol/g (Wu et al., 2009). In our study, the level of VFAs in fresh caecal contents was 45.32 mmol/l. The acid profile of fresh caecal contents in geese was characterized by the highest proportion of acetate, lower proportion of propionate and lowest proportion of butyrate in the total VFA pool, in mutual ratios of 4.7:1.2:1. A similar relationship was reported by Arslan (2003) and Wu et al. (2009).

In fresh content, the level of iso acids such as isobutyrate and isovalerate were 2.06 mol/100mol and 1.79 mol/100mol, respectively. The ratio of acetate to propionate is 4.04, while the ratio of propionate to butyrate is estimated to be 1.30. A similar relationship was obtained in the study of Jamroz et al. (2002).

In the control sample, the value of volatile fatty acids was 286.96 mmol/kg (Table 2). The highest level of VFA was observed in the samples with the addition of 0.15 g *Quillaja saponaria*. On the other hand, the lowest VFA production value was recorded in the samples after application of 0.30 g of saponins extracted from *Quillaja saponaria*.

Table 2. Influence of *Quillaja saponaria* substrates on VFA profile and pH in the cecum

		Substrates		
		K	**D1**	**D2**
Total VFA[1]	$\bar{x}$	286.96	333.04	253.47
	SD	123.18	99.04	75.39
Individual VFA. mol/100mol				
Acetic acid	$\bar{x}$	60.08**	55.32	52.92**
	SD	6.74	6.23	3.46
Propionic acid	$\bar{x}$	19.13	21.45	22.76
	SD	3.83	3.99	3.64
Isobutyric acid	$\bar{x}$	2.30	2.75	2.29
	SD	1.18	1.95	0.94

Butyric acid	$\bar{x}$	13.92*	15.95	16.83*
	SD	2.57	4.18	1.84
Isovaleric acid	$\bar{x}$	2.42	2.26	2.80
	SD	1.37	0.98	0.83
Valeric acid	$\bar{x}$	2.15	2.09	2.37
	SD	0.78	0.34	0.79
A: P	$\bar{x}$	3.31**	2.67	2.39**
	SD	1.07	0.70	0.49
P : B	$\bar{x}$	1.41	1.46	1.38
	SD	0.38	0.67	0.32
pH	$\bar{x}$	6.19	6.23	6.17
	SD	0.77	0.75	0.77

K, control group; D1, supplement sasponins 0.15 g; D2, supplement sasponins 0.30 g; SD. standard error of the mean; 1 mmol/L of undiluted caceum fluid; A:P. acetic to propionic acid ratio; P:B. propionic to butyric acid ratio, **; statistical trends,*; values differ significantly between groups ($P < 0.05$)

In our study, along with the saponin addition, a similar statistical trend was observed in the reduction of acetate production and the reduction of acetate to propionate ratio between the control and D2 groups. The additive in the D2 group resulted in an increase ($P < 0.05$) in butyrate production with respect to the control group.

The available literature does not report results of studies describing the effect of saponin application on the profile of VFAs in caecal contents. Most of the studies concerned dairy cattle. In the *in vitro* fermentation of rumen contents, the application of saponins resulted in a decrease in acetate production and an increase in propionate production, which affects the A to P ratio (Patra et al., 2012). According to Kang et al. (2016), butyrate production increases in 24 h of fermentation in the rumen contents of cows when saponins are added.

The active acidity (pH) in the caecal contents of geese ranges from 6.31 to 6.50 and depends on the type of fiber in the feed (He et al., 2015). The pH value may depend on the level of VFAs production and ammonia in the digestive tract (Strik et al., 2016).

In the experiment conducted, the pH value of fresh caecal content was 6.68±1.11, and it decreased slightly after 8 h of incubation with the addition of saponins (Table 2). The observed effect may be due to the increase in the production of VFAs in group D1, while in group D2 the

level of VFAs decreased, with a concomitant decrease in ammonia concentration.

Geese, due to their highly developed caecum and high proportion of fiber in the ration, produce 1.5*10-3 kg of methane per goose per production cycle, which is a hundred times more when compared proportionally to the chicken broiler (Wang and Hung, 2008). Ammonia also belongs to the group of harmful gases produced by animals. It is a product of bacterial breakdown of uric acid, and is largely derived from the deamination of dietary protein amino acids and endogenous nitrogen, which is commonly used as a preferential nitrogen source by carbohydrate-fermenting bacteria in the caecum (He et al., 2015).

The production of gaseous ammonia in a poultry house can adversely affect the health of birds (Nazeer et al. 2002). Birds are more sensitive to ammonia than other animals. This gas in excessive concentrations can damage internal organs and cause infection of the air sacs. Research on the effects of ammonia on the traits and development of poultry is very important for the improvement of farming environment and food safety (Xing et al., 2016). The use of appropriate feed additives that reduce the emission of gases including ammonia in the feed ration of animals contributes to improving animal welfare and performance (Lynch et al., 2007). After application of 0.30 g saponins from *Quillaja saponaria* the ammonia content was at 25.25 mmol/l (Table 3).

Table 3. Influence of *Quillaja saponaria* substrates on gas production and ammonia in the cecum

		Substrates		
		K	**D1**	**D2**
Ammonia [1]	$\bar{x}$	31.20	32.51	25.25
	SD	16.92	12.47	11.58
Gas production [2]	$\bar{x}$	603.75	692.02	697.65
	SD	160.70	156.51	147.55
Methane [2]	$\bar{x}$	65.10	74.42	70.46
	SD	17.12	10.28	13.73

K, control group; D1, supplement sasponin 0.15 g; D2, supplement sasponin 0.30 g; SD, standard error of the mean; [1] mmol/L of undiluted caecum fluid; [2] mmol/L of undiluted caceum fluid

In the study of He et al. (2015), similar values of ammonia concentration were reported (around 21 mmol/l). The concentration of ammonia produced in the analyzed section of the gastrointestinal tract was maintained at the lowest level in the group with the addition of 0.30 g saponins, and at the highest level when 0.15 g of the extract was used. In the caecum, gas production - including methane - increased under the influence of saponin substrate. In cattle, Unnawong et al., (2021) reported a reduction in ammonia concentration *in vitro* after the application of saponins in the feed ration. Also in cattle, Wei- Line Hu (2005) reported an increase in gas emissions in vitro fermentation, but showed a reduction in methane and ammonia production.

CONCLUSION

The results obtained indicate that there was no negative effect of *Quillaja saponaria* extract on the *in vitro* fermentation profile in the caecum of geese; these results may encourage *in vivo* studies to analyze the effect of saponins on physiological processes occurring in the gastrointestinal tract of geese. The most desirable effects were obtained in the D2 group in which 0.30 g of saponins were applied, due to a decrease in ammonia and acetate production with simultaneous butyrate production.

REFERENCES

Arslan, C. (2003): Bulky feeds in the intensive fattening of goslings, II. Effects of alfalfa, grass and sugar beet pulp on abdominal fat pattern and caecal volatile fatty acid composition in geese. Revue de Médecine Vétérinaire 154, 11, 667-671.

Chen, Y.H., Lee, S.M., Hsu, J.C., Chang, Y.C., Wang, S.Y. (2014): Methane Generation from the Intestine of Muscovy Ducks, Mule Ducks and White Roman Geese. Aerosol and Air Quality Research 14: 323-329.

Duszynska-Stolarska O. (2016): Fizjologiczne podstawy żywienia gęsi: Hodowca Drobiu. Uniwersytet Technologiczno-Przyrodniczy w Bydgoszczy 05, 25-27.

Fleck, J., Betti, A., da Silva, F., Troian, E., Olivaro, C., Ferreira, F., Verza, S. (2019): Saponins from Quillaja saponaria and Quillaja brasiliensis: Particular Chemical Characteristics and Biological Activities. Molecules 24(1), 171.

He, L. W., Meng, Q. X., Li, D. Y., Zhang, Y. W., Ren, L. P. (2015): Influence of feeding alternative fiber sources on the gastrointestinal fermentation, digestive enzyme activities and mucosa morphology of growing Greylag geese. Poultry Science 94(10), 2464–2471.

Hu, W-L., Yue-ming W.U., Jian-xin LIU, Yan-qiu, G., Jun-an YE (2005): Tea saponins affect in vitro fermentation and methanogenesis in faunated and defaunated rumen fluid. Journal of Zhejiang University SCIENCE 6B (8):787-792.

Jamroz, D., Wiliczkiewicz, A., Orda, J., Wertelecki, T., Skorupinska, J. (2002): Aspects of development of digestive activity of intestine in young chickens, ducks and geese. Journal of Animal Physiology and Animal Nutrition 86(11-12), 353–366.

Jayanegara, A., Wina, E., Takahashi, J. (2014): Meta-analysis on Methane Mitigating Properties of Saponin-rich Sources in the Rumen: Influence of Addition Levels and Plant Sources. Asian-Australasian Journal of Animal Sciences 27(10), 1426–1435.

Józefiak, D., Rutkowski A. (2003): A note on changes in the concentration of short-chain fatty acids in the caecal content of laying hens due to addition of fresh grass to the diet. Animal Feed Science 12(1):181–187.

Kang, J., Zeng, B., Tang, S., Wang, M., Han, X., Zhou, Ch., Yan, Q., He, Z., Liu, J., Tan, Z. (2016): Effects of Momordica charantia Saponins on In vitro Ruminal Fermentation and Microbial Population Asian Australas. Journal of Animal Science 29, 4 : 500-508.

Kirchgessner, M., Eder, K., Müller, H.L., Jamroz, D. (1999): Zur energetischen Bewertung von Nicht-StärkePolysacchariden beim Geflügel. Animal Physiology and Animal Nutrition 1, 51-55.

Kolling, G. J., Stivanin, S. C. B., Gabbi, A. M., Machado, F. S., Ferreira, A. L., Campos, M. M., Fischer, V. (2018): Performance and methane emissions in dairy cows fed oregano and green tea extracts as feed additives. Journal of Dairy Science 101(5), 4221–4234.

Li, M., Zhou, H., Xu, T., Zi, X. (2019): Effect of cassava foliage on the performance, carcass characteristics and gastrointestinal tract development of geese. Poultry Science 1;98(7):3081.

Lu, J., Kong, X. L., Wang, Z. Y., Yang, H. M., Zhang, K. N., Zou, J. M. (2011): Influence of whole corn feeding on the performance, digestive tract development, and nutrient retention of geese. Poultry Science, 90(3), 587–594.

Luebert, F. (2013): Taxonomy and distribution of the genus Quillaja Molina (Quillajaceae). Feddes Repertorium 124(4), 157–162.

Lynch, M. B., Sweeney, T., Callan, J. J., & O'Doherty, J. V. (2007): The effect of dietary barley level on volatile fatty acid concentration and manure ammonia emissions in finishing pigs. Livestock Science 109(1-3), 236–239.

Mazanowski, A. (2000): Rearing performance of quadruple crossbreds produced using White Kołuda and regional varieties of geese. Annals of Animal Science - Roczniki Naukowe Zootechniki 27, 4. 65-83, 21.

Mroz, Z., Koopmans, S.-J., Bannink, A., Partanen, K., Krasucki, W., Overland, M., Radcliffe, S., (2006): Carboxylic acids as bioregulators and gut growth promoters in nonruminants. In: R. Mosenthin, J. Zentek, T. Żebrowska (Editors). Biology of Nutrition in Growing Animals Elsevier 81-133.

Murawska, D. (2013): The effect of age on the growth rate of tissues and organs and the percentage content of edible and inedible components in Koluda White geese. Poultry Science 92(5), 1400–1407.

Patra, A. K., Stiverson, J., Yu, Z. (2012): Effects of quillaja and yucca saponins on communities and select populations of rumen bacteria and archaea, and fermentationin vitro. Journal of Applied Microbiology 113(6), 1329–1340

Strik, D. P. B. T. B., Domnanovich, A. M., Holubar, P. (2006): A pH-based control of ammonia in biogas during anaerobic digestion of artificial pig manure and maize silage. Process Biochemistry 41(6), 1235–1238.

Unnawong, N., Anusorn, Ch., Sarong So, S. (2021): Influence of Supplementing Sesbania grandiflora Pod Meal at Two Dietary Crude Protein Levels on Feed Intake, Fermentation Characteristics, and Methane Mitigation in Thai Purebred Beef Cattle. Veterinary Science 8(2), 35.

Wang, Z. Y., Yang, H. M., Lu, J., Li, W. Z., Zou, J. M. (2014): Influence of whole hulled rice and rice husk feeding on the performance, carcass yield and digestive tract development of geese. Animal Feed Science and Technology 194, 99–105.

Wereńska, M., Haraf, G., Wołoszyn, J., Goluch, Z., Okruszek, A., Teleszko, M. (2021): Fatty acid profile and health lipid indicies of goose meat in relation to various types of heat treatment. Poultry Science. Volume 100, Issue 8, 101237.

Westendarp, H. (2005): Saponins in nutrition of swine, poultry and ruminants. Dtsch Tierarztl Wochenschr 112(2):65-70.

Wu, L. Y., Fang, Y. J., Tan, R. B., Shi, K. J. (2009): A Comparison of Cecal Microflora and Volatile Fatty Acid Concentration in Goslings Fed Diets Supplemented with or without a DriedBacillus subtilisCulture. Journal of Applied Animal Research 36(2), 231–234.

Xing, H., Luan, S., Sun, Y., Sa, R., Zang, H. (2016): Effects of ammonia exposure on carcass traits and fatty acid composition of broiler meat. Animal Nutrition 2(4), 282–287.

Ye, M., Sun, L., Yang, R., Wang, Z., Qi, K. (2017): The optimization of fermentation conditions for producing cellulase of Bacillus amyloliquefaciens and its application to goose feed. Royal Society Open Science 4(10), 171012.

THE IMPACT OF NUTRITION ON THE CONTENT OF IMMUNOGLOBULINS IN COLOSTRUM AND MILK OF SOWS – A REVIEW.

EVA MIXTAJOVÁ, MICHAL ROLINEC, DANIEL BÍRO, BRANISLAV GÁLIK, MIROSLAV JURÁČEK, MILAN ŠIMKO, ONDREJ HANUŠOVSKÝ, MÁRIA KALÚZOVÁ

Institute of Nutrition and Genomics, Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovakia

Corresponding email address: xmixtajova@uniag.sk

ABSTRACT

The aim of this review is to provide summary information on selected feed additives that would help to improve the immunological value of colostrum and sow's milk and thus provide better passive immunity to the new-born piglet. As piglets are dependent on maternal immunoglobulins (Ig) after birth, it is important that they are taken up as much as possible in the colostrum. That is why it is good to know about suitable additives that have shown immunomodulatory effects in several studies and have been able to increase the content of immunoglobulins in colostrum and milk when fed. In one part of this review, we focused on the addition of fatty acids that positively affected the levels of immunoglobulins G, A and M in sow mammary gland secretions. Whether fed in the form of soybean, coconut or palm oil, or directly as conjugated linoleic acid, immunoglobulin levels have increased by up to 30% in some cases. The addition of hemp and soybean oil to gestating and lactating sows significantly increased the immunoglobulin content. Likewise, the feed ration of sows enriched with grape seed polyphenols increased the colostrum IgG and IgM levels. The addition of fermented rapeseed meal to the sows' diet increased the IgG content in the colostrum. Likewise, by feeding active dried yeast, a positive effect on IgG and IgA concentrations in colostrum and sow milk was demonstrated. Although the addition of essential oils to the feed ration of gestating and lactating sows did not increase the levels of

immunoglobulins, it was demonstrably reduced the oxidative stress of the sows and improved the performance of the piglets.

Keywords: Immunoglobulins, colostrum, milk, sows

INTRODUCTION

The nutritional and immunological significance of colostrum and milk for the survival of a new-born piglet has been well described by several authors (Le Dividich et al., 2005; Farmer et al., 2009; Hurley, 2015; Theil et al., 2012; Hurley et al., 2013). Because piglets are born with low energy stores in the body and without serum immunoglobulins due to the epitheliochoric nature of pig placenta, the new-born piglet must obtain maternal immunoglobulin G (IgG) from the ingested colostrum for passive immune protection until its immune system is completely developed (Le Dividich et al., 2005). The highest intensity of immunoglobulin absorption was recorded between 4-12 hours after birth and then tended to decrease (King'ori, 2012). The piglet can absorb intact IgG only before the intestinal occlusion gut closure, which occurs in the first 24 hours of a piglet's life and is stimulated by colostrum intake (Le Dividich et al., 2005). While colostrum is important for the survival of piglets (Le Dividich et al., 2005), sow's milk is a limiting factor influencing the growth rate of piglets (Quesnel et al., 2015). Immunoglobulins secreted in colostrum and milk are a major factor in providing immune protection to the neonate. Immune cells and immunomodulatory factors contained in colostrum and milk play an important role in responding to pathogens and help the maturation of the immune system in pigs (Salmon et al., 2009). There is extensive species variability in how and when immunoglobulins are transmitted to the neonate, as well as in the mechanisms by which neonatal immunoglobulins are affected (Butler a Kehrli, 2005). Immunoglobulins found in colostrum or milk are the same as those found in blood or mucosal secretions. Specific antibodies pass into the colostrum by active passage directly from the mother's bloodstream. Only part of the immunoglobulin is synthesized by B-lymphocytes directly in the mammary gland (Rolinec et al., 2012). The most abundant immunoglobulins in sow mammary gland secretions are IgG, IgA and IgM (Hurley et al., 2013; Klobasa et al., 1987). In the first hours after

birth, IgG is the most abundant, but then its content decreases by up to 85 % and at the end of lactation IgA, which is the predominant antibody from the third day of lactation, is the most abundant (Rolinec, 2009). Immunoglobulins received from colostrum in the first days after birth are used in the immunological defense of piglets up to 20-25 days (Kanka et al, 2014). The nutrient as well as the immunological composition of colostrum and milk is different both between breeds and influenced by various factors such as nutrition, number of litters, health status and physical condition (Theil et al., 2012; Quesnel et al., 2015; Picone et al., 2018; Nuntapaitoon et al., 2020). Due to the need for colostrum and milk from piglets for survival, immune resistance and growth after weaning, it is important to know the factors influencing the production of colostrum and milk from sows. This will allow the development of management systems that increase the sow's milkiness and improve the composition of colostrum and milk, increase the survival of piglets and the ability to grow weaners (King'ori, 2012). Based on these findings on the effect of nutrition on the immunological composition of colostrum and sow's milk, we decided to summarize in this review several studies dealing with the effect of different types of additives on the immunoglobulin content.

Additives containing polyphenols

Different types of fruits, berries and pomace contain high concentrations of polyphenols, making them anti-cancer, antimicrobial, antioxidant and immunomodulatory effects in vertebrates (Sehm et al, 2011; Süli et al., 2014; Ivanišová et al., 2019). In our selected studies, the authors (Wang et al., 2019; Wang et al., 2020) fed grape pomace to weaners and the extract of grape seed polyphenols to gestating and lactating sows. In both cases, an increase in immunoglobulins was observed. An increase of IgM and IgG in colostrum was detected and Wang et al. (2019) attribute the increase in IgG in sow colostrum to elevated levels of progesterone, which plays an active role in the transfer of IgG from plasma to colostrum. And it was in the experimental group of sows that they also found higher levels of progesterone, which they attribute to polyphenol.

Additions of fatty acids/ Fatty acid additives

Various animal studies have confirmed that the addition of various additives rich in long chain fatty acids to feed rations of lactating sows increased the milk fat, fatty acid content of milk and immunoglobulin content in colostrum, milk or blood of sows and piglets (Bai et al., 2017; Sun et al., 2020). Fatty acids contained in feed can affect the immune components of colostrum and milk such as immunoglobulins (Vodolazska and Lauridsen., 2020). Likewise, the fatty acid profile of newborn piglets is influenced by the intake of maternal fatty acids during pregnancy and lactation (Rook et al., 2001). Bai et al. (2017) fed soybean, coconut and palm oil and their triple combination to gestating and lactating sows. It was by adding three different types of oils and their combination that they monitored the effect of fatty acids on the content of immunoglobulins in colostrum and milk. There was no statistical difference in IgG, IgA and IgM content between groups for colostrum or milk. However, the content of IgG and IgA in blood plasma was significantly higher in the group fed soybean oil or a combination of oils compared to other groups of sows. According to them, the different effect of the individual oils can be caused by the different number of carbon chains of the individual fatty acids added to the feed rations of sows. The addition of coated omega 3 fatty acid from linseed oil also had a positive effect on IgG levels in colostrum and milk (Sun et al., 2020). They showed a significant difference in this study on day 16 after farrowing. Conjugated linoleic acid (CLA) is the collective name for linoleic acid isomers, and the modulatory effects on the immune system have also been confirmed for these isomers. Rossi et al., (2004) confirmed in their study that the addition of CLA to the feed ration of gestating and lactating sows has a positive effect on the concentration of immunoglobulins in both colostrum and milk. In the experimental groups, IgG values increased by up to 30% compared to the control. As a result, they confirmed the immunomodulatory effects of these isomers. Likewise, Bontempo et al. (2004) confirmed the positive effect of CLA administration to gestating and lactating sows on IgG levels in colostrum and milk. It is by adding polyunsaturated fatty acids to the diet of gestating and lactating sows that more fatty acids get through the colostrum and milk to the piglets than through the placenta. And since

fatty acids are important in both prenatal and postnatal growth and the development of immunity, increasing their content in feed rations is an important factor influencing the new-born. Another fatty acid additive was hemp oil, which is an ideal source of stearidonic acid. Vodolazska and Lauridsen (2020) compared the effect of hemp and soybean oil and their combinations on immunoglobulin concentrations in colostrum and sow's milk. The mean IgG concentration in the experimental group with hemp oil (ratio of basic diet to oil 95: 5) was more than 50% lower compared to the group of sows fed soybean oil (95: 5) and more than 60% lower compared to the group a combination of both oils was fed (50:50). However, overall levels of all three immunoglobulins monitored (G, A, M) were lower in the hemp oil group than in the other two groups.

Other additives

Another suitable alternative to the feed additive for improving the immunoglobulin content of colostrum and milk may be rapeseed meal. It appears to be one of the possible alternatives to soy protein. And it was the feeding of rapeseed meal that they focused on in an experiment by Grela et al., (2019). Since rapeseed contains a certain amount of antinutritional substances (glucosinolates), it has been shown that these are partially removed by fermentation. In the experiment, they focused on assessing the effect of feeding fermented rapeseed meal (FRM) depending on the physiological period and reproductive cycle on production parameters, digestibility, but also the content of immunoglobulins in colostrum. The experimental groups were fed a different proportion of FRM (it replaced soybean meal) according to the stage of pregnancy and lactation, namely 4% or 9%. The content of IgG and IgA was significantly higher in the experimental groups compared to the control groups. They found the interaction between the addition of FRM and the reproductive cycle, as well as the effect of the reproductive cycle itself on the IgG content in the colostrum. The authors attribute the increase in the level of immunoglobulins in sow colostrum to the response of the immune system to a foreign antigen of microbial origin. In their study, Zanello et al. (2013) focused on the administration of 3 different strains of *Saccharomyces cerevisiae* (SC) and their effect on IgG and IgA concentrations in colostrum and sow milk and to reduce the incidence of diarrhea during lactation. An increase in colostrum IgG

concentration was demonstrated in the experimental groups. By adding SC, the IgA concentration was kept almost the same until day 18. The authors also noted a trend in reducing the incidence of diarrhea in piglets in the SC group. Essential oils are generally considered to be natural, less toxic and especially residual free antibiotic alternatives. Last but not least, they have various beneficial effects and one of them is the effect on the immune system and the stimulation of the body's immune responses (Zhai et al., 2018). Oregano essential oils used in experiments by Ariza-Nieto et al., (2011) and Tan et al., (2005) had no demonstrable effect on the levels of immunoglobulins in the blood of sows and gilts. However, in a 2005 experiment, they showed a positive effect on alleviating oxidative stress in sows and improving piglet performance.

CONCLUSION

Colostrum is an irreplaceable and the only source of nutrients in the first hours of piglets after birth. Therefore, the breeder should ensure, as far as possible, its quality and quantity through proper nutrition of the sow in the pre-parturition and lactation periods. Since piglets are born without serum immunoglobulins and their immune system is still evolving, it is important that they take colostrum as soon as possible after birth. And it is the immunoglobulins received from colostrum and milk that provide them with passive immune protection. Therefore, their content in colostrum and milk is a very important factor in assessing quality. Although colostrum is only available to piglets for a short period of time, its chemical composition changes significantly due to its conversion to milk. As has been shown in several studies, the content of immunoglobulins in colostrum and milk can be influenced by feeding gestating and lactating sows. Additions of various types of oils, fatty acids, yeasts or fermented feeds have increased the content of immunoglobulins in colostrum and milk. It is the increase in the content of individual immunoglobulins that presupposes their higher intake in piglets. This will increase their chances of survival and better protection against various pathogens.

ACKNOWLEDGEMENT

This study was supported by the Grant Agency of the Slovak Ministry of Education, Science, Research and Sport and the Slovak Academy of Sciences, project no. 1/0474/19.

REFERENCES

Ariza-Nieto, C., Bandrick, M., Baidoo, S. K., Anil, L., Molitor, T. W., & Hathaway, M. R. (2011): Effect of dietary supplementation of oregano essential oils to sows on colostrum and milk composition, growth pattern and immune status of suckling pigs. Journal of Animal Science, 89(4), 1079-1089.

Bai, Y. S., Wang, C. Q., Zhao, X., Shi, B. M., & Shan, A. S. (2017): Effects of fat sources in sow on the fatty acid profiles and fat globule size of milk and immunoglobulins of sows and piglets. Animal Feed Science and Technology, 234, 217-227.

Bontempo, V., Sciannimanico, D., Pastorelli, G., Rossi, R., Rosi, F., &Corino, C. (2004): Dietary conjugated linoleic acid positively affects immunologic variables in lactating sows and piglets. The Journal of nutrition, 134(4), 817-824.

Butler, J.E. - Kehrli, M.E., Jr. (2005): Immunoglobulins and immunocytes in the mammary gland and its secretions, In Mucosal Immunology, 3rd edn., Vol. 2, J.Mestecky, M. Lamm, W. Strober, J. Bienenstock, J.R. McGhee and L. Mayer, eds., Elsevier, Amsterdam, 1764–1793.

Butler, J.E. - Kehrli, M.E., Jr. (2005): Immunoglobulins and immunocytes in the mammary gland and its secretions, In Mucosal Immunology, 3rd edn., Vol. 2, J.Mestecky, M. Lamm, W. Strober, J. Bienenstock, J.R. McGhee and L. Mayer, eds., Elsevier, Amsterdam, 1764–1793.

Farmer, C., & Quesnel, H. (2009): Nutritional, hormonal, and environmental effects on colostrum in sows. Journal of Animal Science, 87(suppl_13), 56-64.

Graham, H., Pettersson, D. (1992): A note on the effect of a beta-glucanase and a multi-enzyme on production in broiler chicks fed a barley-based diet. Swedish Journal of Agricultural Research 22, 39-42.

Grela, E. R., Czech, A., Kiesz, M., Wlazło, Ł., &Nowakowicz-Dębek, B. (2019): A fermented rapeseed meal additive: Effects on production performance, nutrient digestibility, colostrum immunoglobulin content and microbial flora in sows. Animal Nutrition, 5(4), 373-379.

Hurley, W.L. (2015): Composition of sow colostrum and milk. 2015. In: Farmer, C.: The gestating and lactating sow. Wageningen Academic Publishers, NL, 193-229.

Hurley, W.L., Theil P.K. (2013): Immunoglobulins in Mammary Secretions. In: McSweeney P., Fox P. (eds) Advanced Dairy Chemistry. Springer, Boston, MA.

Ivanišová, E., Terentjeva, M., Kántor, A., Frančáková, H., Kačániová, M. (2019): Phytochemical and antioxidant profile of different varietes of grape from the Small Carpathians wine region of Slovakia. Erwerbs. Obstbau. 61, 53–59.

Kanka, T., Rolinec, M., Bíro, D., Šťastný, P. (2014): Analýza pasívne jimunizácie prasiatok v postnatálnom období. Nitra: Slovenská poľnohospodárska univerzita, ISBN 978-80-552-1181-7

King'ori, A. M. (2012): Sow lactation: Colostrum and Milk Yield: a Review. Journal of Animal Science. 2(6), 525-533.

Klobasa, F., Werhahn, E., Butler, J. E. (1987): Composition of Sow Milk During Lactation. Journal of Animal Science, 64(5), 1458–1466.

Le Dividich, J., Rooke, A., Herpin, P. (2005): Nutritional and immunological importance of colostrum for the new-born pig. The Journal of Agricultural Science, 143, 469-485.

Nuntapaitoon, M., Juthamanee, P., Theil, P. K., Tummaruk, P. (2020): Impact of sow parity on yield and composition of colostrum and milk in Danish Landrace× Yorkshire crossbred sows. Preventive Veterinary Medicine, 181, 105085.

Picone, G., Zappaterra, M., Luise, D. Trimigno, A., Capozzi, F., Motta, R.D., Costa, L.N., Bosi, P., Trevisi, P. (2018): Metabolomics characterization of colostrum in three sow breeds and its influences on piglets' survival and litter growth rates. J Animal Sci Biotechnol, 9, 23.

Quesnel, H., Farmer, C., Theil, P.K. (2015): Colostrum and milk production. In C Farmer (ed.), The gestating and lactating sow. Wageningen Academic Publishers, The Netherlands, pp. 173-192.

Rolinec, M. (2009): Postnatálnatvorbaimunoglobulínovprasiat: PhD Diss. Nitra: SPU. 107s.

Rolinec, M., Biro, D., Stastny, P., Gálik, B., Šimko, M., Juráček, M. (2012): Immunoglobulins in colostrum of sows with porcine reproductive and

respiratory syndrome – PRRS. 2012. Journal of Central European Agriculture. 13, p. 303-311.

Rooke, J. A., Sinclair, A. G., & Edwards, S. A. (2001): Feeding tuna oil to the sow at different times during pregnancy has different effects on piglet long-chain polyunsaturated fatty acid composition at birth and subsequent growth. British Journal of Nutrition, 86(1), 21-30.

Rossi, R., Pastorelli, G., Bontempo, V., &Corino, C. (2004): Effects of dietary conjugated linoleic acid (CLA) on immunoglobulin concentration in sow colostrum and piglet serum. Veterinary research communications, 28(1), 241-244.

Salmon, H., Berri, M., Gerdts, V., &Meurens, F. (2009): Humoral and cellular factors of maternal immunity in swine. Developmental & Comparative Immunology, 33(3), 384-393.

Sehm, J., Treutter, D., Lindermayer, H., Meyer, H. H. D., Pfaffl, M. W. (2011): The Influence of Apple- or Red-Grape Pomace Enriched Piglet Diet on Blood Parameters, Bacterial Colonisation, and Marker Gene Expression in Piglet White Blood Cells. Food and Nutrition Sciences, 02(04), 366–376.

Süli, J., Homzová, K., Sobeková, A., & Hrušková, T. (2014): Fyziologické účinky polyfenolov a ich metabolitov v strave. Diabetologie Metabolismus Endokrinologie Výživa, 17(3), 162-170.

Sun, H. Y., & Kim, I. H. (2020): Coated omega-3 fatty acid from linseed oil positively affect sow immunoglobulin G concentration and pre-weaning performance of piglet. Animal Feed Science and Technology, 269, 114676.

Tan, C., Wei, H., Sun, H., Ao, J., Long, G., Jiang, S., & Peng, J. (2015): Effects of dietary supplementation of oregano essential oil to sows on oxidative stress status, lactation feed intake of sows, and piglet performance. BioMed Research International

Theil, P. K., Nielsen, M. O., Sørensen, M. T., Lauridsen, C. (2012): Lactation, milk and suckling. In K. E. B. Knudsen, N. J. Kjeldsen, H. D. Poulsen, & B. B. Jensen (Eds.), Nutritional physiology of pigs: with emphasis on Danish production conditions Videncenter for Svineproduktion, Landbrug&Fødevarer.

Vodolazska, D., &Lauridsen, C. (2020): Effects of dietary hemp seed oil to sows on fatty acid profiles, nutritional and immune status of piglets. Journal of animal science and biotechnology, 11(1), 1-18.

Wang, R., Yu, H., Fang, H., Jin, Y., Zhao, Y., Shen, J., ... & Zhang, J. (2020): Effects of dietary grape pomace on the intestinal microbiota and growth performance of weaned piglets. Archives of animal nutrition, 74(4), 296-308.

Wang, X., Jiang, G., Kebreab, E., Yu, Q., Li, J., Zhang, X., ... & Dai, Q. (2019): Effects of dietary grape seed polyphenols supplementation during late gestation and lactation on antioxidant status in serum and immunoglobulin content in colostrum of multiparous sows. Journal of animal science, 97(6), 2515-2523.

Zanello, G., Meurens, F., Serreau, D., Chevaleyre, C., Melo, S., Berri, M., ... & Salmon, H. (2013): Effects of dietary yeast strains on immunoglobulin in colostrum and milk of sows. Veterinary immunology and immunopathology, 152(1-2), 20-27.

Zhai, H., Liu, H., Wang, S., Wu, J., &Kluenter, A. M. (2018): Potential of essential oils for poultry and pigs. Animal Nutrition, 4(2), 179-186.

THE EFFECT OF FEED PARTICLE SIZE IN BROILERS CHICKENS ON DIGESTIVE TRACT MORPHOLOGY – A PRELIMINARY REPORT

JAKUB NOVOTNÝ, LUCIE HORÁKOVÁ, MICHAL ŘIHÁČEK, DANA ZÁLEŠÁKOVÁ, ONDŘEJ ŠŤASTNÍK, 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: jakub.novotny@mendelu.cz

ABSTRACT

The influence of feed particle size on broiler chickens´ gastrointestinal organs were evaluated. Broiler males of Ross 308 were divided into 3 experimental groups – Coarse, Medium and Fine. The differences between the diets were in feed particle size. The feed particle size was evaluated by dry sieving. Geometric Mean Diameter (1,111.26 vs. 959.89 vs. 730,48, respectively) and Geometric Standard Deviation (1,085.50 vs. 847.48 vs. 604.12, respectively) of diets were calculated. The different feed mixture had an influence on weight of gizzard and colon. The bigger gizzard weight ($P < 0.05$) was found in groups with larger particle size (Coarse and Medium group).

Keywords: poultry nutrition; Ross 300; animal diets; Geometric Mean Diameter; Geometric Standard Deviation

INTRODUCTION

The physical structure of the diet is determined by the size and shape of the individual particles. Size can be defined as fineness of feed grinding (AMERAH *et* al., 2007). DAVIS et al. (1951) used the general terms "fine, medium and coarse" to describe particle size. The specific size was described by WOLF et al. (2012) as coarse (> 1.4), medium (0.8–1.4), fine (0.4–0.8) and very fine (< 0.4). The particle size is usually determined by dry sieving of representative sample for ten minutes on

the set of sieves. (BAKER and HERMAN, 2002). GMD (Geometric Mean Diameter also d_{gw}) and GSD (Geometric Standard Deviation, also S_{gw}) are used to describe particle size (LENTLE *et* al., 2006). GMD indicates the trough particle size (mm or um) and GSD indicates particle size uniformity. The lower GSD means the higher uniformity (AMERAH *et* al., 2007). The GMD and GSD can be calculated using formulas published by ASABE (2008). SAFAA et al. (2009) noticed the higher intake of coarse feed mixture in comparison with fine feed mixture. AMERAH et al. (2007) found that broilers chickens fed a mixture of fine particles wheat had lower weight gains and more limited feed intake than fed groups mixtures with a higher proportion of medium or coarse particles. Feed particle size may also influence the gastrointestinal tract. Several studies observed feeding with coarse mixture increase the relative gizzard weight (NIR *et* al., 1994 a,c; ENGBERG *et* al., 2002; PÉRON *et* al., 2005; AMERAH *et* al., 2007). The aim of this study is determined the influence of different feed particle size in broilers chickens´ diet on digestive tract morphology

MATERIAL AND METHODS

Animals and experimental conditions

In 5 replicates, a total of 90 one day old male broiler chickens of Ross 308 were randomly divided into 3 different experimental groups (in total 30 chickens per feeding group) with 6 birds per cage. The lighting program, temperature and humidity was set according to the technological instruction (AVIAGEN, 2018). Broilers were fed with experimental starter diets until 10 days of age. Chickens were fed with experimental grower diets from 11th day until 35th day of age. The chickens were fed *ad libitum.* The feed intake of each group was daily recorded. The body weight was regularly noticed. The experimental lasted 35 days. At the end of the trial, broilers were slaughtered, and the selected digestive tract section and liver were weighted and measured.

Experimental diets

In the trial three diets differing only their particle size were used. The first experimental group (the Coarse) was fed with the most of coarse particle size, the Medium group had a lower amount of coarse parts and

the Fine group had fine feed particle size in diet. The ingredients in these diets were used in same amount to ensure the isocaloric and isonitrogenous diets. Compounds and chemical composition of used diets is shown in table 1.

Table 1. Composition of experimental diets and chemical analysis (dry matter)

	STARTER			GROWER		
Component	**Coarse**	**Medium**	**Fine**	**Coarse**	**Medium**	**Fine**
Maize (g/kg)	331.65	331.65	331.65	366.4	366.4	366.4
Soybean meal (g/kg)	438.0	438.0	438.0	396.0	396.0	396.0
Wheat (g/kg)	130.0	130.0	130.0	153.2	153.2	153.2
Rapeseed oil (g/kg)	52.0	52.0	52.0	40.0	40.0	40.0
Premix* (g/kg)	30.0	30.0	30.0	30.0	30.0	30.0
Limestone milled (g/kg)	5.5	5.5	5.5	4.0	4.0	4.0
Monocalcium phosphate (g/kg)	7.7	7.7	7.7	5.9	5.9	5.9
DL-Methionine (g/kg)	2.0	2.0	2.0	1.5	1.5	1.5
Wheat gluten (g/kg)	3.1	3.1	3.1	-	-	-
Chromium oxide (g/kg)	-	-	-	3	3	3
ME_N (MJ/kg)*	12.42	12.42	12.42	12.3	12.3	12.3
Crude protein (g/kg)	231.9	239.6	234.4	215.4	215.1	209.9
Ether extract (g/kg)	71.2	72.4	71.7	73.7	73.1	73.5
Crude fibre (g/kg)	38.2	36.1	36.6	47.6	46.9	42.4
Crude ash (g/kg)	62.7	65.9	64.7	61.3	60.2	60.6

Legend: **Premix for starter contains** (per kg): L-lysine 2.34 g; DL-Methionine 2.4 g; Threonine 0.99 g; calcium 5.25 g; phosphorus 1.95 g; sodium 1.44 g; copper 15 mg; iron 84 mg; zinc 99 mg; manganese 99 mg; iodine 0.99 mg; selenium 0.18 mg; retinol 13,500 IU (international units); calciferol 5,001 IU; tocopherol 45 mg; phylloquinone 1.5 mg; thiamine 4.2 mg; ri-boflavin 8.4 mg; pyridoxin 6 mg; cobalamin 30 µg; biotin 0.21 mg; niacinamid 36 mg; folic acid 1.8 mg; calcium pantothenate 13.5 mg; cholin chloride 180 mg. **Premix for grower contains** (per kg): L-lysine 2.58 g; DL-Methionine 2.52 g; Threonine 1.47 g; calcium 5.04 g; phosphorus 1.65 g; sodium 1.38 g; copper 15 mg; iron 75 mg; zinc 99 mg; manganese 99 mg; iodine 0.9 mg; selenium 0.36 mg; retinol 9,900 IU (international units); calciferol 5,001 IU; tocopherol 45 mg; phylloquinone 1.5 mg; thiamine 4.2 mg; ri-boflavin 8.4 mg; pyridoxin 6 mg; cobalamin 28.8 µg; biotin 0.18 mg; niacinamid 36 mg; folic acid 1.71 mg; calcium pantothenate 13.35 mg; cholin chloride 180 mg. * Apparent metabolize energy, calculated value.

The structure of grower diets was evaluated by dry sieving using a separator Retch AS 200 Control. A representative sample of 100 g of each diet was passed for 10 minutes trough the set of sieves with 3 mm, 2 mm, 1.5 mm, 1 mm and 0.3 mm mesh size. An amplitude was set to 1.8 mm/g. After shaking process, the amount of particle sizes retained on each sieve was determined by subtracting the weight of the sieve and the retained feed from the blank weight of the sieve. The GMD and GSD were calculated.

Sample collection and statistical analysis

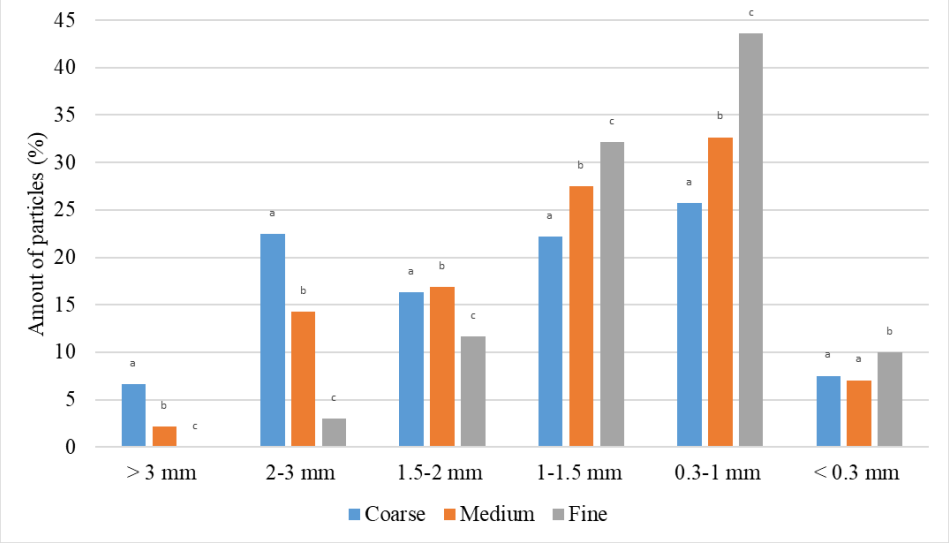
The digestive tract parts and liver were weighed and calculated per kg of live weight. *Duodenum*, *Jejunum*, *Ileum*, Colon and *Cecum* were also measured. Data has been processed by Microsoft Excel (USA) and StatSoft Statistica (USA). It was used one-way analysis of variance (ANOVA). For evaluate statistically differences between groups was used the Sheffé´s test and $P < 0.05$ was regarded a level of statistically significant difference.

RESULTS AND DISCUSSION

Diets analysis

The differences between particle size distribution in the Coarse, Medium and Fine grower diet are shown in graph 1.

Graph 1. Particle size distribution in grower diets



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

From the graph 1 is obvious that there are statistically significant differences in feed particle size between used diets. Differences in amount of particle on sieve were found on each sieve ($P < 0.05$). That proves variety of mixtures. Values for GMD and GSD were calculated based on the weight fraction on the individual sieves. Data are shown in table 2.

Table 2. GMD and GSD of grower diets

	n	**GMD ± GSD (µm)**
Coarse	10	1,111.26 ± 1,085.50[a]
Medium	10	959.89 ± 847.48[b]
Fine	10	730.48 ± 604.12[c]

[a,b,c] means statistically significant differences ($P < 0.05$); n – number of cases; GMD – Geometric mean diameter; GSD – Geometric Standard Deviation

Values of GMD correspond to values from a trial from EGE *et* al. (2019) dealing with the similar issue. They calculated values of GMD were 707 µm vs, 1096 µm.

In the trial, there were not found statistically significant differences between values concerning feed consumption (P > 0.05). The average daily feed intake per trial was 86.47 g/bird in the Fine group, 82.40 g/bird in the Medium group and 85.29 g/bird in the Coarse group.

Table 3 is concerning about chosen segments of digestive tract.

Table 3. Weight of gastrointestinal organ and liver

	Coarse	**Medium**	**Fine**
n	15	15	15
		per kg of BW	
		Mean ± SE	
Crop (g)	3.05 ± 1.90	2.93 ± 0.12	3.39 ± 0.24
Proventriculus (g)	3.73 ± 0.15	3.59 ± 0.11	3.31 ± 0.12
Gizzard (g)	14.97 ± 0.71^{a}	14.56 ± 0.41^{a}	11.84 ± 0.59^{b}
Duodenum (g)	5.88 ± 0.25	6.12 ± 0.24	6.26 ± 0.33
Duodenum (mm)	141.77 ± 7.39	152.25 ± 5.55	139.09 ± 7.39
Jejunum (g)	11.36 ± 0.58	10.94 ± 0.36	10.41 ± 0.44
Jejunum (mm)	330.33 ± 9.09	344.84 ± 12.50	320.15 ± 13.68
Ileum (g)	7.75 ± 0.39	8.09 ± 0.33	7.82 ± 0.31
Ileum (mm)	343.15 ± 11.60	353.45 ± 11.69	342.70 ± 14.64
Colon (g)	1.27 ± 0.04^{a,b}	1.20 ± 0.04^{b}	1.46 ± 0.09^{a}
Colon (mm)	42.43 ± 2.60	39.77 ± 2.08	37.01 ± 1.96
Cecum (g)	3.82 ± 0.16	3.45 ± 0.14	3.62 ± 0.08
Cecum (mm)	81.62 ± 2.57	80.33 ± 3.00	79.03 ± 2.61
Liver (g)	22.32 ± 0.44	22.28 ± 0.42	23.11 ± 0.46

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

Acording to the Table 3, the influence of feed particle size had an effect (P <0.05) on weight of proventriculus and colon. Other examined digestive tract organs were without significant differences. NIR *et* al. (1994 a,b) in their trial found that larger particles in diet for poultry had a positive effect on the development of the muscular stomach, especially its weight. In these experiments found out that groups of poultry fed coarse and intermedium coarse physical structure of the feed mixture had the gizzard about 26% and about 41%, respectively larger.

CONCLUSION

The influence of feed particle size on broiler chickens´ parameters were evaluated. The diets with statistically significant different particle size (coarse, medium and fine) had no influence on feed consumption and most of the parts of digestive tract of chickens. The statistically significant ($p < 0.05$) higher weight of gizzard was found in groups with coarse and medium particle size in diet. Weight of the colon was higher in Fine group in comparison with the medium group. It can be concluded despite the different structure of feed mixture, no effect on monitored markers was recorded except the weight of gizzard.

ACKNOWLEDGEMENT

The project was supported by the Ingernal Grant Agency of Faculty of AgriScience (Mendel University) no. AF-IGA2021-IP026

REFERENCES

Amerah A.M., Ravindran V., Lentle R.G., Thomas D.G. (2007): Feed particle size: Implications on the digestion and performance of poultry. Poultry Science, 63.3, 439–455.

Asabe: Standard (2008): Method of determining and expressing fineness of feed materials by sieving. American Society of Agricultural and Biological Engineers, St. Joseph, US.

Aviagen Group 2018: Technological procedure for broiler Ross. Available at: http://eu.aviagen.com/language-mini-site/show/cz. Last modified July 24, 2019.

Baker S., Herrman T. (2002): Particle size. MF–2051 Feed Manufakturing. Department of Grain Science and Industry, Kansas State University, 5 pp.

Davis R.L., Hill E.G., Sloan H.J., Briggs G.M. (1951): Detrimental effect of corn of coarse particle size in rations for chicks. Poultry Science, 30.3, 325–328.

Ege G., Bozkurt M., Koçer B., Tüzün A.E., Uygun M., Alkun G. (2019): Influence of feed particle size and feed form on productive performance, egg quality, gastrointestinal tract traits, digestive enzymes, intestinal morphology, and nutrient digestability of laying hens reared in enriched cages. Poultry Science, 98.9, 3787–3801.

Engeberg R.M., Hedenann M.S., Jensen B.B. (2002): The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. British Poultry Science, 43.4, 569–579.

Lentle R.G., Ravindran V., Ravindran G., Thomas D.V. (2006): Influence of feed particle size on the efficiency of broiler chickens fed wheat-based diets. Poultry Science, 43.2, 135–142.

Nir I., Hillel R., Shefet G., Nitsan Z. (1994a): Effect of grain particle size on performance. 2. Grain texture interactions. Poultry Science, 73, 781–791.

Nir I., Twina Y., Grossman E., Nitsan Z. (1994b): Quantitative effects of pelletingon performance, gastrointestinal tract and behaviour of meat-type chickens. British Poultry Science, 35,589–602.

Nir I., Shefet G, Aaroni Y. (1994): Effect of particle size on performance. 1. Corn. Poultry Science, 73.1, 45–49.

Péron. A., Bastianelli D., Oury F.X., Gomez J., Carré B. (2005): Effects of food deprivation and particle size of ground wheat on digestibility of food components in broilers fed on a pelleted diet. British Poultry Science, 46.2, 223–230.

Safaa H.M., Jiménez-Moreno E., Valencia D.G., Frikha M., Serrano M.P., Mateos G.G. (2009): Effect of main cereal of the diet and particle size of the cereal on productive performance and egg quality of brown egg-laying hens in early phase of production. Poultry Science, 88.3, 608–614.

Wolf P., Arlinghaus M., Kamphues J. Sauer N., Mosenthin R. (2012): Einfluss der Partikelgröße im Futter auf die Nährstoffverdaulichkeit und Leistung beim Schwein. Übersichten zur Tierernährung, 40, 21–64.

IMMUNE RESPONSE OF RAINBOW TROUT (ONCORHYNCHUS MYKISS) FED WITH FEED ENRICHED BY AUTOCHTONOUS PROBIOTIC LACTOBACILLI

MAREK RATVAJ[1], IVANA CINGEĽOVÁ MARUŠČÁKOVÁ[1], PETRA SCHUSTEROVÁ[1], ADRIÁNA FEČKANINOVÁ[2], PETER POPELKA[3], JAN MAREŠ[4], DAGMAR MUDROŇOVÁ[1]

[1]Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice, Slovakia

[2]Department of Pharmaceutical Technology, Pharmacognozy and Botany, University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81, Košice, Slovakia

[3]Department of Food Hygiene, Technology and Safety, University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81, Košice, Slovakia

[4]Department of Zoology, Fisheries, Hydrobiology and Apiculture, Mendel University, Zemedelska 1, Brno, Czech Republic

Corresponding email address: marek.ratvaj@student.uvlf.sk

ABSTRACT

Our aim was to study effect of feed that has been enriched with autochthonous probiotic *Lactobacillus plantarum* R2 on relative gene expression of immunologically important molecules in rainbow trout. This is a partial work and samples are still being analyzed. Experimental fish were divided into three groups of 10 individuals and each group was separated in its tank. First and second group received our probiotic feed; first group continually during whole experiment and second group cyclically. Third was control group, thus it received just commercial feed. After 7 weeks, samples of distal intestine were collected and change in relative gene expression was measured. Molecules that were

studied were interleukin 1, interleukin 8, CD4, CD8 and immunoglobulin M. The gene expression was assessed using quantitative real time PCR with β-actin as a reference gene. Relative expression of interleukin 1 and 8 did not change significantly although interleukin 8 showed decreased expression in both experimental groups as compared to control. Expression of CD4 was increased in both experimental groups with significant change in continually fed group. CD8 showed slight increase in continual group and slight decrease in cyclical group. Slight increase of expression for immunoglobulin M was also measured in both experimental groups. Overall, lower expression for genes in cyclically fed group might be result of temporary change in feed that stressed fish. We conclude that continually administered probiotic feed positively affected immunity of fish by enhancing numbers of CD4 and CD8 cells and immunoglobulin M while not triggering inflammatory response.

Keywords: cytokines; microbiome; gene expression

INTRODUCTION

The topic of fish health is becoming more and more actual because of increasing consumption as well as production of fish. The most dynamic growth can be seen in fish reared on farms. Aquaculture is the fastest growing sector in production of fish and its growth is expected to not slow down in coming years (FAO, 2020). Inevitably, with increased production of fish reared in monoculture, the level of stress in these fish increases. This causes worse reproduction (Billard et al., 1981), appetite (Conde-Sieira et al., 2018) and also immune response against pathogens (Pickering & Pottinger, 1989). Probiotics, which have been in use for decades in mammals, seem like a suitable tool for compensating any negative effects that affect fish in aquaculture (Merrifield et al., 2010). There are already many bacterial strains which have been studied to various degree (Nayak, 2010), as well as commercially available probiotic supplements for fish. Our goal was to confirm probiotic potential of our autochtonous strain – *Lactobacillus plantarum* R2, previously isolated from gut of rainbow trout (Fečkaninová et al., 2019). In this work we focused on the immunomodulatory potential of the strain.

MATERIAL AND METHODS

Probiotic feed was prepared at University of Veterinary Medicine and Pharmacy in Kosice by combining commercially available pelleted feed for fish in aquaculture with protective components and autochthonous probiotic strain *L. plantarum* R2 that was cultivated overnight. All components were then thoroughly mixed and the feed was dried and stored in cold and dry conditions. Concentration of bacteria in final product was measured to be 10^7-10^8 CFU/g of feed. Probiotic feed was then transferred to Mendel University in Brno where it was fed to experimental fish.

Species of fish used in this experiment was rainbow trout (*Oncorhynchus mykiss*). Total number of 1000 fish was involved in experiment and they were divided into three groups. Each group was then separated in three individual tanks with independent circulation. First group received enriched feed continually (CON) during whole time of experiment, second group received probiotic feed in cycles (CYC) with pause (3 weeks) in-between during which they were fed commercial feed. Third group served as control group (CTRL) thus it received commercial feed with protective components. Fish of each group consumed feed with good appetite.

Sampling took place after 7 weeks of feeding fish and for further analysis only specimens with approximately similar physical dimensions were selected. During dissection samples of skin and gills as well as internal organs; head kidney, spleen and part of distal intestine were collected. For this part of experiment only distal intestine was further analyzed. 10 fish from each group were selected. Samples were stored in RNAlater at -20 °C.

Further processing of samples was done at University of Veterinary Medicine and Pharmacy in Kosice. Isolation of RNA was done using Omega E-Z Total RNA Kit (Bio-tech, USA). Purity and concentration of RNA was measured by spectrophotometer Nanodrop 8000 (Thermo Scientific, USA) at 260/280 nm. Isolated RNA was then transcribed into cDNA using Quantitect Reverse Transcription Kit (Qiagen, Germany). PCR reaction was performed in 10-µl reactions, each reaction consisting of the SYBR green master mix (BioRad, USA), 0,5 µM of both primers,

and 40 ng/µL of cDNA. All reactions contained negative control without cDNA template. Relative gene expression was analyzed in samples of cDNA by qPCR using thermocycler iCycler CFX96 (BioRad, USA). During this part of experiment we measured change in relative gene expression for interleukin 1 (IL1), interleukin 8 (IL8), CD4, CD8 and imunoglobulin M (IgM) as $\Delta\Delta C_t$ (± SD) with CFX96 Manager software (BioRad, USA). Sequences of primers used are in table 1. β-actin was used as a reference gene.

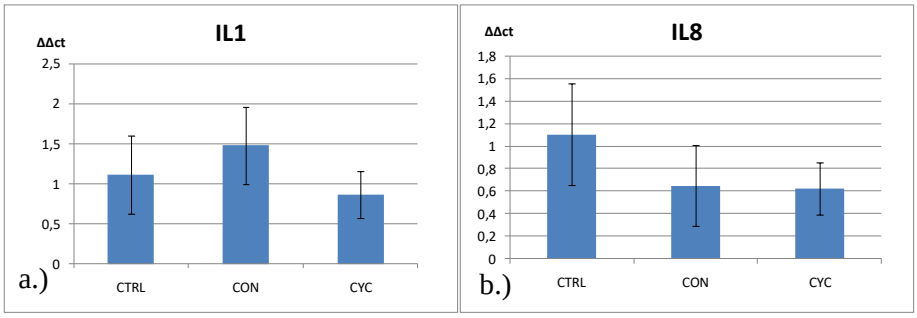
Table 1. Sequences of primers used in qPCR

	Primer sequence	Reference
β-actin F	GGACTTTGAGCAGGAGATGG	(Kim & Austin, 2006)
β-actin R	ATGATGGAGTTGTAGGTGGTCT	
Il-1β F	ACATTGCCAACCTCATCATCG	(Yarahmadi et al., 2016)
Il-1β R	TTGAGCAGGTCCTTGTCCTTG	
Il-8 F	CACAGACAGAGAAGGAAGGAAAG	(Kim & Austin, 2006)
Il-8 F	TGCTCATCTTGGGGTTACAGA	
CD4 F	CCTGCTCATCCACAGCCT	(Li et al., 2019)
CD4 R	CTTCTCCTGGCTGTCTGA	
CD8 F	AGTCGTGCAAAGTGGGA	(Li et al., 2019)
CD8 R	GGTTGCAATGGCATACAG	
IgM F	ACCTTAACCAGCCGAAAG	(Li et al., 2019)
IgM R	TGTCCCATTGCTCCAGTC	

For statistical analysis one way ANOVA test with Tukey multiple comparison test was used in software GraphPad Prism Version 3.00.

RESULTS AND DISCUSSION

Figure 1. Relative gene expression for IL1 (a.) and IL8 (b.). Data presented with ± SD.

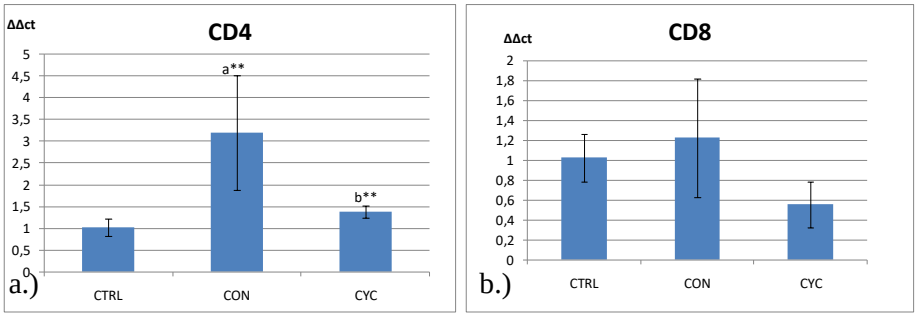


Relative gene expression for interleukins 1 and 8 did not change significantly in either experimental group when compared to CTRL. There was slight increase in expression for IL1 in group that received probiotics continually (figure 1a). IL8 expression was lower in both experimental groups, where it was decreased by same margin, although not statistically significant (figure 1b).

Both IL1 and IL8 are typical proinflammatory cytokines and their synthesis increase in teleosts following viral infection as well as after recognizing molecular patterns associated with pathogens (Seppola et al., 2008). Our results show no significant increase in expression of these cytokines suggesting that bacterial strain we studied (*L. plantarum* R2) did not cause defensive reaction of fish organism and therefore is not pathogenic, which is one of requirements for probiotic bacteria by (FAO/WHO 2002). These results correlate with previous research on cells from gut of rainbow trout after stimulation with probiotics where no significant change in expression of either cytokine was measured (Kim & Austin, 2006) although IL1 was upregulated in spleen and kidney of trout (Panigrahi et al., 2007). IL8 relative expression even decreased suggesting anti-inflammatory effect of *L. plantarum* R2 though Pérez-Sánchez et al. (2011) measured upregulation of expression

for this cytokine in their work. One of the reasons for this could be the difference of bacterial strain.

Figure 2. Relative gene expression for CD4 (a.) and CD8 (b.). Data presented with ± SD. $^{**}p < 0.01$ a – versus CTRL group, b – versus CON group.



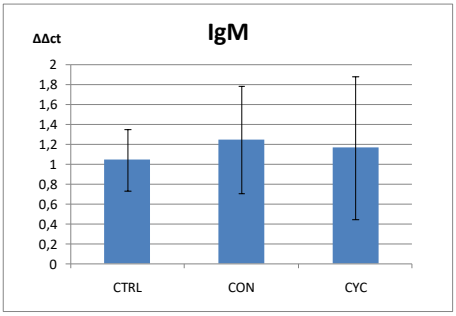
Relative gene expression for CD4 marker was increased in both experimental groups (figure 2a). Significant increase was recorded in CON group when compared to CTRL. Significant change was also observed in CYC group, which had decreased relative gene expression for CD4 when compared to CON group. With CD8 (figure 2b) the situation was similar to expression of IL1; slight increase in relative gene expression in CON group when compared to CTRL and slight decrease in CYC group when compared to CTRL, both without any statistical significance.

CD4 is a transmembrane glycoprotein that can be found in teleosts on surface of T-helper cells (Th) that regulate cytokine response of organism after infection. They belong to several subtypes with different functions (T-helper 1, T-helper 2, T-helper 17, T-helper 9, and regulatory-T cells) (Ashfaq et al., 2019). Which cytokines they produce can only be hypothesized based on mammals. Even though we do not know which subtype is increased specifically in our experimental groups, small changes in expression of pro inflammatory cytokines previously discussed would suggest that there is larger population of regulatory T-cells which are responsible for immune tolerance (Yuan & Malek, 2012)

and since they give signal that *L.plantarum* R2 is not harmful to rest of organism we did not measure increased levels of IL1 and IL8. Another important thing is that the increase in relative gene expression is several times higher in continually fed group, probably because of continual presence and stimulation of intestinal cells by probiotic bacteria and this increased level could provide faster response during infection. In general, in mammals, the increasing ratio of CD4 to CD8 lymphocytes is considered to be a manifestation of immunostimulation, which can also be applied to teleosts, as the function of both subpopulations is the same as in mammals.

Fish CD8 cells are responsible for eliminating transformed cells or cells that are infected by virus and are called cytotoxic T-lymphocytes (Toda et al., 2009). We did not note significant change in relative gene expression for this marker in trout gut, which is expected given that *L.plantarum* R2 does not enter cells neither transforms them. Recorded increase is only within margin of standard deviation although higher concentrations of CD8 cells could provide better defense in case of infection.

Figure 3. Relative gene expression for IgM. Data presented with ± SD.



There was slight increase in gene expression for IgM (figure 3.) in both experimental groups compared to CTRL although again only without statistical significance

In healthy fish, soluble IgM is the most abundant immunoglobulin in all organs and its concentration increases as a response to intestinal bacterial infection (Piazzon et al., 2016). In our case IgM was increased only

slightly and within standard deviation further suggesting that fish organism didn't respond to probiotics as pathogens. On the other hand, imunoglobulins of fish coat symbiotic microbiota present on mucosal surfaces, suggesting their role in immune exclusion of these organisms (WG et al., 2019). In our experiment there was just small increase in relative expression for IgM that would suggest increased production of this Ig for coating new bacteria that were constantly introduced to intestine via feed. Nevertheless, the function of IgM in microbiome homeostasis remains unknown so far.

Overall CYC group showed lower increase in relative gene expression for selected genes. This might be a result of change in feeding, which partially stressed fish.

CONCLUSION

In conclusion; probiotic bacteria *Lactobacillus plantarum R2,* which was supplemented to fish in feed, was recognized by fish immune system as harmless, based on evidence of decreased expression of proinflammatory cytokines, increased expression of CD4 molecules which are linked to cells that provide immune tolerance and slightly increased levels of CD8 and IgM that would destroy these bacteria. Increased levels of CD4 and CD8 cells would also provide faster response in case of infection, enhancing effect of probiotic. Based on these results *L. plantarum* R2 is suitable probiotic strain and its effect on pathogens should be studied further.

ACKNOWLEDGEMENT

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-19-0234.

REFERENCES

Ashfaq, H., Soliman, H., Saleh, M., & El-Matbouli, M. (2019). CD4: A vital player in the teleost fish immune system. In *Veterinary Research* (Vol. 50, Issue 1, p. 1).

Billard, R., Bry, C., & Gillet, C. (1981). Stress, environment and reproduction in teleost fish. In *Stress and fish* (Vol. 4, pp. 185–201).

Conde-Sieira, M., Chivite, M., Míguez, J. M., & Soengas, J. L. (2018). Stress effects on the mechanisms regulating appetite in teleost fish. In *Frontiers in Endocrinology* (Vol. 9, Issue OCT, p. 631).

FAO. (2020). The state of world fisheries and aquaculture 2020. Sustainability in action. In *Food and Agriculture Organization of the United Nations*.

FAO/WHO (2002). Guidelinesfor the evaluation of probiotics in food: joint FAO/WHO working group meeting, London Ontario, Canada, 30 April-1 May 2002. [online] p. 8 Available from http:www.who.int/foodsafety/publications/fs_management/probiotics2/en/index.html (accessed July 19, 2021)

Fečkaninová, A., Koščová, J., Mudroňová, D., Schusterová, P., Cingeľová Maruščáková, I., & Popelka, P. (2019). Characterization of two novel lactic acid bacteria isolated from the intestine of rainbow trout (Oncorhynchus mykiss, Walbaum) in Slovakia. *Aquaculture*, *506*, 294–301.

Kim, D. H., & Austin, B. (2006). Cytokine expression in leucocytes and gut cells of rainbow trout, Oncorhynchus mykiss Walbaum, induced by probiotics. *Veterinary Immunology and Immunopathology*, *114*(3–4), 297–304.

Li, S., Xie, H., Yan, Z., Li, B., Wu, P., Qian, X., Zhang, X., Wu, J., Liu, J., & Zhao, X. (2019). Development of a live vector vaccine against infectious hematopoietic necrosis virus in rainbow trout. *Fish and Shellfish Immunology*, *89*, 516–524.

Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T. M., Bøgwald, J., Castex, M., & Ringø, E. (2010). The current status and future focus of probiotic and prebiotic applications for salmonids. In *Aquaculture* (Vol. 302, Issues 1–2, pp. 1–18)

Nayak, S. K. (2010). Probiotics and immunity: A fish perspective. In *Fish and Shellfish Immunology* (Vol. 29, Issue 1, pp. 2–14)

Panigrahi, A., Kiron, V., Satoh, S., Hirono, I., Kobayashi, T., Sugita, H., Puangkaew, J., & Aoki, T. (2007). Immune modulation and expression of cytokine genes in rainbow trout Oncorhynchus mykiss upon probiotic feeding. *Developmental and Comparative Immunology*, *31*(4), 372–382

Pérez-Sánchez, T., Balcázar, J. L., Merrifield, D. L., Carnevali, O., Gioacchini, G., de Blas, I., & Ruiz-Zarzuela, I. (2011). Expression of immune-related genes in rainbow trout (Oncorhynchus mykiss) induced by probiotic bacteria during

Lactococcus garvieae infection. *Fish and Shellfish Immunology*, *31*(2), 196–201.

Piazzon, M. C., Galindo-Villegas, J., Pereiro, P., Estensoro, I., Calduch-Giner, J. A., Gómez-Casado, E., Novoa, B., Mulero, V., Sitjà-Bobadilla, A., & Pérez-Sánchez, J. (2016). Differential Modulation of IgT and IgM upon Parasitic, Bacterial, Viral, and Dietary Challenges in a Perciform Fish. *Frontiers in Immunology*, *0*(DEC), 637.

Pickering, A. D., & Pottinger, T. G. (1989). Stress responses and disease resistance in salmonid fish: Effects of chronic elevation of plasma cortisol. *Fish Physiology and Biochemistry*, *7*(1–6), 253–258.

Seppola, M., Larsen, A. N., Steiro, K., Robertsen, B., & Jensen, I. (2008). Characterisation and expression analysis of the interleukin genes, IL-1β, IL-8 and IL-10, in Atlantic cod (Gadus morhua L.). *Molecular Immunology*, *45*(4), 887–897.

Toda, H., Shibasaki, Y., Koike, T., Ohtani, M., Takizawa, F., Ototake, M., Moritomo, T., & Nakanishi, T. (2009). Alloantigen-specific killing is mediated by CD8-positive T cells in fish. *Developmental and Comparative Immunology*, *33*(4), 646–652.

WG, K., YY, Y., S, D., ZY, H., LG, D., JF, C., F, D., XT, Z., X, L., HY, X., KF, M., JG, S., & Z, X. (2019). Pharyngeal Immunity in Early Vertebrates Provides Functional and Evolutionary Insight into Mucosal Homeostasis. *Journal of Immunology (Baltimore, Md. : 1950)*, *203*(11), 3054–3067

Yarahmadi, P., Miandare, H. K., Fayaz, S., & Caipang, C. M. A. (2016). Increased stocking density causes changes in expression of selected stress- and immune-related genes, humoral innate immune parameters and stress responses of rainbow trout (Oncorhynchus mykiss). *Fish and Shellfish Immunology*, *48*, 43–53.

Yuan, X., & Malek, T. R. (2012). Cellular and molecular determinants for the development of natural and induced regulatory T cells. *Human Immunology*, *73*(8), 773–782.

POTENTIAL USE OF BEE BREAD IN NUTRITION

NIKOLETA ŠIMONOVÁ[1], ANNA KALAFOVÁ[1], RUDOLF DUPÁK[1], MONIKA SCHNEIDGENOVÁ[1], EMÍLIA HANUSOVÁ[2], CYRIL HRNČÁR[3], JURAJ ČUBOŇ[4], PETER HAŠČÍK[4], MARCELA CAPCAROVÁ[1]

[1] Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovak Republic

2 National Agricultural and Food Center, Hlohovecká 2, 951 41 Lužianky, Slovak Republic

[3]Department of Small Animal Science, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovak Republic

[4]Department of Technology and Quality of Animal Products, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovak Republic

Corresponding email address: xsimonovan1@uniag.sk

ABSTRACT

The present study was conducted to evaluate the activity of liver enzymes of male Japanese quails by adding bee bread into feed in different concentrations. A total of 40 male Japanese quails were divided into four groups according to administered bee bread into feed mixture HYD 11, which was given *ad libitum*, as follows: P1 (n = 10) 2 g/kg of bee bread, P2 (n = 10) 4 g/kg, P3 (n = 10) 6 g/kg, and the control without additives (K) for 8 weeks. The groups were kept under the same conditions and parameters were analysed using an automatic clinical device Biolis 24i Premium. We noticed slight increase in alanine aminotransferase (ALT) in P2 (0.064 ± 0.043 μkat/l) compared with P3 (0.026 ± 0.015 μkat/l), and in enzyme aspartate aminotransferase (AST) between P3 (4,39 ± 1,06 μkat/l-1) compared to P2 (7,78 ± 1,64 μkat/l-1), although not statistically significant (P > 0.05). We can conclude, that bee bread did not have a significant (P > 0.05) effect on the liver enzymes

by addition into feed, nevertheless, the results can be used for further examination.

Keywords: bee bread; beehive products; Japanese quails; liver enzymes

INTRODUCTION

Bee bread is a product from the beehive which has unique properties. Nutrients from bee bread are well absorbed while digestion due to the process of fermentation of bee pollen, honey, wax, and bee excretions in honeycombs in beehives. It is characterized by its antibacterial, antiallergic, hepatoprotective, and antitumor properties, but has not been well studied yet (Barene et al., 2015; Mărgăoan et al., 2019). Bee bread is also a powerful antioxidant (Bharti et al., 2018). It also contains building and protective substances (proteins, fats, carbohydrates, vitamins, minerals, polyphenols, and flavonoids) (Milojkovic, 2018; Yucel et al., 2017; Urcan, 2017). The liver is a major organ of metabolism in the animal body that is involved in the detoxification and regulation of various biochemical functions. The liver is the most sensitive organ to oxidative damage because it is rich in oxidizable substances. Hepatic enzymes, especially AST and ALT, can indicate hepatocellular damage (Ehrmann et al., 2010; Giannini et al., 2005). Japanese quails *(Coturnix japonica)* were used as an animal model in the study of various diseases, metabolism of various substances (Jatoi et al., 2013; Hanusova et al., 2016; Huss et al., 2008). Japanese quail is characterized by a rapid growth rate, fast sexual maturity, and short generation interval (Hanusova et al., 2013). The aim of this study was to evaluate changes in liver enzymes by adding bee bread into feed of Japanese quails, which allows a better understanding of effects of bee bread and it´s possible effects by adding into feed.

MATERIAL AND METHODS

Japanese quails were housed in the Research Institute of Animal Production in Lužianky. From the 35th day of age, they were divided into a four-storey cage system (Venturi, Italy) with a temperature of 21 ± 2 ° C and 64 ± 2 % humidity. The experimental conditions were continuously monitored. A total of 40 male Japanese quails were divided

into four groups as follows: P1 (n = 10) received bee bread (National Botanical Garden of the National Academy of Sciences, Kyiv, Ukraine) at a dose of 2 g/kg, P2 (n = 10) 4 g/kg and P3 (n = 10) 6 g/kg feed mixture. Control group K (n = 10) was without the addition of bee bread. Feeding mixture HYD 11 (Tekro, Slovak Republic) was given to each group together with bee bread and water *ad libitum.* The composition of the feed mixture is presented in Table 1.

Table 1 The composition of feed mixture HYD 11

Composition	Declared quality features
corn 32 %	nitrogenous substances min 200 g/kg
extracted soybean meal 19.2 %	fibre max 60 g/kg
wheat 15 %	ash max 160 g/kg
CaCO3 10 %	ME min 11,7 MJ/kg
rapeseed meal 7 %	lysine min 7,5 g/kg
sunflower meal 4.5 %	methionine and cysteine min 6 g/kg
animal fat 4 %	linoleic acid min 10 g/kg
malt flower 3 %	Ca min 35 g/kg
monocalcium phosphate 1 %	P min 5 g/kg
premix additives 1 %	Na min 1,6 g/kg
NaCl 0.3 %	
fish meal 3 %	

At the age of 8 weeks, quails were slaughtered and blood samples were taken, centrifuged at 3000 rpm for 30 minutes. Selected liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) were analysed in the blood serum by biochemical analyser Biolis 24i Premium (Tokyo Boeki MediSys Inc., Japan) using analytical kits from Randox (Randox Laboratories Ltd, Crumlin, UK). The data were analysed using the One Way ANOVA test by using GraphPad Prism 8 statistical

software (GraphPad Software Inc., La Jolla, CA, USA). Differences between treatments were tested for significance at $P \leq 0.05$.

RESULTS AND DISCUSSION

Therefore, we have noticed slight changes in liver enzymes, the changes have not been statistically significant ($P > 0.05$) in any of enzyme. The differences have been noticed in AST between P3 (4.39 ± 1.06 µkat/l) compared to P2 (7.78 ± 1.64 µkat/l). Slight increase of ALT was noticed in P2 (0.064 ± 0.043 µkat/l) compared with P3 (0.026 ± 0.015 µkat/l. We also noticed slight increase in GGT in P3 (0.06 ± 0.06 µkat/l) compared to other groups, but differences remained insignificant ($P > 0.05$). Results of ALP did not show any statistically significant differences ($P>0.05$) between groups (Table 2). All of the values were in the range of reference values for Japanese quails (Scholtz et al., 2009).

Table 2 Results of liver enzymes in experimental groups

Parameter	K	P1	P2	P3	P value
AST µkat/l	5.31 ± 1.99	5.75 ± 1.65	7.78 ± 1.64	4.39 ± 1.06	P = 0.0626
ALT µkat/l	0.04 ± 0.00	0.03 ± 0.004	0.06 ± 0.04	0.02 ± 0.01	P = 0.159
GGT µkat/l	0.02 ± 0.01	0.01 ± 0.005	0.03 ± 0.03	0.06 ± 0.06	P = 0.382
ALP µkat/l	1.73 ± 0.85	2.19 ± 0.58	2.13 ± 1.20	3.10 ± 0.62	P = 0.248

K - control group without adding bee bread, P1 - bee bread 2 g/kg, P2 - bee bread 4 g/kg, P3 - bee bread 6 g/kg. Values shown as mean ± S.D.

Generally, we can conclude that high doses (6 g/kg) of bee bread might have positive effects on lowering AST. Similarly, Čeksterytė et al. (2012) noted a reduction of AST while treating patients with liver disease with beebread supplement mixed with honey. Yıldız et al. (2013) concluded potential hepatoprotective effects of chestnut bee pollen on liver enzymes AST and ALT in rats. We monitored slight decrease in

ALT in group P2 compared with P3, although not statistically significant $P > 0.05$. Bakour et al. (2021) found that bee bread was effective in restoring biochemical parameters including liver enzymes harmed due to titanium nanoparticles reactivity in rats. We assume that our results in comparison with other authors might be influenced by the age of quails, the breed of quails, as well as the composition of the administered bee bread. Determining the dose of bee bread used in experiment is due to limited number of studies a big problem

CONCLUSION

We can conclude that the application of bee bread into the feed of Japanese quails did not influence the activity of liver enzymes (AST, ALT, GGT, ALP), nevertheless, high doses (6 g/kg) of bee bread might have positive effects on lowering activity of AST, ALT. Further examination is needed.

ACKNOWLEDGEMENT

The project was supported by the VEGA 1/0144/19 a APVV 19/0243 and the Operational Program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund.

REFERENCES

Bakour, M., Hammas, N., Laaroussi, H., Ousaaid, D., Fatemi, H. E., Aboulghazi, A., ... & Lyoussi, B. (2021). Moroccan Bee Bread Improves Biochemical and Histological Changes of the Brain, Liver, and Kidneys Induced by Titanium Dioxide Nanoparticles. BioMed Research International, 2021.

Barene, I., Daberte, I., Siksna, S. (2015). Investigation of bee bread and development of its dosage forms. Medicinos teorija ir praktika 60, 16-22.

Bharti, U., Kumar, N. R., & Chaudhary, D. (2018). Modulatory Prospective of Bee Pollen against Oxidative Stress Induced by Drugs: A Review. Research Journal of Pharmacy and Technology, 11(12), 5648-5652.

Čeksterytė, V., Balžekas, J., Baltuškevičius, A., & Jurgevičius, E. (2012). The use of beebread-honey mixture in the treatment of liver diseases in alcohol-dependent patients. Chemical Technology, 60(2), 62-66.

Ehrmann, J. et al. (2010): Hepatologie. 1.vyd. Praha: Grada. 590 s. ISBN: 978-80-247- 3118-6.

Giannini, E. G., Testa, R., & Savarino, V. (2005). Liver enzyme alteration: a guide for clinicians. Cmaj, 172(3), 367-379.

Hanusová, E. – Baumgartner, J. – Hetényi, L. – Hanus, A. (2013): Prepelica japonská. Nitra: Publikácie CVŽV. ISBN 978-80-89418-30-5

Hanusová, E., Hrnčár, C., Hanus, A., & Oravcová, M. (2016). Egg traits in Japanese quails. Acta Fytotechnica et Zootechnica, 19(5).

Jatoi, A. S., Sahota, A. W., Akram, M., Javed, K., Jaspal, M. H., Hussain, J., ... & Mehmood, S. (2013). Effect of different body weight categories on the productive performance of four close-bred flocks of Japanese quails (Coturnix coturnix japonica). JAPS, Journal of Animal and Plant Sciences, 23(1), 7-13.

Mărgăoan, R., Stranț, M., Varadi, A., Topal, E., Yücel, B., Cornea-Cipcigan, M., ... & Vodnar, D. C. (2019). Bee collected pollen and bee bread: Bioactive constituents and health benefits. Antioxidants, 8(12), 568.

Milojkovic, V. (2018). Bee Bread (Perga)—The Source of Health, Vitality and Longevity. Proceedings of the Apiquality & Apimedica.

Scholtz N, Halle I, Flachowsky G, Sauerwein H (2009) Serum chemistry reference values in adult Japanese quail (Coturnix coturnix japonica) including sex-related differences. Poult Sci 88: 1186-1190

Urcan, A., Marghitas, L. A., Dezmirean, D. S., Bobis, O., Bonta, V., Muresan, C., Margaoan, R. (2017). Chemical Composition and Biological Activities of Beebread-Review. Bulletin of the university of agricultural sciences & veterinary medicine Cluj-Napoca. Animal Science & Biotechnologies, 74(1).

Yıldız, O., Can, Z., Saral, Ö., Yuluğ, E., Öztürk, F., Aliyazıcıoğlu, R., ... & Kolaylı, S. (2013). Hepatoprotective potential of chestnut bee pollen on carbon tetrachloride-induced hepatic damages in rats. Evidence-based complementary and alternative medicine, 2013.

Yucel, B., Topal, E., & Kosoglu, M. (2017). Bee products as functional food. Superfood and Functional Food-An Overview of Their Processing and Utilization, 15-33.

EFFECT OF SACCHAROMYCES CEREVISIAE APLLICATION ON PHYSIOLOGICAL AND MICROBIOLOGICAL PARAMETERS IN DOGS

LUCIA ŠTEMPELOVÁ[1], VIOLA STROMPFOVÁ[1], IVANA KUBAŠOVÁ[1], DAGMAR MUDROŇOVÁ[2], MARTINA TAKÁČOVÁ[3], BARTOSZ GĄSOWSKI[2], KLAUDIA ČOBANOVÁ[1], ALADÁR MAĎARI[3]

[1]Centre of Biosciences of the Slovak Academy of Sciences, Institute of Animal Physiology, Šoltésovej 4-6, 040 01 Košice, Slovakia
[2]University of Veterinary Medicine and Pharmacy, Department of Microbiology and Immunology, Komenského 73, 041 81, Košice, Slovakia
[3]University of Veterinary Medicine and Pharmacy, Small Animal Clinic, Komenského 73, 041 81, Košice, Slovakia

Corresponding email address: stempelova@saske.sk

ABSTRACT

One of the most commonly studied yeast species in humans or animals is *Saccharomyces cerevisiae* especially in its live cells form. Scarce knowledge on its hydrolysate product effects in dogs forced us to test diet supplemented with hydrolysed brewery *S. cerevisiae* **(**at a dose 0.3 % of the diet) for 14 days to healthy adult dogs**.** Twenty German Shepherds were randomly divided into 2 groups: control and experimental, ten dogs in each. The experiment lasted 42 days (blood and faeces sample collection at days 0, 14, 28, and 42). The results of this straightforward experiment showed significant increase in the abundance of bifidobacteria (day 14), lactic acid bacteria (day 42) and clostridia (day 42). The faecal pH was significantly increased at day 28. In blood serum, the concentration of triglyceride and cholesterol decreased (day 42) while activities of alanine aminotransferase (at day 14) and aspartate aminotransferase significantly increased (at days 28 and 42). Activities of these enzymes were above reference range top in 7 dogs (ALT) and 4 dogs (AST). Haematological parameters and activity of phagocytes as well as on percentage of lymphocyte subsets $CD4^+$,

$CD8^+$, $CD4^+CD8^+$, $CD21^+$ were not changed during the experiment. The important point of these results is their onset mostly in the post-supplementation period. The observation of some unexpected effects emphasizes the need for reassessment to use yeasts products for dogs but further studies using different doses are necessary.

Keywords: *Saccharomyces cerevisiae;* dog; serum biochemistry; faecal analysis; heamatology

INTRODUCTION

Saccharomyces sp. is unicellular eukaryotic which converts carbohydrates to carbon dioxide and alcohols by fermentation. Despite *Saccharomyces* is not regularly detected genus in canine intestine, several species of this genus have been studied as probiotics however in dogs very rarely so far (Aktas et al., 2007; D´Angelo et al., 2018). According results of human clinical trials, some strains of *Saccharomyces cerevisiae* and *Saccharomyces boulardii* can be used to treat acute stress-related intestinal dysmotility (West et al., 2016), to prevent antibiotic or *Clostridium difficile* induced diarrhea, and to attenuate acute gastroenteritis (rotoviral and bacterial) and traveller's diarrhea (Czerucka et al., 2007). In animals, detoxification of bacterial toxins, improvement of digestibility and animal performance, antagonism suppression of pathogenic bacteria, stimulation of immune system and increase of activity-specific enzyme bacteria have been reported (Elghandour et al., 2019). This yeast is frequently used as nutritional supplement and source of B-complex vitamins and amino acids also for animals (Yalcin et al., 2010; Chollom et al., 2017).

The aim of this study is to determine microbiological, biochemical, haematological parameters and faecal characteristics in healthy dogs after their diet was supplemented with 0.3 % of brewery *S. cerevisiae* hydrolysate for 14 days. There is still lack of studies comprehensively analyzing safety and effects of daily administration of yeast preparations to healthy dogs.

MATERIAL AND METHODS

Animals and diet

Adult dogs of German Shepherds breed (n=20; 2 males, 18 females) were randomly divided into 2 experimental groups (ten dogs in each group): to the C group (control, 2 males, 8 females; weight 31.6±3.1 kg; age 4.3±2.8; the E group supplemented with Progut (experimental group, 10 females; weight 31.5±3.0 kg; age 5.9±2.8. The dogs were administered hydrolyzed brewery *S. cerevisiae* (processed with a patented EP 1387620 hydrolysis process, incorporated in the product PROGUT, Suemen Rehu, Finland) daily during the feeding time (14 days) at a dose 3 g per 1000 g of diet. The chemical composition of lysate is following: moisture 6.0 %, crude protein 36.0 %, crude fat 2.0 %, ash 21.5 %, crude fiber 0.2 %, non-fibre extracts 34.3 %, calcium 0.2 %, phosphorus 3.7 %, sodium 1.3 %, lysine 1.6 %, methionine + cysteine 1.3 %, threonine 1.2 %.

Analysis of faecal samples

Fresh faecal samples were collected in air-tight containers at days 0, 14, 28 and 42 during morning individual walking. Microbial populations in samples were determined according to the standard microbiological method using the following selective media: MacConkey's agar, M-Enterococcus agar, MRS (all from Becton and Dickinson, Cockeysville, USA); TOS-propionate agar with mupirocin-selective supplement, *Clostridium difficile* agar base with addition of defibrinated horse blood and *Clostridum difficile* selective supplement, *Aeromonas* medium agar base (all from Oxoid, Basingstoke, UK) and *Pseudomonas* agar (Himedia Laboratories, Mumbai, India). The results were expressed as $\log_{10}$ CFU per gram of faeces. Fresh faecal samples were visually scored (FS-faecal score) according to the following system: 1 = hard dry faeces; 2 = hard, formed stool; 3 = soft, formed and moist stool; 4 = soft, unformed stool; 5 = watery liquid. The pH values (pH Meter, Hanna Instruments, Smithfield, Rhode Island, USA) were measured immediately. The dry matter (DM) content was determined after drying to constant weight at 105 °C (Ecocell, BMT Medical Technology s.r.o, Brno, Czech Republic).

Analysis of blood samples

Blood samples (from *vena cephalica antebrachii*) were collected at days 0, 14, 28 and 42 into EDTA tubes for haematology, with heparin for immunological analyses and without anticoagulant for determination of biochemical parameters.

Haematological parameters were analysed using the Cell-Dyn 3700 (Abbott Laboratories, Chicago, Illinois, USA). Biochemical parameters in blood serum were determined using colorimetric methods (Spectrophotometer UV-2550 Shimadzu, Kyoto, Japan) with kits (Randox Laboratories Ltd., Crumlin, UK) for the following parameters: total protein (TP245), albumin (AB 362), urea (UR 107), triglyceride (TR 210), cholesterol (CH 200), glucose (GL 2623), alanine aminotransferase (AL 100), calcium (CA 590), inorganic phosphorus (PH 1016). The pH values of blood with EDTA anticoagulant was measured using portable pH7+DHS portable pH-meter with XS 2 Pore electrode (XS Instruments, Carpi, Italy).

Immunological analyses

Immunological analyses included testing of phagocytic activity and phenotypying of selected lymphocyte subsets in heparinized blood. Phagocytic activity was assassed by the Phagotest® (Orpegen Pharma, Frankfurt, Germany) according to the guidelines. The flow cytometric analysis was performed using a six color BD FACSCantoTM flow cytometer equipped with blue (488 nm) and red (633 nm) lasers (Becton Dickinson Biosciences, San José, California, USA). Data were evaluated in the BD FACS DivaTM software. Selected lymphocyte subsets were identified by direct staining with a combination of conjugated anti-dog monoclonal antibodies (MoAb): CD4-FITC / CD8a-R-PE / CD21-Alexa Fluor® 647 (ThermoFisher Scientific, Rockford, Illinois, USA). 50 µL of heparinized blood was incubated with 8 µL anti-CD4 / anti-CD8a Antibody coctail (clones: YKIX302.9 / YCATE55.9) and 4 µL anti-CD21 MoAb (clone: LT-21) for 20 min in the dark at laboratory temperature. After incubation, 1 mL of lysing solution (BD FACS Lysing Solution, BD Biosciences, USA) was added to the tubes, then the tubes were incubated for 20 min in the dark at laboratory temperature and the content of the tubes was washed twice with 1 mL PBS (MP Biomedicals, France). Before measurement, 100 µL PBS solution was

added to the tubes. The analyses were carried out on a above described flow cytometer. Proportions of lymphocytes are expressed in percentage.

Statistical analysis

The results are expressed as the mean ± standard deviation. Statistical analyses were performed using GraphPad Prism software (version 6.0). Repeated-measures ANOVA (Tukey post-test) with the level of significance set at $P<0.05$ was used to evaluate the experimental groups of dogs.

RESULTS AND DISCUSSION

The addition of *S. cerevisiae* hydrolysate to the diet of dogs for 14 days resulted in significant changes of several tested genera of bacteria but almost all changes was observed in the post-treatment period (Table 1).

Table 1 Faecal microbial populations (log CFU/g) and faecal characteristics of the dog supplemented with *S. cerevisiae* autolysate (for 14 days, E group) and of the control dogs (C group)

Microorganims	Group	Day				S.D.	*P*- value
		0	14	28	42		
***Bifidobacterium* spp.**	C	3.39	3.84	3.41	4.24	1.38	0.4496
	E	3.42^{a}	$\mathbf{5.28^{b}}$	3.29^{ac}	$\mathbf{4.96^{bc}}$	1.47	0.0020
***Clostridium* spp.**	C	7.79	8.18	8.33	8.15	0.80	0.4560
	E	7.43^{a}	7.95^{ab}	8.23^{ab}	$\mathbf{8.75^{b}}$	1.3	0.0314
***Enterococcus* spp.**	C	4.4	4.35	4.55	4.60	0.82	0.3674
	E	4.19	4.60	5.66	5.17	1.8	0.0224
LAB	C	8.23	7.73	7.90	8.15	0.85	0.4357
	E	7.82^{a}	7.15^{a}	8.43^{ab}	$\mathbf{8.46^{b}}$	1.3	0.0055
hodnota pH	C	6.22	6.35	6.37	6.41	0.28	0.2762
	E	6.13^{a}	6.23^{a}	$\mathbf{6.55^{b}}$	6.46^{ab}	0.28	0.0130

S.D. standard deviation

^{a,b,c} $P<0.05$

Results of our study concerning faecal microbiology outlined significant increases in the abundance of Gram-positive bacteria during (bifidobacteria) or in the post-treatment period (lactic acid bacteria, enterococci and clostridia). Similarly, Lin et al. (2019) reported greater abundance of *Bifidobacterium* sp. in *S. cerevisiae* fermentation product-

supplemented dogs. Bifidobacteria degrade hexose sugars through a particular metabolic pathway, termed the "bifid shunt", where the fructose-6-phosphoketolase enzyme plays a key role.

This enzyme is considered to be a taxonomic marker for the family of Bifidobacteriaceae (Felis et al., 2007). In contrast, application of live yeast to beagle dogs at a dose $2.9x10^8$ CFU/g caused decrease of *Escherichia coli* and enterococci in faeces compared to control group (Lin et al., 2019). A consistency of faeces scored visually (faecal score) and faecal dry matter were not significantly changed compared to baseline (FS remains optimal between 2.5-3.0, $P>0.05$, Table 1). The measurement of pH values of faecal samples revealed an increase with the highest values detected at day 28 (by 0.4 compared to day 0, $P<0.05$).

Table 2 Haematological parameters of the dogs supplemented with S. cerevisiae hydrolysate for 14 days (E group) and of the control dogs

Parameter	Group	Day				S.D.	*P* value
		0	14	28	42		
Red blood cell count (G/L)	C	7.27	7.51	7.48	7.49	0.35	0.3502
	E	7.30	7.51	7.39	7.41	0.70	0.6142
White blood cell count (T/L)	C	10.08	9.73	10.22	9.01	2.52	0.1002
	E	11.69	11.28	10.00	10.33	2.74	0.1992
Haemoglobin (g/L)	C	161.6	168.8	170.9	170.4	9.82	0.1609
	E	158.3	162.6	165.02	165.1	13.8	0.2465
Haematocrit (L/L)	C	0.503	0.522	0.526	0.517	0.025	0.1548
	E	0.501	0.517	0.505	0.506	0.037	0.3258
Neutrophils count (G/L)	C	6.85	6.64	7.15	6.08	1.76	0.1414
	E	7.85	7.80	6.52	6.82	2.35	0.2057
Lymphocyte count (G/L)	C	1.66	1.60	1.53	1.46	0.49	0.1549
	E	1.86	1.75	1.66	1.65	0.42	0.1532
Monocyte (G/L)	C	0.91	0.82	0.93	0.89	0.29	0.4865
	E	1.26	0.96	1.05	1.11	0.53	0.2008
Eosinophils (G/L)	C	0.56	0.53	0.51	0.49	0.35	0.6425
	E	0.62	0.65	0.68	0.66	0.30	0.8493
Basophils (G/L)	C	0.10	0.13	0.10	0.08	0.06	0.2557
	E	0.09	0.12	0.10	0.11	0.07	0.5032
Blood pH	C	7.56	7.55	7.58	7.57	0.05	0.6242
	E	7.56	7.54	7.54	7.52	0.05	0.4531

S.D. standard deviation

Haematological parameters were not affected significantly after the application of *S. cerevisiae* hydrolysate to experimental group of dogs ($P>0.05$, Table 2). Detection of blood pH did also not reveal any significant differences. Significant decrease of white blood cell counts detected Lin et al. (2019) in adult dogs which received *S. cerevisiae*

fermentation product. Although we observed also lower white blood cell count especially at day 28 (by 1.7 T/L) compared to baseline, the difference was not significant.

Evaluation of serum biochemical parameters showed decreasing trend in lipid profile after hydrolysate treatment compared to initial levels (Table 3). Significant decreases of serum triglyceride and cholesterol in our experimental dogs is in agreement with several studies testing effects of yeast hydrolysate on egg quality in laying hens (Bolakali et al., 207; Yalcin et al, 2010). In these studies, serum cholesterol and triglyceride but also egg yolk cholesterol decreased significantly at a dose 0.2, 0.3 and 0.4 % of yeast autolysate in the diet of hens. In our study, some negative effects we detected on the activity of hepatic enzymes alanine aminotransferase (ALT) and aspartate aminostranferase (AST). Both enzymes were increased over reference range in 7 (ALT) and 4 dogs (AST).

Table 3 Serum biochemical parameters of the dogs supplemented with S. cerevisiae hydrolysate for 14 days (E group) and of the control dogs (C group)

Parameter	Group	Day				S.D.	P-value
		0	14	28	42		
Total protein	C	68.3	71.1	70.1	67.9	5.11	0.3453
	E	72.9	72.8	74.1	72.9	5.17	0.7925
Albumin (g/L)	C	37.8	38.4	39.1	39.7	3.1	0.3850
	E	40.1	38.6	39.5	41.9	6.5	0.5153
Urea (mmol/L)	C	6.56	6.51	6.72	6.79	0.79	0.6369
	E	6.68	6.79	8.29	7.44	1.59	0.0258
Glucose (mmol/L)	C	4.62	4.53	4.76	4.67	0.52	0.6652
	E	4.52	4.39	4.70	4.65	0.44	0.0970
Triglyceride (mmol/L)	C	1.06	1.00	1.01	0.96	0.14	0.2934
	E	1.12^{ab}	1.11^{a}	1.08^{ab}	1.01^{b}	0.24	0.0764
Cholesterol (mmol/L)	C	5.97	5.66	5.69	5.65	0.73	0.5172
	E	6.04^{a}	5.62^{ab}	5.46^{ab}	5.02^{b}	1.48	0.0546
Alanine aminotransferase (µkat/L)	C	0.266	0.303	0.297	0.317	0.072	0.2734
	E	0.259^{a}	0.411^{b}	0.512^{ab}	0.428^{ab}	0.279	0.0763
Aspartate aminotransferase (µkat/L)	C	0.177	0.227	0.202	0.246	0.077	0.1226
	E	0.200^{a}	0.177^{a}	0.350^{b}	0.342^{b}	0.131	0.0003
Alkaline phosphatase (µkat/L)	C	1.71	1.34	1.26	1.40	0.72	0.6921
	E	1.83	1.02	1.32	1.56	1.31	0.1613
Calcium (mmol/L)	C	2.54	2.59	2.44	2.57	0.13	0.7672
	E	2.52	2.61	2.54	2.50	0.26	0.3795
Phosphorus (mmol/L)	C	1.53	1.66	1.68	1.71	0.20	0.1425
	E	1.72^{ab}	1.75^{ab}	1.80^{a}	1.52^{b}	0.27	0.1570

S.D. standard deviation

^{a,b} different letter indicate significant differences ($P<0.05$) over time in the same group

No statistically significant differences in phagocytic activity summary, phagocytic activity of neutrophils and monocytes and mean fluorescent intensity was detected. Similarly, populations of cytotoxic and helper T lymphocytes and B lymphocytes were not significantly changed after yeast hydrolysate application (P>0.05).

CONCLUSION

The inclusion of 0.3 % of yeast hydrolysate to dog diet was associated with effects such as increase in the abundance of bifidobacteria, lactic acid bacteria and enterococci, faecal pH and decrease of serum lipid profile (triglyceride, cholesterol). Whereas not all results were desirable (increase of clostridia, liver enzyme activities), it is necessary to reconsider the use of yeast hydrolysate for longer time to dogs. Further testing with different doses will bring us more information.

ACKNOWLEDGEMENT

The project was supported by the Slovak National Scientific Grant Agency VEGA Grant no. 2/0006/20 – The skin microbiome in dogs under physiological and pathological conditions.

REFERENCES

Aktas M.S., Borku M.K., Ozkanlar Y. (2007): Efficacy of *Saccharomyces boulardii* as a probiotic in dogs with lincomycin induced diarrhoea. Bull Vet Inst Pulawy, 51, 365-369.

Bolakali M., Irak K., (2017): Effect of dietary yeast autolysate on performance, slaughter, and carcass characteristics, as well as blood parameters, in quail of both genders. South Afr J Anim Sci, 47, 460-470.

Czerucka D., Piche T., Rampal P. (2007): Review article: yeast as probiotics -- *Saccharomyces boulardii*. Aliment Pharmacol Ther, 26, 767–778.

D´Angelo S., Fracassi F., Bresciani F., Galuppi R., Diana A., Linta N., Bettini G., Morini M., Pietra M. (2018): Effect of *Saccharomyces boulardii* in dog with chronic enteropathies: double-blinded, placebo-controlled study. Vet Rec, 182:258.

Elghandour M.M.Y., Tan Z.L., Abu Hafsa S.H., Adegbeye M.J., Hreiner R., Ugbogu E.A., Cedillo Monroy J., Salem A.Z.M. (2019): *Saccharomyces cerevisiae* as a probiotic feed additive to non and pseudo-ruminant feeding: a review. J Appl Microbiol, 128, 658-674.

Felis G.E., Dellaglio F. (2007): Taxonomy of Lactobacilli and Bifidobacteria. Curr Issues Intest Microbiol, 8, 44-61.

Chollom P.F., Agbo E.B., Doma U.D., Okojokwu O.J., Yisa A.G., (2017): Nutritional value of spent brewers´ yeast (*Saccharomyces cerevisiae*): A potential replacement for soya bean in poultry feed formulation. Researcher 9, 70-74.

Lin C.Y., Alexander C., Steelman A.J., Warzecha C.M., de Godoy M.R.C., Swanson K.S. (2019): Effects of a *Saccharomyces cerevisiae* fermentation product on fecal characteristics, nutrient digestibility, fecal fermentative end-products, fecal microbial populations, immune function, and diet palatability in adult dogs. J Anim Sci 97, 1586-1599.

West C., Stanisz A.M., Wong A., Kunze W.A. (2016): Effects of *Saccharomyces cerevisiae* or *boulardii* yeasts on acute stress induced intestinal dysmotility. World J Gastroenterol, 22, 10532–10544.

Yalcin S., Yalcin S., Cakin K., Eltan Ö. (2010): Effects of dietary yeast autolysate (*Saccharomyces cerevisiae*) on performance, egg traits, egg cholesterol content, egg yolk fatty acid composition and humoral immune response of laying hens. J Sci Food Agric 90, 1695-1701.

FEEDING HORSES SUFFERING WITH EQUINE ASTHMA

ALICA TVRDÁ, PAVEL NAĎ, ANDREJ MARCIN

Department of Animal Nutrition and Husbandry, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Slovak Republic

Corresponding email address: tvrda.alica@gmail.com

ABSTRACT

The severe equine asthma (SEA) affects an estimated 10 - 20% of horses in the Northern Hemisphere and other temperate climates. It occurs primarily in adult horses, usually 6 years of age or older. Therefore, it is very important to be able to recognize asthma in time and start the appropriate therapy. Feeding horses with asthma is specific and requires adjustment of the feed ration.

Keywords: nutrition; steaming; hay; fatty acid; horses

ANATOMY OF THE RESPIRATORY SYSTEM

The main function of the respiratory system is to supply oxygen and remove carbon dioxide from the blood. Air is delivered into the lungs through a series of conductive airways that transfer ambient air to the alveoli, where oxygen and carbon dioxide are exchanged. These conducting airways include the nostrils, nasal cavity, pharynx, larynx, trachea, bronchi, and bronchioles (Robinson et al., 2007). Apart from the first few centimeters, the bronchi are completely surrounded by the lung parenchyma. Unlike the lungs of most other mammals, the horse's lungs are not divided into distinct lobes. (Robinson and Sorenson, 1978; Robinson, 1982). Gas exchange occurs in the alveolar ducts and alveoli, which are both characterized by an extensive pulmonary capillary network, so that there is a huge vascular surface for the diffusion of oxygen and carbon dioxide. Blood enters the pulmonary capillaries from the right ventricle through the pulmonary arteries and returns to the left atrium through the pulmonary veins. The bronchial circulation, which is

a branch of the systemic circulation, also provides nutrients to the bronchi, large vessels and the pleura (Robinson et al., 2007).

SEVERE EQUINE ASTHMA

Asthma is a chronic non-infectious inflammatory obstructive disease of the lower respiratory tract. The terms that have been used to describe this syndrome are numerous; reccurent airway obstruction (RAO), chronic obstructive pulmonary disease (COPD), heaves and broken wind (Lavoie, 2007). The name of horse asthma is applied worldwide since 2015 (Bezděková, 2020).

While SEA is observed in horses that are stabled and fed hay, a similar clinical presentation is observed in some horses when pastured; the condition is reversible by housing affected horses in a dust-free stable. This syndrome has been called summer-pasture-associated obstructive pulmonary disease (SPAOPD) and shares many clinical and pathological similarities with asthma (Laurent et al., 2004). Other types of SEA does occur, including incuced by heat, pollen and smoke. Other potential triggers of equine asthma are exposure to traffic-related air pollution such nitrogen dioxide, and fine particles. However, Couëtil (2004) claims that the evidence supporting these particles as aeroallergens is currently only indirect.

During disease exacerbation, horses present exercise intolerance, increased tracheobronchial exudate, mild to severely increased respiratory efforts, and occasionally cough (Laurent et al., 2004). Clinical signs are exacerbated by inhalation of dust particles present in the stables, especially those associated with hay feeding. Therefore, SEA is regarded as a disease of domestication.

When stabled, horses susceptible to asthma develop a marked and persistent airway inflammation it is believed to be a consequence of the immune response and genetic predispositions. During exacerbation, bronchospasm is a major cause of airflow limitation, as indicated by the rapid and significant (60–70%) improvement in lung function observed following the administration of bronchodilators. In addition to inflammation, a characteristic sign of heaves is the remodelling of the airway wall. A result of airway remodelling is incompletely reversible,

or even irreversible airway obstruction, bronchial hyper-responsiveness and an accelerated decline in lung function (Leclere at al., 2011).

MODIFICATIONS OF FEEDING SCHEDULE

One of the first changes that needs to be made involves horse's dietary forage. The worst culprit is what the horses are eating (Conrad, 2021).

Most importantly, do not feed dry hay. Because hay is the No. 1 source of aeroallergens, asthmatic horses should never be fed dry hay (Lesté-Lasserre, 2020).

To feed hay, it is necessary soak it before feeding. Hay soaking involves completely immersion the ration for 30 minutes, not just watering it down with a hose. Other hay alternatives include oiled hay such as the Nutri-Foin system, haylage, and pasteurized hay (Oke, 2021).

Steaming hay can be done instead of soaking hay. Couëtil (2004) says molds will persist if the hay is not fully steamed to the inside of the bale. Furthermore, the steamers need to be thoroughly cleaned so that no mold remains. Commercial steamers take about 1 hour per bale, and some owners might find the cost prohibitive.

Experiment of 2015 from Moore-Colyer et al. showed steaming in the commercial steamer reduced airborne respirable particles (ARP) and microbial contamination by 99%. Steam treatment preserved mineral and protein contents but reduced water-soluble carbohydrate by 18.3%. Steaming with a commercial steamer is a realistic long-term strategy for reducing ARP and microbial contamination, while conserving mineral and protein content in hay and is thus suitable for providing hygienically clean forage to stabled horses.

Bezděková (2020) recommends haylage. Steaming prefers over soaking. Lastly, she recommends feeding granulated or pelleted hay, especially because of the financial demands.

Feeding long forage to stabled horses can help maintain normal time budgets by satisfying the animal's innate need to chew. Daily nutrient requirements can be supplied by quality hay or haylage, although many owners think haylage is too energy dense to be offered ad libitum and thus prefer to feed horses a higher fiber lower energy forage such as grass hay (Moore-Colyer et al., 2014).

The degree of lower airway inflammation (% neutrophils in bronchoalveolar lavage) was significantly lower when horses were housed indoors and bedded on shavings versus straw or when steamed hay versus dry hay (Dauvillier et al., 2018).

Finally, feeding omega-3 fatty acids is recommended as they can help control the inflammatory response (Thunes, 2019). Omega-3 are polyunsaturated fatty acids. These are substances that the horse cannot make on its own and must absorb in food. They are also called essential fatty acids (EFA). This group includes docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Dietary fats serve as carriers for fat-soluble vitamins A, D, E, and K. The horse needs a balance of Omega-3's and Omega-6's to function at an optimal level, but the exact amounts of Omega-3 : Omega-6 is not known. Omega-3 fatty acids control the inflammatory response in the body and have positive health benefits. Omega-6 fatty acids aid pro-inflammatory processes and excessive levels of Omega-6, relative to Omega-3, may increase the probability of a number of diseases.

Pasture grasses and hay, although including only 2% to 3% fat, have greater concentrations of Omega-3 than Omega-6 fatty acids. Higher levels of Omega-6 than Omega-3 fatty acids are in cereal grains, such as corn and oats, along with sunflower and safflower seeds. Rice bran, soybean and canola oils are higher in Omega-3 and lower in Omega-6 content than corn oil. The largest amount of Omega-3's, with higher levels of Omega-3 than Omega-6 fatty acids are in oils from soy, flax, and canola seeds. The most concentrated plant source of Qmega-3 fatty acids is flaxseed oil, and it is six times richer than fish oils in Omega-3 (Hygain, 2013).

Research has shown that long-term administration of DHA to horses has a positive effect on reducing the inflammatory response in horses with asthma. The positive effect was demonstrated at a dose of 1.5 to 3 grams of DHA per day for at least 2 months (Řízková Horáková, 2020).

If horses are turned out on pasture, Omega-3's are probably not indicated because grass pasture is usually a good source of Omega-3. Further, haylage is a good source of Omega-3's, so owners may not need to supplement if feeding this roughage either (Couëtil, 2004).

CONCLUSION

It is not possible to create a sterile environment for the horse, the goal is to reduce exposure to inhalation particles so as to minimize the symptoms of asthma in the horse. The most important is dust-free feeding of roughage. Haylage, pasture and steamed hay are among the recommended feeding methods. Supplementation of omega-3 fatty acids has a positive effect on the control of airway inflammation. Furthermore, adequate ventilation and air flow in stables, use commercial dust-free wood shavings, cardboard, or other low-dust options for bedding, storage of hay and straw outside the stables, turn horses out for at least 12 hours a day, groom horses outdoors, and clean stables when horses are turned out. These principles must be followed throughout the life of an asthmatic horse. Appropriate drug therapy is a matter of course.

REFERENCES

Bezděková B. (2020): Onemocnení dýchacího aparátu v praxi. Blansko, s. 89

Conrad SE. (2021): Breathing Easy: Managing Horses With Asthma. The horse. https://thehorse.com/19375/breathing-easy-managing-horses-with-asthma/

Dauvillier J., Woort TF., Van Erck-Westergren E. (2018): Fungi in respiratory samples of horses with inflammatory airway disease. J Vet Intern Med.; 1–8. https://doi.org/10.1111/jvim.15397

Hygain (2013): Essential Omega 3, 6 and 9 fatty acids in horses. https://hygain.com.au/blogs/library/omega-3-6-9-fatty-acids-horses

Kellon E. (2017): Hay Steamers. Equisearch. For People Who Love Horses. https://www.equisearch.com/articles/hay-steamers

Laurent L. Couëtil and Kenneth W. Hinchcliff (2004): Non-infectious diseases of the lower respiratory tract. Equine Sports Medicine and Surgery; 613-656. https://doi.org/0.1016/B978-0-7020-2671-3.50033-7

Lavoie J. Chronic obstructive pulmonary disease. In: Robinson N. (1997): ed. Current therapy in equine medicine. Philadelphia: WB Saunders; 1997; 431–435.

Leclere M., Lavoie-Lamoureux A., Lavoie JP. (2011): Heaves, an asthma-like disease of horses. Respirology. https://doi. org/10.1111/j.1440-1843.2011.02033.x.

Lesté-Lasserre C. (2020): Owners Reluctant to Change Asthmatic Horses' Environments. The horse. https://thehorse.com/188841/

owners- reluctant-to-change-asthmatic-horses-environments/

Moore-Colyer1 JSM., Lumbis K., Longland A., Harris P. (2014): The Effect of Five Different Wetting Treatments on the Nutrient Content and Microbial Concentration in Hay for Horses. PLOS ONE | DOI:10.1371/journal.pone.0114079 November 26, 2014

Moore-Colyer JSM., Taylor LEJ., James R. (2015): The Effect of Steaming and Soaking on the Respirable Particle, Bacteria, Mould, and Nutrient Content in Hay for Horses. Journal of Equine Veterinary Science 39 (2016); 62–68

Oke S. (2020): Low-Dust Forages Essential for Asthmatic Horses. The horse. https://thehorse.com/195307/aaep-2020-low-dust-forages-essential -for-asthmatic-horses/

Oke S. (2021): The INS and OUTS of Severe Equine Asthma. The horse. https://thehorse.com/features/equine-asthma/

Robinson NE., Furlow WP., Dixon PM., Schumacher J. (2007): Anatomy of the Respiratory System. Equine Respiratory Medicine and Surgery; 3–17

Robinson NE. (1982): Some functional consequences of species differences in lung anatomy. Advances in Veterinary Science and Comparative Medicine 26: 1–33

Robinson NE., Sorenson PR. (1978): Collateral flow resistance and time constants in dog and horse lungs. Journal of Applied Physiology 44: 63–68

Řízková Horáková D. (2020): Imunitní systém a krmné doplňky. Dromy. https://www.dromy.cz/imunitni-system-a-krmne-doplnky/

Thunes C. (2019): Feeding Horses With Equine Asthma. The horse. https://thehorse.com/171810/feeding-horses-with-equine-asthma/

INFLUENCE OF FATTY ACIDS ON *IN VITRO* RUMEN FERMENTATION

MARIANA VADROŇOVÁ[1,2], MIROSLAV JOCH[1,2], KATEŘINA JOCHOVÁ[1,2], ALENA VÝBORNÁ[2], PETR HOMOLKA[1,2]

[1]Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, Prague 16500, Czech Republic
[2]Institute of Animal Science, Prague-Uhříněves, Czech Republic

Corresponding email address: vadronovam@af.czu.cz

ABSTRACT

This *in vitro* study evaluated the effect of four medium-chain fatty acids (MCFA, i.e., caprylic (C8:0), capric (C10:0), lauric (C12:0), and myristic acid (C14:0)), used alone or in binary combinations, together with nitrate (NT) on rumen fermentation and methane (CH_4) production. Using a 24 h batch incubation, the experiment showed that all the MCFA+NT treatments reduced ($P<0.001$) CH_4 production; the most potent treatment being C10:0+NT and the least effective C14:0+NT. Notably, the treatments had no effect ($P=0.051$) on apparent dry matter disappearance (aDMd) and net production of volatile fatty acids (nVFA), which shows that fermentation of feed was not adversely influenced. These results show the benefit of combining lower doses of feed additives with complimentary modes of action to enhance rumen fermentation.

Keywords: medium chain fatty acid; nitrate; methane production

INTRODUCTION

The world population continues to grow and with it the demand for animal-sourced food; there is an estimated increase of 80% in demand by 2050 for beef (Honan et al., 2021). Ruminants gain energy from the digestion of plant fibres in the rumen, where various microorganisms degrade feedstuff and produce volatile fatty acids (VFA), carbon dioxide (CO_2), and hydrogen (H_2) (Qomariyah et al., 2020). VFA are used as the

main source of energy for the animal, and CO_2 is reduced to methane (CH_4) by methanogens, with methanogenesis, therefore, functioning as an electron sink for the entire ruminal ecosystem (Friedman, Jami, & Mizrahi, 2017). However, the CH_4 produced during fermentation means a significant loss of energy (Elghandour et al., 2019) and contributes to the phenomenon of global warming (Black, Davison, & Box, 2021). To enhance rumen fermentation, various strategies have been tested, and among them is the use of feed additives (Troy et al., 2015). Medium chain fatty acids (MCFA) can directly affect methanogens and inhibit protozoa (Soliva et al., 2003). Furthermore, Troy et al., 2015 suggested nitrate (NT) could provide an alternative H_2 sink to outcompete methanogens. Methanogens may be even directly suppressed by NT´s intermediate, nitrite (Zhou, Yu, & Meng, 2012).

Therefore, the objective of this study was to test the effects of individual MCFA and their binary combinations in addition with NT. We hypothesized that two feed additives with complementary modes of action would be more effective than single additives, and by using them combined in lower doses, detrimental effects on health of the animal would be avoided.

MATERIAL AND METHODS

The effects of four MCFA (C8:0, C10:0, C12:0, and C14:0, i.e., caprylic, capric, lauric, and myristic acid, respectively; purity ≥ 99%) used alone (500 mg/l) or in binary combinations (250 mg/l + 250 mg/l) together with NT (sodium nitrate as a source of NT; 5 mM) on rumen fermentation and CH_4 production were evaluated using a 24-h batch culture incubation. The experimental substrate (corn silage (300 g/kg), alfalfa silage (300 g/kg), and barley (400 g/kg) on DM basis) was ground to pass a 1–mm screen and subsequently weight (200 mg) into sterile CO_2-flushed serum bottles. After obtaining the rumen fluid from two early lactation Holstein cows, it was strained through a stainless steel sieve (250 µm; Retsch, Haan, Germany), pooled in equal portions and mixed with a medium (1:3, v/v; preparation described by Menke et al., 1979) to prepare a culture fluid. The culture fluid was gassed with CO_2 and kept for 10 min at 39°C before the bottles were filled up. By pipetting of 200 µl of filter

sterilized distilled water and ethanol stock solutions, sodium nitrate and MCFA, respectively, were added to the bottles to reach the desired concentration in the culture fluid. The control and blank bottles contained the equivalent amount of water and ethanol, so any effects on fermentation of the solvents were compensated for. In all incubation, only CO_2 was the initial headspace gas phase. Afterwards, using a bottle top dispenser (Calibrex 530 salutae, Socorex, Switzerland), 20 ml of the culture fluid was dispensed into each serum bottle and the bottles were gassed with CO_2. After sealing, all the serum bottles were placed in a temperature-controlled water bath (SW 22, Julabo, Germany) kept for 24 h at 39°C with a shaking frequency of 90 rpm. After the incubation, total volume of produced gas was estimated from the headspace gas pressure using Boyle's Law equation (Romero-Pérez & Beauchemin, 2018). The headspace gas pressure was measured via manometer (Traceable, Fisher Scientific, Pittsburgh, USA). Using a 23-gauge needle, the headspace gas was sampled by displacement into a pre-filled tube with distilled water. In accordance with Joch et al., 2019, the concentration of CH_4 in the headspace and of each VFA in the cultures was measured by gas chromatography, and apparent dry matter disappearance (aDMd) was determined gravimetrically. Finally, as described by Weatherburn, 1967, NH_3-N concentrations in the cultures were determined by the indophenol method. The analysis of the main effect of treatment was carried out via the PROC MIXED procedure of SAS (SAS Enterprise Guide 6.1, SAS Institute Inc., Cary, NC, USA) according to a randomized complete block design, using run (n = 2) as the blocking factor. Technical replicates were averaged per run before statistical analysis.

RESULTS AND DISCUSSION

The effects of MCFA+NT on *in vitro* rumen fermentation characteristics are presented in Table 1 and Table 2. Notably, all of the treatments reduced ($P<0.001$) total gas production (mL/gDMi) (TGP) and showed the ability to suppress ($P<0.001$) CH4 production when expressed as CH4 production per aDMd, per net production of VFA (nVFA), and relatively per TGP. No treatment affected ($P<0.001$) aDMd nor nVFA, which is in agreement with Soliva et al., 2003, and Yanza et al., 2020.

Previously, when C10:0 alone was tested, a 23% (Goel et al., 2009) and a 17% (Dohme et al., 2001) decrease in VFA production was reported.

An increase in pH was recorded in all treatments; however, the average pH of the control sample was 5.89 and the highest value was 6.25 (C10:0+NT; an increase by roughly 6%), therefore, the parameter remained in the physiological range of the rumen (5.5–7.0; Krause & Oetzel, 2006). As the vast majority of molar proportions of VFA was not altered ($P<0.001$) and the net NH_3-N concentrations increased ($P=0.002$) in all treatments, it can be assumed that NT served as an alternative electron acceptor to methanogenesis, as NT is reduced to nitrite and finally to ammonia in the rumen (Marais et al., 1988). The treatments C10:0+NT and C8:0/C10+NT elevated ($P<0.001$) NH_3-N concentrations the most prominently, with an increase of 94.1% and 84.4%, respectively.

The treatment C10:0+NT was the most potent CH_4 inhibitor; it reduced ($P<0.001$) methanogenesis by 37.2% (mL/g aDMd). In previous studies, capric acid (C10:0) had either no effect on CH_4 production (Dohme et al., 2001), or a dose dependent effect, where 0.4 and 0.6 mg/mL incubation medium decreased methanogenesis by 44% and 88%, respectively (Goel et al., 2009). Currently, C10:0+NT was the only treatment that affected ($P<0.001$) molar proportions of VFA; butyrate and valerate increased ($P<0.001$) by 31.4% and 32.1%, respectively, at the expense of acetate which decreased ($P<0.001$) by 8.5%. This shift towards butyrate production was observed by Meng et al., 2010 when NT was supplemented. This is in line with a proposition, that the production of butyrate is alternative to methanogenesis (Panyakaew et al., 2013). The decrease in acetate production in C10:0+NT may have aided the decrease in methanogenesis, as the formation of acetate generates H_2 (Zhao et al., 2015). Contrary to our results, Latham et al., 2016 and Zhao et al., 2015 concluded that NT increases acetate production at the expense of butyrate production. Furthermore, in the present experiment, C10:0 enhanced the effects of other MCFA in the

Table 1. Effects of medium-chain fatty acids (MCFA) and nitrate (NT) on *in vitro* gas and methane production, apparent dry matter disappearance (aDMd), ammonia-N concentration, and pH

Treatment	TGP†	Methane				aDMd‡	Net NH_3-N	pH
	(mL/g DMi§)	(mL/g DMi)	(% TGP)	(mL/g aDMd)	(mol/mol nVFA¶)	(g/g)	(mg/100 mL)	
Control	303.4	36.9	12.2	58.3	0.218	0.636	13.5	5.89
Nitrate	294.3*	30.9*	10.5*	50.2*	0.184	0.620	20.8*	6.04*
C8 + NT	289.5*	28.3*	9.8*	43.4*	0.164	0.654	20.7*	6.04*
C10 + NT	269.3*	23.2*	8.6*	36.6*	0.162	0.642	26.2*	6.25*
C12 + NT	286.7*	28.2*	9.8*	45.0*	0.161	0.631	21.0*	6.11*
C14 + NT	294.7*	31.3*	10.6*	53.1*	0.183	0.592	23.1*	6.06*
C8/C10 + NT	278.9*	25.9*	9.3*	40.5*	0.201	0.644	24.9*	6.17*
C8/C12 + NT	288.1*	27.3*	9.5*	41.9*	0.179	0.657	22.2*	6.11*
C8/C14 + NT	294.4*	29.9*	10.2*	46.6*	0.171	0.645	24.0*	6.04*

C10/C12 + NT	274.0*	24.3*	8.9*	39.1*	0.149	0.628	23.9*	6.16*
C10/C14 + NT	283.1*	26.7*	9.4*	41.8*	0.156	0.640	22.1*	6.12*
C12/C14 + NT	288.4*	28.7*	9.9*	46.7*	0.161	0.617	22.1*	6.09*
SEM	3.26	0.79	0.19	1.54	0.0063	0.0071	1.32	0.026
P-value	<0.001	<0.001	<0.001	<0.001	0.190	0.051	0.002	0.001

† Total gas production; ‡ apparent dry matter disappearance; § dry matter incubated; ¶ net production of volatile fatty acids

* Means within a column differ significantly (P < 0.05) from corresponding control (0 mg/L). SEM, standard error of the mean

Control (no MCFA, no NT), NT (5 mmol/l of sodium nitrate), C8 + NT (500 mg/l of C8 + 5 mmol/l of sodium nitrate), C8/C10 + NT (250 mg/l of C8 + 250 mg/l of C10 + 5 mmol/l of sodium nitrate) etc.

Table 2. Effects of medium-chain fatty acids (MCFA) and nitrate (NT) on *in vitro* volatile fatty acids production and proportion

Treatment	nVFA† (mmol/L)	Molar proportion of VFA (mol/100 mol)						A:P‡
		Acetate	Propionate	Butyrate	*iso*-butyrate	Valerate	*iso*-valerate	
Control	66.6	60.1	19.7	11.8	0.7	5.3	2.4	3.1
NT	65.8	62.4	19.1	10.4	0.8	4.9	2.4	3.3
C8 + NT	68.1	61.4	20.4	10.9	0.9	4.2	2.3	3.0
C10 + NT	56.6	55.0*	18.7	15.5*	1.0	7.0*	2.8	3.0
C12 + NT	68.5	61.2	19.6	10.5	0.9	5.4	2.3	3.2
C14 + NT	69.0	61.8	19.5	10.3	0.8	5.2	2.4	3.2
C8/C10 + NT	56.2	60.5	18.3	12.9	0.1	5.6	2.6	3.3
C8/C12 + NT	62.8	59.1	20.5	11.6	1.0	5.3	2.4	2.9
C8/C14 + NT	69.4	60.3	20.7	10.7	1.5	4.5	2.4	2.9

C10/C12 + NT	64.4	56.7	21.0	12.3	1.6	6.0	2.5	2.7
C10/C14 + NT	67.1	58.9	20.5	11.4	1.6	5.3	2.3	2.9
C12/C14 + NT	70.5	59.6	20.8	10.3	1.7	5.3	2.3	2.9
SEM	2.39	0.48	0.33	0.31	0.14	0.24	0.11	0.06
P-value	0.287	0.013	0.105	<0.001	0.227	0.001	0.163	0.205

† Net production of volatile fatty acids; ‡ acetate:propionate

Control (no MCFA, no NT), NT (5 mmol/l of sodium nitrate), C8 + NT (500 mg/l of C8 + 5 mmol/l of sodium nitrate), C8/C10 + NT (250 mg/l of C8 + 250 mg/l of C10 + 5 mmol/l of sodium nitrate) etc.

binary mixtures. And, on the other hand, when mixed, all the MCFA reduced the ability of C10:0 to decrease CH_4 formation.

As the most effective inhibitor of methanogenesis, C10:0+NT was followed by the treatment C10:0/C12:0+NT (-32.9%; $P<0.001$). The effect of treatments further decreased in the following order: C8:0/C10:0+NT > C10:0/C14:0+NT > C8:0/C12:0+NT > C8:0+NT > C12:0+NT > C8:0/C14:0+NT > C12:0/C14:0+NT > C14:0+NT. The lack of a stronger effect of C12:0/C14:0+NT was surprising to the authors, as this combination of MCFA is believed to be the most effective (Dohme et al., 2001, Machmüller et al., 2006). These authors did not add NT to the treatments; therefore, it could be assumed that C12:0 and C14:0 have a similar mode of action as NT, and their effects aren't additive. Differing proportions of these MCFA in a mixture resulted in a 96% and an 87% reduction in methanogenesis, where the ratio of C12:0 and C14:0 was 2:1 and 1:1, respectively (Soliva et al., 2004). The addition of C14:0 to NT did not further enhance the CH_4 suppressing effect, in fact, CH_4 production was 5% higher than when NT was incubated alone. Soliva et al., 2003 reported no effect of C14:0 on methanogenesis, and Dohme et al., 2001 achieved a 12% decrease in CH_4 production.

CONCLUSION

The magnitude of CH_4 suppression of MCFA depends on their type and combination. In our study C10:0+NT was the most effective inhibitor of methanogenesis. NT appears to have functioned as an electron acceptor, which might have prevented a shift in molar proportions of VFA. These results show the benefit of combining lower doses of feed additives with complimentary modes of action to enhance rumen fermentation.

ACKNOWLEDGEMENT

This study was supported by the National Agency for Agricultural Research (NAZV; grant number QK1920037) and institutional support of the Ministry of Agriculture of the Czech Republic (MZE-RO0718).

REFERENCES

Black, J. L., Davison, T. M., & Box, I. (2021): Methane Emissions from Ruminants in Australia: Mitigation Potential and Applicability of Mitigation Strategies. Animals, 11, 951.

Dohme, F., Machmüller, A., Wasserfallen, A., & Kreuzer, M. (2001): Ruminal methanogenesis as influenced by individual fatty acids supplemented to complete ruminant diets. Letters in Applied Microbiology, 32, 47–51.

Elghandour, M. M. Y., Khusro, A., Adegbeye, M. J., Tan, Z., Abu Hafsa, S. H., Greiner, R., Ugbogu, E. A., Anele, U. Y., & Salem, A. Z. M. (2019): Dynamic role of single-celled fungi in ruminal microbial ecology and activities. Journal of Applied Microbiology.

Friedman, N., Jami, E., & Mizrahi, I. (2017): Compositional and functional dynamics of the bovine rumen methanogenic community across different developmental stages. Environmental microbiology, 19, 3365–3373.

Goel, G., Arvidsson, K., Vlaeminck, B., Bruggeman, G., Deschepper, K., & Fievez, V. (2009): Effects of capric acid on rumen methanogenesis and biohydrogenation of linoleic and α-linolenic acid. Animal, 3, 810–816.

Honan, M., Feng, X., Tricarico, J., & Kebreab, E. (2021): Feed additives as a strategic approach to reduce enteric methane production in cattle: Modes of action, effectiveness and safety. Animal Production Science.

Joch, M., Kudrna, V., Hakl, J., Bozik, M., Homolka, P., Illek, J., Tyrolová, Y., & Vyborna, A. (2019): In vitro and in vivo potential of a blend of essential oil compounds to improve rumen fermentation and performance of dairy cows. Animal Feed Science and Technology, 251, 176–186.

Krause, K. M., & Oetzel, G. R. (2006): Understanding and preventing subacute ruminal acidosis in dairy herds: A review. Animal feed science and technology, 126, 215–236.

Latham, E. A., Anderson, R. C., Pinchak, W. E., & Nisbet, D. J. (2016): Insights on alterations to the rumen ecosystem by nitrate and nitrocompounds. Frontiers in microbiology, 7, 228.

Marais, J. P., Therion, J. J., Mackie, R., Kistner, A., & Dennison, C. (1988): Effect of nitrate and its reduction products on the growth and activity of the rumen microbial population. British journal of nutrition, 59, 301–313.

Machmuller, Andrea. (2006): Medium-chain fatty acids and their potential to reduce methanogenesis in domestic ruminants. Agriculture, ecosystems & environment 112, 107–14.

Meng, Q., Zhou, Z., Cui, Z., & Ren, L. (2010): Effect of nitrate addition level on in vitro ruminal fermentation characteristics and microbial efficiency. Scientia Agricultura Sinica, 43, 3418–3424.

Menke, K., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979): The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. The Journal of Agricultural Science, 93, 217–222.

Panyakaew, P., Goel, G., Lourenço, M., Yuangklang, C., & Fievez, V. (2013): Medium-chain fatty acids from coconut or krabok oil inhibit in vitro rumen methanogenesis and conversion of non-conjugated dienoic biohydrogenation intermediates. Animal Feed Science and Technology, 180, 18–25.

Qomariyah, N., Retnani, Y., Jayanegara, A., Wina, E., & G. Permana, I. (2020): Influence of Biochar and Liquid Smoke Additives from Cacao-Pod Husks on In vitro Ruminal Fermentation Characteristics. Advances in Animal and Veterinary Sciences, 9.

Romero-Pérez, A., & Beauchemin, K. A. (2018): Estimating gas volume from headspace pressure in a batch culture system. Canadian Journal of Animal Science, 98, 593–596.

Soliva, Carla R, Leo Meile, Ida K Hindrichsen, Michael Kreuzer, & Andrea Machmuller. (2004): Myristic acid supports the immediate inhibitory effect of lauric acid on ruminal methanogens and methane release. Anaerobe 10 (5): 269–76.

Soliva, C., Hindrichsen, I., Meile, L., Kreuzer, M., & Machmüller, A. (2003): Effects of mixtures of lauric and myristic acid on rumen methanogens and methanogenesis in vitro. Letters in Applied Microbiology, 37, 35–39.

Troy, S., Duthie, C., Hyslop, J., Roehe, R., Ross, D., Wallace, R., Waterhouse, A., & Rooke, J. (2015): Effectiveness of nitrate addition and increased oil content as methane mitigation strategies for beef cattle fed two contrasting basal diets. Journal of Animal Science, 93, 1815–1823.

Weatherburn, M. (1967): Phenol-hypochlorite reaction for determination of ammonia. Analytical chemistry, 39, 971–974.

Yanza, Y. R., Szumacher-Strabel, M., Jayanegara, A., Kasenta, A. M., Gao, M., Huang, H., Patra, A. K., Warzych, E., & Cieślak, A. (2020): The effects of

dietary medium-chain fatty acids on ruminal methanogenesis and fermentation in vitro and in vivo: A meta-analysis. Journal of animal physiology and animal nutrition.

Zhao, L., Meng, Q., Ren, L., Liu, W., Zhang, X., Huo, Y., & Zhou, Z. (2015): Effects of nitrate addition on rumen fermentation, bacterial biodiversity and abundance. Asian-Australasian Journal of Animal Sciences, 28, 1433.

Zhou, Z., Yu, Z., & Meng, Q. (2012): Effects of nitrate on methane production, fermentation, and microbial populations in in vitro ruminal cultures. Bioresource Technology, 103, 173–179.

THE EFFECT OF CRUDE PROTEIN DEFICIENCY IN DIET ON BLOOD BIOCHEMICAL PARAMETERS IN BROILER CHICKENS

DANA ZÁLEŠÁKOVÁ, JAKUB NOVOTNÝ, MICHAL ŘIHÁČEK, LUCIE HORÁKOVÁ, ONDŘEJ ŠŤASTNÍK, 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: xzalesa4@mendelu.cz

ABSTRACT

The influence of crude protein deficiency on blood biochemical parameters of fattened chickens of the Ross 308 hybrid combination was evaluated. The chickens were divided into two groups with two different diets – control (with a standard level of crude protein corresponding to the recommended content) and test (with a 30% deficiency of crude protein in comparison with control). From the 14th to 35th day of age, the control group reached a higher body weight ($P<0.05$) compared to the test group (389 vs. 278 g; 1,875 vs. 1,694 g). Significant differences ($P<0.05$) between control and test were found in the following blood parameters: albumin (15.238 vs. 14.188 g/l), cholesterol (2.821 vs. 3.623 mmol/l), triglycerides (0.489 vs. 0.683 mmol/l) and globulin fractions α-2 glob (0.181 vs. 0.199%), β-2-glob (0.056 vs. 0.064%) and γ-glob (0.087 vs. 0.121%).

Keywords: poultry nutrition; crude protein; blood biochemical parameters; metabolism; Ross 308

INTRODUCTION

Blood analysis is widely used in the medicine of large livestock, it is not commonly used in poultry medicine due to the absence of a large part of reference physiological values (SILVA et al., 2007). In the evaluation of the level of nutrition through biochemical blood parameters, it is essential to evaluate those parameters that we rank as the basic indicators

of metabolism. The existence of the organism depends not only on the intake of nutrients, but also on the ability to use them and on the subsequent exclusion of end products of metabolism and undigested feed residues (JELÍNEK et al., 2003). Metabolism is regulated in the body by neurohumoral and metabolic regulatory mechanisms that stabilize the internal environment and homeostasis of the body. The basic functions of metabolism include the conversion of ingested nutrients into the body's own substances (tissues, organs), the creation and storage of energy storage forms and the ability to convert it to work (DOUBEK et al., 2017). Nitrogenous substances play a very important role in the nutrition of broiler chickens. Thus, in biochemical analysis of poultry blood, we can evaluate several substances as indicators of nitrogen metabolism – total protein and ammonia level in the blood, uric acid, creatinine, etc. The available studies suggest that the level of total protein and ammonia in the blood, together with the reduction of crude protein in the diet decrease (ALAGAWAY et al., 2014; HERNÁNDEZ et al., 2012), even when replaced by amino acids (ALAGAWAY et al., 2014). Uric acid is directly related to the amount of nitrogenous substances ingested in the diet, as suggested by HERNÁNDEZ et al. (2012). Its decrease in crude protein deficiency in feed may be due to reduced availability of glycine for uric acid synthesis in the liver (NAMROUD et al., 2008). The experiment of HERNÁNDEZ et al. (2012) also showed a lower blood glucose level in chickens fed a rich-protein diet. The level of creatinine in a low crude protein diet increases which is confirmed by several studies (NDAZIGARUYE et al., 2019; ARCZEWSKA-WŁOSEK et al., 2018). The aim of this study is to determine whether and how crude protein deficiency in the diet affects selected biochemical parameters of the blood in fattened broilers.

MATERIAL AND METHODS

Animals and experimental diets

In 4 replicates, a total of 48 broiler chickens, 1-day-old hybrid Ross 308, were chosen for the experiment. Chickens were randomly divided into 2 different experimental groups with 24 birds per feeding group (6 birds per cage). Temperature and humidity were measured and recorded at regular intervals, using an automatic sensor according to the

technological instructions (AVIAGEN, 2018). The health of the chickens was checked regularly. Non-pelleted feed mixtures were used for feeding. Chickens were fed ad libitum every day and had a constant supply of water. The feed mixtures were replenished at the same time every day, residues were regularly collected and weighed. The body weight was noticed regularly every week. There were used 2 different diets for starter and grower in the trial. The composition of the feed control diets for starter and grower were recommended nutritional requirements of the relevant category according to the recommended nutrient content in feed mixture for Ross 308 chickens (AVIAGEN, 2019). The second experimental diet was designed to contain approximately 30% of nitrogen deficiency in starter and grower. Composition and nutrient content of these mixtures are shown in table 1.

Table 1. Composition and nutrient content of feed mixtures

Components (g/kg)	Starter		Grower	
	C	T	C	T
Wheat	195	382	224.7	400.1
Maze	290	352	330	348
Soybean meal	430	195	323.3	175
Limestone milled	6	6.5	4.4	4.9
Rapeseed oil	41	20	45	30
Vitamin-mineral premix[1,2]	30	30	30	30
Monocalcium phosphate	8	8	7.3	7.3
L-Lysine	-	5.8	0.3	4.5
DL-Methionine	-	0.7	-	0.2
Wheat gluten	-	-	35	-
AME_N (MJ)[3]	12.55	12.55	12.97	12.97
Dry matter	860	860	860	860
Crude protein	227.75	159.09	212.12	146.34
Ether extract	57.77	37.25	69.79	47.76
Crude fiber	26.48	20.29	24.91	19.73
Ash	65.22	51.8	52.39	50.34

Legend: [1]Vitamin-mineral premix for starter contains (per kg): L-lysine 2.34 g; DL-Methionine 2.4 g; Threonine 0.99 g; calcium 5.25 g; phosphorus 1.95 g; sodium 1.44 g; copper 15 mg; iron 84 mg; zinc 99 mg; manganese 99 mg; iodine 0.99 mg; selenium 0.18 mg; retinol 13,500 IU (international units); calciferol 5,001 IU; tocopherol 45 mg; phylloquinone 1.5 mg; thiamine 4.2 mg; riboflavin 8.4 mg; pyridoxin 6 mg; cobalamin 30 µg; biotin 0.21 mg; niacinamid 36 mg; folic acid 1.8 mg; calcium pantothenate 13.5 mg; cholin chloride 180 mg. [2]Vitamin-mineral premix for grower contains (per kg): L-lysine 2.58 g; DL-Methionine 2.52 g; Threonine 1.47 g; calcium 5.04 g; phosphorus 1.65 g; sodium 1.38 g; copper 15 mg; iron 75 mg; zinc 99 mg; manganese 99 mg; iodine 0.9 mg; selenium 0.36 mg; retinol 9,900 IU (international units); calciferol 5,001 IU; tocopherol 45 mg; phylloquinone 1.5 mg; thiamine 4.2 mg; ri-boflavin 8.4 mg; pyridoxin 6 mg; cobalamin 28.8 µg; biotin 0.18 mg; niacinamid 36 mg; folic acid 1.71 mg; calcium pantothenate 13.35 mg; cholin chloride 180 mg. [3]Apparent metabolize energy, calculated value. C – control group; T – test group with crude protein deficiency.

The experiment lasted for 35 days. At the end of the trial, broilers were weighted, slaughtered and the blood samples were collected. After slaughter, the carcasse weight was recorded and the yield of breast and thigh muscles was measured.

Sample collection and data processing

The feed intake was calculated daily, the weight changes were measured weekly.

The blood samples were collected into heparinized tubes after the slaughtering. Plasma was collected after centrifugation (15 minutes, 3,000 rpm) till 2 hours after blood collection and then it was frozen (-20 °C) until biochemical examination. The following parameters were determined using standardized biochemical methods using Erba Lachema (Czech Republic) commercial sets on the Ellipse automatic biochemical analyzer (AMS Spa, Italy) in blood plasma samples: enzymes activity AST – aspartate aminotransferase; GGT – gamma-glutamyltransferase; ALT – alanine aminotransferases; ALP – alkaline phosphatase and LD – lactate dehydrogenase. As other markers of hepatic metabolism, nitrogen and fat metabolism, was determined concentrations of the total bilirubin – Bili, TG – triglycerides, cholesterol, urea, creatine kinase, creatinine, TP – total protein, albumin and globulin fractions determined by protein electrophoresis. The content of globulins (total protein minus albumin) and albumins to globulins ratio were calculated.

The carcass weight, yield of breast and thigh muscle were weighed and calculated per kg of live weight.

The obtained data were processed in StatSoft Statistica, version 12.0 and Microsoft Excel. Analysis of Variance (ANOVA) with a one-way design using the general linear model was performed and the level of significance was established at $P<0.05$.

RESULTS AND DISCUSSION

The feed intake and live weight

The average feed intake from the control group compared to test group didn't show any significant differences ($P>0.05$), as shown in Table 2.

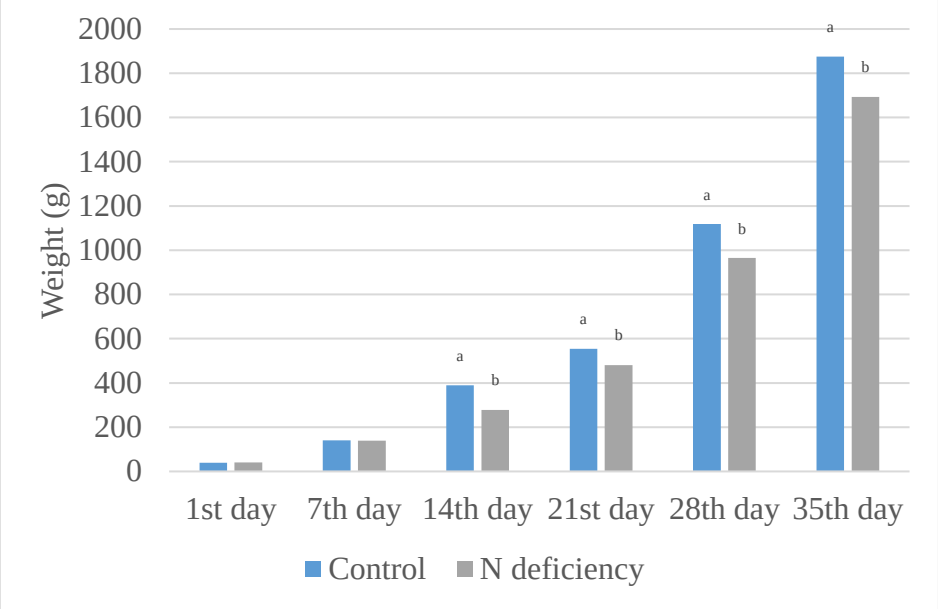
Table 2. Average feed intake of chickens

Group	n	Average feed intake per experiment (g)			Average feed intake per day (g)		
		Mean ± SE					
C	4	2430.21	±	50.58	114.86	±	1.445
T	4	2477.15	±	139.21	121.67	±	3.977

Legend: There were found no statistically significant differences ($P>0.05$). n – number of cases; C – control group; T – test group with crude protein deficiency; SE – Standard error

Nevertheless, the crude protein depleted group showed a slightly higher average total and daily feed intake compared to the control group. However, none of the groups met the target set by AVIAGEN publication Ross 308: Performance Objectives (2019) which is 3,290 g per 35-days-old hybrid. Although the feed intake didn't show statistically significant changes, weight of the chickens from the second week of age between groups was distinct, as shown in Figure 1.

Figure 1. Average weight of chickens during the trial



Legend: [a, b] means there were found statistically significant differences (P<0.05).

14-day-old chicken from control group (n=12) weighed an average of 389 g, chicken from the test group weighed 278 g. This contrast was further exacerbated at the end of the experiment, when a 35-day-old chicken from the control group weighed 1,875 g and chicken from the test group weighed 1694 g on average. None of these values meet the targets reported by AVIAGEN (2019), where the average chicken weight of this hybrid combination should be approximately 2,235 g at 35 days of age.

As demonstrated by MALOMO et al. (2013), chickens fed a higher proportion of crude protein (20–22% in the diet) showed higher feed intake compared to groups fed a lower proportion of crude protein (16–18%), which wasn't observed in our experiment. However, a statistically significant difference (P<0.05) was recorded in weight gain, where chickens fed a higher proportion of crude protein reached a higher weight compared to chickens fed a lower proportion of crude protein. Also, the experiment of BREGENDAHL et al. (2002) showed a close relationship between different dietary crude protein levels and carcass yield,

including live weight. A similar trend was observed in the study of MAIA et al. (2021), where chickens fed diet with higher proportion of crude protein (22%) showed higher body weight gains compared to chickens fed diet with lower proportion of crude protein (19%). Similar to our case, in this experiment was noticed a slight increase of feed intake in chickens fed a lower proportion of crude protein compared to the control group. The slightly higher feed intake with simultaneously lower average body weight in chickens fed a test diet in our experiment may be caused by absence of sufficient essential amino acids needed for muscle growth and formation.

Blood biochemical parameters

The aim of this study was to determine whether and how crude protein deficiency in the diet affects selected biochemical parameters of the blood of fattened broilers – in our case mainly the parameters used in the assessment of nitrogen metabolism. Obtained results are shown in Table 3.

Table 3. Blood biochemical parameters

Group	C	T
n	8	8
	Mean ± SE	
ALT (µkat/l)	0.31 ± 0.034	0.296 ± 0.020
AST (µkat/l)	3.753 ± 0.276	4.024 ± 0.591
GMT (µkat/l)	0.230 ± 0.015	0.241 ± 0.014
ALP (µkat/l)	92.325 ± 13.500	153.9 ± 28.404
LD (µkat/l)	17.336 ± 2.071	23.23 ± 5.935
CK (µkat/l)	221.578 ± 38.615	310.355 ± 104.751
Tbili (µmol/l)	4.835 ± 1.709	6.422 ± 2.271
Urea (mmol/l)	1.616 ± 0.174	1.371 ± 0.136
Creat (µmol/l)	30.8 ± 1.068	29.738 ± 1.033
UA (µmol/l)	226.75 ± 30.534	234.688 ± 17.44
TP (g/l)	30.95 ± 0.889	33.763 ± 1.557
Alb (g/l)	**15.238 ± 0.403^{a}**	**14.188 ± 0.580^{b}**
Glob (g/l)	15.713 ± 0.626	19.575 ± 1.182
Alb/Glob	**0.978 ± 0.04^{a}**	**0.737 ± 0.039^{b}**
Glu (mmol/l)	14.271 ± 0.553	13.609 ± 0.512

Chol (mmol/l)	**2.821 ± 0.117[a]**	**3.623 ± 0.182[b]**
TG (mmol/l)	**0.489 ± 0.033[a]**	**0.683 ± 0.044[b]**
α-1 glob (%)	0.042 ± 0.0026	0.046 ± 0.0030
α-2 glob (%)	**0.181 ± 0.0028[a]**	**0.199 ± 0.0047[b]**
β-glob (%)	0.140 ± 0.0027	0.148 ± 0.0075
β-2-glob (%)	**0.056 ± 0.0027[a]**	**0.064 ± 0.0025[b]**
γ-glob (%)	**0.087 ± 0.0078[a]**	**0.121 ± 0.0086[b]**

Legend: [a,b] means there were found statistically significant differences (P<0,05). ALT alanine aminotransferases; AST – aspartate aminotransferase; GMT – gama-glutamyltransferase; ALP – alkaline phosphatase; LD – lactate dehydrogenase; CK – creatine kinase; Tbili – total bilirubin; Creat – creatinine; UA – uric acid; TP – total protein; Alb – albumin; Glob – globulin; Glu – glucose; Chol – cholesterol; TG – triglycerides; α-, β-, γ-glob – globulines; n – number of cases; SE – Standard error

Experiments of ALAGAWAY et al. (2014) and HERNÁNDEZ et al. (2012) suggest that the level of total protein in the blood decreases with the reduction of crude protein in the diet, which, however, our experiment didn't confirm. In contrast, a small but statistically inconclusive increase in blood TP was noted in the deficient group, which was also observed by MAIA et al. (2021). The main product of nitrogen metabolism in birds is uric acid. It's content in blood plasma and faeces should increase with increasing nitrogen intake in feed (THRALL et al., 2012), which wasn't confirmed in our experiment. Another important indicator of nitrogen and energy metabolism is also creatine kinase, which acts as a catalyst for reversible phosphorylation, whose increased activity indicates skeletal muscle damage or increased physical exertion (DOUBEK et al., 2010). In our study its value was higher in the test group, but the changes weren't statistically significant. However, demonstrable differences were detected in the results of blood parameters of albumin, cholesterol, triacylglycerols and several globulin fractions (highlighted in the Table 3). Albumins in blood plasma primarily maintain osmotic pressure and evaluate the synthetic function of the liver (DOUBEK et al., 2010). Also, it can serve as protein source under feed restricted conditions (YANG et al., 2009). In our study, their proportion was higher in the control group compared to the test group, which also confirms MALOMO et al. (2013). Changes in the

concentration of globulins and their fractions, which were higher in the test group ($P<0.05$), may indicate liver disease (KRAFT and DURR, 2001). It could be confirmed by higher activity of liver enzymes (ALT, AST, GMT, LD) but, however, in our case weren't observed significant changes ($P>0.05$) in the liver enzymes activity between groups. Plasma TG levels were significantly higher in the test group ($P<0.05$). This detection was confirmed by ROSEBROUGH et al. (1999) who used a diet with a lower proportion of nitrogenous substances while preserving the energy. The opposite trend was observed in the experiment of JARIYAHATTHAKIJ et al. (2018). The low protein level also led to increase cholesterol, which confirms ABD-ELSAMEE et al. (2020) in their study.

CONCLUSION

Feed intake, live weight and blood biochemical parameters were monitored in broiler chickens. Our experiment didn't show any statistically significant differences in feed intake during the 35-days-long fattening period between the control group and the test group. Statistically significant differences were observed in live weight – since 14th day of age the control group have reached higher weight gain compared to the test group. Differences in blood biochemical parameters between control and test group were detected in albumins, cholesterol, triglycerides and globulin fractions. In the present study, the obtained data indicate that early nutrient reduction could affect the protein and lipid metabolism, which was reflected in the changes of several blood metabolites.

ACKNOWLEDGEMENT

The project was supported by the Internal Grant Agency of Faculty of AgriScience (Mendel University) no AF-IGA-2021-IP064.

REFERENCES

Abd-Elsamee, M. O., Motawe, H. F. A., Selim, M. M., Mohamed, H., & Elsherif, R. (2020). Effect of Different Dietary Crude Protein Levels and Citric

Acid on Broiler Chickens' Performance, Carcass Characteristics, Intestinal Morphology, and Blood Components. World, 10(3), 362-374.

Alagawany, M., El-Hack, M. E. A., Laudadio, V., & Tufarelli, V. (2014). Effect of low-protein diets with crystalline amino acid supplementation on egg production, blood parameters and nitrogen balance in laying Japanese quails. Avian Biology Research, 7(4), 235-243.

Alagawany, M., El-Hack, M. E. A., Laudadio, V., & Tufarelli, V. (2014). Effect of low-protein diets with crystalline amino acid supplementation on egg production, blood parameters and nitrogen balance in laying Japanese quails. Avian Biology Research, 7(4), 235-243.

Aviagen Group 2018: Broiler ROSS: Management Handbook. Available at: https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf

Aviagen Group 2019: Broiler ROSS: Nutrition Specifications. Available at: https://en.aviagen.com/assets/Tech_Center/Ross_Broiler/RossBroilerNutritionSpecs2019-EN.pdf

Aviagen Group 2019: Broiler ROSS: Performance Objectives. Available at: http://eu.aviagen.com/tech-center/download/1339/Ross308-308FF-BroilerPO2019-EN.pdf

Bregendahl, K., Sell, J. L., & Zimmerman, D. R. (2002). Effect of low-protein diets on growth performance and body composition of broiler chicks. Poultry science, 81(8), 1156-1167.

Doubek, J., Matalová, E., Váňová, I., Tomenedálová, J., Kuchařová, V., Veselá, I., Celá, P. (2017). Přehled fyziologie I pro VFU Brno (2nd edition). Brno: Tribun EU s.r.o., 216 p. ISBN 978-80-263-1184-3

Doubek, J., Šlosárková, S., Řeháková, K., Bouda, J., Scheer, P., Piperisová, I., ... & Matalová, E. (2010). Interpretace základních biochemických a hematologických nálezu u zvířat. Noviko s.r.o.

Hernández, F., López, M., Martínez, S., Megías, M. D., Catalá, P., & Madrid, J. (2012). Effect of low-protein diets and single sex on production performance, plasma metabolites, digestibility, and nitrogen excretion in 1-to 48-day-old broilers. Poultry Science, 91(3), 683-692.

Jariyahatthakij, P., Chomtee, B., Poeikhampha, T., Loongyai, W., & Bunchasak, C. (2018). Effects of adding methionine in low-protein diet and subsequently fed low-energy diet on productive performance, blood chemical

profile, and lipid metabolism-related gene expression of broiler chickens. Poultry science, 97(6), 2021-2033.

Jelínek, P., & Koudela, K. (2003). Fyziologie hospodářských zvířat. Mendelova zemědělská a lesnická univerzita.

Kraft, W., & Dürr, U. M. (2001). Klinická laboratórna diagnostika vo veterinárnej medicine. Hajko & Hajková.

Maia, R. C., Albino, L. F., Rostagno, H. S., Junior, M. L. X., Kreuz, B. S., Silva, R. L., ... & Calderano, A. A. (2021). Low crude protein diets for broiler chickens aged 8 to 21 days should have a 50% essential-to-total nitrogen ratio. Animal Feed Science and Technology, 271, 114709.

Malomo, G. A., Bolu, S. A., & Olutade, S. G. (2013). Effects of dietary crude protein on performance and nitrogen economy of broilers. Sustainable Agriculture Research, 2.526-2016-37765.

Namroud, N. F., Shivazad, M., & Zaghari, M. (2008). Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. Poultry science, 87(11), 2250-2258.

Rosebrough, R. W., McMurtry, J. P., & Vasilatos-Younken, R. (1999). Dietary protein effects on the broiler's adaptation to triiodothyronine. Growth, development, and aging: GDA, 63(3), 85-98.

Silva, P. R. L., Freitas Neto, O. C., Laurentiz, A. C., Junqueira, O. M., & Fagliari, J. J. (2007). Blood serum components and serum protein test of Hybro-PG broilers of different ages. Brazilian Journal of Poultry Science, 9, 229-232.

Thrall, M. A., Weiser, G., Allison, R. W., & Campbell, T. W. (Eds.). (2012). Veterinary hematology and clinical chemistry. John Wiley & Sons.

Yang, Y. X., Guo, J., Yoon, S. Y., Jin, Z., Choi, J. Y., Piao, X. S., ... & Chae, B. J. (2009). Early energy and protein reduction: effects on growth, blood profiles and expression of genes related to protein and fat metabolism in broilers. British Poultry Science, 50(2), 218-227.

GROWTH INTENSITY AND PRODUCTION PARAMETERS OF PHEASANTS FEEDED BY DIET WITH DIFFERENT PROTEIN LEVELS

JOZEF LAZAR[1], LUKÁŠ BUJŇÁK[2], PAVEL NAĎ[2]

[1]Department of Breeding and Diseases of Game, Fish and Bees, Ecology and Cynology, [2]Department of Animal Nutrition and Husbandry, University of veterinary medicine and pharmacy in Košice, Komenského 73, 041 81, Slovak Republic

Corresponding email address: pavel.nad@uvlf.sk

ABSTRACT

The effect of the addition of protein supplements (soybean meal and sunflower meal) to triticale on the production parameters of young pheasants was studied. Use of both protein supplements as a part of concentrated feed (triticale) led to significantly higher body weight ($P < 0.001$, resp. $P < 0.01$), improved feed conversion ratio and lower mortality of young pheasant in comparison to control group. In all groups, the live weight of pheasant roosters was on average 300 g higher than hens. Higher weights in cocks compared to hens were also found in the pectoral muscle, thigh muscle, wings and trunk. Only the weight of abdominal fat was higher in hens compared to cocks in all groups. The increase of protein concentration in young pheasant nutrition positively affected their development before their release into the free nature.

Keywords: pheasant chickens, production, triticale, soybean meal, sunflower meal

INTRODUCTION

The success of livestock farming is affected by the level of nutrition. This also applies to the breeding of feathered game released into the wild. For the survival of artificially bred pheasants after release into the free nature, their weight is important. In the first stages, the pheasant chickens are intensively fed complete feed mixtures, in the pre-release phase they are fed green fodder supplemented with cereals in an effort to bring their nutrition closer to natural conditions (Kokoszynski, 2014). Triticale is

characterized by a higher protein content than most cereals. It has more favorably balanced amino acids than wheat and rye. It contains a higher proportion of lysine and sulfur amino acids. The energy value of triticale is similar to that of wheat and higher than that of rye. A high proportion of triticale can be used in poultry diets without any side effects (Leeson and Summers, 1997). Soybean meal is a quality protein feed in the diets of all kinds of animals. Due to the content of antinutritive substances, soy must be heat treated before feeding. Sunflower meal can be a very useful component of compound feed as a protein supplement, especially in areas where soybeans are not grown. The sunflower from which the extracted meal is produced is suitable in compound feeds, especially during washing, because it has a beneficial effect on the color saturation and gloss of feathers (Leeson and Summers, 2005). The aim of the study was to monitor the effect of the addition of soybean and sunflower extracted meal to cereals (triticale) on the growth intensity, conversion of the mixture and production parameters during the 3rd phase of breeding pheasant chickens.

MATERIAL AND METHODS

600 pheasant chickens, 12 weeks old, were included in the experiment. Pheasants were divided into 3 groups of 200 chickens, in equal proportions hens and roosters. The chickens were housed in aviaries with access to green forage and the addition of concentrated feed *ad libitum*. Group T was supplemented with crushed triticale throughout the observed period. In group T+SB, triticale was combined with soybean meal in an amount of 10% in the feed. In group T+SF a 12% supplement of sunflower meal was added to the triticale. The weight of randomly selected pheasant chickens (n = 50, 25 roosters and 25 hens) and feed consumption were recorded at 2-week intervals. Chicken mortality was monitored daily. The content of nutrients in concentrated feeds (dry matter, crude protein, ash, fat, fiber and nitrogen-free extractives) was determined according to Commission Regulation (EC) No. 152/2009. The feed conversion factor was calculated. At week 22 of follow-up, 25 males and 25 females from each group were slaughtered, defeathered and eviscerated, and their carcasses were dissected. Yield and percentage of

breast, thighs, wings, torso and abdominal fat were monitored. The obtained results were evaluated statistically (ANOVA).

RESULTS AND DISCUSSION

The results of the analysis of feed are given in table 1. The analysis shows that triticale alone cannot provide a sufficient need for proteins. Compared to the classic feed mixture for pheasant breeding in the last phase (BŽ3), where a protein concentration of 18% is required (Mohelský, 2013), triticale alone provides 46 g less protein. By adding 10% soybean meal in the T+SB group, we achieved a higher protein content by 16 g compared to the conventional feed mixture, and with the addition of 12% sunflower meal in the T+SL group, the protein level was just below 18%.

Table 1 Nutrient contents in supplementary diets of pheasants in the last phase of growth (g.kg-1 dry matter)

Parameter/Group	T	T+SB	T+SF
dry matter	863,5	865,9	865,8
crude protein	134,6	196,0	173,6
fat	16,3	18,0	12,7
crude fibre	33,3	40,2	66,9
ash	19,6	29,6	27,5
nitrogen-free extract	796,2	716,2	719,3
metabolizable energy MJ	12,54	12,56	12,10

T - triticale; T+SBEM – 90% triticale + 10% soybean meal; T+SFEM – 88% triticale + 12% sunflower meal; MJ - mega joule

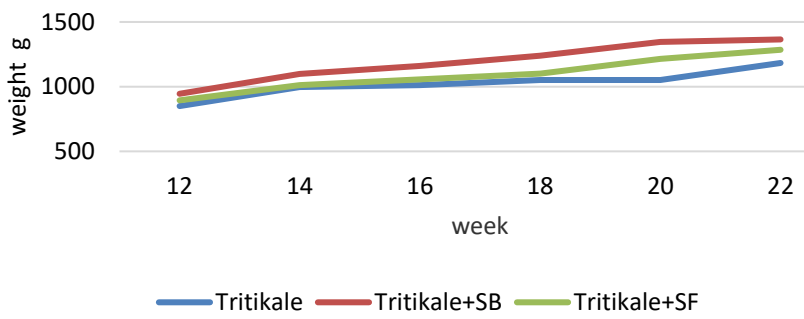
Monitoring the weight of the pheasant chickens at week 22 of rearing showed the highest average weight (1365 g) in the group of chickens fed a mixture composed of 90% triticale and 10% soybean meal. The difference compared to the control group was statistically significant ($P < 0.001$). Pheasants chickens supplemented with a feed mixture based on triticale and sunflower meal had a weight of 13.9% higher at week 22 compared to the control group ($P < 0.01$). Kokosinsky et. al. (2011) in a pheasant experiment recieved a diet containing 27.0% crude protein (CP) and 11.8 MJ metabolizable energy (ME) to 4 weeks, 23.5% CP and 12.1 MJ ME from 5 to 8 weeks, and 17% CP and 11.5 MJ ME from 9 weeks,

reached in 20 weeks body weight 1355 g in cocks and 998 g in hens. In our experiment at 22 weeks, the cocks weighed 1420 g, 1480 g, resp. 1455g and hens 946 g, 1251 g resp. 1115 g depending on the composition of the diet they received in the group.

At week 22 of rearing chicken pheasants, the highest weight gains were recorded in chickens supplemented with a triticale diet supplemented with soybean meal (Graph 2), which represented a weight increase of 64% compared to the control group. In the experiment in the group supplemented on triticale with the addition of sunflower meal, the weight gains were 44.7% higher than in the control group.

Fig. 1 shows the growth curve of pheasant chickens. Throughout the experiment, the growth intensity was higher in the experimental groups than in the control group, which confirmed the justification for the inclusion of plant protein feeds in the form of sunflower and soybean meal to the triticale diet.

Fig. 1. Growth curve of pheasant chickens



Karasková et al. (2012) when monitoring the fattening of young pheasants with commercial compound feeds, the highest daily gains were registered in 6 to 12 weeks of breeding.

The best feed conversion (0.65) in our experiment was found in the group of tritical fed pheasants with the addition of soybean meal. The feed conversion coefficient in the group fed with triticale and sunflower meal was 0.82, and the highest (1.18) was in the group fed only triticale.

The reared pheasant chickens were in good health during the experiment. The mortality of pheasant chickens in the experimental groups was lower (3%) than in the control group (6.5%) fed only with triticale.

Similar to our experiment when using a mixture based on triticale and sunflower resp. soybean meal, a positive effect of feeding pheasants with a mixture on the weight of pheasants at the age of 24 weeks was noted by Kuzniacka et al. (2010). Sage et al. (2002) found higher average weight gain and better feed conversion in pheasants of cocks and hens from 10 to 16 weeks of age fed a granular mixture with a high protein content compared to pheasants fed only cereals. Dordevic et al. (2010) confirmed a favorable relationship between increasing dietary protein concentration and feed conversion.

The average live body weights, the carcass weights and the weights and the percentage of each part are given in table 2.

Table 2. Body weight, dressing percentage and carcass composition of pheasant

Trait		**T**		**T+SB**		**T+SF**	
Body weight	g	1183*	±127	1365,3*	±68,62	1285*	±85,57
Carcass weight	g	697,3	±72,6	799,3	±44,59	768,7	±46,81
Dressing percentage	%	58,9	±0,65	58,6	±0,48	59,8	±0,75
Brest muscles	g	229,2	±11,8	244,3	±9,50	248,3	±6,36
	%	19,3	±0,20	17,9	±0,30	19,3	±0,47
Leg muscles	g	213,7	±9,85	245,7	±5,31	234,1	±8,77
	%	18,1	±0,27	17,9	±0,16	18,2	±0,02
Wings	g	37,9	±1,80	40,3	±1,50	39,2	±1,41
	%	3,2	±0,05	2,9	±0,05	3,1	±0,05
Abdominal fat	g	26,3	±7,53	37,3	±7,39	19,5	±3,97
	%	2,22	±0,71	2,7	±0,58	1,5	±0,31
Remainder of carcass	g	135,7	±16,9	185,7	±9,16	164,4	±12,48
	%	11,4	±0,32	13,6	±0,46	12,8	±0,46

T - triticale; T+SB – 90% triticale + 10% soybean meal; T+SFEM – 88% triticale + 12% sunflower meal; * - statistically significant

There was a statistically non-significant difference between body weights of pheasant roosters (1420, 1480 and 1455 g, respectively). However, in pheasant hens, a difference in weight ($P < 0.001$) was found between the group fed only triticale (946 g) and the group fed triticale with the addition of soybean meal (1252 g). A significant difference ($P < 0.01$) in weight was found also between the triticale-only group and the triticale-fed group supplemented with sunflower meal (1115 g).

Differences in another parameters were not statistically significant among the groups. Slaughter yield was below 60% in the all groups. Kokoszynski et al. (2014) achieved a 10% higher yield in pheasant chickens at 16 weeks of breeding.

Significant differences in weights were found within gender. Pheasant roosters were on average 300g heavier than hens. Higher weight in the cocks was also found in the pectoral muscle, thighs, wings and torso. With abdominal fat, weights in all groups were significantly higher in hens compared to cocks. Identical findings are also reported by Kokoszyński et al. (2011). In addition to abdominal fat, they also found significantly higher weight in the skin with subcutaneous fat in pheasant hens compared to faucets.

CONCLUSION

The level and quality of nutrition in the breeding of young pheasants is an important factor in their preparation for release into the wild. In our experiment in the third phase of breeding young pheasant chickens (12 - 22 weeks), in aviaries with access to green forage, *ad libitum* was added concentrated feed in the form of shredded triticale. In the experimental aviaries, triticale was added with the addition of soybean meal (10%) and the addition of sunflower meal (12%), respectively. The group of pheasants supplemented with the addition of triticale and soybean extracted meal proved to be the most optimal breeding variant in our experiment. The results of the work confirmed the justification of the combination of cereals with protein core feeds of plant origin in the last phase of breeding young pheasants. At the end of the experimental period before release into the wild, the pheasants were sufficiently developed with good flying ability and fully colored.

REFERENCES

Dordevic M., Pekeč S., Popovic Z., Dordevic N.: Influence of dietary protein levels on production resuls and mortality in pheasants reared under controlled conditions. Acta Veterinaria (Beograd), 2010, Vol. 60, No 1, 79-88.

Karasková K., Straková E., Suchý P., Zapletal D. Jambor M.: Analysis of fatteting performance and carcass value indicators of the common pheasant (*Phasianus colchicus L.)*, Maso International Brno, 2012, 2, 143-146

Kokoszyński D., Bernacki Z., Korytkowska H., Wilkanowska A.: Effect of different feeding regimens for game pheasants on carcass composition, fatty acid profile and mineral content of meat. European Poultry Science, 2014, 78: 1-10.

Kokoszyńsky D., Bernacki Z., Cisowska A.: Growth and development of young game pheasants *(Phasianus colchicus)*, Archiv Tierzucht, 2011, 54, 1, 83-92

Kuzniacka J., Adamski M.: Growth rate of body weight and measurements in pheasants reared up to the 24th week of life. Archiv Tierzucht, 2010, 53(3): 360-367.

Leeson S., Summers J. D.: Commercial Poultry Nutrition, 2nd edn. University Books, Guelph, Canada, 1997, s. 355.

Leeson S., Summers J. D.: Commercial poultry nutrition, 3rd edition. Department of Animal and Poultry Science University of Guelph, Ontario, Canada, 2005, ISBN 0-9695600-5-2

Mohelský M.: Výživa a krmení pernaté zvěře. Myslivost, 2013, 6, s. 34 – 37.

Sage R. B., Putaala A., Woodburn M. I. A.: Comparing Growth and Condition in Post Release Juvenile Common Pheasants on Different Diets. Poultry Science 2002, 81: 1199-1202

NutriNET 2021

Proceedings of reviewed scientific papers

Editors: MVDr. Lukáš Bujňák, PhD.

doc. MVDr. Naď Pavel, PhD.

Published and printed by:

The University of Veterinary Medicine and Pharmacy in Košice

Year of publishing: 2021

Number of copies: 100

Number of pages: 157

ISBN: 978-80-8077-713-5


THE UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN KOŠICE

NutriNET