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EFFECTS OF HERBAL PREPARATION ON HEALTH INDICATORS IN CALVES AT AN EARLY AGE

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ABSTRACT

Phytobiotics (= plants, plant products and other bioactive compounds) are used for the prevention and treatment of animals. Herbal preparations have myriad range of effects on health. Legislation prefers phytobiotics and other additives to using of antibiotics, because the applications of phytobiotics have any risk of antibiotic residues. Special attention in this regard is devoted to cattle.

The aim of diploma thesis was to evaluate the effect of herbal tincture FAUNAKOMPLEX® in Holstein calves from birth to eight weeks of age. A number of health problems occur in this period, since the immune system still developing. Various factors influencing health status of calves were examined. The sufficient degree of colostral protection has a huge impact this time. Calves were divided in two groups – experimental impact of FAUNAKOMPLEX® on the incidence of various diseases, course and intensity of disease. The costs of the drugs were also compared.

There were found differences in incidence of diseases (the overall incidence of diseases was shorter by 30 days in the experimental group) and differences in drug costs (costs of drugs were 45% lower in experimental group). Differences were found in the connection of intake degree of colostrum. The result show that the experimental group of calves receiving FAUNAKOMPLEX® had overall lower incidence of diseases and higher resistance, thus the application of this phytobiotic could represent opportunity to strengthen animal health in practice.

Keywords: Phytobiotics; herbs; FAUNAKOMPLEX®; disease prevention; immunity;

INTRODUCTION

Feed additives are specifically active substances. Appropriate amount of feed additives is added to ration. Additives protect the health against

adverse effects. Importance of additives increases in concentrations farms where animals need to be especially protected against disease and other stress factors (Zeman et al., 2006).

Plants, plant products and other bioactive compounds are re-used as a natural additive (= phytobiotics). It is very important to focus on determining the specific chemical compound (tannins, saponins, essences and other active substances) and to identification the factors that determine the concentration of active ingredients. Determining of chemical and identification of factors are very difficult. The concentration and effectiveness of the bioactive agents may be influenced by numerous factors (e.g., plant varieties, the influence of season and climate, temperature, humidity, light, soil composition, storage, processing, etc.) (Salem et al., 2012).

Plants have a great range of effects on health status. E.g. herbs contribute to reducing inflammatory processes (prevention of disease). These herbs can reduce inflammatory interleukins IL 6 level and can increasing anti-inflammatory interleukins IL 10 level (Mueller et al., 2010).

The effects of phytobiotics can be used in cattle farming. They were evaluated the effects of herbal mixtures on feed intake, performance, udder health, ruminal fermentation and blood biochemical indicators in the study of high numbers of somatic cells in milk. The results showed that the addition of the herbal blend decreased somatic cells in milk. There has also been an increase in feed intake, improve feed digestibility and increase in milk production (Hashemzadeh-Cigari et al., 2014).

Phytobiotics can increase the efficiency of fermentation in the rumen (eg. strengthening of the positive aspects of N metabolism, reducing methane production, reducing the risk of bloat and acidosis). Reducing methane production will be better feed conversion. Klevenhusen et al. (2012) examined phytobiotics impact on reducing methane production in the rumen fermentation. Some phytobiotics can have antimicrobial and antioxidant effects. This leads to a strengthening of the immune system, improving bowel function, and integration of, the thus increases the tolerance of the animals on the oxidative or thermal stress. Phytobiotics may increase the intake and digestibility, and improve the performance of animals (Salem et al., 2012).

Bovine animals have syndesmochondrial type of placenta, so the immune substances from the mother's blood cannot penetrate into the bloodstream of the fetus before birth. Immunity calves dependent on sufficient intake of colostrum and on passive transport

of immune substances through the wall of colostrum in the first hours after birth (passive immunization). (Slosarkova et al., 2011).
Nutrition has a significant effect on immune function. Insufficient or excessive intake of nutrients can impact immune status adverse and susceptibility of various pathogens may dangerous. Specified food should correspond to genetic factors and environmental influences. It is important also create a suitable temperature, light, microbial and social environment. Nutrition, optimal environment and good health are the basic physiological factors. Deficiency of one factor can cause stress (Broucek a Soch, 2008).
There is a real risk of illness caused by microorganisms that are resistant to antibiotics. Particular attention in this regard must be paid to cattle, especially cows, because they usually antibiotics done very often. The consumption of antibiotics in cows high and in general practice are applied in some routes of administration of antibiotics (at drying off cows applied intramammary antibiotics often massive scale), where it is medically absolutely unjustifiable and are thus inducing conditions for easy emergence of resistance bacteria (Hofirek et al., 2009).
Evaluate the effect of herbal tinctures FAUNAKOMPLEX® on the health status of calves at an early age was the aim of this study. FAUNAKOMPLEX® is used to strengthen the immune system, especially in adult animals. The question is whether we can expect these positive effects also in calves that do not yet have a fully developed immune system and they are after birth dependent on colostral immunity. Animal health is equivocal term.
Impact of FAUNAKOMPLEX® on the incidence, duration and severity of enteritis and respiratory syndrome in calves was assessed in this study. The reason for this is the fact that enteritis (inflammation of the intestine) is one of the most occurring diseases in calves at an early age. Another common problem is the occurrence of respiratory syndrome, and to a lesser extent, there are also other diseases such as e.g. navel infections, dehorning infection and myopathy. They were also assessed economic aspects (costing a total treatment, and weight gain).

MATERIAL AND METHODS

Three drops of herbal tincture FAUNAKOMPLEX® was administered to the experimental group of calves (from birth though 8 weeks) orally into clean drinking water (when calves accepted only milk diet, the tincture was served into the milk diet). If calf from control

or experimental group had health disorders, conventional treatment was initiated. It was observed: general health, incidence of disease (pneumonia, navel, myopathy, etc.), diarrhea (days and intensity), the state of mucous membranes and natural body openings.
Female calves of Holstein breed of cattle (a total of 30 pieces of calves) from birth to 8 weeks of age were ranked into experiment. The calves were divided into two groups: control (15 pieces) and experimental (15 pieces). Calves after first connection of colostrum were moved to outside individual booths with numbers.
Calves accepted milk mixture (colostrum and powdered milk 3 x 2l/day), starter and water (ad libitum).

Nutrition:

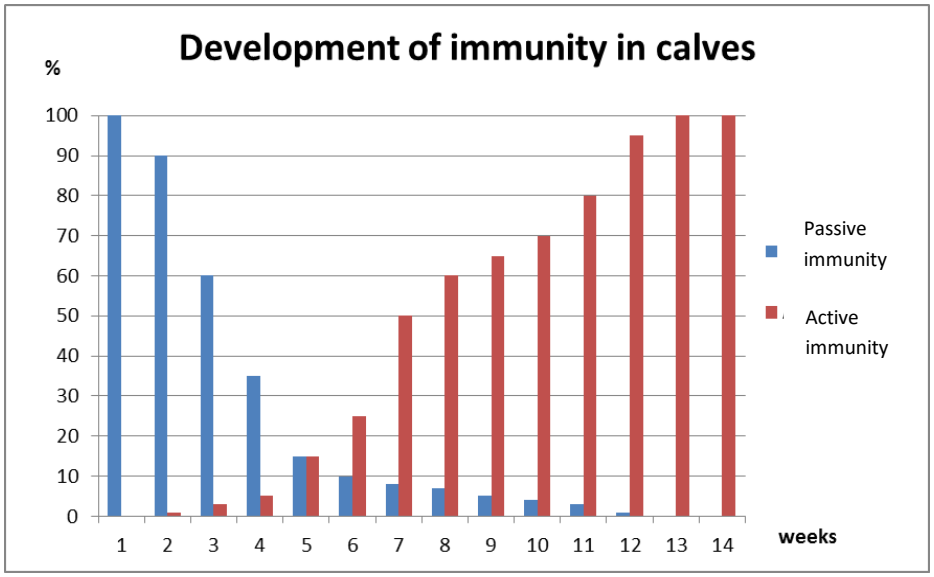
- From first to third day of age: colostrum and milk 3x 2l/day
- From fourth to fourteenth day of age: starter and milk with milk substitute in ratio 1:1 3 x 2l/day
- From fifteenth day to one month of age: starter and milk substitute 2x 2l/day, hay

Calves were weaned from milk diet in one month of age. Heifers were transferred to the joint stable in two months of age.
Statistical calculations were performed in Graphpad Prism statistical program version 5.01 (GraphPad Software, La Jolla, CA, USA) using the unpaired t-test. Tables and graphs were drawn in Microsoft Office Excel 2007. Statistically significant differences were determined in control and experimental groups with the following parameters: total number of days of illness, number of days of the occurrence of enteritis, the number of days the incidence of respiratory syndrome.

RESULTS AND DISCUSSION

Occurrence of several types of diseases (enteritis, respiratory syndrome and other disorders of health) was detected during this monitoring. Both groups peaked occurrence sick animals within 2 and 3 weeks after birth. It is known that the active antibody production begins at the calf until 2-3 weeks after birth due to lack ability to transmit placental cotyledons antibody fetus (Langel et al. 2015). Length and development depending on passively acquired immunity while developing its own (active) the immune system are shown in graph 1 (Illek, 2015).
For both groups of calves was reported incidence of the disease. The most common cause disruption of the calf's health status was enteritis. It was also observed incidence respiratory syndrome, or other diseases (infection navel infection after dehorning, myopathy).

Graph 1: Development of immunity in calves.



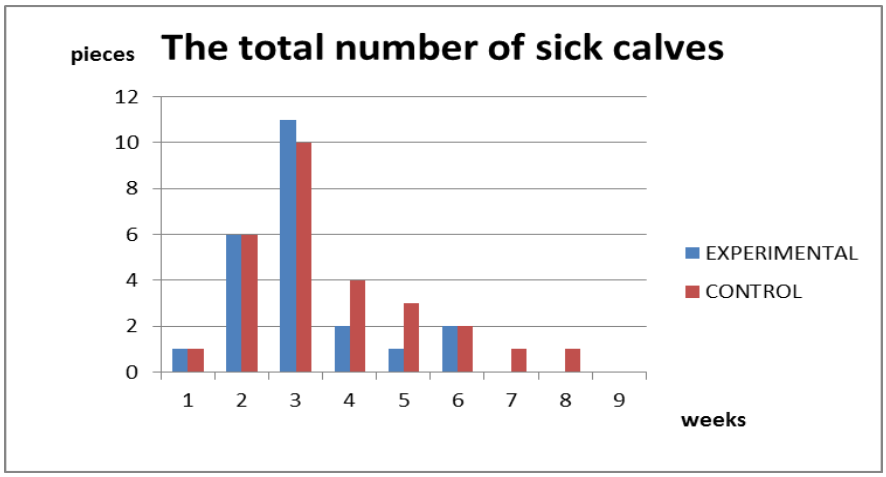
Incidence of disease in calves depends on many factors discussed above. Diarrheal diseases occur mostly in the first 14 days of life, bronchopneumonia in this period less. Respiratory diseases most occur in the age of about one month. Weaning and moving to group housing also effect on occurrence of enteritis and respiratory syndrome (Davidek, 2015).
Table 1 summarizes the total sick days broken into enteritis, respiratory syndrome and other diseases. Enteritis was compared the average duration of health problems. For respiratory syndrome has also determined the average duration of health problems.

Table 1: Summarized incidence of various diseases and the cost of drugs

EXPERIMENTAL GROUP						
Total sick days	Total enteritis days	Average duration enteritis	Total respiratory syndrome days	Average duration respiratory syndrome	Total other diseases days	Total costs of drugs
142 days	**106 days**	7.07 days	**28 days**	5.6 days	**8 days**	**2 081 CZK**

CONTROL GROUP						
Total sick days	Total enteritis days	Average duration enteritis	Total respiratory syndrome days	Average duration respiratory syndrome	Total other diseases days	Total costs of drugs
172 days	**101 days**	7.21 days	**47 days**	7.83 days	**24 days**	**3 784 CZK**

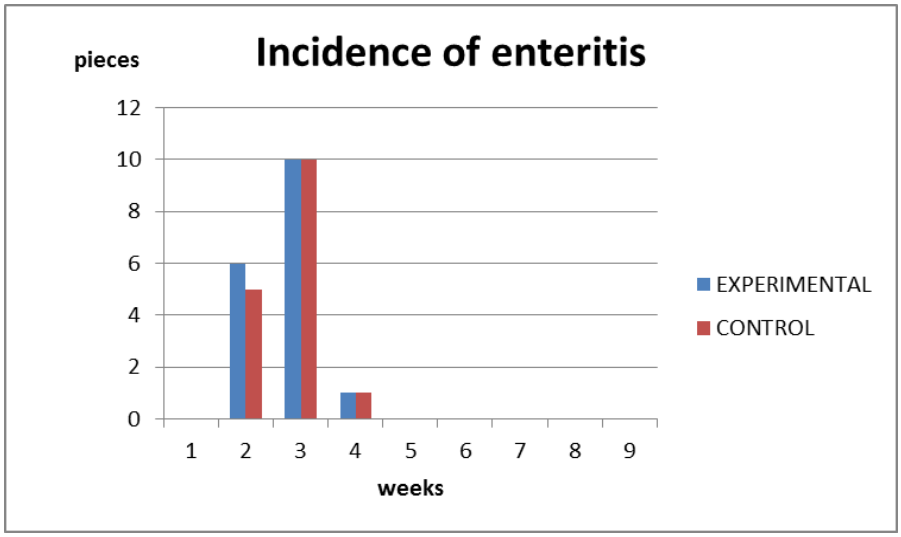
Graph 2: The total number of sick calves



ENTERITIS

Enteritis accounted a major share in the incidence of the disease. As seen in Graph 2, the occurrence of enteritis was bound from second to fourth week. Like in the case of the total number of sick animals, the incidence of reported enteritis in the second and third peak incidence. Enteritis incidence of neonatal calves (under the age of 3 weeks) did in the experimental group of animals 34% while control 32%. Bostedt a Jung (2003) report, that the average incidence of enteritis in neonatal calves is typically around 36%. Statistically significant difference was not between experimental and control group.

Graph 3: Incidence of enteritis

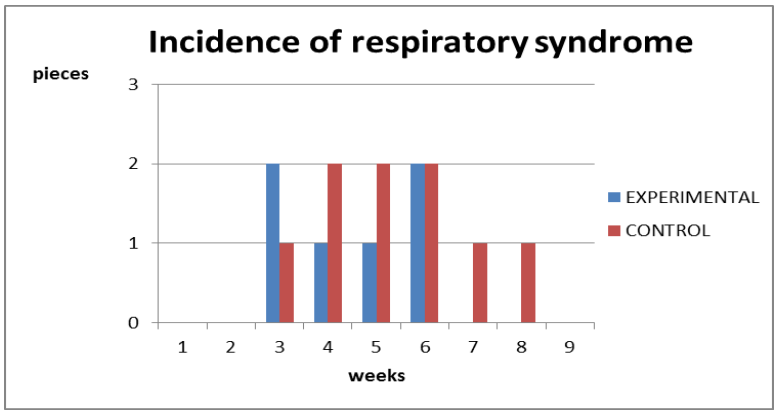


Graph 3 represents the length of the disease in individual animals of experimental and control groups. The sum of the incidence of enteritis days for each group is summarized in Table 1.

RESPIRATORY SYNDROME

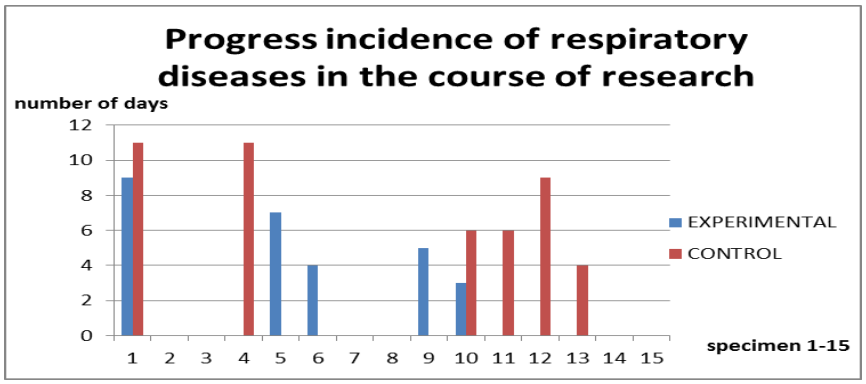
Incidence of respiratory syndrome in calves occurred in 26% during the first three weeks of age (Jung a Bostedt, 2003). Prevalence of respiratory syndrome varies during the year: April 84%, May 32%, in June 8% and July 0%. Incidence of respiratory diseases observed animals are consistent with those found Illek et al. (2013). Results were not observed statistically significant difference in the number of sick calves (5 pieces and 6 pieces of experimental control group), but it is a noticeable difference in the average duration of respiratory problems in the experimental group lasted for respiratory problems, on average, 5.6 days and the control problems persisted on 2 days longer, i.e. 7.83 days (see. Table 1). Respiratory syndrome of experimental group occurred only until sixth week, while the control until eighth week.

Graph 4: Incidence of respiratory syndrome



Graph 4 expresses the length of the disease in individual animals of experimental and control group. Sum days the incidence of respiratory disease in each group is summarized in Table 1.

Graph 5: Progress incidence of respiratory diseases in the course of research



Graph 5 records the occurrence and duration of respiratory disease in specimen calves from experimental and control group.

OTHER DISEASES

Other diseases against enteritis and respiratory syndrome occur to a much lesser extent. Navel infections are frequent. Incidence of navel infection is about 15% (Jung a Bosted, 2003). The first signs of infection, inflammation of the navel are found at 2-3 days old calves, the navel stump rope is not dry and is moist (Nemecek, 2009). Incidence of infections after dehorning was also observed during follow-up. The pain caused from cauterizing causes animal stress (cortisol flooding), which affects the immune system of animals adversely (Stafford and Mellor, 2005). The results show,

that the incidence of other diseases in the total number of days was different. The control group was three times higher than the experimental group which was administered FAUNAKOMPLEX®. Other diseases occurred generally in the experimental group 8 days while in the control group 24 days (see. Table 1).

ECONOMIC ASPECTS

The level colostral immunity calves have long term effects on morbidity and mortality of calves to weaning as well as after. Furthermore, colostral immunity have effect on feed conversion, weight gain, age at first calving and even in milk production in the first and second lactation (Slosarkova et al., 2011). Health status of calves during first weeks of life is very important (effect on costs of treatment, weight gain, feed conversion, productive and reproductive performance etc.) from economic point, which must be taken into account. Economic losses caused deaths and necessary deductions calves are made up of many respiratory diseases (47%), also disorders of the digestive tract made 9%. The financial loss of death and deduction is about 2 500 CZK of newborn Holstein calf and approximately 9 000 CZK of six months old calf (Kvapilik, 2010). The difference cost of drugs between the experimental and control groups was significant. Cost of drug was by 45% (1703 CZK) below in the experimental group. Cost of antibiotics (engemycin, flumixin) increased mainly in the control group. Total daily weight gain was in the experimental group higher on average 16 g in compared with control. Incidence of respiratory diseases was reduced in the experimental group, so the application of FAUNAKOMPLEX® could reduce costs of respiratory therapy and also reduce the number of compulsory slaughtering calves having respiratory difficulties, which are the most common cause of emergency slaughter.

CONCLUSION

The aim of the thesis was to evaluate the effect of herbal tinctures FAUNAKOMPLEX® on health indicators calves of Holstein cattle from birth to eight weeks of age. In this work were reported the following results:

Total number of sick days (all diseases) by experimental group was 30 days shorter in compared with control group. This fact was reflected

in the cost of drugs. In the control group increased the cost of medicines.

ENTERITIS: Effect on incidence, duration, or intensity of enteritis disease was not significant.

RESPIRATORY SYNDROME: It was observed a difference of respiratory disease occurrence between control and experimental group. The respiratory diseases occur by experimental group from third to sixth week and by control group from third to eighth week.

Length of respiratory disease in experimental group was an average two day shorter than in control group.

OTHER DISEASES: Noticeable difference of the occurrence of other diseases was observed between experimental and control groups. Other diseases occurred threefold less by experimental group compared with control group. Total number of days by experimental group was only 8 days while the control 24 days.

ECONOMIC ASPECTS: Between experimental and control groups was observed significant difference in the cost of drug (control group had higher cost of antibiotics). The total experimental group cost of drug was by 45% less in compared with control group. The animals also had higher weight gains on average 16 g per day.

It should be noted some important facts in the overall evaluation of benefits of herbal tincture FAUNAKOMPLEX®:

- it can rule out the placebo effect
- it would be needed carry out this experiment on higher number of animals in the future
- it is necessary to consider the individuality of animals and to minimize "noise" internal and external factors.

It can conclude that the experimental group of calves receiving FAUNAKOMPLEX® showed overall lower incidence of disease and higher resistance, therefore the application of this phytobiotics could represent one of the ways to strengthen animal health in practice.

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EFFECT OF LACTATION NUMBER ON RUMINAL PH OF DAIRY COWS

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ABSTRACT

The aim of the study was to continuously monitored reticuloruminal pH with e-bolus for 15 weeks of lactation and found the differences in the ruminal pH between cows in the second (n=3) and third lactation (n=4) at school experimental farm in Oponice. Animals were fed once with Total Mix Ratio *ad libitum* (between 4:00 a.m. and 5:00 a.m. h) and milked 3 times per day (6:00 a.m., 12:00 a.m. and 6:00 p.m.). The bolus which was implemented through *Esophagus* measured pH and temperature values every 15 minutes (96 data points per day) with accuracy +/-0.1 pH. Data were downloaded and collected with HathorHBClient v. 1.8.1 and statistically evaluated with IBM SPSS v. 20.0 (One-way ANNOVA, Tukey Test, Indipendent Samples T-Test). The pH mean of dairy cows in the 2nd lactation was 6.18±0.33 and in the 3rd lactation 6.46±0.41. Statistical differences between lactation weeks and lactation numbers were found ($p<0.05$). The most frequented pH values in the both groups was in the interval 6.2-6.8 (2nd lactation 45.64%, 3rd lactation 52.02%). Values under pH 5.8 formed 13.07% (2nd lactation) and 5.38% (3rd lactation cows).

Keywords: bolus; continuous monitoring; ruminal environment; cattle

INTRODUCTION

Modern feeding strategies have changed from primarily forage-based to progressively more readily fermentable carbohydrate (RFC) feedstuffs in dairy rations to meet the increasing milk production of high-producing animals. These practices favour the use of silages with a high acid content, low fiber diets with reduced particle size, and high levels of concentrates (Peyraud and Apper-Bossard, 2006). In a survey of field nutritionists, dietary starch concentration in rations for lactating

dairy cattle that supported both positive production and health were between 15 to 25% of ration DM (Hall and Eastridge, 2014). High-producing dairy cattle require large amounts of dietary energy to meet the demands of increased milk production. To accommodate this energy requirement, it has often been economical for producers to feed large amounts of cereal grains to provide energy to rumen microbes and their host. Cereal grains contain large quantities of highly fermentable carbohydrates that can result in a build-up of organic acids in the rumen and reduce rumen buffering (Kleen et al., 2003; Stone, 2004), causing a depression in rumen pH. Subacute ruminal acidosis (SARA) is frequently occurring metabolic disease which is caused by depression of pH under 5,5 for more than 3 hours daily (Kleen et al., 2003; Stone, 2004; Gozho et al. 2005). The aim of the study was to continuously monitored reticuloruminal pH with e-bolus for 15 weeks of lactation and found the differences in the ruminal pH between cows in the second and third lactation.

MATERIAL AND METHODS

Measured data from 7 dairy cows of Holstein breed (average age 3.57) in cooperation with the University Experimental Farm in Oponice during 15 weeks of lactation were collected. Selected cows have average milk production 10 175 kg per lactation with 3.94% of fats, 3.10% of crude proteins and 4.7% of lactose. Cows were divided into two groups according to lactation on 2^{nd} lactation cows (n=3) and 3^{rd} lactation cows (n=4). Animals were fed once with Total Mix Ratio (DMI – 24.45 kg; 153.86 MJ/kg NEL; 15.74% CP; 24.35% NDF; forage to concentrate ratio - 53:47) *ad libitum* (between 4:00 a.m. and 5:00 a.m. h) and milked 3 times per day (6:00 a.m., 12:00 a.m. and 6:00 p.m.). Every dairy cow has implemented farm bolus for continual data measuring which was implemented through *esophagus* orally with the use of special balling gun. The bolus pH and temperature values were measured every 15 minutes (96 data points per day) with accuracy ±0.1 for pH. Used boluses (eCowDevon, Ltd., Great Britain) are characteristic with its small dimensions (135 × 27 mm) and weight 207 g. Data were downloaded with the handset with antenna and dongle connected with USB dongle connector with the radio frequency 434 MHz in the milking parlour. Collected data were summarized with HathorHBClient v. 1.8.1 and statistically evaluated with IBM SPSS v. 20.0 (One-way ANNOVA, Tukey Test, Indipendent Samples T-Test).

RESULTS AND DISCUSSION

Results and descriptive statistics are shown in the Table 1 and Table 2. Overall, average pH of dairy cows during experiment was 6.36±0.41.

Table 1 Differences in the pH mean between dairy cows during lactation

Week of lactation	Second Lactation			Third Lactation		
	pH mean	Standard Deviation	Standard Error	pH mean	Standard Deviation	Standard Error
4	6.44[a]	0.28	0.01	6.19[a]	0.26	0.01
5	6.47[a]	0.28	0.01	6.13[b]	0.35	0.01
6	6.44[a]	0.25	0.01	6.07[c]	0.41	0.01
7	6.38[b]	0.23	0.01	6.15[ab]	0.31	0.01
8	6.18[c]	0.25	0.01	6.33[d]	0.25	0.01
9	6.25[d]	0.22	0.01	6.32[de]	0.26	0.01
10	6.16[c]	0.22	0.01	6.29[e]	0.30	0.01
11	6.16[c]	0.22	0.01	6.39[f]	0.23	0.00
12	6.15[c]	0.19	0.01	6.43[g]	0.28	0.01
13	6.00[e]	0.27	0.01	6.44[g]	0.38	0.01
14	6.01[e]	0.27	0.01	6.46[h]	0.41	0.01
15	5.99[e]	0.23	0.01	6.75[i]	0.34	0.01
16	5.71[f]	0.24	0.01	6.84[j]	0.34	0.01
17	5.89[g]	0.26	0.01	6.79[k]	0.41	0.01
18	5.78[h]	0.19	0.01	6.84[j]	0.33	0.01

Different letters in the columns indicate significant differences. The mean difference is significant at the 0.05 level (Tukey Test). The pH mean of dairy cows in the 2nd lactation was 6.18±0.33. In comparison with pH of dairy cows in the 3rd lactation (6.46±0.41) it was 4.35% less (p=0.000). Mertz et al. (2009) found mean pH in the 1st lactation 6.43±0.28, 2nd lactation 6.87±0.19 and 3rd lactation 6.40±0.40. Mean pH in the group of multiparous cows was 5.84±0.06 and in the group of primiparous cows 5.95±0.06 (Meakawa et al., 2002). Bowman et al. (2003) determined higher pH in the group of primiparous cows (5.67) than multiparous (5.58). Maulfair et al. (2013) observed the lowest pH values from 5.28 to 5.59 and the highest values of pH from 6.69 to 6.95. Another research recorded average pH values from 5.69 to 6.50 (Krause et al., 2009) and from 5.90 to 6.60 (Křížová et al., 2011).

Table 2 Descriptive statistics of pH mean according to lactation number of dairy cows

Lactation Number	pH mean	Standard Deviation	Standard Error	Min.	Max.
2nd	6.18[a]	0.33	0.00	5.18	7.39
3rd	6.46[b]	0.41	0.00	5.30	7.57

Different letters in the columns indicate significant differences. The mean difference is significant at the 0.05 level (Indipendent Samples T-Test).

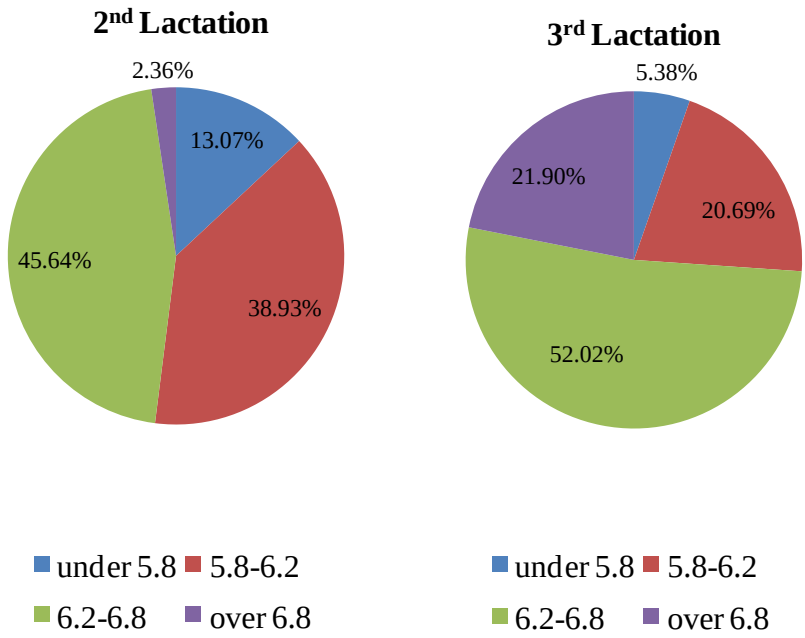
Dairy cows in the 2nd lactation had ruminal pH for the first three observed weeks from 6.44±0.28 to 6.47±0.28. After that was found sharp drop to 6.18±0.25 in the 8th week of lactation. In comparison with 4th week of lactation was ruminal pH less by 4.34% (p=0.000). Weak increase (p=0.000) of pH was determined in the 9th lactation week to 6.25±0.22. Then was detected statistically significant decrease (p=0.000) of ruminal pH and pH mean was between 10th and 12th week of lactation from 6.15±0.19 to 6.16±0.22. Next lactation weeks (13th week 6.00±0.27; 14th week 6.01±0.27; 15th week 5.99±0.23) had statistically significant lower (-2.77%; p=0.000) ruminal pH in comparison with 12th week of lactation. In the 16th week of lactation was detected the lowest mean pH of dairy cows in the 2nd lactation (5.71±0.24). After comparison of this ruminal pH with maximal pH mean in the 5th week of lactation it was 11.72% lower (p=0.000). In the next lactation week (17th week) was found statistically significant increase (+3.21%; p=0.000) to 5.89±0.26. After that was found light drop (-1.84%; p=0.000) to 5.78±0.19. From the 4th week of lactation to 18th week of lactation declined ruminal pH of 2nd lactation cows by 10.18% (p=0.000).

Dairy cows in the 3rd lactation had mean pH in the 4th week of lactation 6.19±0.26. After that was determined slight decrease (-1.86%; p=0.000) to 6.07±0.41 in the 6th lactation week. Then ruminal pH rose up gradually by +1.24% (p=0.000) to 6.15±0.31 (7th week of lactation) and by +3.04 (p=0.000) to 6.33±0.25 (8th week of lactation). After that was found small drop (-0.61%; p=0.000) to 10th week of lactation to 6.29±0.30. Mean ruminal pH of 3rd lactation dairy cows between 12th and 14th lactation week was from 6.43±0.28 to 6.46±0.41. In comparison with 10th week of lactation was ruminal pH higher (+2.69; p=0.000). Then was found sudden increase (+4.37; p=0.000) to 6.75±0.34 in the 15th lactation week. Maximal ruminal pH mean of 3rd lactation cows was determined in the 16th and 18th week of lactation

(6.84±0.34). After comparison with minimal pH mean in 6th lactation week it was higher (+12.67; p=0.000). From the 4th week of lactation to 18th week of lactation went up average ruminal pH by +10.58%.

The most frequented pH values in both groups was in the interval 6.2-6.8. Ideal pH for all rumen microbes is from 6.2 to 7.0 (Barber et al., 2010). In the 2nd lactation dairy cows it was 45.64% and in the 3rd lactation cows 52.02%. On average, 2nd lactation cows spent in this interval 10 hours and 57 minutes per day and 3rd lactation cows 12 hours and 29 minutes per day. Interval between pH 5.8-6.2 was represented by 38.93% (9 hours and 20 minutes per day) in the 2nd lactation cows and 20.69% (4 hours and 58 minutes per day) in the 3rd lactation cows 52.02%. On average, 2nd lactation cows spent in this interval 10 hours and 57 minutes per day and 3rd lactation cows 12

Figure 1 Frequency of pH intervals according to lactation number



hours and 29 minutes per day. Interval between pH 5.8-6.2 was represented by 38.93% (9 hours and 20 minutes per day) in the 2nd lactation cows and 20.69% (4 hours and 58 minutes per day) in the 3rd lactation cows. Ruminal pH below 6.2 cause slow fibre digestion (Barber et al., 2010). During experiment were affected two dairy cows by metabolic disorders. One of dairy cows with subacute ruminal acidosis was in the group of 2nd lactation cows and second with ketosis in the group of 3rd lactation cows. This fact was reflected in the

frequency of pH occurrence. Values of pH under 5.8 formed 13.07% (3 hours and 8 minutes per day) and over 6.8 only 2.36% (33 minutes per day) in the group of 2nd lactation cows. On the other side in the group of 3rd lactation cows were pH under 5.8 represented by 5.38% (1 hour and 17 minutes per day) and over 6.8 even 21.90% (5 hour and 15 minutes per day). Multiparous cows (47.63 % of measured data) remained pH under 5.8 about 1.2 hour per day longer then primiparous cows (37.18 % of measured data) (Meakawa et al., 2002). Similar results found Bowman et al., (2003). In their study were multiparous cows per day under pH 5.8 longer (6.8 hour daily) then primiparous cows (5.4 hour daily). AlZahal et al. (2007) monitored ruminal pH below 5.6 for 5 hours daily and during another experiment found AlZahal et al. (2008) pH values in the rumen under 5.6 for 1 hour daily. Average daily pH values under 6.0 took 4 hours and 33 minutes of a day (Keunen et al., 2002).

CONCLUSION

During lactation pH mean in the group of 2nd lactation cows decreased from 6.44±0.28 to 5.78±0.19 (-10.18%; p=0.000). On the other side pH mean in the group of 3rd lactation cows increased from 6.19±0.26 to 6.84±0.33 (+10.58%; p=0.000). Total pH mean for monitored period was in the group of 2nd lactation cows 6.18±0.33 and 3rd lactation cows 6.44±0.41. Average pH was statistically significantly higher in the group of 3rd lactation cows (4.35%; p=0.000). Frequency of determined pH were the most represented in the in the interval 6.2-6.8 (45.64% 3rd lactation, 52.02% 2nd lactation). The second largest interval was 6.2-6.8 in the both groups (38.93% and 20.69%). Frequency of measured pH under 5.8 was higher in the group of 2nd lactation cows (13.07% vs. 5.38%) and on the other side over 6.8 higher in the group of 3rd lactation cows (2.36% vs. 21.90%).

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THE EFFECT OF BACTERIAL INOCULANT ON NUTRIENTS COMPOSITION OF GRASS SILAGES

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ABSTRACT

The aim of the study was to analyze the effect of biological additive on nutrient composition and nutritional value of grass silages. Grass mass of arable land was ensiled in silage bags. Laboratory analysis of silage samples was carried out at 8 weeks of fermentation, 6 and 12 months of storage. Into grass silage in experimental group was added biological additive, consisted of mixture of homofermentative and heterofermentative lactic acid bacteria, before ensiling. Additive included strains *Lactobacillus brevis* and *Lactobacillus plantarum*. There were found significant ($P<0.05$) differences between control and experimental group in contents of dry matter and fat in grass silage samples. Higher content of dry matter and lower content of fat was detected after addition of bacterial inoculant. Significantly ($P<0.05$) higher contents of NEL, NEG and PDIE were found in the experimental groups of silage. In the case of production efficiency, tendency ($P>0.05$) of a higher milk production potential was observed in all experimental samples of silage. Biological supplementation had positive impact especially on content of dry matter in grass mass and grass silage, nutritional value and production efficiency of grass silage.

Keywords: additives; fermentation; lactic acid bacteria; storage

INTRODUCTION

Additives generally improve silage quality (Nysand and Suokannas, 2012). Silage additives are also used to improve the fermentation (Lád et al., 2006) and for reduction of dry matter losses and preservation of nutrients during fermentation (Jaster, 1994). The reasons for which the additives apply into silage are inhibition of growth of aerobic microorganisms (*Listeria monocytogenes*), undesirable anaerobic

microorganisms (*Enterobacteria, Clostridia*), inhibition of activity of plant and microbial proteases and deaminases, improving the stocks of fermentable substrates for lactic acid bacteria, the addition of beneficial microorganisms, improvement ensilability, nutritional value, utilization of nutrients and are nutrient source (Jaster, 1994; Buxton et al., 2003). Inoculant strain should be able to promote a rapid decline in the pH, survive throughout the fermentation process and improve the aerobic stability (Saarisalo et al., 2007). Inoculants containing homofermentative lactic acid bacteria (LAB) aim at improving silage preservation by accelerating and enhancing the initial phase of the conservation process (fermentation) through fermentation of water-soluble carbohydrates into lactic acid, with a consequent rapid drop in pH. However, a concern of these inoculants is that they have not shown improved aerobic stability of silage (Hu *et al.* 2009; Kristensen *et al.* 2010). Silage inoculants based on heterofermentative LAB have been introduced more recently and shown positive effects on aerobic stability of various types of silages (Kleinschmit and Kung 2006; Kung *et al.* 2007; Hu *et al.* 2009; Kristensen *et al.* 2010) .

The aim of this study was to determine the effect of bacterial inoculant on changes in nutrient composition of grass silage.

MATERIAL AND METHODS

The experiment was realized in practical conditions in the farm PD Suché Brezovo in 2013. Grass mass of arable land was cut at the beginning of bloom and ensiled in silage bags. The content of dry matter was 22.7% in fresh grass mass. Length of ensiled grass mass was 50 mm. Weight of cubic meter of silage was 550 kg in bag. Laboratory analysis of silage samples was carried out at 8 weeks of fermentation, 6 and 12 months of storage. The number of samples was three from each sampling. Into grass silage in experimental group was added biological additive, consisted of mixture of homofermentative and heterofermentative lactic acid bacteria, before ensiling. Control samples of grass silage were without additive. Additive included strains *Lactobacillus brevis* and *Lactobacillus plantarum.* Inoculant contained $2x10^5$ CFU per 1 gram. Dose of preservative was 4g/t of silage. At first was dissolved in water and then applied by using applicator Ziegler (type-FDG). Chemical analysis was conducted at the Laboratory of quality and nutritional value of feeds (Excelent Center for Agrobiodiversity Conservation and Benefit) at the Department of Animal Nutrition (Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra). Nitrogenous value (PDIN, PDIE in

g/kg dry mater (DM)), energy (NEL, NEG in MJ/kg DM) and production efficiency were determined by calculation according to Sommer (1994). After collecting complete analyze results of experiment, it was assessed the impact of inoculant on process of fermentation and changing nutrients in silage. Differences between groups were analyzed with one-way analysis of variance (ANOVA) by using the statistical programme SPSS 20.0. Results were evaluated by using Tukey test. Values with different superscripts within a column are significant at $P<0.05$. Relationships between nutrients were assessed on the base Pearson correlation coefficient.

RESULTS AND DISCUSSION

Results of nutrients composition of grass mass and silage are shown in Table 1 and 2. There were found significant ($P<0.05$) differences in content of dry matter (DM) and fat in grass mass before ensiling. Contents of DM, crude fiber and organic matter were higher in experimental group and crude protein, ash and fat were lower after addition of biological additive. Significant ($P<0.05$) higher content of DM was detected in the experimental samples of silage in all analyzes. The optimal content of dry matter in grass silages should be 30-40% (Doležal et al., 2012). Guo et al. (2013) compared grass silages with 31% dry matter without and with the addition of *L. plantarum + L. buchneri* but they did not observe statistically significant differences in dry matter and crude protein content. The optimal mean concentration of crude protein in grass silage is approximately 160 g/kg DM, although it can range from 39 to 282 g/kg DM (Merry et al., 2000). In our trial, content of crude protein was higher only after 6 months of storage (146.2 g/kg of DM) in the experimental group. Tendency ($P>0.05$) of a higher content of ash was found in the experimental groups of silage. After supplementation of bacterial inoculant there was observed ($P<0.05$) lower content of fat in grass silage. Jalč et al. (2009) found higher content of dry matter (from 222. 8 g to 246.6 g/kg), crude protein (from 126.4 g to 139.9 g/kg DM), lower content of ash (from 78.1 g to 75.9 g/kg DM), fiber (from 409.5 g to 348.3 g/kg DM), fat (from 24.9 g to 24.5 g/kg DM) after addition *Lactobacillus plantarum*. Winters et al. (2001) reported positive effect of bacterial inoculants on content of DM and crude protein.

Table 1. Content of nutrients in grass mass before ensiling

		DM	CP	CF	Ash	Fat	OM
		%	% of DM				
Control	Mean	42.11[a]	14.3	33.53	11.6	2.38[a]	88.4
	S.D.	1.06	0.07	0.54	0.36	0.01	0.36
Experiment	Mean	45.18[b]	14.29	34.78	11.19	2.21[b]	88.81
	S.D.	1.00	0.07	1.14	0.37	0.02	0.37

DM: dry mater, CP: crude protein, CF: crude fiber, OM: organic matter, S.D.: standard deviation. Values with different superscripts within a column are significant at $P<0.05$.

Table 2. Content of nutrients in grass silage

			DM	CP	CF	Ash	Fat	OM
			%	% of DM				
8 weeks	Control	Mean	40.82[ab]	15.65	31.90	10.74	2.85[a]	89.26
		S.D.	0.5	1.67	0.96	0.06	0.01	0.06
	E	Mean	43.2[b]	14.49	33.42	10.86	2.7[b]	89.14
		S.D.	0.59	0.07	0.73	0.42	0.02	0.42
6 months	Control	Mean	41.16[ab]	14.27	32.19	10.72	2.71[b]	89.28
		S.D.	0.2	0.05	0.57	0.21	0.02	0.21
	E	Mean	43.32[b]	14.62	32.72	10.87	2.62[c]	89.13
		S.D.	0.53	0.39	0.84	0.34	0.03	0.34
12 months	Control	Mean	40.60[a]	14.2	33.18	10.71	2.61[c]	88.29
		S.D.	0.5	0.2	1.02	0.25	0.01	0.25
	E	Mean	42.56[b]	14.13	32.31	11.15	2.58[c]	88.85
		S.D.	0.61	0.03	1.27	0.02	0.01	0.02

DM: dry mater, CP: crude protein, CF: crude fiber, OM: organic matter, S.D.: standard deviation, E: experiment. Values with different superscripts within a column are significant at $P<0.05$.

Results of nutritional value of grass mass and silage are shown in Table 3 and 4. Negative effect ($P>0.05$) of biological additive was found in contents of NEL, PDIN and PDIE in grass mass. Content of NEG was higher in the experimental group before ensiling. There were observed statistically significant ($P<0.05$) differences between control and

experimental group in content of NEL after 8 weeks and 12 months and NEG after 12 months of storage in grass silage. Tendency (P>0.05) of a higher content of PDIE was recorded in all analyzes of experimental groups. Jančová (2009) and Zurek and Wróbel (2006) found similar values of NEL, NEG, PDIN and PDIE. Doležal and Hejduk (2002) also reported higher value NEL in inoculated silage. There were found contents of NEL 6.1 and 6 MJ/kg of DM in grass-white clover and grass-red clover silages (Steinshamn and Thuen, 2008). Jančová (2014) observed in grass silage with dry matter content 361.07 g/kg value NEL 5.52 MJ/kg, NEG 5.37 MJ/kg of DM, PDIN 71.49 g/kg of DM and PDIE 69.64 g/kg of DM. Winters et al. (2001) reported positive effect of bacterial inoculant on values of NEL and NEG. In the study Burke et al. (2007), were found contents of PDIN 104 g/kg of DM and PDIE 75 g/kg of DM in grass silage. There were detected also relations between basic nutrients and nutritional value of grass silage. Significant (P<0.05) Pearson correlation coefficient was found only between content of DM and NEL (r = 0.819).

Results of production efficiency of grass silage are shown in Figure 1. There were not found significant (P>0.05) differences between control and experimental group. The highest production efficiency was observed after 6 months of storage and the highest differences between control and experimental group were recorded after 12 months of storage in grass silage. Similar results of production efficiency were presented by Zurek and Wrobel (2006) and Herkeľ et al. (2015). Jančová (2014) reported production efficiency of grass silage 1.74 kg.

Table 3. The nutritional value of the grass mass before ensiling

		NEL	NEG	PDIN	PDIE
		MJ/kg of DM		g/kg of DM	
Control	Mean	4.98	4.78	89.18	78.12
	S.D.	0.16	0.04	0.17	0.05
Experiment	Mean	4.5	4.79	88.02	77.96
	S.D.	0.15	0.06	0.04	0.16

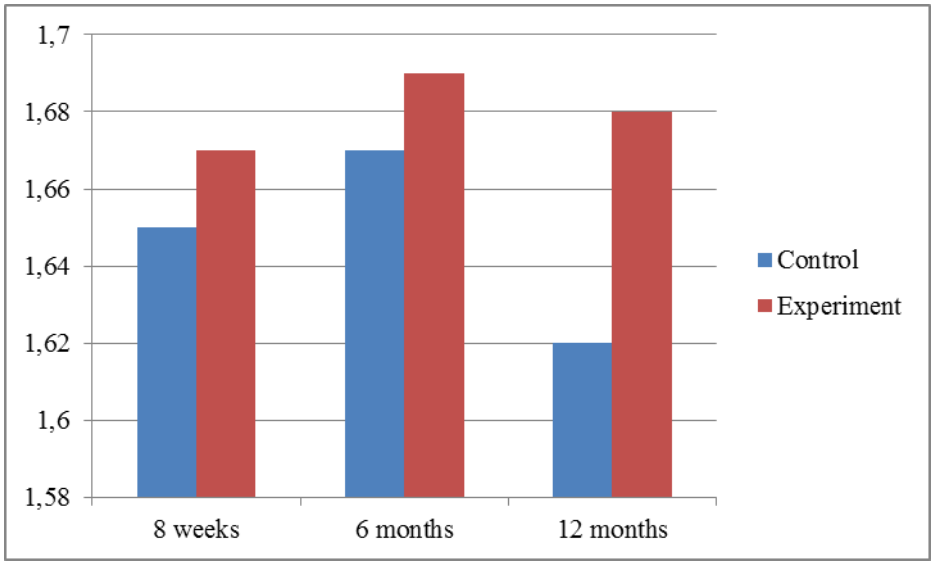
NEL: net energy for lactation, NEG: net energy for gain, PDI: true protein digested in the small intestine, S.D.: standard deviation. Values with different superscripts within a column are significant at P<0.05.

Table 4. The nutritional value of the grass silage

			NEL	NEG	PDIN	PDIE
			MJ/kg of DM		g/kg of DM	
8 weeks	Control	Mean	5.22[a]	5.12[a]	88.08	73.56[ab]
		S.D.	0.11	0.02	0.60	0.62
	Experiment	Mean	5.29[b]	5.16[a]	91.09	74.01[b]
		S.D.	0.10	0.03	5.91	0.07
6 months	Control	Mean	5.28[ab]	5.19[ab]	88.24	71.50[a]
		S.D.	0.06	0.03	0.63	0.59
	Experiment	Mean	5.35[bc]	5.22[b]	87.31	72.84[ab]
		S.D.	0.10	0.05	1.05	0.54
12 months	Control	Mean	5.12[b]	5.12[ab]	86.88	72.05[ab]
		S.D.	0.03	0.05	0.70	1.00
	Experiment	Mean	5.33[c]	5.21[c]	87.32	73.58[b]
		S.D.	0.08	0.02	0.90	0.87

NEL: net energy for lactation, NEG: net energy for gain, PDI: true protein digested in the small intestine, S.D.: standard deviation. Values with different superscripts within a column are significant at $P<0.05$.

Figure 1. Production efficiency of grass silage (kg)



CONCLUSION

Biological additive (*Lactobacillus brevis* and *Lactobacillus plantarum*) lead to an increase of dry matter, crude fiber and organic matter in grass mass, dry matter and ash in grass silage. Differences in content of dry matter were statistically significant ($P<0.05$). Evidently ($P<0.05$) lower content of fat was found in the experimental groups before and after ensiling. Bacterial inoculant had positive ($P<0.05$) effect on NEL, NEG and PDIE in grass silage. Higher values ($P>0.05$) of production efficiency were found in the experimental samples of silage.

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NOTICEABLE CHANGES IN A FATTY ACID PROFILE OF THE WHOLE BODY OF PHEASANTS IN RELATION TO THEIR AGE

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ABSTRACT

The objective of our work was to evaluate the effect of the age of pheasant chicks (*Phasianus colchicus*) on contents of selected fatty acids in whole pheasant bodies fattened till the age of 118 days. In the experiment, 232 chicks of common pheasants were used. Pheasants were fed *ad libitum*. In the course of the experiment, the required number of pheasants was randomly selected at 10-day intervals. After one-day fasting, pheasants were stunned and killed by bleeding. Thereafter, the whole pheasant bodies with the blood were homogenized. The concentrations of fatty acids in the form of methyl-esters were specified by gas chromatography, analyzer GC-2010 (Shimadzu, Japan) with a flame ionization detector. The proportions of fatty acids were determined in % of total fatty acids in samples. The age of pheasant chicks had a significant effect on the content of all selected fatty acids. Significant changes in the proportion of fatty acid groups were recorded mainly at 20, 40 and 60 days of age. By 20 days of age the proportion of monounsaturated fatty acids decreased considerably in relation to the increase in the content of polyunsaturated fatty acids. The content of monounsaturated fatty acids increased between day 20 and day 40, causing a simultaneous decrease in polyunsaturated fatty acids. From day 40, the monounsaturated fatty acid content increased gradually till the end of fattening, whereas the reverse trend was observed in the saturated fatty acid content in this

period. As for polyunsaturated fatty acids, their presence increased from day 40 to day 60, followed by a gradual decrease till the end of fattening. Our findings indicate the varying ability of pheasants to digest and utilize particular fatty acid with varying efficiency throughout their growth.

Keywords: pheasant; fatty acid; whole body; intensive rearing

INTRODUCTION

The contents of fat and fatty acids (FA) are affected by many factors. The most important factors are the species, health status, age, gender etc. (Suchý et al., 2009). Karásková (2012) state that chemical composition of whole pheasant bodies is considerably changed during an ontogenetic development, while chemical composition of whole pheasant bodies is described in the available literature only partially. While published works concerning the chemical composition of pheasants mainly deal with the composition of their breast and thigh muscles (Večerek et al., 2005; Straková et al., 2006; Straková et al., 2010; Nuernerg et al., 2011).

The aim of our work was to evaluate the effect of the age of pheasants on the contents of selected FA in their whole bodies up to the age of 118 days.

MATERIAL AND METHODS

A total of 232 chicks of common pheasants (*Phasianus colchicus*) were used in the experiment. Pheasants were housed on a deep litter floor under the controlled temperature and light regime. Pheasants were fed *ad libitum*. A 3-phase feeding program (starter, grower, and finisher) was used. The starter (CP – crude protein 208.9 g/kg, ME – metabolize energy 14.8 MJ/kg) was fed to chicks till the age of 15 days, the grower (CP – 193.0 g/kg, ME – 15.2 MJ/kg) was fed from 16 to 30 days of age and the finisher (CP – 229.3 g/kg, ME – 15.7 MJ/kg) was fed until the end of the rearing period.

In the course of the experiment, the required number of pheasants was selected at random at 10-day intervals (on days 1, 10, 20, 31, 40, 49, 60, 70, 80, 90, 101, 110 and 118 of age). Pheasants used in all samplings were always a group consisting of both sexes that were randomly selected. After one-day fasting, pheasants were stunned by a blow to the head and killed by bleeding. Thereafter the whole pheasant bodies (including all feathers, skin, viscera and blood) were homogenized.

After sample homogenization, water was separated; fat was extracted and then esterified to prepare a sample for analysis. The concentrations of FA in the form of methyl-esters were specified by gas chromatography, analyzer GC-2010 (Shimadzu, Japan) with a flame ionization detector. The contents of fatty acids are expressed in % of total FA in samples.

Statistical analysis was performed using Statistica CZ, version 10. ANOVA analysis was used to study the differences in FA composition of the whole body of pheasants.

RESULTS AND DISCUSSION

The age of pheasant chicks had a significant effect on the content of selected FA.

The most present FA of the saturated fatty acids (SFA) group was the palmitic acid (C16:0), its highest content was found on day 1 of age. Its content thereafter decreased through day 20 of age ($P < 0.01$), subsequently increased on day 40 in value 24.13 % of total FA ($P < 0.05$), followed by a decrease on day 80. At the end of rearing its content increased ($P < 0.01$). The second most represented FA from the SFA was the stearic acid (C18: 0). A significant increase in its content occurred on days 20 and 40 of age ($P < 0.01$), followed by a gradual decrease towards the end of rearing (Figure 1).

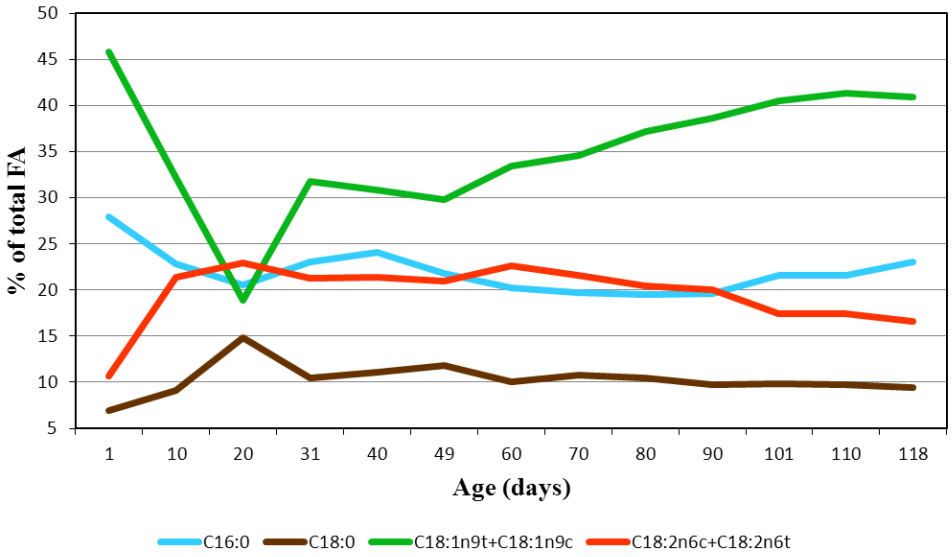
The most represented FA from the monounsaturated fatty acids (MUFA) was the elaidic acid+oleic (C18:1n9t+C18:1n9c). Their content was the highest after hatching, then it demonstrably decreased till day 20 of age after which it significantly increased till the end of the experiment.

The second most present FA of MUFA group was the palmitoleic acid (C16:1). Similarly to C18:1n9t + C18:1n9c, the highest content of C16:1 was found on day 1 of age with a subsequent minimum on day 20 ($P < 0.05$); thereafter there was an increase on day 31, followed by a decrease between days 60 and 90 of rearing.

The most represented FA from the polyunsaturated fatty acids (PUFA) was the lenoleic+linolelaidic acid (C18:2n6c+C18:2n6t). Their lowest presence was found on day 1 of age ($P < 0.01$), their contents increased rapidly as early as on day 10 and remained at a similar level till day 90

Figure 1. The most represented FA (palmitic, stearic, elaidic+oleic, and lenoleic+linolelaidic) from all FA

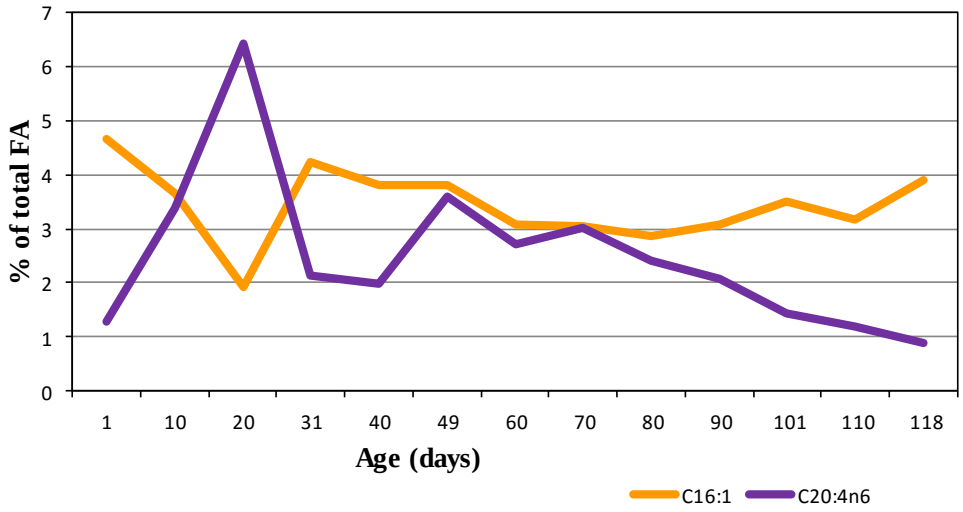
of age, after which they decreased slightly at the end of rearing. The



second most present FA of PUFA group was the arachidonic acid (C20:4n6). Its content culminated on day 20 ($P < 0.01$), with a subsequent decrease on day 40, a demonstrable increase on day 49 ($P < 0.01$) and a subsequent gradual decrease till the end of fattening (Fig.2). Significant changes in the proportion of FA groups were seen mainly on day 20, 40 and 60 of chicks' age. Till day 20 of age the proportion of MUFA decreased considerably, which also related to the considerable increase in the PUFA content. In addition, the SFA content increased only slightly and from day 20 to day 40 it remained virtually unchanged. Compared to the previous period, MUFA contents increased, which conversely caused a reduction in proportion of PUFA. From day 40, the MUFA content increased gradually till the end of fattening ($P < 0.01$), whereas the reverse trend occurred in the SFA proportion (except for day 118). The PUFA content increased from day 40 to 60 with a subsequent constant gradual decrease till the end of fattening. increased with age, the PUFA content decreased. This corresponds to the results of our study in pheasants from 20 days of age. Similarly, Poureslami et al. (2010) stated that the content of PUFA in various parts of broiler chickens decreased with their age. On the other hand, Baeza et al. (2000) found in duck meat that age did not have an effect on contents of SFA and MUFA up to 90 days of age. In addition,

Firuge 2. The course of the content of selected FA (palmitoleic and arachidonic) during the pheasants´ rearing

Komprda et al. (2002) found in turkey meat that as the MUFA content Zelenka et al. (2003) found that digestibility of all SFA and MUFA (with the exception of C20:1 n9) decreased demonstrably from day 9 to



day 42 of age in broiler chickens, whereas in this period digestibility of PUFA was higher than that of both SFA and MUFA.

CONCLUSION

The age of pheasants had a significant effect on the contents of all evaluated FA. Significant changes in the proportion of FA groups were seen mainly on day 20, 40 and 60 of chicks' age. Our findings indicate the varying ability of pheasants to digest and utilize particular FA with varying efficiency throughout their growth.

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THE EFFECT OF FEEDING WHEAT WITH BLUE ALEURONE ON PERFORMANCE PARAMETERS AND ANTIOXIDANT CAPACITY OF BROILERS

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ABSTRACT

The aim of this study was to evaluate effect of feeding of wheat with blue aleurone layer to performance parameters and antioxidant capacity of broiler chickens. Sixty chickens were divided into two equal groups Control and Blue. The experimental group received feed mixture containing 38.2% of wheat with blue aleurone layer. Average feed consumption, antioxidant capacity and carcass yield were evaluated. The feeding wheat with blue aleurone did not affect significantly monitored parameters.

Keywords: Ross 308; coloured wheat; carcass yield; FRAP

INTRODUCTION

Wheat is an important cereal grain in the human nutrition, providing protein, carbohydrates, and dietary fiber (Zeven, 1991). In recent years, minor bioactive compounds, for example, polyphenols and carotenoids, have attracted more interest from research and food manufacture like as health-promoting and disease-preventing effects (Jacobs and Steffen, 2003). Anthocyanins are the largest group of water soluble natural pigments that give red, violet, and blue colour to many fruits, vegetables, and cereal grains. Anthocyanins have some beneficial health effects concerning oxidative damage, detoxification enzymes, and the immune system (Choia et al., 2007; Prior and Wu, 2006). It is well known that herbal anthocyanins are functioning as antioxidants and they have antibacterial and anticarcinogenic effects (Abdel-Aal and Hucl, 1999). Anthocyanins in wheat and barley are found either in the

pericarp or in the aleurone layer, causing purple and blue hues of kernel colour (McIntosh et al., 2003). These substances are found in plants in glycosylated forms, generally linked with glucose, galactose, arabinose, rhamnose, xylose, and fructose (Hosseinian and Beta, 2007). Cyanidin 3-glucoside is the most abundant anthocyanin, followed by peonidin 3-glucoside. They represent approximately 31% and 16%, respectively of the total anthocyanins (Abdel-Aal et al., 2006). Cyanidin is responsible for purple colour in plants (Lee et al., 2005).

Given the above findings, it is verified the hypothesis that anthocyanins of blue wheat will have a positive effect on antioxidant capacity and performance parameters of chickens.

MATERIAL AND METHODS

The experiment was performed with cockerels of Ross 308 hybrid (n = 60) which were fattened on conventional deep litter system. Wood shavings were used as bedding material. The adaptive period when the chickens received a commercial feed mixture BR1 was carried out to day 12 of the chicken's age.

The trial was conducted from day 13 to day 37 of chicken's age. Room temperature and humidity were controlled and set according to Technological procedure for ROSS 308 (Aviagen Group, 2014). Lighting system was 16 hours light and 8 hours dark. Cockerels were divided into two equal groups Control and "UC 66049" (UC). The experimental group received feed mixture containing 38.2% of wheat with blue aleurone layer UC. The feed mixture of control group contained common wheat "Vánek". Feed mixture was prepared on the basis of "Recommended nutrient content in poultry diets and nutritive value of feeds for poultry" (Zelenka et al., 2007). The chemical composition of both experimental wheats is shown in Table 1. The composition of feed mixtures is shown in Table 2. The chickens were fed *ad-libitum.*

Table 1. Chemical composition of wheats (as fed)

	Control	UC
Dry matter (g/kg)	883.4	881.2
Crude Protein (g/kg)	118.6	163.4
Crude Fat (g/kg)	13.4	12.4
Crude Fibre (g/kg)	24.0	22.7
Crude Starch (g/kg)	614.3	566.9
Crude Ash (g/kg)	12.3	19.3
Cyanidin-3-glucoside (mg/kg)	5.10	47.64

Table 2. Composition of feed mixture (g/kg)

Component	Control	UC
Wheat	382	382
Maize	243	273
Soybean extruded	190	190
Soybean meal	105	95
Premix*	30	30
Rapeseed oil	20	20
Wheat gluten	19	0
Monocalciumphosphate	7	7
Limestone ($CaCO_3$)	4	4
Chemical composition (per kg of diet)		
Dry matter	880	880
Crude protein	187.4	190.3
Crude Fat	78.3	71.7
Crude Fibre	28.8	29
Crude Ash	51.8	55.7

* lysine 60.0 g; methionine 75.0 g; methionine + cysteine 75.0 g; calcium 195.0 g; phosphorus 55.0 g; sodium 46.0 g; copper 4.0 mg; zinc 3.70 mg; tocopherol 1.50 mg; biotin 6.0 mg per kg. Retinol 450 IU; calciferol 166.70 IU

During the trial, health status was evaluated daily, whereas feed intake and live weight were measured every week. Mean feed consumption per one chicken was calculated. At the end of experiment 6 birds were selected randomly from each group and slaughtered. Carcass yield was calculated from eviscerated carcase without neck, abdominal fat and giblets as a percentage of live weight. Leg meat represents sum of deboned thigh without skin and deboned drumstick without skin as a percentage of live weight. Percentage of breast meat was calculated without skin and bone removed, as a percentage of live weight.

Another five chickens from each group were slaughtered and their blood was taken at 37 days of age. Total antioxidant capacity of the plasma samples was determined by the Ferric reducing/antioxidant power assay (FRAP) (Benzie and Strain, 1996). The FRAP reagent containing 2,4,6-tripyridyltriazine (TPTZ)/ferric chloride/acetate buffer was prepared by mixing ten volumes of acetate buffer (300 mM, pH 3.6) with one volume of TPTZ (40 mM dissolved in 40 mM HCl) and one volume of ferric chloride (20 mM in water). For determination of the antioxidant capacity, 96-well microtiter plate was used. To each well, 10 µl of sample was added to 200 µl of FRAP reagent. The

mixture was vortexed and incubated for 8 min at 37°C. Then the absorbance at 593 nm was measured using Tecan Infinite M200 PRO microplate reader (Tecan; Mannedorf, Switzerland). All samples were run in triplicate. Results were calculated using the standard curve with ascorbic acid. All chemicals were purchased from Sigma-Aldrich (Prague, Czech Republic).

The data were processed by Microsoft Excel (Microsoft, USA) and Statistica version 12.0 (StatSoft, CZ). We used one-way analysis (ANOVA). To ensure evidential differences Scheffe´s test was applied at $P < 0.05$.

RESULTS AND DISCUSSION

Mean body weights in control group was higher compare to experimental group during the experiment. The results were not statistically significant (Table 3). According to the technological procedure for ROSS 308, the average body weight of cockerels should be 2,493 g at 37 days of age (Aviagen Group, 2014). On the other hand, in our earlier experiments with coloured wheats (Karásek et al., 2014;

Table 3. Average bodyweight of chickens during the trial (g)

Week of trial	n	Control				UC			
		Mean ± standard error							
1	30	289	±	3.4	[a]	284	±	2.6	[a]
2	30	473	±	6.4	[a]	464	±	5.3	[a]
3	30	961	±	12.3	[a]	938	±	15.9	[a]
4	30	1597	±	22.6	[a]	1475	±	33.3	[a]
5	30	2352	±	34.4	[a]	2232	±	47.4	[a]

[a] – statistically non-significant differences ($P > 0.05$)

Šťastník et al., 2014) worsen performance parameters of chickens were not detected. Feed conversion ratio is reported in Table 4.

Table 4. Feed conversion ratio (kg/kg)

Group	Control	UC
Feed conversion ratio	1.69	1.76

The difference of carcass yield, breast meat and leg meat yield were not statistically significant (Table 5). Carcass yield stated in the Technological procedure for ROSS 308 (Aviagen Group, 2014) is

72.08% for 2,200 g live weight. Our results are slightly lower according to Technological procedure for ROSS 308 (Aviagen Group, 2014) and according to values which states Šťastník et al., (2014).

Table 5. Carcass yield (% of live weight)

Group	n	Carcass			Breast meat			Leg meat		
		Mean ± standard error								
Control	6	69.9	±	0.98	19.7	±	1.06	14.9	±	0.44
UC	6	69.2	±	0.36	20.3	±	0.52	14.2	±	0.44

Our results are lower by 1.07 and 1.77% respectively, than present in The manual for the hybrid Ross 308 (Aviagen Group, 2014).

Antioxidant capacity measured by FRAP method are in Table 6. The experimental group shows a higher antioxidant capacity then control group, but results are not significant (P = 0.52). Nevertheless, in our earlier trial (Karásek et al., 2014) significant differences was found in the antioxidant capacity of liver tissue of chickens Ross 308 measured by Free Radicals method.

Table 6. Antioxidant capacity by FRAP method (ascorbic acid concentration μm)

	n	Mean ± standard error		
Control	5	3.8	±	0.34
UC	5	4.3	±	0.31

CONCLUSION

The feeding of wheat with blue aleurone had no effect on performance parameters and antioxidant capacity.

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THE DEGRADABILITY OF CRUDE PROTEIN APPLICATED TO THE DIET OF RUMINANTS

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ABSTRACT

The aim of this study is determined degradability of crude protein by Cornell system (CNCPS; Cornell Net Carbohydrate and Protein System). According Cornell system is possible to described different degrading part of crude protein into five fractions (A, B1, B2, B3 and C). These fractions represent soluble and insoluble crude protein. The highest value of soluble protein has rye grass silage (128.0 g/kg of DM) and the smallest value has corn silage (24.0 g/kg of DM). But in average were the values of soluble protein higher for silages (75.4 g/kg of DM) while SOLP of meadow foxtail were in average 35.6 g/kg of DM. Insoluble protein, included especially nitrogen insoluble in neutral and acid detergent, has the highest value for meadow foxtail (161.0 g/kg of DM).

Keywords: degradability; crude protein; soluble protein; insoluble protein; ruminants

INTRODUCTION

One of the systems, which discussed this topic, is Cornell system. This system evaluates the crude protein of feeds based on their degradability and divides the crude protein into various fractions (A, B1, B2, B3 and C). The Cornell system including into the evaluation of crude protein brings more information about the protein digestion and also better formulation of feed ruminants rations, both important for milk and meat production (Fox et al., 2004; Ghoorchi and Arbabi, 2010).

Fraction A constitutes NPN (Bovera et al., 2003) and is separated by using trichloroacetic acid (TCA). The fraction B is protein considered as true protein (Alzueta et al., 2001) which is further divided into 3

parts as fraction B1 (rapidly degraded protein), B2 (intermediately degraded protein), B3 (slowly degraded protein) (Alzueta et al., 2001; Polat el al., 2014). The fraction B1 is expressed by estimating the true protein soluble in a buffer. The fraction B2 is known as neutral detergent soluble protein estimated as the difference between buffer insoluble protein and neutral detergent insoluble nitrogen (Tham et al., 2008). Sniffen at al. (1992) says that some fraction B2 fermented in the rumen and some fraction B2 escape into the intestines. This depends on the relative speed of digestion and the possibility of passage (Sniffen et al., 1992). Fraction B3 is insoluble in neutral detergent, but soluble in the acidic detergent. The fraction C is referred as acid detergent insoluble nitrogen (Bovera et al., 2003), measured by estimating nitrogen in ADF residue (Parashuramulu et al., 2013). This fraction includes lignified nitrogen and Maillard products and is largely unavailable to the animal (Krishnamoorthy et al., 1982). Sniffen et al. (1992) says that the models, which destined to assess utilization of dietary crude protein by ruminant animals, it is assumed that most of the soluble crude protein is completely degraded in the rumen, and changing proportions of the insoluble fractions escape ruminal degradation depending on the interactive effects of digestion and passage (Sniffen et al., 1992). Nitrogen fractions are differ in rate and extent of ruminal degradation, the proportions of these different crude protein fractions are believed to influence the amounts of ruminally degraded and escape crude protein consumed by animals (Van Soest, 1994).

MATERIAL AND METHODS

For this study were used feedstuffs commonly fed to ruminants in the Czech Republic, which are represented by concentrates (n = 4; clover silage, corn silage, rye grass silage, grass silage) and roughages (n = 2*;* meadow foxtail 1st miter, meadow foxtail 2nd miter) (Table 1).
The fresh feedstuffs were dried at 50°C according to Harazim et al. (1999). Dried material was subsequently milled to pass through a 1 mm sieve for laboratory analyses. Dry matter (DM) was determined by drying at 103°C for 4 hours. Ash was determined over combustion at 550°C for 6 hours according to the AOAC (2005). Crude protein (CP) was analysed according to the Kjeldahl method (nitrogen × 6.25) and ether extract (EE) was analyzed by Soxtec extraction with petroleum ether. Ash-free concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the methods described by Van Soest et al.

(1991). Chemical analyses of crude protein content were described by Licitra et al. (1996).
Protein degradation and solubility were described by Cornell system into 5 fractions, while fraction A and B1 represented soluble protein (SOLP) and fraction B2, B3 and C represented insoluble protein (IP). The calculations (Kelzer et al., 2010) are as follows: fraction A = NPN; fraction B1 = SOLP – NPN; fraction B2 = CP – SOLP – NDIN; fraction B3 = NDIN – ADIN; fraction C = ADIN.
ADIN = nitrogen insoluble in acid detergent, CP = crude protein, NDIN = nitrogen insoluble in neutral detergent, NPN = non-protein nitrogen, SOLP = soluble crude protein.
Data were statistically analyzed in the SAS 9.3 GLM (SAS Institute, 2003) procedure (PROC GLM) and PROC CORR procedure was evaluated by correlation coefficients between the observed variables. Test statistically significant differences were evaluated by Scheffe analysis.

RESULTS AND DISCUSSION

The chemical compositions of evaluated samples are presented in Table 1. Estimated CP value averaged 154.9 % of DM. As expected, the CP of meadow foxtail decreased and the contents of NDF, ADF and ADL increased as plant maturity advanced 1st miter (sample 5) and 2nd miter (sample 6). Contrary to other silages had grass silage the highest content of ADF (409.7 g/kg of DM) and ADL (103.7 g/kg of DM). The content of EE varied from 17.0 to 34.1 g/kg of DM.

Table 1. Chemical composition (g/kg of DM) of estimated samples.

Samples		CP	NDF	ADF	ADL	EE	Ash
1	Clover silage	179.8	423.1	380.3	52.9	17.5	94.4
2	Corn silage	69.5	434.4	259.9	28.0	28.1	44.8
3	Rye grass silage	192.8	364.2	257.8	21.1	26.4	192.2
4	Grass silage	161.3	459.4	409.7	103.7	24.4	73.9
5	Meadow foxtail*	205.2	579.5	278.7	55.0	34.1	76.8
6	Meadow foxtail**	120.5	762.5	414.4	193.3	17.0	48.6

For abbreviations see the text. *1st miter **2nd miter

Amount of SOLP and IP (Table 2) were evaluated as a complex of CP. SOLP represents fraction A plus fraction B1. The highest SOLP value was found for rye grass silage (128.0 g/kg of DM). The highest content of SOLP was for silages (averaged 75.4 g/kg of DM) in contrary to

SOLP of meadow foxtail (averaged 35.6 g/kg of DM). This trend can be possible because fermented crops, as silages as, have a higher amount of NPN (fraction A) (Van Soest, 1994), which constitutes part of SOLP. This also declared publications of Givens and Rulquin (2004) and Slottner and Bertilsson (2006). Purwin et al. (2012) has pointed out, that during ensiling plant proteases and peptidases hydrolyze most of the plant protein to free amino acids, ammonia and other forms of NPN.

IP (Table 2) includes fraction B2, B3 and C. As expected, the highest values of IP have a meadow foxtail (averaged 127.4 g/kg of DM), while silages were in average 75.5 g/kg of DM. IP includes also NDIN and ADIN, shows a clear influence ($P < 0.05$) of ADF and ADL in relation to NDIN (Table 3).

Scheffe test was conducted by the test of statistically significant differences ($P < 0.05$). Between estimated feed samples were found statistically significant differences (Table 2) for individual nitrogen fractions and their composition ($P < 0.05$).

Table 2. Determination of nitrogen fractions and their composition (g/kg of DM) of estimated samples.

Samples	ADIN	NDIN	SOLP	IP	A	B1	B2	B3
1	25.4^{b}	65.7^{a}	81.2^{b}	98.6^{b}	69.3^{b}	12.0^{a}	$32.9^{c,d}$	40.4^{b}
2	5.1^{f}	13.8^{e}	24.0^{e}	45.6^{d}	22.8^{d}	$1.2^{b,c}$	31.7^{d}	8.8^{d}
3	19.3^{c}	23.3^{d}	128.0^{a}	64.8^{c}	114.7^{a}	13.3^{a}	41.5^{b}	4.0^{e}
4	63.2^{a}	64.4^{a}	68.2^{c}	93.1^{b}	68.1^{b}	0.15^{c}	28.7^{d}	1.2^{e}
5	15.9^{d}	50.5^{c}	44.4^{d}	161.0^{a}	34.2^{c}	$10.2^{a,b}$	110.5^{a}	34.6^{c}
6	8.3^{e}	53.7^{b}	26.7^{e}	93.7^{b}	22.0^{d}	$4.8^{a,b,c}$	$40.0^{b,c}$	45.5^{a}

For abbreviations see the text. ^{a,b,c,d,e,f} different letters indicate statistically significant difference between the feeds ($P < 0.05$; Scheffe test)

Table 3 shows correlations between chemical compositions. A strong interaction ($P < 0.05$) was found between DM and content of ADF, ADL, ash, ADIN and NDIN. Significant correlation effect ($P < 0.05$) was also declared for CP in relation to ash, SOLP and IP.

Table 3. Correlation coefficients of estimated chemical variables (g/kg of DM).

	Original DM	CP	EE	NDF	ADF	ADL	Ash	ADIN	NDIN
CP	0.027								
EE	-0.020	0.198							
NDF	-0.429	-0.182	-0.244						
ADF	**-0.617**	-0.025	**-0.758**	0.460					
ADL	**-0.579**	-0.211	**-0.568**	**0.862**	**0.792**				
Ash	**0.574**	**0.613**	0.105	**-0.572**	-0.393	-0.491			
ADIN	**-0.643**	0.343	-0.072	-0.299	0.497	0.080	0.087		
NDIN	**-0.604**	0.442	-0.441	0.337	**0.826**	**0.521**	-0.248	**0.568**	
SOLP	0.436	**0.654**	-0.040	**-0.659**	-0.182	-0.459	**0.951**	0.332	-0.030
IP	-0.404	**0.648**	0.299	0.425	0.150	0.186	-0.156	0.115	**0.608**

For abbreviations see the text.
Bold numbers represent statistical significance $P < 0.05$.

CONCLUSION

The degradability of crude protein in ruminant nutrition is worldly discussed topic. In that case is a request to extend the knowledge about the digestion of essential nutrients occurring in the feedstuffs. One of the systems, which discussed this topic, is Cornell system. It is possible to formulate content of crude protein as soluble and insoluble crude protein or as individual nitrogen fractions. The highest value of SOLP was found for rye grass silage. This result can be explained by the presence of the NPN as a part of SOLP. IP shows the highest value for 1st miter of meadow foxtail.

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SEA BUCKTHORN PELLETS IN DIETS FOR LAYING HENS AND THEIR EFFECT ON THE AMOUNT OF FATTY ACIDS CONTAINED IN EGG YOLK

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ABSTRACT

The aim of the study was to find out the effect of sea buckthorn pellets, which were added to the feed mixture for laying hens. It was observed, how the supplement of sea buckthorn pellets affects the amount of fatty acids in egg yolk of laying hens. One kg of sea buckthorn pellets contained: 212.7 g of crude protein, 145.8 g of crude fat, 177.6 g of crude fiber, 442.1 g of nitrogen-free extract substances and 21.7 g of ash. Gross energy was 23.6 MJ/kg. The amount of fatty acids obtained in sea buckthorn pellets and in egg yolks of laying hens in study was determined by gas chromatography. 100 g of sea buckthorn oil contained: 61.69 g of fatty acids, 14.14 g of saturated fatty acids, 47.55 g of unsaturated fatty acids, 22.49 g of monounsaturated fatty acids, 13.63 g of n-6 fatty acids and 11.43 g of n-3 fatty acids. Laying hens (Isa Brown) were placed in 4 groups (8 hens in each group). It was created 1 control group (without addition of sea buckthorn pellets) and 3 experimental groups (feed mixture with addition of 2 %, 5 % and 10 % of sea buckthorn pellets). Length of experiment was 4 weeks. Every week 40 eggs of each group were taken. The results show that addition of sea buckthorn pellets in feed mixture for laying hens has favourable effect on amount of fatty acids in egg yolk. During experiment was detected higher content of saturated fatty acids, monounsaturated fatty acids and n-3 fatty acids in egg yolks of laying hens in experimental groups. The results show, that addition of sea buckthorn pellets in feed mixture for laying hens highly significantly increased ($P \leq 0.01$) quantity of fatty acids in egg yolks.

Key words: *Hippophae rhamnoides*; chemical composition; n-3 fatty acids; n-6 fatty acids; feed mixture for laying hens

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides*) belongs to the family *Elaeagnaceae*. It is dioecious, thorny tree, which reaches a height of 3 to 4 m (Li and Beveridge, 2003). Its natural habitat is in areas of Central Asia and northwest Europe (Suryakumar and Gupta, 2011). Its fruits are small and have oval shape, ripe berries have a colour from dark yellow to reddish. Fruits of sea buckthorn have a look of drupe. Berries are juicy and rich in oil content. The green coloured leaves have trichomes on their surface, which give them a silver tint. The leaves are narrow, lanceolate and they have alternating arrangement (Li and Beveridge, 2003; Suryakumar and Gupta, 2011). Whole plant contains very valuable bioactive compounds, especially its fruits. Sea buckthorn is very important source of natural antioxidants (ascorbic acid, tocopherols, carotenoids and flavonoids). It is also very significant source of proteins, vitamins (especially source of vitamin C), mineral substances and lipids (Christaki, 2012). Unsaturated fatty acids (UFA) are the most abundant fatty acids (FA) in sea buckthorn. Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are represented in the highest amount in oil which was obtained from sea buckthorn seeds. Palmitooleic acid (16:1n-7) dominates in oil, which was obtained from whole sea buckthorn fruits (Yang and Kallio, 2001). Sea buckthorn is very rich source of other essential FA from the group of n-3 FA, n-6 FA, n-7 FA and n-9 FA (Solcan et al., 2012). It is also very important source of substances such as carbohydrates, organic acids and flavonoids (Christaki, 2012). Sea buckthorn has a big number of valuable compounds, which have positive influence on health of organisms. Sea buckthorn is still more used in human and animal nutrition for its positive effects on health of organisms.

MATERIAL AND METHODS

This work was based on requirements of sea buckthorn growers, who cultivate sea buckthorn for obtaining juice of its fruits. During obtaining of juice from sea buckthorn berries waste products are formed and we are trying to find a suitable use for them, especially in the field of animal nutrition.

Growers farm is on 4 ha area, where on 1 ha is cultivated around 1,000 plants. Every year is harvested only one half of plantation. During

harvesting of 1 ha area 1,500 l of sea buckthorn juice is obtained and around 570 kg of sea buckthorn waste products.
Amount of individual FA (g/kg) in sea buckthorn pellets and content of FA in egg yolks were determined by gas chromatography. FA were determined by the device GC 2010 GAS CHROMATOGRAPH SHIMADZU (firm - Shimadzu Japan). Preparation of the sample is based on the extraction of the sample by the method of the Soxhlet (sea buckthorn pellets) or extraction in a solvent (egg yolks), transesterification of the sample under the cooler, isooctane phase and injection of the sample into the analyser.
The experiment was carried out on laying hens Isa Brown. 32 laying hens were included in the experiment. Laying hens were placed in 4 groups, each group contained 8 laying hens. It was created 1 control group (K) and 3 experimental groups (P1, P2 and P3). Laying hens were kept in enriched cages in accredited stable of Department of Animal Nutrition at the University of Veterinary and Pharmaceutical Sciences Brno. Laying hens of control group were feed by feed mixture without addition of sea buckthorn pellets. Laying hens of experimental groups received feed mixture with addition of sea buckthorn pellets (group P1 received 2% addition, group 2 received 5% addition and P3 received 10% addition). Duration of experiment was 4 weeks. Every week 40 eggs from each group were collected. Over the course of the experiment 160 eggs of each group were obtained.
Chemical analysis included determining the content of individual FA in sea buckthorn oil. By chemical analysis of sea buckthorn were detected these saturated fatty acids (SFA): C12:0, C14:0, C16:0, C17:0, C18:0, C20:0 and C22:0, these monounsaturated fatty acids (MUFA): C16:1, C17:1, C18:1, C20:1 and C22:1, these FA of the group n-6 FA: C18:2n6, C18:3n6, C20:2n6 and C22:2n6 and these FA of the group n-3 FA: C18:3n3, C20:5n3 and C22:6n3.
Within chemical analysis of fat in egg yolk was determined amount of FA, quantity of SFA, UFA, amount of MUFA, polyunsaturated fatty acids (PUFA) and quantity of n-6 FA and n-3 FA.

RESULTS AND DISCUSSION

Chemical analysis of nutrient of sea buckthorn is shown in Table 1. On the basis of chemical analysis it was found that sea buckthorn pellets contain valuable nutrients, which can be used in animal nutrition.

Table 1. Chemical analysis of sea buckthorn pellets. Results are presented in 100% dry matter.

Nutrient content	Units	Amount
Crude protein	g/kg	212.7
Crude fat	g/kg	145.8
Crude fiber	g/kg	177.6
Nitrogen-free extract substances	g/kg	442.1
Ash	g/kg	21.7
Calcium	g/kg	1.2
Phosphorus	g/kg	2.6
Magnesium	g/kg	0.9
Zinc	mg/kg	18.3
Cuprum	mg/kg	9.7
Iron	mg/kg	32.1
Gross energy	MJ/kg	23.6

During chemical analysis of sea buckthorn oil was determined amount of individual FA. Results of qualitative analysis of fat confirm, that oil obtained from sea buckthorn pellets has very interesting composition of individual FA.

100 g of sea buckthorn oil contained: 61.69 g of FA, 14.14 g of SFA, 47.55 g of UFA, 22.49 g of MUFA, 13.63 g of n-6 FA and 11.43 g of n-3 FA.

See buckthorn oil has relatively low representation of SFA. Palmitic acid (C16:0) is the most dominant SFA. It was detected 12.420 g of palmitic acid in 100 g of sea buckthorn oil. Stearic acid (C18:0) is also very important SFA, which was analysed in sea buckthorn oil. It was detected 1.22 g of stearic acid in 100 g of sea buckthorn oil. Other SFA are represented in very low amount or they were not detected at all.

For sea buckthorn oil is characteristic especially high amount of MUFA. Sea buckthorn oil has high content of palmitooleic acid (C16:1). High quantity of palmitooleic acid is typical for sea buckthorn oil unlike other vegetable oils. Oleic/elaidic acid (C18:1n9t + C18:1n9c) is also obtained in high quantity in sea buckthorn oil. Other MUFA are represented in the footsteps, or they are not present in sea buckthorn oil at all.

Sea buckthorn oil is very good source of PUFA of group n-6 FA. Linoleic/linolelaidic acid (C18:2n6c + C18:2n6t) is dominant acid of n-

6 FA, other FA of this group are represented only in small amount or they are not included.
From the dietary point of view sea buckthorn is suitable source of PUFA of group n-3 FA. Linoleic acid (C18:3n3) is contained in the largest amount of n-3 FA. Other FA from n-3 FA are represented only in small amount or they are absent.
Chemical analysis of fat in egg yolk contains amount of FA, quantity of SFA and UFA and ratio between UFA and SFA, amount of MUFA,
Table 2. The content of detected FA in sea buckthorn oil. Results are presented in g/100 g of sea buckthorn oil.

FA	formula	g/100 g
LAURIC ACID	C12:0	0.010
MYRISTIC ACID	C14:0	0.100
PALMITIC ACID	C16:0	12.420
HEPTADECANOIC ACID	C17:0	0.140
STEARIC ACID	C18:0	1.220
ARACHIDIC ACID	C20:0	0.230
BEHENIC ACID	C22:0	0.020
PALMITOLEIC ACID	C16:1	11.320
***cis*-10-HEPTADECANOIC ACID**	C17:1	0.010
OLEIC ACID/ELAIDIC ACID	C18:1n9t + C18:1n9c	11.030
***cis*-11-EICOSENOIC ACID**	C20:1n9	0.110
ERUCIC ACID	C22:1n9	0.020
LINOLEIC ACID/LINOLELAIDIC ACID	C18:2n6c + C18:2n6t	13.140
γ-LINOLENIC ACID	C18:3n6	0.110
***cis*-11,14-EICOSADIENOIC ACID**	C20:2n6	0.230
***cis*-8,11,14-EICOSATRIENOIC ACID**	C20:3n6	0.040
***cis*-13,16-DOCOSADIENOIC ACID**	C22:2n6	0.110
α-LINOLENIC ACID	C18:3n3	11.370
***cis*-5,8,11,14,17-EICOSAPENTAENOIC ACID**	C20:5n3	0.010
***cis*-4,7,10,13,16,19-DOCOSAHEXAENOIC ACID**	C22:6n3	0.050

PUFA, ratio between MUFA and PUFA, representation of n-3 FA, n-6 FA and ratio between n-6 FA and n-3 FA. The results demonstrate that

there were changes in some of observed parameters of fat in egg yolk. The amount of FA were higher in egg yolks of laying hens in experimental groups in compared with the control group of laying hens. We found an increase amount of FA, SFA, MUFA and n-3 FA in experimental groups. These changes and their statistical difference are presented in Table 3 and Table 4.

Table 3. Results of amount of FA in egg yolk, the amount of SFA and UFA in egg yolk and ratio between UFA and SFA. Results are presented in g/100 g of fat of egg yolk.

	Σ FA (g/100 g of fat)	**SFA (g/100 g of fat)**	**UFA (g/100 g of fat)**	**UFA/SFA**
K	75.687	25.462^{A}	50.225	1.976^{A}
P1	75.641	25.687	49.955	1.946
P2	76.139	26.508^{B}	49.632	1.874^{B}
P3	76.073	26.325^{B}	49.749	1.895^{B}

$^{A, B}$ a highly significant difference ($P \leq 0.01$)

Table 4. Results of amount of MUFA and PUFA in egg yolk, ratio between MUFA and PUFA, amount of n-3 FA and n-6 FA in egg yolk and their mutual ratio. Results are presented in g/100 g of fat of egg yolk.

	MUFA (g/100 g of fat)	**PUFA (g/100 g of fat)**	**MUFA/PUFA**	**n-6 FA (g/100 g of fat)**	**n-3 FA (g/100 g of fat)**	**n-6 FA /n-3 FA**
K	37.538^{A}	12.688^{A}	2.973^{A}	11.603^{A}	1.085	14.859
P1	38.817^{B}	11.138^{B}	3.514^{B}	10.042^{B}	1.096	13.185
P2	39.091^{B}	10.541^{B}	3.808^{B}	9.372^{B}	1.169	11.677
P3	38.256	11.493^{B}	3.415^{B}	10.298^{B}	1.195	13.917

$^{A, B}$ a highly significant difference ($P \leq 0.01$)

CONCLUSION

The results of experiment confirm that sea buckthorn pellets can be suitable component in feed mixture for laying hens, especially for their: unique FA composition of sea buckthorn oil, favourable effect on amount of FA in egg yolk, especially for representation of SFA in egg

yolk , beneficial effect on amount of MUFA in egg yolk and favourable influence on quantity of n-3 FA in egg yolk.

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ANALYSIS OF THE NUTRITIONAL EFFECT ON THE QUANTITY AND COMPOSITION OF COW MILK

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ABSTRACT

Feed management is one of the principal tools by which the productionand composition of milk by dairy cows can be modulated in short term. From this study we conclude that the ratio of fat-to-protein percentage, and milk urea and protein are a valuable indicator of lipomobilisation and the negative energy balance status in postpartum cows for the evaluation of nutritional status and herd health programs on dairy farms.

Keywords: glucogenic; lipogenic nutrients; fermentation; rumen; milk;

INTRODUCTION

Profitability of the dairy herds is limited by the level and economic effectiveness of milk production. The period of early lactation is often characterized by metabolic changes and severity of negative energy balance (NEB) and related metabolic disorders in dairy cows. Balanced composition of nutrients, structure of TMR and optimal ruminal fermentation allow nutritional influencing of the amount and components of milk also the profile milk fatty acids that influence human's health. The source of carbohydrate has a large impact on the dry matter intake (DMI), fiber digestion, concentration of milk fat and nitrogen (N) utilization (Cabrita et al. 2006, Oba & Allen 2003). In ruminants, lipogenic nutrients originate either from fiber that stimulates the ruminal production of acetate and butyrate or from dietary fat, or are derived from body reserves. Glucogenic nutrients originate from starch which escaped from rumen degradation or gluconeogenesis from propionic acid, glucogenic amino acids and lactic acid, are the main contributors to gluconeogenesis in ruminants (Brockman, 2005). Fermentation of starch by microorganisms in the rumen produces high

amounts of propionic acid, in addition to other volatile fatty acids. Propionic acid is a major substrate for glucose synthesis in the liver, which, in turn, fuels lactose synthesis in the mammary gland. However, while starch digestion in the rumen helps support milk volume, excessive amounts of starch digestion in the rumen can negatively affect excessive energy metabolism and dry matter intake, neutral detergent fiber (NDF) digestibility, resulting in reduced milk and milk fat production, in lactating dairy cow (Allen et al., 2009, Weakley and Reutzel, 2013). Level of milk production and milk composition is influenced most significantly by acetic and propionic acids, and therefore the most effective application of the nutritional effect can be reached by management of the ruminal production of lipogenic and glucogenic nutrients by the composition of TMR (Jenkins 2013). The mobilisation of body reserves by releasing of fatty acids involves disbalance of metabolites of lipogenic and glucogenic nutrients. The total amount of the acetic and butyric acids produced in the rumen are in a direct relationship to the intake and digestibility of NDF from forages (Bernardini a kol. 2010), while amount of carbohydrates influence the level of fermentation in the rumen (pH, VFA) as well as serum values of glucose, NEFA, BHBA, and the level of triglycerides in the liver (Hall et al.2010). Nutrition, nutrient composition of daily ration and nutritional imbalances have important effect on digestive, metabolic and production responses, its serve as a main tool of regulation of the amount and composition of milk.

The aim of study was to determine the effect and relationship of nutrition – rumen fermentation – intermediate metabolism and milk yield and composition in dairy cows.

MATERIAL AND METHODS

The present study was carried in feeding experiments of Holstein dairy herd in the lowland (farm N) and Simmental dairy herd of the highland (breeding N) breeding conditions. Diets were fed as a total mixed ration (TMR) *ad libitum* at 08:00 a.m. and 06:00 p.m. The ingredient and chemical composition of the diets and daily intake of nutrients are showed in Table 1. Samples of TMR were analyzed for dry mater (DM), crude protein (CP), acid and neutral detergent fibre (ADF, NDF), fat, ash and starch according to conventional methods (Committee regulation ES No.152/2009 of 27.1. 2009). Non-fibre carbohydrates (NFC) was calculated by difference (100− (CP + (NDF–NDF bound protein) + ash + ether extract)).

Samples of rumen content intended for analysis of fermentative and synthesizing capacity of the rumen were taken 4-5 hours after morning feeding by stomach cannulas. VFA in the rumen content were determined in a two-capillary isotachophoretic analyser EA100 (VILLA LABECO, Slovak Republic). The pH of the rumen content was determined potentiometrically with portable electronic pH-meter (JP SELECTA, Spain). The level of NH_3 in the samples of rumen content was determined by a Kjeldahl-N using a 2300 Kjeltec Analyser Unit (Foss Tecator AB, Hoganas, Sweden).
Blood samples collected 6 hour after the morning feeding to monitor serum metabolites. Analysis of metabolites of serum: glucose, blood urea nitrogen (BUN), albumin, cholesterol were performed using commercially available kits on biochemical analyser "Ellipse". Non-esterified fatty acids (NEFA) were according to Ducomb). ß-hydroxybutyric acid (BHBA) was determined in a two-capillary isotachophoretic analyser EA100 (VILLA LABECO, Slovak Republic). Cows were milked 2 times per day and milk yield was recorded. Milk samples were collected from each milking once per month and analysis of milk composition were done by Plemenárska služba SR.

RESULTS AND DISCUSSION

The nutrient composition of samples of TMR for both farms is summarized in Table. 1. The nutrient composition of TMR is evaluated by comparing the nutrient content of the ration (TMR) with the recommended nutrient by production phases for dairy cows on farms in our conditions. Chemical analysis of TMRs showed no difference in mainly nutrients as CP, EE, NDF and ADF, except of NFC and starch. Lower concentrations of starch and NFC were determined in TMRs on farm P, where alfalfa silage was substituted by clover-grass silage. Feeding different types of TMRs didn't affect concentration of VFA, acetic acid, lactic acid of rumen (Table 2). However, the concentration of propionic and butyric acid were higher and pH were lover in cows on farm P than farm N ($P < 0.001$). In this farm N the increase propionic acid production was affected by source of starch (corn grain) and time of starch degradation rate in the rumen. Corn grain is used as the main dietary starch source in dairy cow diets. However, wheat grain has been used in dairy cow diets as a partial substitute for corn grain, in the highland breeding conditions. The starch of non-treated corn is slightly digested in rumen than wheat starch. The ruminal ammonia

Table 1. Content of feed nutrients in the post-partum and peak of lactation daily rations

	POST PARTUM (until 21 DIM)		**PEAK OF LACTATION (21-100 DIM)**	
Composition of feed ration	**Farm N**	**Farm P**	**Farm N**	**Farm P**
Corn silage kg	18	13,1	26	17
Alfalfa silage kg	9		6	
Clover -grass silage kg		11		14
Alfalfa hay kg	0,8		1	
Barley straw kg		0,8		1
Grain mixture kg	7	5,5	11	7
Content of nutrients	**Chemical composition of TMR**			
Dry matter (DM) $g.kg^{-1}$	383	441,6	383	441,6
CP $g.kg^{-1}$ DM	169.1	171	169.1	171
Fat $g.kg^{-1}$ DM	40.9	38.7	40.9	38.7
NDF $g.kg^{-1}$DM	351.2	366.8	351.2	366.8
ADF $g.kg^{-1}$ DM	215,8	209	215,8	209
Starch $g.kg^{-1}$DM	228	199	228	199
NFC $g.kg^{-1}$DM	361.4	350	361.4	350
NEL MJ..kg^{-1}DM	6.62	6.6	6.62	6.6
Daily intake of nutrients				
DM kg	14.0	15.5	22.5	20.2
NEL MJ	92.7	102.3	148.9	127.3
CP g	2367	2650	3804,7	3373
NDF g	4917	5685	7902	7286
Starch g	3192	3084	5130	3973

DM - dry matter; CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; NFC – non-fibre carbohydrates; NEL – net energy for lactation; TMR – total mix ration

concentrations in all groups and individually (79 %) in cows were above the upper physiological level.

In the animals on the farm N was observed: fermentation of carbohydrates with a balanced pH 6.22 at the lower physiological level. Analyzed level of VFA in average 120.6 ± 11.9 $mmol.l^{-1}$, and rate of acetic (C_2 71.6 $mmol.l^{-1}$) and propionic acid (C_3 31.9 $mmol.l^{-1}$) production. The ratio of C_3: C_2 - an average of 1: 2.3 in rumen indicates the sudden transition to a concentrated type of postpartum TMR, balanced character of rumen fermentation with high production VFA. Concentrated type of TMR modifies rumen fermentation towards to the risk of rumen acidification.

Table 2. The level of rumen fermentation of lactating dairy cows

	POST PARTUM (until 21 DIM)				PEAK OF LACTATION (21-100 DIM)			
	Farm N		Farm P		Farm N		Farm P	
Rumen content	**x**	**SD**	**x**	**SD**	**x**	**SD**	**x**	**SD**
pH	6.22***	0.1	7.04***	0.1	6.32	0.6	6.81	0.1
NH_3 mg.100 ml^{-1}	30.9	4.2	24.9	6.9	28.9	6.1	29.5	3.5
Lactic acid $mmol.l^{-1}$	17.5	0.9	19.9	3.7	18.0	3.5	21.8	2.9
Acetic acid $mmol.l^{-1}$	71.6	6.5	74.5	5	74.3	22.1	79.1	6.9
Propionic ac. $mmol.l^{-1}$	31.9**	5.5	20.4**	2.1	31.2	13.8	22.1	3.2
Butyric acid $mmol.l^{-1}$	17.1	1.4	12.0	0.8	17.5	6.1	12.4	1.2
Σ VFAs $mmol.l^{-1}$	120.6	11.9	106.9	7.2	122.9	41.5	113.7	9.9
Acetic acid %	59.5	2.7	69.7	1.1	61.4	3.4	69.6	2.1
Propionic acid %	26.3	2.7	19.1	1.2	24.4	3.3	19.5	2.1
Butyric acid%	14.2	0.7	11.2	0.4	14.2	0.9	10.9	0.2
C_2:C_3	2.3	0.3	3.7	0.2	2.6	0.5	3.6	0.5

*NH_3 - ammonia; VFAs – volatile fatty acids; C_3 - propionic acid; C_2 - acetic acid; SD – standard deviation; DIM – days in milk, ***P <0.001, ** P<0.01*

Analysis of energy metabolism of the blood**:** - observed values of the energy metabolism of glucose (3.65 $mmol.l^{-1}$), on the middle third of the reference values for all animals. Blood levels of NEFA (0.41 $mmol.l^{-1}$), cholesterol (4.33 $mmol.l^{-1}$) were observed within the physiological range in all animals confirm of metabolically compensated lipomobilisation. The analyzed values of ß-hydroxybutyric acid - BHBA (0.91 $mmol.l^{-1}$) confirm increased ketogenesis and expression of subclinical ketosis. Analysis of the liver enzymes AST, GGT and bilirubin in average on the middle third of references value in all animals with confirming optimal functional condition without metabolic load of liver in dairy cows after calving.

In the animals on the farm P by the analysis of the rumen and blood parameters was confirmed: In the group of dairy cows after calving – the analysis of fermentation confirmed rumen alkalosis with pH 7.04. Rumen synthesis of VFA 106.9 ± 7.2 $mmol.l^{-1}$ and rate of acetic acid (C_2 74.5 $mmol.l^{-1}$), and propionic acid production (C_3 20.4 $mmol.l^{-1}$) with the ratio C_3: C_2 - an average of 1: 3.7 confirms the type of fermentation of voluminous type of TMR with limited adaptation on the concentrated type of rumen fermentation after parturition.

Table 3. Evaluation of blood markers of dairy cows

	POST PARTUM (until 21 DIM)				PEAK OF LACTATION (21-100 DIM)			
	Farm N		Farm P		Farm N		Farm P	
Blood serum	**x**	**SD**	**x**	**SD**	**x**	**SD**	**x**	**SD**

Glucose	mmol.l^{-1}	3.65	0.3	3.87	0.2	3.44	0.4	3.77	0.4
Urea	mmol.l^{-1}	5.64	0.4	4.57	0.9	6.15	0.74	5.97	0.7
Albumin	g. l^{-1}	34.18	1.8	35.65	2.6	35.28	2.26	38.31	2.66
Cholesterol	mmol.l^{-1}	4.33**	0.8	2.71**	0.3	5.4	0.62	5.01	0.9
NEFA	mmol. l^{-1}	0.41	0.1	0.7	0.3	0.29	0.07	0.28	0.15
BHBA	mmol. l^{-1}	0.91	0.2	0.68	0.2	1.2**	0.37	0.5**	0.11
AST	µkat. l^{-1}	1.49	0.3	1.79	0.2	1.69	0.16	1.73	0.27
GGT	µkat. l^{-1}	0.46	0.1	0.45	0.1	0.53	0.04	0.42	0.06
Bilirubín	µmol. l^{-1}	3.6*	0.6	9.1*	4	3.17	0.64	4.62	1.74

NEFA – non-esterified fatty acids; BHBA – beta-hydroxybutyric acid; AST – aspartate aminotransferase; GGT - gamma-glutamyl transferase, ***P <0.01*, ** P<0.05*

Evaluation of the analysed indicators of blood serum confirmed: - significantly negative energy balance with increased lipid mobilization (NEFA 0.7 mmol.l^{-1}). During the NEB following early lactation in the present study (Farm N), showed only a small change in BHBA concentration compared with cows on farm P (0.68 vs. 0.91 mmol.l^{-1}). This can be explained by the lower utilization of NEFA as a substrate for ketogenesis, and higher proportion of NEFA was utilized for hepatic uptake of lipids and showing the strong metabolic load of liver (bilirubin 9.1 umol.l^{-1}) in dairy cows in periparturient phase.

In cows at first phase of lactation –This higher rumen propionate suggests that more glucose precursors could be supplied, and supplying more glucose precursors such as propionate might inhibit hepatic gluconeogenesis by amino acid deamination. Thus, corn grain in the diet might decrease N losses by decreasing deamination, and higher utilization of protein into milk protein synthesis. The increased propionate due to the replacement of corn grain could be attributed to the high starch content and optimal corn starch degradability compared with rapid digested wheat grain (Hoffman and Shaver 2011).

In the present study, the ruminal propionate and starch digestibility were higher for diets with corn and alfalfa silage (farm N) than for corn and clover-grass silage TMR (farm P). Different source of starch grain and leguminous silages in the TMR didn't showed a significant effect on the serum glucose, albumin and urea concentration however, a tendency to an increased concentration of ketogenesis (BHBA) in group N, but tendency to an increased concentration of NEFA were observed. Excessive uptake of NEFA by the liver may develop fatty liver. Increased concentrations of bile constituents (bilirubin 9.1 umol.l^{-1}) and hepatic enzymes in plasma (AST, GGT) indicate dysfunction of liver in cows during postpartum period (group P).

Milk Yield and milk Composition: Throughout lactation, daily milk production follows a well-established lactation curve. Milk fat and milk protein follow the inverse of the lactation curve, mainly due to the

dilution effect. It is important to review the lactation cycle of the dairy cow to fully understand the different production and energy demands made on a cow throughout her cycle. In farm N milk yield started with 31.2 kg/d and the fat to protein ratio peaked at 1.58 mean in 12.3 d postpartum and declined to relatively constant values around 1.3 for the whole lactation period.

Table 4. Evaluation of milk production on the Farm N

Days of lactat.	Cows	% Cows	Ø DIM	Milk kg/day	Fat %	Proteins %	Fat/Protein
0-40	15	5.6	12.3	31.22	5.00	3.17	1.58
41-100	62	23.2	71.3	31.48	4.12	3.12	1.32
101-200	70	26.2	150.5	26.97	4.32	3.34	1.29
201-305	57	21.3	246.5	21.34	4.35	353	1.23
Over 305	63	23.6	433.1	18.47	4.79	3.57	1.34
Total	**267**	**100.00**	**211.5**	**25.05**	**4.43**	**3.37**	**1.31**

In Farm P cows showed a moderate lower milk yield 20.2 kg/d in 19.3 d postpartum and the fat to protein ratio 1.43 persist on to relatively constant values around 1.4 for the 305 d lactation period. NEB is associated with the rise of some metabolites such as NEFA and BHBA, and on the other hand a decrease of glucose and cholesterol. Increased milk fat occurs in cows in NEB or ketosis, presumably because of the increased availability of BHBA and fatty acids for milk fat synthesis. However, if the butterfat levels fall and the fat: protein ratio is low, this suggests problems with rumen health and possible subclinical acidosis.
Several studies have shown a correlation between energy levels and milk fat: protein ratio (Eichner 2004). These studies indicate that the ideal range for milk fat: milk protein ratio is 1 - 1.25. A milk fat: protein ratio greater than 1.5 is considered a risk factor for metabolic problems. There are two mechanisms responsible for increase in milk fat: protein ratio. The first mechanism is an increase in milk fat due to negative energy balance. The second mechanism is a decrease in milk protein as the result of a lack of energy in the ration and/or decreased voluntary dry matter intake.

Table 5. Evaluation of milk production on the Farm P

Days of lactat.	Cows	% Cows	Ø DIM	Milk kg/day	Fat %	Proteins %	Fat/Proteins
0-40	15	6.5	19.1	20.20	5.11	3.56	1.43

41-100	39	16.9	75.2	24.70	4.65	3.33	1.40
101-200	50	21.7	14.,6	24.70	4.96	3.42	1.45
201-305	59	25.6	252.3	17.67	5.36	3.81	1.41
Over 305	67	29.1	397.1	12.26	4.98	4.02	1.24
Total	**230**	**100,00**	**225.4**	**18.98**	**5.03**	**3.69**	**1.36**

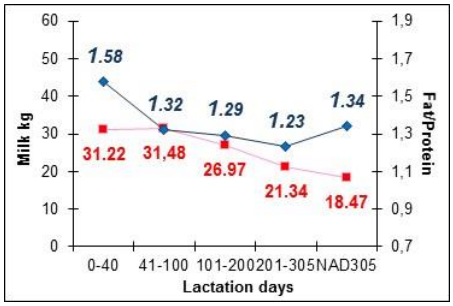
In the both farms we confirmed NEB in the analysed production phases. When cows are in negative energy balance (or ketosis), milk protein levels tend to drop and butter fat levels often rise (cow mobilizes body condition, some of the fat is diverted into the milk) (Gross et al., 2011). So a high fat: protein ratio suggests that the cows may be suffering from negative energy balance.

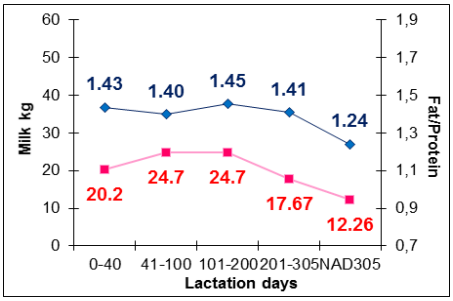
Milk production curve rapidly rises in early postpartum period and reaches maximum in the first third of lactation, whereas animal dry matter consumption rises slowly and cannot follow increased needs for the nutrients. Therefore, dairy cows enter a period of negative energy balance (NEB), which leads to mobilization of body reserves, mainly fat, to balance the deficit between food energy intake and production requirements (Huer et al., 1999; Opsomer et al., 2000).

Evaluation of urea in milk of dairy cows. Measurement of milk urea (MU) can be a useful diagnostic tool in evaluating the efficiency of diets and is also good indicator of ammonia levels in the rumen. Milk urea is related to the ratio between energy that is available for the microorganisms and degradable (soluble) protein of TMR (Hopkins and Whitlow 2001). High concentration of MU can suggest excess ruminal ammonia, due to overfeeding degradable protein and/or not enough fermentable carbohydrate, while low MU levels indicate low ruminal ammonia levels or insufficient dietary protein. Production of milk protein is limited mainly by the energy requirements of the ruminal biomass. Various studies have demonstrated both urea synthesis and urea-N recycling to correspond to dietary N intake (Martineau et al., 2011). The graphical representation of protein versus urea of milk can simultaneously evaluate the equilibrium that exists

Fig.1.Evaluation of milk production on the Farm N

Fig. 2. Evaluation of milk production on the Farm P





between protein and energy intakes by TMR. We confirm the milk urea concentrations outside of the recommended levels in 70 % of the animals; it signifies that there is an imbalance between the rumen soluble carbohydrates and protein needed for microbial synthesis. The excess urea in the milk coming from the excess of ammonia in the rumen not only can result in excessive degradability of protein as well as the lack of fermentable energy: in this last case, due to the limitation of energy, the milk protein was low at the farm N in agreement with the literature (Eichner 2004, Recktenwald et al., 2014).

Fig. 3. Relationship of protein and urea in milk in the Farm N

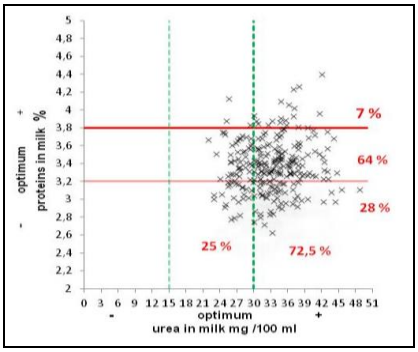
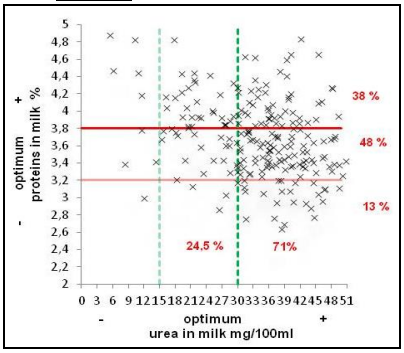


Fig. 4 Relationship of protein and urea in milk in the Farm P



CONCLUSION

The monitoring and analysis of change of milk components can be good indicator for evaluation TMR and change of condition of rumen metabolism. Milk urea nitrogen measurement can be a useful diagnostic tool to help formulate diets that use protein and energy more efficiently.

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ORGANIC MATTER DIGESTIBILITY AND NEUTRAL DETERGENT FIBER OF ITALIAN RYEGRASS DURING THE VEGETATION

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ABSTRACT

The aim of this experiment was analyse the differences of the content of neutral detergent fiber and organic matter digestibility in the fodder of italian ryegrass during the vegetation. Next aim was the evaluation of relation between neutral detergent fiber and organic matter digestibility. Experimetn was conducted with the collaboration of cultivation station Vetrov (Oseva uni a.s.). 48 samples of the dry fodder of italian ryegrass were included in experiment. Italian ryegrass was grown on land Pahorky. The sampling was conducted at eight dates of harvest during the vegetation in 2014: 13.6., 20.6., 27.6., 3.7., 11.7., 17.7., 25.7. a 1.8.

Analyses were performed in the laboratory of Faculty of Agriculture of University of South Bohemia in Ceske Budejovice. Organic matter digestibility was determined by pepsin-cellulase in vitro method according to Mika et al. (2009) with using inexpensive Czech cellulases in Ankom Daisy Incubator. The determination of the neutral detergent fiber was conducted in Ankom Fiber Analyzer. The results was evaluated by software Statistica.

The statistically significant differences of the content of neutral detergent fiber and organic matter digestibility was found between the dates of harvest (Conf. Interval 0,95). The content of neutral detergent fiber ranged from 37,55% to 55,42%. The lowest content of neutral detergent fiber had samples from 27.6.2014 and the highest content samples from 1.8.2014. The organic matter digestibility was ranged from 68,68% to 90,42%. The highest digestibility had samples from 13.6.2014 and the lowest had samples from 1.8.2014. The results showed the relation between neutral detergent fiber and organic matter

digestibility. Using correlation analysis was found statistically significant correlation coefficient -0,8318.

KEY WORDS
Italian ryegrass; organic matter digestibility; neutral detergent fiber

INTRODUCE

Fodder plants are important componens of feeds for ruminant animals whether in the fresh green form or in the conserved form like silage or hay. The fodder plants can be fed separately or like the part of TMR (total mixed ration). Italian ryegrass is very often used fodder plant. Italian ryegrass is sown above all on arable land and on intensively farmed temporary meadows where it provides high yield of very quality feed (OSEVA UNI).

Italian ryegrass (Lolium multiflorum) is loosely tufted grass from family Poaceae. The tillering is intravaginal. Leaf blades are soft, smooth, flat, generally hairless, front side is glossy and reverse side is ribbed. Fertile stalks are 30-100 cm tall. A flowerhead is up 25 cm long and it contains of small, stalkless spikelets that are alternate to one another along the main flowering stem. A caryopsis is greenish gray. The weight of thousands seeds is 1,8 - 2,1 g (SKLADANKA, 2005). Italian ryegrass is very responsive to nitrogen fertilization (PAVINATO et al., 2014).

One of the basic indicators of forage quality is OMD (organic matter digestibility). In vivo and in vitro methods are used to the determination of OMD in practice. In vivo methods are demanding becouse the group of experimental animals is necessary. It is reason to frequent use of in vitro method. In vitro methods are based on the principle of the laboratory simulation of the digestion in the presence of appropriate enzymes or inokulum from rumen. The shortcoming of pepsin-cellulase method is the high price of proteolytic enzymes. This shortcoming was eliminated by new metgod based on the use of cheap Czech cellulases. While 1 g of cellulase from Trichoderma viride is worth 2600 CZK, 1 g of Czech cellulase from Trichoderma reesei is worth only 20 CZK (MIKA, 2009). Czech celullases from Trichoderma reesei are 130x cheaper than cellulases from Trichoderma viride.

While older sources did not recommend in vitro pepsin-cellulase method for the determination of organic matter digestibility of individual plants, but rather for the comparison between the plants (NOCEK, 1988), new researches shows high correlation between in vitro and in vivo methods and recommend in vitro pepsin-cellulase

method as reliable method for the determination of digestibility (JANCIK., 2007).

The sacharides are the most important source of energy in feed. Quantity, quality and mutual ratio of structural and nonstructural sacharides provide significant information about the supply of animal organism by structural fiber that influences digestibility of feed (KOUKOLOVA and HOMOLKA, 2008). Neutral detergent fiber (NDF) is structural sacharides - the walls of cells constituted mainly by cellulases, hemicellulases and lignin. NDF serves as food for the ruminal microorganisms and it has a role in stimulating digestive tract. The lack of NDF in feed causes the decrease of feed intake, the excessive content NDF in feed has similar efect and also the digestibility of feed is lower. The sufficient amount of NDF in feed for milk cows is important for milk quality, because the main of the precursors of milk fat is acetic acid that arises during the ruminal fermentation of NDF. According to McCullough (1994) the milk cows in the first faze of lactation need 30% - 33% NDF in dry matter of feed, cows in the second faze of lactation need 30% - 36 % NDF in dry matter of feed, cows in the third faze of lactation needs 34% - 40% NDF in dry matter of feed and dry cows need 37% - 45 % (KUDRNA, 1998). Not only the content of NDF is important but the size of particles is very important too. Too minced fiber does not stimulation digestive tract sufficiently. Too fine feed causes acidosis (ZEMAN et al., 2006).

The date of harvest has very significant effect on quality of fodder. Optimal harvest time is time when the plant contains the ideal amount of high digestible nutrients. The regularly monitoring of nutrient composition in plants is appropriate. The nutrients loss and the decrease of digestibility during growing season are significant. Therefore, even a few days delay in the harvest has negative effect on the digestibility of feed (TATARCIKOVA, 2007). But the economic efficiency have to be considered when choosing harvest time. It is necessary choose harvest time when plants has sufficient yield of forage and suitable composition - it usually is before flowering. Later the digestibility declines sharply (HEJDUK et al., 2013). Optimal date of harvest changes every year depending on the weather.

MATERIAL AND METHODS

The experiment was conducted on samples italian ryegrass. Ryegrass was grown on land Pahorky, LPIS (Land Parcel Identification System) 8607/2, altitude 610 m, potato production type, oat production subtype, brown soil type, light soil, pH 6,1, seeding17.04.2015, fertilization

Table 1 - The effect of vegetation phase to the content of fiber and the digestibility of organic matter (DOLEZAL et al., 2013):

Date of harvest	Growth stage	Fiber in dry matter (%)	OMD (%)
Very early	Before heading	Less than 22	More than 78
Medium early	Heading	22-25	73-78
Medium late	Beginning of flowering	26-28	66-72
Late	End of flowering	29-32	60-65
Very late	Overaged forage	More than 32	Less than 60

dates:16.4. LOVOFERT LAV 27 150 kg/ha and 15.5. LOVOFERT LAV 27 27 200 kg/ha.. The sampling was conducted at eight dates of harvest during the vegetation in 2014: 13.6., 20.6., 27.6., 3.7., 11.7., 17.7., 25.7. a 1.8.. Analyses were done in the laboratory Faculty of Agriculture, University of South Bohemia in the České Budějovice.

Determined of OMD was carried by pepsin-cellulase method according to MIKA et al. (2009). Result has been reported as OMD_{cel} (organic matter digestibility by pepsin-cellulase method). The samples were dried, milled on the mill with a sieve of 1 mm and exposed to the proteolytic enzyme for 24 hours at 40 °C (the first stage of fermentation). Thereafte the samples were for 24 hours exposed to cellulase solution in acetate buffer at 40 °C (the second stage of fermentation). Fermentation samples weighed carried out in plastic bags F57. Insoluble residues after the first and the second stages were always washed with water, dried and in the end of the second stage were dried to constant weight at 105 °C, weighed and incinerated at 550 °C. The content OMD_{cel} was calculated.

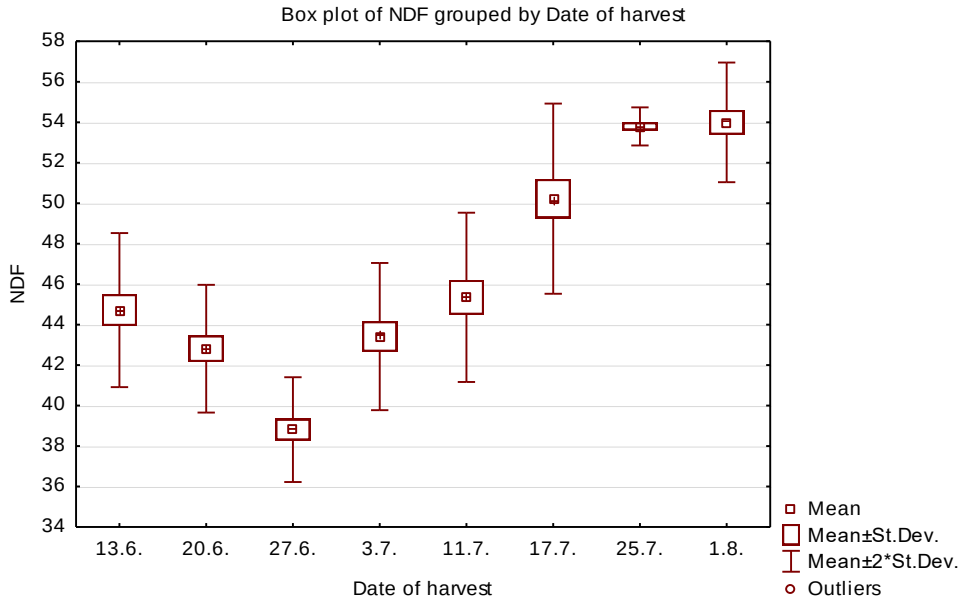
NDF was determined as the residue of cell walls (cellulose, hemicellulose and lignin) after the hydrolysis of sample in the neutral solution of sodium lauryl sulfate in Ankom Fibre Analyzer. The results was evaluated using software Statistica - function Anova, Tukey test and correlation analysis.

RESULTS AND DISCUSSION
NDF:

Table 2 - descriptive statistics NDF (%) by date of harvest in 2014

Harvest	Mean	Min	Max	St. Dev.
13.6.	44.72	42.96	46.51	1.90
20.6.	42.81	41.33	44.29	1.58
27.6.	38.81	37.55	40.08	1.29
3.7.	43.41	41.55	45.08	1.82
11.7.	45.35	43.41	47.35	2.09
17.7.	50.22	48.01	52.59	2.35
25.7.	53.80	53.26	54.30	0.47
1.8.	53.99	52.55	55.42	1.48

Table 2 shows the descriptive statistics of NDF. NDF content ranged from 37,55% to 55,42%. Statistically significant differences was found between dates of harvest (see table 3).. The lowest content of neutral detergent fiber had samples from 27.6.2014 and the highest content



Graph 1 - NDF (%) by date of harvest in 2014 samples from 1.8.2014. The mean od NDF was 46,64. Similar value of NDF found out Jancik (2007) by experiment with perennial ryegrass. His experiment shows the mean value of NDF 47,4%. The content of NDF diminished during the first three dates of harvest, then the content began to increase. This is in line with the sources which mention increasing the proportion of cell walls in forage during growing old (COBLENTZ et al., 1998, CONE et al., 1999, JANCIK et al., 2008

Table 3 - Matrix of statistical significance differences NDF between the dates of harvest

Date of harvest	13.6.	20.6.	27.6.	3.7.	11.7.	17.7.	25.7.	1.8.
13.6.		*0.5375*	**0.0001**	*0.8852*	*0.9981*	**0.0002**	**0.0001**	**0.0001**
20.6.	*0.5375*		**0.0052**	*0.9986*	*0.1968*	**0.0001**	**0.0001**	**0.0001**
27.6.	**0.0001**	**0.0052**		**0.0010**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
3.7.	*0.8852*	*0.9986*	**0.0010**		*0.5206*	**0.0001**	**0.0001**	**0.0001**
11.7.	*0.9981*	*0.1968*	**0.0001**	*0.5206*		**0.0005**	**0.0001**	**0.0001**
17.7.	**0.0002**	**0.0001**	**0.0001**	**0.0001**	**0.0005**		**0.0171**	**0.0099**
25.7.	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0171**		*1.0000*
1.8.	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0099**	*1.0000*	

OMD_{cel}

Table 4 - descriptive statistics OMD_{cel} (%) by date of harvest in 2014

Harvest	Mean	Min	Max	St. Dev.
13.6.	87.92	86.35	90.42	1.46
20.6.	84.38	80.15	86.64	2.65
27.6.	85.96	84.07	87.12	1.06
3.7.	85.46	84.50	87.33	1.08
11.7.	80.48	78.48	81.33	1.10
17.7.	78.15	75.96	81.49	1.98
25.7.	73.95	71.54	76.12	2.04
1.8.	71.01	68.68	72,80	1.48

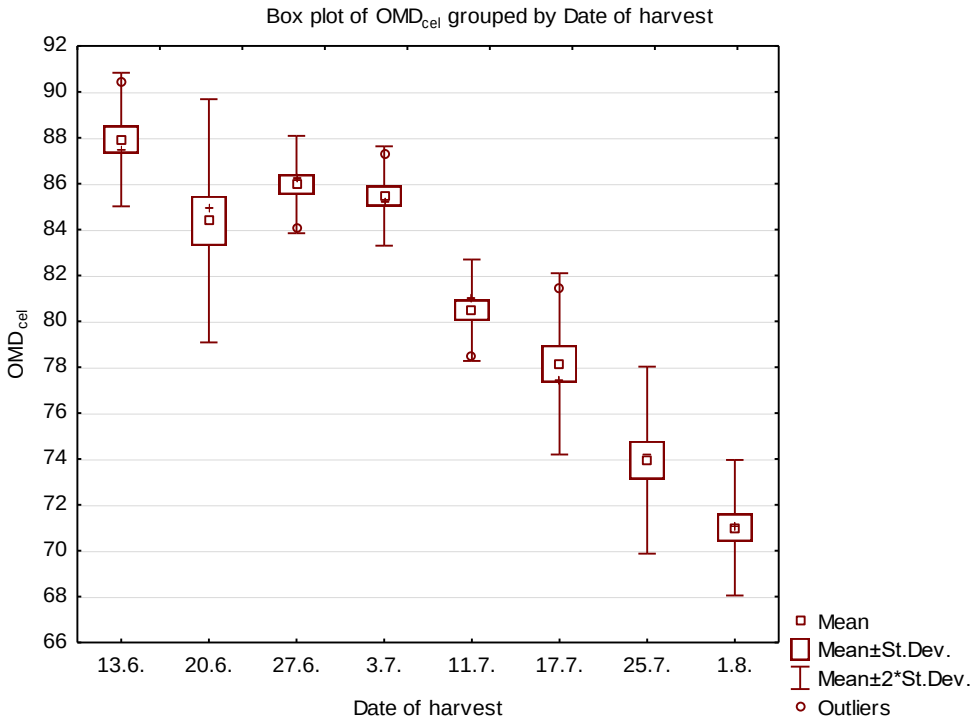
Table 4 shows the descriptive statistics of OMD_{cel}. It ranged from 8,68% až 90,42%. Statistically significant differences was found between dates of harvest (see table 5).The lowest content of OMD_{cel}

had samples from 1.8.2014 and the highest content samples from 13.6.2014. The mean of OMD_{cel} was 80,91. Similar experiment was done by Valente (2000), the digestibility also declined during the vegetation.

Graph 2 - OMD_{cel} by date of harvest in 2014

Table 5 - Matrix of statistical significance differences OMD_{cel} between the dates of harvest

Date of harvest	13.6.	20.6.	27.6.	3.7.	11.7.	17.7.	25.7.	1.8.
13.6.		**0.0167**	*0.4905*	*0.2178*	**0.0001**	**0.0001**	**0.0001**	**0.0001**
20.6.	**0.0167**		*0.7354*	*0.9509*	**0.0062**	**0.0001**	**0.0001**	**0.0001**
27.6.	*0.4905*	*0.7354*		*0.9996*	**0.0002**	**0.0001**	**0.0001**	**0.0001**
3.7.	*0.2178*	*0.9509*	*0.9996*		**0.0003**	**0.0001**	**0.0001**	**0.0001**
11.7.	**0.0001**	**0.0062**	**0.0002**	**0.0003**	.	*0.2726*	**0.0001**	**0.0001**
17.7.	**0.0001**	**0.0001**	**0.0001**	**0.0001**	*0.2726*		**0.0026**	**0.0001**
25.7.	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0026**		*0.0774*
1.8.	**0,0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	*.,0774*	

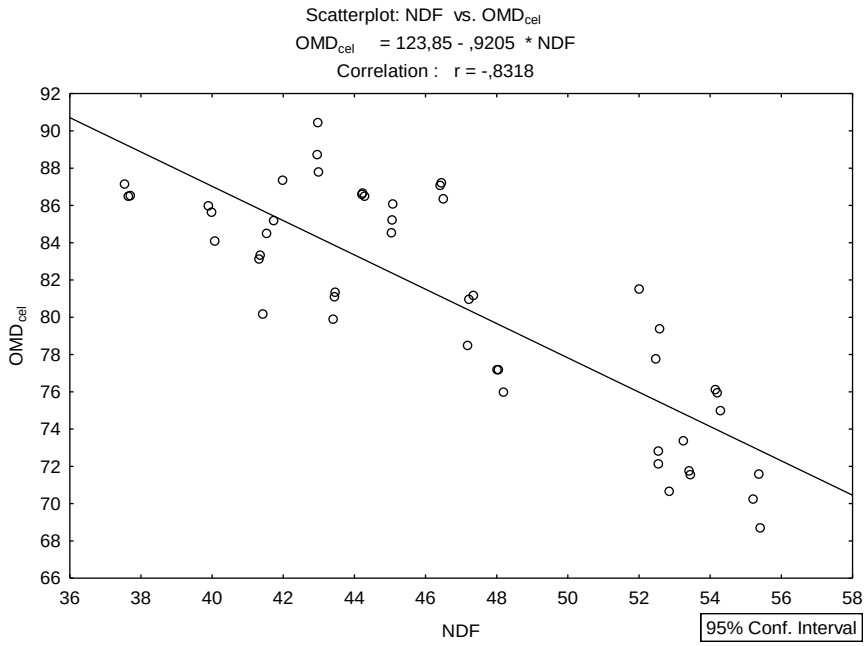


The negative correlation between NDF and OMD_{cel} was found by

correlation analysis. Correlation coeficient is -0,83 (Conf. Interval 0,95). Correlation between NDF a OMD_{cel} is evident on Graph 3. Similar correlation coeficient (-0,82) between NDF and OMD_{cel} also stated Koukolova (2008).

Relation between NDF and OMD_{cel}

Graph 3 - correlation between NDF and OMD_{cel}



CONCLUSION

This experiment showed statistically significant differences in the content of NDF and organic matter digestibility. NDF increased and digestibility declined during the growing season in accordance with literature sources. Analysis of correlation showed statistically significant negative correlation between NDF and OMDcel. Optimal harvest date for make feed for milk cows was 13.6.2014 when forage had favourable content of NDF and high organic matter digestibility. The trial have been supported by GAJU 020/2013/Z-

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LUPINE SEEDS IN DIETS FOR BROILER CHICKENS FOR FATTENING

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ABSTRACT

The main objective of this experimental study was to determine how diets containing lupin meal affect the chemical composition of breast and thigh muscles in broiler chickens. The diets tested in experimental groups P1 and P2 differed as follows: in group P1, one third of nitrogen-containing substances (NSs) from extracted soybean meal was replaced with NSs from lupin meal; in group P2, two thirds were replaced compared to the control group. As far as chemical composition is concerned, chickens receiving the lupin-containing feed showed a significant ($P \leq 0.01$) increase in the ash content in breast muscles. On the contrary, in thigh muscles in group P2, the ash content decreased significantly ($P \leq 0.01$). The content of calcium showed an increasing trend in both breast and thigh muscles in both experimental groups. In contrast, the content of magnesium in chicken muscles in both experimental groups decreased. These differences were significant ($P \leq 0.01$) only in thigh muscles. The replacement of soybean meal with lupin meal is reflected in the amino acids composition of muscle in experimental groups of broiler chickens. Our results show that lupin seed is a suitable substitute for NSs contained in soybean extracted meal. It is considered optimal to replace up to one third of NSs contained in soybean meal with lupin seed. Higher inclusion rate of lupin meal in diets may reduce the growth intensity of chickens, particularly the yield of breast muscles. Due to substantial inter-varietal differences, it is necessary to optimize individual nutrients, particularly amino acids when formulating lupin-containing diets.

Keywords: *Lupinus albus;* lupin meal; broiler chickens; chemical composition of breast and thigh muscles

INTRODUCTION

Growing lupine in recent years is subject of worldwide interest and becoming an interesting commodity. At present in the Czech Republic encounter crop surfaces of this prospective crop. In the cultural varieties of lupine seeds of nutrition terms, positively evaluate low content of anti-nutritional factors, eg. chinolizidin alkaloids or antitrypsin factor for that instance. Cultural lupine varieties can be divided into three basic groups. It is a group of narrow-leaved varieties (*Lupinus angustifolius*), a group of white varieties (*Lupinus albus*) and a group of yellow varieties (*Lupinus luteus*). Within each group of varieties, there are considerable differences in nutrient composition. These differences are due to the content of crude protein, fat, as well as other nutrients. In the Czech Republic for cultivation for feed purposes the most appropriate group of white lupine varieties, because of the protein content, which is comparable to the protein content in soya beans, some varieties of white lupine have a higher protein content than soybeans. In addition, the white varieties can be relatively easily grown in soil and climatic conditions in the Czech Republic and are more resistant to fungal diseases. Ecologically speaking, lupins are promoted because most lupin varieties are not genetically modified. From a nutritional point of view, the seed of cultivars of the genus *Lupinus* is a protein enriched raw material used as feed. In this context we are mainly interested in the content of crude protein, wherein the protein quality is determined by the content of individual amino acids. The experimental work was to determine how diet containing lupine meal will affect the chemical composition of pectoral and thigh muscle of broiler chickens focusing on amino acid spectrum.

MATERIAL AND METHODS

Unlike the control group, the experimental feeding mixtures contained lupin meal that replaced one third (P1) or two thirds (P2) of nitrogen-containing substances (NSs) of extracted soybean meal. The experiment used the variety *Amiga* from a group of white flowering lupins. The chickens were fed on deep litter in an accredited experimental livestock stable with controlled lighting, temperature, and feed zoohygienical technological regime. Chickens were fed according to the standard operating procedur efor the fattening of Ross 308 broiler chickens. There were 3 groups of Ross 308 sexed chickens, control group K (35 females and 35 males), experimental groups P1 (35 females and 35 males) and groups P2 (35 females and 35 males). In order to meet the amino acid requirements, the diets with lupin meal

were supllemented with synthetic amino acids L-lysine, L-threonine *and D, L-methionine.*

Muscles (breast, thighs) obtained from the carcass analysis were subjected to chemical analysis. Crude protein was determined in muscles (N x 6.25). Nitrogen was determined according to the Kjeldahl method using the Buchi analyser (Centec automatika, spol. s.r.o.). The content of crude fat was determined using ANKOM^{XT10} Fat Analyzer (O.K. SERVICE BioPro). The content of ash was determined by gravimetrically after incineration at a temperature of 550 °C at pre-defined conditions. The contents of calcium, phosphorus and magnesium were determined after the incineration of a sample of meat. Calcium and magnesium were determined in chloride extract using chelatometry (chelaton 3 - di - NA EDTA), while phosphorus was determined photometrically at 445 nm. The gross energy of muscles was determined calorimetrically using AC 500 (LECO). The amino acids spectrum was determined using AAA 400 (INGOS Praha). The chemical values of muscles presented in this paper are expressed per 100% dry matter. The results obtained were processed by the statistical programme Unistat CZ version 5.6 for Excel which evaluated the mean values and their differences multiple comparisons using the Tukey-HSD test, at significance levels of $P \leq 0.01$ and $P \leq 0.05$.

RESULTS AND DISCUSSION

The performance indicators determined show that extracted soybean meal in diets used for the fattening of broiler chickens can be replaced with lupin-seed meal without significantly affecting the live weight of chickens during fattening. Live weight in groups P1 and P2 decreased slightly (non-significantly) at the end of the fattening period. Both experimental groups (P1 and P2) also exhibited lower feed conversion (1.82 kg and 1.87 kg) compared to the control chickens (1.70 kg). The results are in agreement with the findings of RothMaier and Kirchgessner (1994) or Schams-Schargh et al. (1994) who reported that lupin included in a diet up to 20% did not significantly affect the performance indicators of fattening chickens. It is apparent that the lower content of amino acids in feeding mixtures in the experimental groups of chickens in the last phase of the fattening period, i. e. from Day 30, was associated with a reduction in the average live weight and the lowered conversion of feeding mixtures.

The nutritional value, based on the chemical composition of foodstuffs is one of the major indicators in defining the quality of food. Part of the experimental work was therefore to determine whether the administered would affect the chemical composition of breast and thigh

muscles, which are an important part of human nutrition. These results of chemical analyses show that no significant differences in the average content of basic organic substances (crude protein and crude fat) were observed among the groups. The results show that the substitution of soybean meal with lupin meal did not affect the above-mentioned indicators of meat quality. Differences were confirmed only for minerals, as assessed on the basis of ash content. When evaluating gross energy based on the results, we concluded that the died did not have a significant effect on the energy value in both breast and thigh muscles. These results are in agreement with those reported by Lettner and Zollitsch (1995), Sitko and Čermák (1998) and Teixeira and Dos (1995) who showed that meat quality indicators did not differ between the groups.

Chemical composition of breast and thigh muscles in broiler chickens on Day 40 of the fattening period in 100% dry matter are provided in Tables 1 and 2.

Table 1. Chemical composition of breast and thigh muscles in females of broiler chickens on Day 40 of the fattening period in 100% dry matter

Indicator	**Breast muscle**			**Thigh muscle**		
(g/kg)	**K**	**P1**	**P2**	**K**	**P1**	**P2**
Crude protein	220,47	221,81	219,33	176,41	181,07	175,58
Crude fat	19,69	14,36	16,12	80,06	84,33	85,50
Ash	$11,33^{A}$	$12,11^{B}$	$12,23^{B}$	$10,47^{a}$	$10,95^{b}$	10,59
Calcium	$0,474^{a}$	$0,529^{b}$	0,515	$0,401^{A}$	$0,477^{B}$	$0,491^{B}$
Phosphorus	2,28	2,34	2,30	1,99	1,96	2,00
Magnesium	$0,365^{A}$	$0,413^{B}$	$0,307^{C}$	$0,343^{A}$	$0,306^{B}$	$0,277^{C}$
Gross energy (MJ/kg)	5,94	5,80	5,83	7,42	7,54	7,55

$^{A, B, C}$ statistically highly significant differences ($P \leq 0.01$); $^{a, b, c}$ statistically significant differences ($P \leq 0.05$)

Breast muscles showed a highly significant increase ($P \leq 0.01$) in the ash content in experimental chickens of females in both groups P1 and

P2 compared to control chickens. This increase was accompanied with a non-significant increase in the content of calcium and phosphorus and a decrease in the content of magnesium. A different trend was observed in thigh muscles of males, with a highly significant ($P \leq 0.01$) decrease detected in the ash content in group P2 that received the diet with the highest inclusion of lupin. This decrease in the ash content in thigh muscles corresponded to a highly significant ($P \leq 0.01$) decrease of the content of magnesium in group P2 of males compared to controls and group P2.

Table 2. Chemical composition of breast and thigh muscles in males of broiler chickens on Day 40 of the fattening period in 100% dry matter

Indicator	**Breast muscle**			**Thigh muscle**		
(g/kg)	**K**	**P1**	**P2**	**K**	**P1**	**P2**
Crude protein	210,97	205,55	211,41	179,57	165,51	177,84
Crude fat	16,15	14,94	17,61	64,47	65,17	76,14
Ash	11,46	11,62	11,65	$10,88^{A}$	$10,77^{A}$	$10,12^{B}$
Calcium	0,516	0,510	0,526	0,445	0,463	0,482
Phosphorus	2,25	2,28	2,35	$1,95^{A}$	$1,92^{A}$	$2,07^{B}$
Magnesium	0,357	0,385	0,373	0,304	0,295	0,273
Gross energy (MJ/kg)	5,65	5,61	5,66	6,76	6,90	7,27

$^{A, B, C}$ statistically highly significant differences ($P \leq 0.01$); $^{a, b, c}$ statistically significant differences ($P \leq 0.05$)

Similar to breast muscles, calcium content in thigh muscles also increased in both experimental groups P1 and P2. Unlike breast muscles, in thigh muscles of females these differences were a highly significant ($P \leq 0.01$).

Amino acids composition of breast and thigh muscles in broiler chickens on Day 40 of the fattening period in 100% dry matter are provided in Tables 3 and 4.

Table 3. Amino acids composition of breast and thigh muscles in females of broiler chickens on Day 40 of the fattening period in 100% dry matter

Amino acids (g/kg)	Breast muscle		Thigh muscle	
	K	P 2	K	P 2
Asp	19,53	20,53	16,97[a]	17,86 [b]
Thr	9,52	9,36	8,39	7,88
Ser	8,23	7,91	7,33[a]	6,77 [b]
Glu	28,87[A]	31,93[B]	28,25[A]	30,13 [B]
Pro	9,45	9,74	9,39	9,37
Gly	9,29	9,67	9,96[A]	8,75 [B]
Ala	12,54	12,95	11,62[A]	9,23 [B]
Val	10,95[a]	11,58[b]	9,29[A]	11,08 [B]
Met	5,91	6,26	5,12	5,38
Ileu	10,40	10,48	8,84	9,03
Leu	17,15	17,73	14,92	15,10
Tyr	11,35[A]	9,74[B]	7,71[A]	6,89 [B]
Phe	8,42	8,96	7,03[a]	7,84 [b]
His	10,29	10,32	7,05	7,07
Lys	20,85	20,43	19,06[A]	16,67 [B]
Arg	16,54	15,52	15,20[A]	13,78 [B]

[A, B, C] statistically highly significant differences ($P \leq 0.01$); [a, b, c] statistically significant differences ($P \leq 0.05$)

As far as amino acids composition is concerned of muscles of females, chickens receiving the lupin-containing feed showed a highly significant ($P \leq 0.01$) increase in the content of glutamic acid in breast muscles, in thigh muscles also valine. On the contrary, in breast muscles in group P2, the content of tyrosine decreased highly significantly ($P \leq 0.01$), in thigh muscles in group P2 also the content of lysine, alanine and arginine.

Breast muscles of males in experimental groups showed a highly significant ($P \leq 0.01$) increase in the content of proline, glycine and methionine, on the contrary, was observed a highly significant ($P \leq 0.01$) decreased in content of tyrosine. In thigh muscles in group P2 was observed a highly significant ($P \leq 0.01$) increase in content of aspartic acid, serine, glutamic acid, glycine, methionine and leucine.

Table 4. Amino acids composition of breast and thigh muscles in males of broiler chickens on Day 40 of the fattening period in 100% dry matter

Amino acids (g/kg)	Breast muscle		Thigh muscle	
	K	P 2	K	P2
Asp	18,04	18,86	$14,40^{A}$	$17,42^{B}$
Thr	$8,41^{a}$	$9,15^{b}$	$7,46^{a}$	$8,41^{b}$
Ser	$7,25^{a}$	$7,79^{b}$	$6,46^{A}$	$7,33^{B}$
Glu	26,89	30,09	$24,85^{A}$	$28,97^{B}$
Pro	$8,84^{A}$	$17,14^{B}$	7,47	9,36
Gly	$8,62^{A}$	$9,66^{B}$	$8,88^{A}$	$10,07^{B}$
Ala	11,76	12,45	10,43	11,23
Val	10,41	10,66	8,62	9,05
Met	$5,28^{A}$	$6,40^{B}$	$4,62^{A}$	$5,42^{B}$
Ileu	9,75	9,42	8,05	8,59
Leu	$15,92^{a}$	$17,10^{b}$	$13,43^{A}$	$15,16^{B}$
Tyr	$9,60^{A}$	$7,98^{B}$	$7,43^{a}$	$6,58^{b}$
Phe	7,99	8,34	6,79	7,13
His	9,50	9,84	0	0
Lys	20,53	20,26	6,41	6,49
Arg	$14,49^{a}$	$16,17^{b}$	13,64	14,53

$^{A, B, C}$ statistically highly significant differences ($P \leq 0.01$); $^{a, b, c}$ a statistically significant differences ($P \leq 0.05$), 0 value was not detected

CONCLUSION

The results of the experiment have confirmed the full value of compensation soybean meal with with lupin meal of lupine seeds varieties *Amiga*. Our results are in agreement with those reported by Sitko and Čermák (1998) who showed that performance indicators or feed conversion did not differ between the groups in the classification of lupine seeds in feed mixtures. Experimental work has confirmed the assumption that the lupine seed is a good source of protein for fattening broiler chickens, this statement is consistent with the findings Olkowskeho et al. (2001) who show that the lupine seeds are an important source of protein for poultry. Taking into account the achieved slightly higher weights and lower feed consumption appears to be fed with the addition of lupine seeds as economically beneficial. Our results show that lupin seed is a suitable substitute for NSs contained in soybean extracted meal. It is considered optimal to replace up to one third of NSs contained in soybean meal with lupin seed. Higher inclusion rate of lupin meal in diets may reduce the growth intensity of chickens, particularly the yield of breast muscles. Due to

substantial inter-varietal differences, it is necessary to optimize individual nutrients, particularly amino acids when formulating lupin-containing diets.

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INFLUENCE OF ADMINISTRATION OF *CLOSTRIDIUM BUTYRICUM* MIYAIRI 588 STRAIN ON BROILER PERFORMANCE AND INTESTINAL MICROBIOTA

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ABSTRACT

Miya-Gold® is a feed additive containing viable spores of probiotic *Clostridium butyricum* MIYAIRI 588 strain as an active substance. Its purpose is to stabilize the gut microbiota of the animal. The aim of this research was to evaluate the influence of Miya-Gold on intestinal and crop microbiota of broiler chickens and on their performance. The hypothesis was that individuals fed with Miya-Gold will have higher growth rates and more stable intestinal microbiota. Broiler chickens in the experimental group group were fed from hatching to day 49 (the day of slaughter) with the feed fortified by addition of Miya-Gold in amount of 1 g/1000 g of feed. We found statistically significantly higher ($P < 0.05$) average weights of individuals in Miya-Gold group than in the control group at the day 7, 10, 20 and 49. By the analysis of microbiota we found at the day 42 statistically significantly lower ($P < 0.05$) amounts of *Escherichia coli* in the caecum and *Escherichia coli* and coliform bacteria in the crop and also lower pH in the crop in the Miya-Gold group. The analysis of short-chain fatty acids was also performed and we found statistically significantly higher ($P < 0.05$) amount of butyrate in the caecum of Miya-Gold group. The results indicate the ability of Miya-Gold to affect positively the performance and the intestinal microbiota of broiler chickens.

Keywords: chicken; feed; Miya-Gold; *Escherichia coli*

INTRODUCTION

Intestinal microbiota is an important factor for the health of every individual, protecting the body from several diseases (Guarner et Malagelada, 2003), fermenting not digested carbohydrates by production of short-chain fatty acids, synthesizing some vitamins and metabolising some xenobiotics (Cummings et MacFarlane, 1997).

In commercial poultry production, the development of intestinal microbiota in the chickens is often influenced by modern practices such as facility hygiene, routine medication, and artificial egg incubation, hatching and chick rearing. Consequently, chicks are more susceptible to colonization by bacterial pathogens (Barrow 1992). Related to the above, the use of antibiotics has been a common practice in commercial poultry production as the antibiotics reduced the incidence of diseases. In 2001, the European Union banned the use of antibiotics as animal growth promotants to address increased public concerns over the risk of using dietary antibiotics for animal growth promotion (European Commission 2001). Due to this, novel alternatives to dietary antibiotics came to interest. One of these alternatives is to influence the gut microbiota by usage of probiotics.

According to the new definition, the probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014). Many different strains of bacteria are considered as probiotic, including clostridia. In our study, we used *Clostridium butyricum* MIYAIRI 588 strain as a component of Miya-Gold® feed additive for broiler chickens.

Miya-Gold® is a zootechnical additive containing 5 × 108 CFU/g viable spores of *Clostridium butyricum* as an active agent. The product is authorised in the European Union for use with chickens for fattening. It is authorised for use in many Asian countries both for humans and as feed additive for most animal species. *Clostridium butyricum* MIYAIRI 588 is originally isolated from the soil. The proposed effect of Miya-Gold is improved feed conversion, improved average daily gain and thus higher final body weight in the chickens for fattening (European Food Safety Authority, 2011).

Aim of this study was therefore to evaluate the effectiveness of Miya-Gold in the improvement of feed conversion, average daily gain and final body weight in the chickens for fattening and also to observe the improvement of the stability of the gut and crop microbiota.

MATERIAL AND METHODS

Total of 160 broiler chicken were tested. The broiler chickens were fed with ROSS 308 feed mixture supplemented with addition of 1 g/1000 g of Miya-Gold from 2 to 49 days of life in the experimental group, and with ROSS 308 alone in the control group. The feed mixtures were pelletized in order to reduce the dustiness. Both groups consisted of 80 individuals.
In the nutritionist part of the trial, the average weight, feed conversion and average daily gain were observed at the days 1, 7, 10, 20, 35, 49.

In the microbiological part of the trial, the total counts of anaerobic bacteria, bifidobacteria, lactobacilli, enterococci, *Escherichia coli* and coliform bacteria were analysed at the day 1, 10 and 42 in the crop and caecum of 5 randomly selected individuals. One gram of both crop and caecum contents were sampled immediately after slaughter into the tubes containing anaerobic broth for storage of anaerobic bacteria. The analyses were performed immediately after sampling by anaerobic cultivation for total counts of bacteria and bifidobacteria, microaerophilic cultivation for lactobacilli and aerobic cultivation for enterococci, *E. coli* and coliform bacteria. The evaluation was performed by plate count method after of cultivation at 37 °C for 48 h. Media used for the cultivation of each group of bacteria are shown in table1.
Table 1: Media used for the cultivation of bacteria.

Group of bacteria tested	Cultivation medium
Total counts of anaerobic bacteria	Wilkins-Chalgren anaerobe agar supplemented with soya peptone (W+S)
Bifidobacteria	W+S with addition of mupirocin and 100 μL/100 mL glacial acetic acid (Rada et Petr, 2000)
Lactobacilli	Rogosa agar wit addition of 132 μL/100 mL glacial acetic acid
Enterococci	Slanetz-Bartley agar
Escherichia coli	T.B.X. agar
Coliform bacteria	T.B.X. agar

Statistical evaluation was performed by Statgraphics Centurion XV (Statpoint Technologies, Inc., Warrenton, Virginia, USA) using independent two-sample t-tests.
Additionally, the analysis of short-chain fatty acids was performed by the gas chromatography with Stabilwax®-DA column and hydrogen as a career gas.

RESULTS AND DISCUSSION

Results of average daily gain, average weight and feed conversion ratio are shown in tables 2, 3 and 4.

Average weight (g/day)						
	Day 1	7*	10*	20*	35	49*
Experimental group	44.7	138.1	217.3	810.3	1787.2	3231.7
Control group	44.1	131.0	195.0	760.1	1767.9	2780.9

Table 2. Average weight of the tested broilers. Statistically significant differences between the groups ($P < 0.05$) are indicated by the asterisk (*) symbol.

Daily gain (g)					
	1 to 7	1 to 10	11 to 20	21 to 35	36 to 49
Experimental group	15.56	19.17	59.30	65.13	103.17
Control group	14.48	16.77	56.56	67.19	72.36

Table 3. Average daily gains of the tested broilers.

Feed conversion ratio (kg)							
	Days					To	
Day / groups	1 – 7	1 – 10	11 - 20	21 - 35	36 – 49	35th	49th
Experimental	1.53	1.71	1.70	2.22	2.04	1.79	1.84
Control group	2.09	1.90	1.81	1.82	2.76	1.90	2.08

The results show the ability of Miya-Gold to increase feed conversion ratio, daily gain and thus the weight of the chickens for fattening. The only exception is the average weight at the day 35 which is crucial for chickens for fattening. In this period we found no significant

differences between the groups. This could be caused by the aggressive behaviour which can appear in the certain life period of the chickens (Estevez et al., 2003). The group is then less homogenous and therefore the results can be variable.

The results of microbiological analysis are shown in the tables 5, 6, 7, 8, 9 and 10. Microbiological counts were expressed in log CFU/g of the sample. Additionally, the amounts of short-chain fatty acids in the samples at the day 42 are shown in the tables 11 and 12.

Table 4. Feed conversion ratios of the tested broilers.

Caecum, Day 1	**Control**	**Miya-Gold**
Total counts	10.08 ± 0.26	10.06 ± 0.28
Bifidobacteria	4.92 ± 1.27	5.09 ± 1.09
Lactobacilli	5.91 ± 0.47	6.87 ± 0.91
Enterococci	9.55 ± 0.43	8.90 ± 1.75
E. coli	9.60 ± 0.06	9.03 ± 1.70
Coliforms	8.38 ± 0.06	7.59 ± 1.19

Table 5. Counts of bacteria at the day 1 in the caeca.

Crop, Day 1	**Control**	**Miya-Gold**
Total counts	8.95 ± 0.31	9.01 ± 0.12
Bifidobacteria	-	5.40 ± 0.46
Lactobacilli	5.54 ± 1.72	7.29 ± 0.78
Enterococci	7.73 ± 0.78	7.84 ± 0.28
E. coli	7.90 ± 0.78	7.96 ± 0.75
Coliforms	7.71 ± 0.56	7.60 ± 0.30
pH	4.66 ± 0.23	4.58 ± 0.28

Table 6. Counts of bacteria at the day 1 in the crop.

Caecum, Day 10	**Control**	**Miya-Gold**
Total counts	10.09 ± 0.20	10.25 ± 0.32
Bifidobacteria	9.18 ± 0.23	8.83 ± 1.35
Lactobacilli	9.04 ± 0.19	8.55 ± 0.56
Enterococci	8.64 ± 0.35	7.82 ± 0.61

E. coli*	8.47 ± 0.81	7.29 ± 0.61
Coliforms	7.25 ± 1.05	7.01 ± 0.51
pH*	5.16 ± 0.15	5.70 ± 0.43

Table 7. Counts of bacteria at the day 10 in the caeca. Statistically significant differences between the groups (P < 0.05) are indicated by the asterisk (*) symbol.

Crop, Day 10	Control	Miya-Gold
Total counts	9.32 ± 0.39	9.58 ± 0.46
Bifidobacteria*	5.04 ± 0.39	4.29 ± 0.55
Lactobacilli	8.82 ± 0.11	8.69 ± 0.63
Enterococci	8.08 ± 0.33	8.34 ± 0.63
E. coli	-	-
Coliforms	6.70 ± 0.55	6.40 ± 0.35
pH	4.72 ± 0.11	4.42 ± 0.22

Table 8. Counts of bacteria at the day 10 in the crop. Statistically significant differences between the groups (P < 0.05) are indicated by the asterisk (*) symbol.

Caecum, Day 42	Control	Miya-Gold
Total counts	10.09 ± 0.26	10.13 ± 0.23
Bifidobacteria	9.92 ± 0.36	9.55 ± 0.19
Lactobacilli	8.53 ± 0.31	9.01 ± 0.40
Enterococci*	8.10 ± 0.17	7.55 ± 0.39
E. coli*	8.22 ± 0.64	7.00 ± 0.92
Coliforms	7.06 ± 0.60	6.05 ± 1.10
pH	6.44 ± 0.09	6.04 ± 0.51

Table 9. Counts of bacteria at the day 42 in the caecum. Statistically significant differences between the groups (P < 0.05) are indicated by the asterisk (*) symbol.

Crop, Day 42	Control	Miya-Gold
Total counts	8.74 ± 0.56	9.65 ± 0.33
Bifidobacteria	5.20 ± 0.32	4.73 ± 0.96
Lactobacilli	8.29 ± 0.59	9.04 ± 0.44

Enterococci	7.18 ± 0.43	6.94 ± 0.40
E. coli*	6.73 ± 0.52	5.64 ± 0.47
Coliforms*	6.05 ± 0.75	4.33 ± 0.35
pH*	5.74 ± 0.53	4.72 ± 0.35

Table 10. Counts of bacteria at the day 42 in the crop. Statistically significant differences between the groups ($P < 0.05$) are indicated by the asterisk (*) symbol.

Metabolite	**control**	**Miya-Gold**
acetate	441.52	381.08
propionate	183.42	196.96
isobutyrate	3.37	2.14
butyrate*	103.41	132.12
isovalerate	7.51	4.08
valerate	8.24	4.80
isocapronate*	0.91	2.74
capronate	1.18	0.28
heptanoate	-	-
∑	**749.57**	**724.19**

Table 11. Amounts of short-chain fatty acids produced at the day 42 in the caeca. Statistically significant differences between the groups ($P < 0.05$) are indicated by the asterisk (*) symbol.

Table 12. Amounts of short-chain fatty acids produced at the day 42 in the crop.

Metabolite	**control**	**Miya-Gold**
acetate	59.42	58.79
propionate	1.14	0.27
isobutyrate	2.19	1.85
butyrate	-	-
isovalerate	0.48	-
valerate	2.46	1.25
isocapronate	4.64	3.70
capronate	0.84	2.78

heptanoate	**-**	**-**
∑	**71.16**	**68.63**

The individuals fed with Miya-Gold shown statistically significantly lower presence of *E. coli* and/or coliform bacteria in their caeca and crops and in the association with that they had statistically significantly higher amounts of butyric acid in their caeca. The results clearly show the ability of Miya-Gold to influence the composition of both caecal and crop microbiota. The production of butyric acid can play a key role in the inhibition of pathogenic organisms where some strains of *E. coli* and coliform bacteria can belong in a certain conditions (Van Immerseel et al., 2004).

CONCLUSION

Our results show that administration of the viable spores of *Clostridium butyricum* MIYAIRI 588 strain in the form of Miya-Gold feed additive can positively influence the composition of the chicken caecal and crop microbiota and thus reduce the risk of the infections. Related to this we observed higher growth performance in the chickens fed by Miya-Gold. The potential of Miya-Gold is therefore both in the reduction of the risk of infections and in the improvement of the growth performance of broiler chickens.

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GROWTH OF WEANED RABBITS OF DWARF LOP BREED IN ASSOCIATION WITH DIET

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ABSTRACT

The aim of our study was to evaluate the growth of weaned rabbits of the well-known Dwarf Lop breed. Used rabbits came from the common pet stock. The young rabbits were weaned at 7th week of age. A total of 56 rabbits were divided into 3 groups (namely the group A, the group B and the group C). The rabbits in the experimental groups A and B were fed pelleted diets specially designed for this study. Particularly, the experimental diets varied in the content of the white lupine seeds. The diet C was the foreign commercial pelleted feed (without white lupine seeds) and was used for the control group of rabbits. Timothy hay was offered all rabbits three-times a week. Live weights of the young rabbits were recorded at two-week periods up to the 15th week of their age. We found the significant differences in live weights among all groups of rabbits that received the evaluated diets. In the beginning of the experiment, the young rabbits fed the control diet showed the significantly higher live weight as compared to the both experimental diets ($P < 0.01$). On the 77th day of age, the rabbits fed the diet B showed more intensive growth. No statistical difference in the live weight the control group and the group B was observed from 77th day of age to the end of the experimental period. Moreover, the group B showed better growth performance since the 91st day of age, what points out to the suitable nutrient composition of the diet B. The results of our study bring more accurate specifics of growth of weaned Dwarf Lop breed. The obtained findings indicate that the white lupine seed can be the suitable component in the diets for dwarf rabbits.

Keywords: dwarf rabbit; Dwarf Lop; diet; white lupine; growth

INTRODUCTION

Rabbit becomes a favourite pet animal. Concerning the great amount of the rabbit breeds, the Netherland Dwarf and Dwarf Lop breeds show distinct popularity (González-Redondo and Contéras-Chacón, 2012). Appropriate nutrition and feeding play a key role in both of rabbit health and production (Gidenne *et al.*, 2010). The progress and research in rabbit nutrition is widely studied regarding the meat-type rabbits. Composition of rabbit diets is optimized primarily concering an actual physiological state and/or purpose of rabbits (Maertens, 2010). Recently, also research of alternative ingredients for production of the diets rations is running. White lupine´s protein in the rabbit diet met with successful results in fattening meat-type rabbits (Volek and Marounek, 2009) as well as in nursing does (Uhlířová *et al*., 2014). With respect to an unproduction target of dwarf rabbits stocks, is necessary to take into consideration their specific requirements. Rapid growth and superior weight gains provided by the diets for commercial rabbits is undesirable. On the otherhand, welfare requirements, quality of life, good health state linked with longer livespan should be considered (Prebble, 2014; Proença and Mayer, 2014). No findings have existed regarding an effect of the white lupine seeds in diets on the growth of dwarf rabbits. The aim of our study was to evaluate effect of different diets on the growth of weaned rabbits of Dwarf Lop breed.

MATERIAL AND METHODS

Animals -The study was conducted on a total of 56 animals. Rabbits originated from the common pet stock which performs breeding and showing in according to the guidelines of Czech Small Animal Breeders Association. Only purebreds of Dwarf Lop breed were used in the experiment. The experimental procedures were approved by the Animal Welfare Committee of the University of Veterinary and Pharmaceutical Sciences (UVPS) Brno (no. 15/2015/2230/FVHE).

Experimental design and nutrition - Rabbits were divided into three groups. In the experiment, two types of pelleted feed were designed as experimental diets, specifically the diet A and the diet B. As a control diet was used the foreign commercial pelleted feed diet (diet C) for dwarf rabbits (Berkel-Futter Light 6008, Coesfeld, Germany). Ingredients and chemical composition of all the diets are presented in **Table 1**.

On the 7th week of age were kits weaned from does. The group C received the control diet till the 15th week of age. In the experimental groups (A and B), one-week adaptation to the experimental diets was

performed (Lowe, 2010). Both of experimental groups (A and B) received the specific experimental diets from 8th week till 15th week of age. All rabbits were fed once a day (30 g of diet per kg of live weight per day). Rabbits had unlimited access to drinking water. Timothy hay was offered all rabbits three-times a week with the same amount per one rabbit within all evaluated groups.

On the 11th week of age were kits separated regarding a gender and then they were individually housed. All rabbits were housed at hutches sheltered against climatic conditions. Weights of young rabbits were recorded at two-week periods up to the 15th week of the age. The individual weight of each rabbits was recorded.

Statistical analysis - Obtained results were statistically analyzed using software STATISTICA CZ version 9. One-way variance analysis (ANOVA) was used to determinate differences in the monitored live weights of groups. When ANOVA showed significant differences between the groups, Tuckey´s HSD test was used. The significant differences among evaluated groups are in the text and tables marked as $P < 0.01$ (statistically highly significant) and $P < 0.05$ (statistically significant).

RESULTS AND DISCUSSION

The results of growth performance are given in **Table 2**. On the 49th day of age, initial live weights of weaned rabbits within all the evaluated groups showed distinct differences. The highest live weight was found in the control group ($P < 0.01$), while this tendency persisted to the 63rd day of age. The control group showed the highest live weight (736.4 g) as compared to the experimental groups A and B. Furthermore, the rabbits fed the diet B showed higher live weight than those fed the diet A ($P > 0.05$). The lower values in both of the experimental groups as compared to the control group could be caused by the adaptation to the specific diets. On the 77th day of age, the control group still showed the highest live weight, however, the significant difference in this trait was found in the group C as compared to the experimental group A ($P < 0.05$). It may be pointed out to the group B which showed distinct progress in growth. No significant difference in live weights between the group C (799.0 g) and the group B (740.4 g) was found in this age. Moreover, it can be noted that no statistical difference in the live weights between the control group and the experimental group B was observed from 77th day of age to the end of the experimental period. On the other hand, weaned rabbits fed diet A (half content of white lupine seeds as compared to the diet B)

showed the lowest values of live weight during the entire experimental period. Although the chemical composition of the experimental diet A was similar to the control diet, rabbits of the group A showed different growth performance. We suppose that these results may be associated with the different values of nutrient compositions between these diets. On the 91st day of age, the highest live weight was found in rabbits fed the experimental diet B. In addition, we found the significant difference between live weights of the rabbits fed the diet B (921.2 g) and the diet A (725.0 g). No significant difference among the control group and the group B was found. Overall, the experimental group B showed better growth performance since the 91st day of age. On the 105th day of age, the young rabbits fed the diet B showed statistically the higher live weight as compared to the group A ($P < 0.01$). However, no significant difference between live weights of rabbits fed the control diet and those fed on the diet B existed. Therefore, it can be concluded on the suitable nutrient composition of the diet B. As for the chemical composition, the diet B (a higher content of white lupine seeds) showed the highest contents of proteins as well as the value of ether extract. As for protein content of used diets, both the control diet and the diet A were consistent with the recommendations for pet rabbits according to Lowe (2010). The diet B showed a slightly higher content of proteins. Also, an obvious difference in content of the ether extract was evident within all the evaluated diets. Although values of the ether extract were distinctly different among all the evaluated diets, their values met requirements for pet rabbits (Proença and Mayer, 2014). Recently published works pointed out to the high content of both the ether extract and the proteins in white lupine seeds (Straková *et al.*, 2015; Suchý *et al.*, 2015). The growth tendency of young rabbits were consistent with the findings observed by Dalle Zotte *et al.* (2013) in other dwarf rabbit breed. It can be noted that the values of live weights of the Dwarf Lop in our study were somewhat higher than those published by Zadina (2003) for the Czech breed standard of the Dwarf Lop. In general, this finding confirms that the body growth is mainly affected by the nutrition.

The average live weight of the rabbit kits in relation to litter size is presented in **Table 3**. No statistical difference in live weights among the specific litter sizes was found within this dwarf rabbit breed. On the other hand, Poigner *et al.* (2000) found significant differences in the live weight among the different liter size of meat-type rabbits. The observed litter size of the Dwarf Lop rabbit in our study is in agreement with values that reported Zadina *et al.* (2004).

Table 1. Ingredient and chemical composition of the pelleted diets.

Item	Control diet	Diet A	Diet B
Ingredient (g per kg)			
Alfalfa meal	417.0	350.0	350.0
Wheat bran	226.0	210.0	200.0
Malt sprouts	151.0	0	0
Barley	85.0	50.0	20.0
Oat bran	60.0	0	0
Oat	0	210.0	150.0
White lupine seeds	0	100.0	200.0
Sugar beet pulp	29.0	20.0	20.0
Molasses	19.0	30.0	0
Glycerole	0	0	30.0
Monocalcium phosphate	1.0	15.0	15.0
Calcium carbonate	8.5	10	10
Sodium chloride	3.5	0	0
Mineral premix	0	5	5
Chemical composition in 1 kg of dry matter			
Dry matter (g)	1000.0	1000.0	1000.0
Crude protein (g)	160.5	164.3	187.1
Ether extract (g)	26.8	36.3	41.0
Starch (g)	151.9	204.0	159.0
Crude fibre (g)	173.2	161.3	167.7
Acid detergent fibre (g)	233.6	226.5	224.6
Neutral detergent fibre (g)	420.0	358.1	365.0
Acid detergent lignin (g)	53.0	55.2	48.9
Ash (g)	86.2	94.7	91.4
Ca (g)	8.3	11.9	10.8
P (g)	5.7	8.5	8.5
GE (MJ)	18.3	18.2	18.4
DE (MJ)	10.78	11.49	11.46

GE, gross energy; DE, digestible energy.

Table 2. Effect of different diets on live weight (g) of weaned Dwarf Lop rabbits.

Age (days)	Diet					
	C		A		B	
	x	SEM	x	SEM	x	SEM
49	563.6	± 30.4^{B}	482.4	± 39.6^{A}	484.2	± 60.1^{A}
63	736.4	± 38.8^{B}	483.5	± 80.6^{A}	570.5	± 112.4^{A}
77	799.0	± 36.4^{B}	595.4	± 104.4^{A}	740.4	± 125.8^{A,B}
91	821.7	± 77.4^{a,b}	725.0	± 87.2^{a}	921.2	± 126.4^{b}
105	1080.9	± 85.2^{B}	833.2	± 78.6^{A}	1106.0	± 118.8^{B}

x – Arithmetic Mean, SEM – Standard Error of the Mean,
^{A,B}: $P < 0.01$, ^{a,b}: $P < 0.05$

Table 3. Live weight (g) of Dwarf Lop kits in relation to litter size.

Litter size (number of kits)	Age (days)				
	49	63	77	91	105
	x	x	x	x	x
1	803.0	1090.0	1340.0	1410.0	1520.0
2	640.3	767.5	903.3	1096.3	1236.3
3	480.7	636.6	763.0	791.5	1013.5
4	493.0	605.9	664.2	738.2	997.5
5	486.3	546.2	696.3	800.9	910.0
6	563.2	715.0	764.8	860.2	1120.4

x – Arithmetic Mean

CONCLUSION

It can be concluded that the growth performance of young rabbits of Dwarf Lop breed was affected by types of the diets. Regarding the live weights, we found the significant differences between the control group as compared to the both experimental groups. Besides that, the significant difference in live weights was found in the group A as compared to the group B. It can be concluded that young rabbits of the group B showed favourable growth performance, mainly in the later phase of the experimental period.

From the results of our study follows that seeds of the white lupine can be used as the suitable component of feed for dwarf rabbits. Regarding

the nutrient composition of our designed diets, there is need to perform further more detailed studies on dwarf rabbits.

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THE EFFECT OF FEEDING WHEAT WITH BLUE ALEURONE TO THE BLOOD BIOCHEMICAL PROFILE OF RATS

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ABSTRACT

The aim of present study was to evaluate the effect of feeding of wheat with blue aleurone to the blood biochemical profile of rats. The male rats of Wistar albino strain (n = 15) were divided into 3 equal groups. The first experimental group received diet containing 100% of wheat with blue aleurone layer cultivar "UC 66049" (UC group) and second group was fed with 100% cultivar Skorpion wheat with blue aleurone too (Skorpion group). The diet of third group (control group) contained 100% of common wheat "Vánek". In Skorpion group there was observed significantly higher ($P < 0.05$) liver weight expressed as a percentage of liveweight. The Skorpion group had also the highest value ($P < 0.05$) of the alkaline phosphatase (ALP) enzyme. The lower ($P < 0.05$) blood cholesterol concentration in the UC group fed by color wheat indicates a possible positive effect. The coloured wheat feeding did not have a negative effect on the metabolism of monitored parameters.

Keywords: coloured wheat; anthocyanins; liver enzymes

INTRODUCTION

The purple colour of wheat is caused by anthocyanins accumulated in the pericarp, while the blue colour is generated by anthocyanins in the aleurone layer of the wheat grain (Zeven, 1991). Anthocyanins are produced by plants as secondary metabolites to protect it against environmental stress factors and fungal infections and they also promote human health (Chalker-Scott, 1999; Wallace, 2013; Pojer, *et al.*, 2013). Cyanidin-3-glucoside (CG) has shown responses in experimental models that indicate a potential role in reversing the signs of metabolic syndrome. CG decreased obesity and circulating

triglycerides in an *in vivo* study using laboratory mice (Wei, *et al*, 2011). *In vitro*, CG decreased inflammation in isolated vascular endothelial cells and monocytes and produced an insulin-like effect in human omental adipocytes and 3T3-L1 cells (Luo *et al*., 2012; Scazzocchio, 2011). For proanthocyanidins from grape seed (*Vitis vinifera* L.), it was found that these compounds lower blood cholesterol levels by increasing the excretion of bile acids due to regulation of CYP7A1 (Jiao *et al*., 2010). In this way may behave proanthocyanidins from other sources of vegetable, fruits, etc. and modification of some of the iso-enzymes cytochrome families may affect the metabolism of exogenously administered substances (Kolečkář *et al*., 2012).

The aim of present study was to evaluate the effect of feeding wheat with blue aleurone to the blood biochemical profile of rats.

MATERIAL AND METHODS

The male rats of Wistar albino strain (n = 15) were used in this study. The laboratory rats were divided into 3 equal groups at the age of 6 weeks. Rats were marked by shaving of specific areas and kept in plastic boxes with 5 rats per one. The floor of cages was fitted with filter paper and metal grates. The first experimental group received feed mixture containing 100% of wheat with blue aleurone layer "UC 66049" (UC group) and second group feed mixture of 100% Skorpion wheat with blue aleurone (Skorpion group). The feed mixture of third group (control group) contained 100% of common wheat "Vánek". Differences in crude protein content of used wheats were balanced with wheat gluten. The feed mixtures were pelleted. The rats had *ad libitum* access to the feed and water. Feed consumption was measured. Room temperature and humidity were controlled. Lighting system was 16 hours light and 8 hours dark. Table 1 shows a chemical composition of the diets for rats.

During the trial, health status was evaluated daily. At the end of experiment (at the age of 11 weeks) the experimental rats were anesthetised by using isoflurane. Heparinized blood was collected from the abdominal aorta and then the rats were euthanized. Blood was collected into heparinized tubes and centrifuged for 15 minutes at 3,000 rpm. The separated blood plasma was frozen (-20 °C) until biochemical examination. The liver and kidneys were removed, weighed and frozen for further laboratory analysis.

Table 1. Chemical composition of diets

	Control	Skorpion	UC
Dry Matter (g/kg)	886.6	885.2	886.8
Crude Protein (g/kg)	169.5	169.4	165.8
Crude Fat (g/kg)	19.4	14.7	14.3
Crude Fiber (g/kg)	18.4	20.4	22.4
Crude Ash (g/kg)	13.3	16	19.5
Cyanidin-3-glucoside (mg/kg)	5.09	48.29	47.63

The biochemical profile of blood plasma was analysed with the use of Ellipse (AMS Spa, Italy) analyser. The individual parameters were analysed using individual tests produced by Erba Lachema (Brno, CZ): albumin (Alb 500); total protein (TP 500); AST - aspartate aminotransferase (AST/GOT 500); GGT - gamma-glutamyl transferase (GGT 250); ALP - alkaline phosphatase (ALP AMP 500); ALT - alanine aminotransferase (ALT/GPT 500); LD - lactate dehydrogenase (LDH-L 100); bilirubin (BIL T JG 350); cholesterol (CHOL 250); TG - triglycerides (TG 250); creatinine (CREAT L 500) and Randox, UK: Urea (Urea, cat. No. UR 107).

The data were processed by Microsoft Excel (Microsoft, USA) and Statistica version 12.0 (StatSoft, CZ). The one-way analysis (ANOVA) was used. To ensure evidential differences Scheffe´s test was applied and $P < 0.05$ was regarded as statistically significant difference.

RESULTS AND DISCUSSION

In Skorpion group there was observed a significantly higher ($P < 0.05$) liver weight expressed as a percentage of liveweight (Table 3). The Skorpion group had also the highest value ($P < 0.05$) of the ALP enzyme (Table 4).

Table 2. Live weight and tissues wet weights of rats

Group	Control	Skorpion	UC
n	5	5	5
	mean ± standard deviation		
Live weight (g)	230.54 ± 13.23 [a]	231.80 ± 26.01 [a]	253.77 ± 19.13 [a]
Liver (g)	6.98 ± 0.28 [a]	8.54 ± 1.61 [a]	8.38 ± 0.94 [a]

Kidney (g)	1.99 ± 0.18 [a]	2.00 ± 0.23 [a]	2.19 ± 0.24 [a]
Liver (%)*	3.03 ± 0.21 [a]	3.66 ± 0.35 [b]	3.30 ± 0.16 [ab]
Kidney (%)*	0.86 ± 0.05 [a]	0.86 ± 0.07 [a]	0.86 ± 0.06 [a]

[a, b] – different letters in one line – statistically significant differences ($P < 0.05$)
*Percentage of liver weight or kidney weight from live weight

Table 3. Biochemical blood parameters of rats when fed different wheat

Parameter	Control	Skorpion	UC
n	5	5	5
	mean ± standard deviation		
AST (µkat/l)	1.97 ± 0.21 [a]	1.98 ± 0.64 [a]	1.79 ± 1.66 [a]
GGT (µkat/l)	0.02 ± 0.01 [a]	0.12 ± 0.18 [a]	0.02 ± 0.01 [a]
ALT (µkat/l)	1.02 ± 0.11 [a]	1.10 ± 0.29 [a]	0.74 ± 0.24 [a]
ALP (µkat/l)	3.16 ± 0.53 [ab]	4.70 ± 2.07 [b]	1.92 ± 0.28 [a]
Bili (µmol/l)	12.80 ± 3.51 [a]	8.44 ± 3.32 [a]	11.22 ± 3.14 [a]
LD (µkat/l)	7.64 ± 2.43 [a]	5.50 ± 3.45 [a]	4.69 ± 5.07 [a]
TG (mmol/l)	0.90 ± 0.03 [a]	1.04 ± 0.44 [a]	0.85 ± 0.10 [a]
Chol (mmol/l)	1.59 ± 0.25 [b]	1.48 ± 0.21 [ab]	1.10 ± 0.21 [a]
Urea (mmol/l)	12.29 ± 0.39 [a]	12.03 ± 1.56 [a]	10.59 ± 2.47 [a]
TP (g/l)	60.06 ± 2.43 [a]	61.42 ± 2.35 [a]	50.16 ± 8.60 [a]
Alb (g/l)	31.58 ± 1.00 [a]	32.88 ± 0.71 [a]	27.10 ± 3.84 [a]
Creat (µmol/l)	55.86 ± 14.27 [a]	56.42 ± 9.15 [a]	58.88 ± 6.66 [a]

[a, b] – different letters in one line – statistically significant differences ($P < 0.05$)
AST – asparate aminotransferase; GGT – gama-glutamyltransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; Bili – bilirubin, LD – lactate dehydrogenase; TG – triglycerides, Chol – cholesterol, TP – total protein, Alb – albumin, Creat – creatinine

The AST, GGT, ALT, ALP and LD activities and bilirubin concentration and indicate liver tissue status. The TG and cholesterol concentrations characterized fat metabolism. The urea, TP, albumin and creatinine concentrations indicate nitrogen metabolism of organism.
Based on biochemical characteristics the coloured wheat feeding does not show negative effect on this parameters (excluding higher ALP

activity in Skorpion group). Another significant lower plasma cholesterol concentration was found in UC group in comparison to the control group which also suggests a positive effect of coloured wheat feeding. Other biochemical parameters like nitrogen and fat metabolism did not show significant differences (Table 4).

In an experiment carried out by Bhaswant *et al*. (2015) with added cyanidin-3-glucoside into rats feed, there was not found differences in cholesterol and TG between control and experimental group.

CONCLUSION

The coloured wheat feeding did not have a negative effect on the metabolism of monitored parameters. The lower blood cholesterol concentration in the group fed by coloured wheat indicates a possible positive effect of feeding.

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THE EFFECT OF DIETARY LEVELS OF ZINC AND CALCIUM ON BROILER PERFORMANCE

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ABSTRACT

The experiment was conducted to determine the effect of different zinc and calcium levels in corn-wheat-soybean meal diets on broiler growth and carcass yield. A total of 48 male broiler chicks (Ross 308) were kept in a temperature-controlled room. The experiment started at 11 days of broiler age and chicks were fattened up to 35 days of age. During the trial, chicks were *ad libitum* access to feed and water. They were allotted to 4 dietary treatment groups consisting of 2 replicates per 2x2 factorial arrangement of treatments (2 zinc levels and 2 calcium levels). It was tested total calcium content in the diet in levels of 6 and 9 $g.kg^{-1}$ (supplied by $CaCO_3$) in combination with zinc levels of 148 and 48 $mg.kg^{-1}$ (supplied as ZnO). At the end of the feeding trial, birds were slaughtered and carcass evaluation was performed. The results show that these combinations of levels of zinc and calcium had no significant effect ($P<0.05$) on body weight gains, feed conversion ratio (FCR) or carcass yield. No signs of disorders such as loss of appetite, growth depression or abnormalities of the skin was appeared in chicks. It seems that reduced zinc and calcium levels from 148 to 48 $mg.kg^{-1}$ and from 9 $g.kg^{-1}$ to 6 $g.kg^{-1}$ respectively, not influenced growth performance parameteres of broilers in this study.

Keywords: zinc; calcium; broiler; carcass yield

INTRODUCTION

Calcium (Ca) and zinc (Zn) are essential nutrients for many biochemical pathways, they are necessary for chick growth, feathering, immune system and disease resistance. Broiler diets are routinely supplemented with zinc and calcium, usually in inorganic sources. The nutritional value of mineral sources depends on the composition of the diet, concentration in the feed, interactions with other mineral elements,

and the bioavailability of the element to the chicks (Star et al., 2012). Typical corn-soybean meal based diets provide low level of zinc and the presence of fiber, phytate, and concentrations of other elemements further complicates utilization of zinc by the animal (Collins, Moran, 1999). Excessively high concentrations of dietary Ca may impede the availability of nutrients by the formation of non-absorbable complexes (Abdollahi, 2015). It was described antagonistic effect of calcium on zinc availability and zinc uptake, which is probably inhibited by high calcium:zinc ratio. Based on many studies (Underwood, 1977; Rama Rao et al., 2006; Letourneau-Montminy et al., 2010; Suttle, 2010) EFSA (2014) stated that negative effect of calcium was observed only at very high levels of calcium, so impact through the formation of Ca-phytate-Zn complexes is limited as well as it is unlikely that calcium in the diet considerably reduced zinc uptake.

The National Research Council (1994) estimated the dietary zinc requirement for broilers as 40 $mg.kg^{-1}$ and calcium requirement 9 $g.kg^{-1}$. Recommended values are usually higher than NRC requirements for ensuring adequate performance. According to Ross Broiler Management Handbook (Aviagen, 2014), nutrition recommendation for calcium is 8.7 $g.kg^{-1}$ and for supplementary zinc 110 $mg.kg^{-1}$ from 11 to 24 days of age, and 7.9 $g.kg^{-1}$ of calcium and 110 $mg.kg^{-1}$ of added zinc from 11 to 24 days of age. High zinc concentration in broilers manure spreaded on fields can affect quantity and quality of humus and lead to reduced crop yields. Regulation (EC) No 1334/2003 set maximum total zinc contents for poultry as 150 $mg.kg^{-1}$ complete feed and the FEEDAP Panel propose new maximum content of total zinc in complete feed for poultry (except turkeys for fattening) at the level of 100 $mg.kg^{-1}$ (EFSA, 2014)

MATERIAL AND METHODS

Birds and management

Fourty eight male broiler chicks (Ross 308) in 7 days of age were allotted to 8 balance cages with 6 birds per cage such that each cage had a similar initial weight. The chicks had free access to feed and water throughout feeding trial. The lighting system was set on 18 hours light and 6 hours dark. Birds were marked by wing tags and housed in a room that had a temperature set according to Ross Broiler Management Handbook (2014). Temperature and relative humidity was recorded every day.

The experiment started at 11 days of broiler age and chicks were fattened up to 35 days of age. It consisted of 4 dietary treatments with 2 replications per treatment. Composition of the diet BR2 (broiler grower feed) is shown in Table 1 and it was formulated to meet or exceed NRC (1994) nutritional requirements except zinc and calcium. It was used vitamin-mineral premix containing minimum amount of zinc and calcium and experimental premix with different levels of Zn and Ca.

Table 1. Composition of the diet BR2

Ingredients	$g.kg^{-1}$
Maize	340
Wheat	315
Soybean meal	260
Sunflower oil	40
Vitamin-mineral premix [1]	20
Experimental premix [2]	20
Chromium oxide	5
Nutrient composition	
ME_N (MJ/kg)	12.69
Crude protein	206.6
Lysine	11.9
Methionine	5.8

[1]Supplied per kilogram of premix: lysine 101.7 g, methionine 135.6 g, threonine 51.22 g, calcium 68,30 g, phosphorus 98.19 g, natrium 62.89 g, sulphur 0.39 g, chlorine 119.7 g, copper 752.5 mg, iron 3768.6 mg, zinc 44.73 mg, manganese 6046 mg, cobalt 11 mg, iodine 47.95 mg, selenium 8.96 mg, vitamin A 680000 IU, vitamin D 250000 IU, vitamin E 2250 mg, K_3 74.8 mg, B_1 206.4 mg, B_2 344 mg, B_6 300.4 mg, B_{12} 1999 mg, biotin 11 mg, niacinamid 1793mg, calcium pantothenate 676.2 mg, folic acid 82.8 mg, choline chloride 9000 mg
[2] Content different levels of Zn and Ca according to the dietary treatments

Total content of this minerals in the diets is referred in Table 2. The source of added zinc was ZnO and calcium $CaCO_3$.

Table 2. Total content of zinc and calcium in the groups

Group	**Zinc ($mg.kg^{-1}$)**	**Calcium ($g.kg^{-1}$)**
Group 1	48	6
Group 2	48	9
Group 3	148	6
Group 4	148	9

Body weight of each chicks was measured on the digital scales at the start of experiment (11 d of age), then twice a week in the morning before feeding and at the final day (35 d of age) before slaughter.

Evaluation of carcass quality

At the end of the experiment (35 d of age), broilers were weighed and slaughtered by cervical cutting. Carcasses were weighed, breast and leg meat were cut, skinned and percentages of live body weight were calculated.

Statistical analysis

Data has been processed by STATISTICA.CZ, version 12.0 (CZ). The results were expressed as mean ± standard deviation (SD). It was used one-way analysis (ANOVA). Scheffe´s test was applied to defined statistical differences and differences between groups were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The effects of zinc and calcium levels on performance parameters are shown in Table 3.

Table 3. Effects of different Zn and Ca levels on body weight gain (g/broiler) and feed conversion ratio (FCR) during the trial (11 – 35 d of broilers age)

Group	n	Zinc ($mg.kg^{-1}$)	Calcium ($g.kg^{-1}$)	Weight gain (g)	FCR
Group 1	12	48	6	2026 ± 144	1,50
Group 2	12	48	9	1931 ± 167	1,54
Group 3	12	148	6	1858 ± 173	1,49
Group 4	12	148	9	1849 ± 180	1,57

No significant differences at a level of p <0.05

Table 4 presents feed consumption during the experiment. In all cases, chicks given zinc levels of 48 $mg.kg^{-1}$ were higher weight gains and feed consumption than groups fed 148 $mg.kg^{-1}$ of zinc, nevertheless the best FCR were achieved in group 3 (diet containing 148 $mg.kg^{-1}$ of zinc and 6 $g.kg^{-1}$ of calcium), but there was no significant difference ($P<0.05$) between groups.

Table 4. Effects of different Zn and Ca levels on feed consumption (g/broiler) during the trial (11 – 35 d of broilers age)

Group	n	Zinc ($mg.kg^{-1}$)	Calcium ($g.kg^{-1}$)	Feed consumption (g)
Group 1	12	48	6	3033
Group 2	12	48	9	2968
Group 3	12	148	6	2778
Group 4	12	148	9	2910

Carcass yield are expressed as a percentages of live body weight at the day of slaughter (35 day of age). Breast meat and leg meat was skinned and deboned and yield of these parts was expressed as a percentages of slaughter weight. The results are presented in Table 5 and they are practically similar with no significant differences.

Table 5. Effects of varying zinc and calcium levels on carcass yield (% of live body weight)

Group	n	Carcass	Breast meat	Leg meat	Leg meat (deboned)
Group 1	12	73.9 ± 1.6	21.8 ± 1.4	19.6 ± 1.1	15.3 ± 1.2
Group 2	12	73.9 ± 2.4	20.8 ± 2.3	19.2 ± 1.3	14.9 ± 1.3
Group 3	12	73.1 ± 2.3	20.5 ± 2.3	18.9 ± 1.4	14.8 ± 1.3
Group 4	12	732 ± 1.8	21.0 ± 2.4	19.2 ± 0.7	14.9 ± 0.7

Breast and leg weight values are expressed as skinless.
No significant differences at a level of $p < 0.05$

An early sign of zinc deprivation is loss of appetite and growth depression afterwards (Suttle, 2010). In this study, the highest feed consumption and body weight gains were achieved in group 1 and group 2, so it indicates that the amount of zinc at level of 48 $mg.kg^{-1}$ was acceptable for providing adequate feed intake and growth despite of antagonism between zinc and calcium. An important influence of increased calcium concentrations from 6 to 9 g.kg-1 in the broiler diets with low zinc level was not found. Similar to our results presented here, Abdollahi et al. (2015) observed higher weight gains and feed intake in chicks given diet containing 6 $g.kg^{-1}$ of Ca than diet with 9 $g.kg^{-1}$ of Ca. The negative effect of calcium on zinc intake was observed at very high calcium concentrations. Suttle (2010) and Underwood (1977) stated that poultry is considered less susceptible to high calcium. Increasing zinc content is associated with enhanced

breast meat percentage (Jahanian et al., 2008). In our experiment, zinc levels had no effect on breast muscle yield.

CONCLUSION

In this experiment, different levels of zinc and calcium in the corn-wheat-soybean meal diet were evaluated for their effect on the growth performance of broiler chicks from 11 days up to 35 days of age. Body weight gains, FCR and carcass yield were not affected by zinc and calcium content in the broilers diet.

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