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Department of nutrition, dietetics and animal breeding
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THE GOAL OF THE UNIVERSITY OF VETERINARY MEDICINE AND PHARMACY IN PROVIDING HIGHER EDUCATION AND IN SPECIALIZATION OF VETERINARY PROFESSION

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Changes in production conditions and development of productive and technological systems of animal rearing, production and processing practices, starting from the primary production through technological industrial processing of raw materials and food including food distribution and market, have changed essentially the conventional veterinary procedures and the role of veterinary surgeons. Analysis of the current situation and profile of developmental trends in the field of veterinary practices and veterinary profession places stress on identification of conditions and consideration of influences under which the veterinarians practice their profession.

Veterinary medicine of food-producing farm animals. Rearing conditions of farm animals, increasing density and productivity together with technological innovations in free housing of animals undergo important changes that affect the object and performance of veterinary activities. Currently the following requirements deserve priority:

1. Shift from medical procedures (diagnostics, therapy) focusing on individual animals towards the health of herd, within age, production and reproduction categories.
2. Biological approaches with increased stress on more effective and economic preventive and prophylactic measures in preference to curative chemotherapeutic approaches.
3. Health control programmes for food animal herds together with defining, analysis and management of critical control points, applied to ensure hygiene and safety of food and raw materials of animal origin, as systemic measures providing basis for food safety and concept of certification in agreement with the current trends in food hygiene veterinary practice.
4. Implementation of systems of nutrition prevention of health disorders based on control of quality and health safety of feed, nutrition and feeding procedures according to production and reproduction phases, together with registration and tracking down the feed, integrates veterinary practice into a complex system ensuring on-farm productive health.

Veterinary medicine of companion animals. It is oriented on individual animals and closely models clinical, diagnostic, therapeutic and preventive procedures of human medicine In addition to common biological and medical basis, this involves an important public-social aspect of the man – animal relationship. Clinical practice in the field of companion animals, related research activities and obtaining new knowledge within species and systemic specialisation based on theoretical disciplines from the point of view of cellular – organ – system studies, contribute to further development of medicine of companion animals and thus also to improvement of animal health and quality of human life.

Veterinary medicine involving food hygiene. This is a specific field of veterinary medicine. Due to globalisation of productive and marketing activities involving food commodities, integration of legislation and control procedures in the field of hygiene, health safety and control of food and raw materials and dividing competences among regional, national and multinational institutions, the range and competences of veterinary medicine have extended considerably. Veterinary inspection activities in the field of hygiene and health safety of food, as a part of protection of public against food-borne diseases, together with control of antropozoonoses, are integral parts of public veterinary medicine.

The wide biological basis of profile of pre-graduate education of veterinarians, historically verified unreplaceability of veterinary profession in the field of health, production and reproduction of animals, key role in environmental control and protection, prevention of food-borne diseases and antro-

pozoonoses provide solid basis for scientific branches with interdisciplinary specialization. Production practice and public demands determine internal specialization within veterinary sciences and profession and expansion and orientation outside the common core of veterinary activities. Priority role of our profession is orientation of our activities on the following areas:

- **animal sciences** with specialisation on animal-husbandry, **bio-food** and food production scientific branches,
- **biochemical and biomedical sciences** with orientation on analytical, laboratory and technological practice and wide involvement in biological interpretation of results; **ecology and animal welfare** – as part of environmental protection and management in relation to macroclimate influences. The modern veterinary sciences and practice inevitably include care of the animals from the point of view of microclimate and technological housing conditions, treatment, nutrition and feeding and the derived metabolic and productive diseases of animals.

Based on these intentions we prepared also the project of diversification of higher education in terms of interdisciplinary specialization at the UVMP in Košice for the accredited study programmes on all three levels of higher education.

The University of veterinary medicine and pharmacy, established by the Act of the Slovak National Council No. 1/1950 Coll., initially the Veterinary College in Košice, fulfils its mission in the system of higher education institutions by providing higher pre-graduate and post-graduate veterinary education on the basis of original scientific research in the field of veterinary sciences and pharmacy.

Higher education of the 1st level is provided within the study programmes *Cynology, Safety of feed and food, Man – animal relationship and its use in canistherapy and hipotherapy.*

Higher education of the 2nd level is provided in the accredited doctor study programmes *General veterinary medicine* and *Food hygiene* starting from the academic year 2006/2007, magister study programme *Pharmacy* and starting from the academic year 2010/2011 also in the follow-up magister study programmes *Market and food quality* and *Productive health of animals and environmental protection*.

Higher education of the 3rd level is provided in **17** accredited study programmes in external and internal form at standard length of study of 4 and 5 years, resp. The study programmes of the 3rd level of higher education were accredited within the respective study branches and after their successful completion the graduate is awarded the academic title "philosophiae doctor", abbrev. "PhD.".

Evaluation of effectiveness and quality of education-teaching and scientific research activities. In the EU, veterinary medicine and pharmacy belong among the so-called controlled profession and the respective education has to satisfy the EU Directive 2005/36/ES. Because of this the effectiveness and quality of education-teaching and scientific research activities at the UVMP in Košice is evaluated at the university, national and international levels.

At the university level, the effectiveness and quality of education and scientific research is evaluated regularly at the meetings of the Scientific Board of the UVMP and Rector's Collegium and twice a year at the meetings of the Academic Senate of the UVM with appropriate discussion resulting in resolutions, suggestions and recommendations.

At the national level, in agreement with the Act on higher education, our university is evaluated by the Accreditation Commission of the Slovak Republic which carried out in the past ten years evaluation of the education and research activities, accreditation of study programmes within the approved System of educational branches, accreditation of habilitations and procedures for appointing professors within the approved system of educational branches, complex accreditation and re-accreditation of our university in the period of years 2000–2005 and 2002–2007 in accordance with the Government Decree of the Slovak Republic No. 104/2003 Coll. on accreditation commission.

On the basis of complex accreditation our university was included in the highest category of higher education establishments, the category of university-type higher education establishments. The accredited study programmes, the right to perform habilitation procedures, procedures for appointing professors and, as a follow-up, the evaluation of scientific-research activities, put our university under an obligation to guarantee and develop the fields of veterinary medicine, food and feed safety, environment and pharmacy. On the basis of results of complex accreditation the UVMP in Košice was authorised to carry out research and development for the period up to the following accreditation.

At the international level, the University of veterinary medicine and pharmacy in Košice was subjected to the following evaluations in the years 1996–2011:

a) evaluation by the Education Committee of the European Association of Establishments for Veterinary Education (EAEVE) with the seat in Brussels (1996–1997),
b) evaluation of approximation of education in the field of veterinary medicine between the Slovak Republic and EU Commission – TAIEX (2002),
c) re-evaluation by the Education Committee of the EAEVE with the seat in Brussels (2005–2006),
d) institutional evaluation of our university by the European University Association (EUA) (2006–2007).

On the basis of results of evaluation of approximation of education in the field of veterinary medicine between the Slovak Republic and the EU, the EU Commission – TAIEX, based on evaluation carried out in 2002, issued a document that the University of veterinary medicine and pharmacy in Košice meets the EU standards and the Diploma awarded by the University of veterinary medicine and pharmacy in Košice is recognized in EU member states.

Upon the decision of the European Commission for veterinary education of 20th April, 2011, the University of veterinary medicine and pharmacy in Košice has been put on the *"EAEVE list of visited and approved establishments"* which comply with the requirements of the Directive 200/36/ES of the European parliament and of the Council on the recognition of professional qualifications.

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SYSTEM OF VETERINARY CONTROL OF HEALTH, QUALITY OF PRODUCTION AND ECOLOGY OF DAIRY COW BREEDING

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ABSTRACT

Cattle health surveillance is essential for protecting public health, enhancing access to international markets for animals and their products, and improving animal health, production and welfare. The objectives of animal health programmes include the support of economic growth and stakeholder livelihoods. Animal health surveillance systems typically include the following broad system components: passive reporting, active clinical surveillance, sentinel monitoring, outbreak investigation, biological or laboratory testing, target epidemiological studies and border inspection. Veterinary authorities should clearly identify animal health objectives and review animal health surveillance information needs.

Key words: animal health; health programmes; welfare

INTRODUCTION

The surveillance of animal health status is a limiting factor of protection of public health and by contributing to good health, protection of animals and their welfare, it supports the international trade in animals, their products and foods. The health programmes at the level of herds are an integral part of management systems focused on increasing the genetic potential of herds, their performance and economic effectiveness of animal breeding. An active control of animal health comprises scientifically well-founded complex procedures which generate in the food chain from-farm-to-table information on health and production risks, risk management and the creation of national and international health programmes.

The aim of the study was to introduce the contents, orientation and application of the system of veterinary health control implemented within national health programmes in cattle holdings.

RESULTS AND DISCUSSION

In 20011, in the field of animal health, the SVFA of the SR fulfilled the duties arising from the Act No. 39/2007 Coll. on Veterinary Care as amended. The main tasks involve preparation of management tools, direction and coordination of executions of the state administration performed by the Regional Veterinary and Food Administrations (8 RVFAs, cancelled since November 1, 2011) and District Veterinary and Food Administrations (40 DVFAs) in the field of the control of compliance with the requirements on identification and registration of animals, classification of holdings, regions and areas from the viewpoint of the occurrence of certain animal diseases, for the implementation of programmes for the control, monitoring and eradication of diseases and for the control of compliance with the requirements for operation of animal assembly centres, animal markets, collection centres for trade in animals, insemination centres, animal holdings and other facilities for breeding and keeping of animals, subject to the approval or authorization by the veterinary administration authority. In 2011, the SVFA of the SR in the field of animal health elaborated and submitted to the Ministry of Agriculture and Rural Development of the Slovak Republic for the approval totally 14 national programmes for eradication, control or surveillance of animal diseases in the Slovak Republic, out of which the plan of surveillance of bluetongue, eradication of infectious bovine rhinotracheitis (IBR/IPV) and the programme of the prevention, monitoring and control of some transmissive spongiform encefalopathies (TSE) are intended for cattle.

The SVFA of the SR within the competencies of the Plan for the protection of the state territory of the Slovak Republic performs controls in respect to the observance of requirements for classification of holdings, regions and areas connected with veterinary requirements for movement of animals based on certain health statutes. This includes the plan of diagnostic actions and vaccinations covered from the state budget of by a breeder, for 12 groups of animals (cattle, pigs, sheep, goats, poultry, horses, fish, bees, wild

animals, all animal species, wild birds and zoo animals) with the total number of 103 types of preventive and diagnostic actions.

Classification of holdings, regions and areas

The Slovak Republic has continued to maintain the status of a country officially free of bovine tuberculosis, bovine brucellosis, enzootic bovine leukosis and ovine brucellosis (*Brucella melitensis*). To maintain the status, totally 70 530 tuberculinations of cattle, 68 956 serological examinations for bovine brucellosis and 65 780 examinations for enzootic bovine leucosis were performed in the Slovak Republic during the year 2011. Totally 21 800 sheep and 724 goats over 6 months of age and all breeding rams were examined for ovine brucellosis (*Brucella melitensis*). In order to retain the recognized status and for the reason of preventing a spreading of animal diseases, the SVFA of the SR elaborated the Plan for the protection of the state territory of the Slovak Republic 2011 and the methodical guidance Classification of holdings, measures for gradual introduction of surveillance network and health requirements upon movement of live animals and germinal products – guidance for the year 2011 of December 22, 2010.

Eradication, control and surveillance of animal diseases in the Slovak Republic

Infectious bovine rhinotracheitis

In the year 2011, the monitoring of IBR/IPV in cattle holdings was carried out based on the approved eradication programme – Plan of eradication of infectious bovine rhinotracheitis (IBR/IPV) in Slovakia in the year 2011. The aim of the programme is to eradicate cattle holdings from IBR/IPV on the whole territory of the Slovak Republic. Recovery of holdings will improve a health status and trade barriers in the domestic as well as in foreign trade will be eliminated.

Bluetongue

The targeted monitoring of bluetongue (hereinafter referred to as the "BT") in the Slovak Republic is aimed on the surveillance and disease monitoring within the whole territory of the Slovak Republic, which serves also for a declaration that the Slovak Republic is without the occurrence of this disease and for the data collection on the risk assessment of the introduction of this disease into the territory of the Slovak Republic, observance of principles of prevention against introduction of this disease and application of restrictive and tightened measures upon movement of animals from prohibited zones and through prohibited zones.

Since April 1, 2008, the serological monitoring of BT in holdings of sentinel (serologically negative) animals has been performed. Within this programme totally 100 cattle farms/holding were selected and in each of them 10 animals were chosen being once a month subject to a serological blood examination for BT. Since 1. 04. 2008 also the entomological monitoring of BT has been performed in the Slovak Republic in 8 selected cattle holdings (in the sphere of competence of each RVFA one holding).

Transmissive spongiform encefalopathies (TSE)

By the Commission Decision No. 2007/453/EC of 29 June, 2007, establishing the BSE status of Member States, third countries or regions thereof according to their BSE risk, the Slovak Republic is included among countries with a controlled BSE risk. In the Slovak Republic, the monitoring of BSE in cattle was performed from July 1, 2001, to June 30, 2011 on all healthy slaughtered bovine animals over 30 months of age and on all dead and emergency slaughtered bovine animals over 24 months of age.

By Commission Implementing Decision No. 2011/358/EU of 17 June 2011, amending Decision 2009/719/EC, the Slovak Republic was included in the group of the EU member states for which it was permitted to revise their annual BSE monitoring programmes. Since July 1, 2011, the Slovak Republic increased the age limit of bovine animals which must be examined for BSE as follows:

- from the category of bovine animals intended for examination of animals over 30 months of age to 72 months of age of animals,
- from the category of bovine animals intended for examination of animals over 24 months of age to 48 months of age of animals.

Control of the protection of animals bred for farming purposes

In the course of the year 2011 totally 1 963 inspections in 17 960 holdings of individual farm animals were performed, out of which 1 071 controls focused on the control of welfare in holdings of cattle and calves, 338 controls in pig holdings, 331 inspections on sheep and goat farms and 134 controls on holdings of laying hens and chickens bred for meat production. During these controls, totally 152 violations of individual provisions were found out and 148 measures for removal of deficiencies and 4 sanctions were imposed.

In conclusion the study deals with the system of the health status control, production, protection and welfare of dairy cows from the viewpoint of the economic effectiveness of farming, health safety of animals, their products and health guarantees upon entry the national and international market. The surveillance of dairy cow health is based on the principle from farm to table, so that the biological peculiarities of productive and reproductive performance and dairy cow welfare were scientifically taken into consideration.

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CONTROL AND MANAGEMENT OF NUTRITION AND PRODUCTIVE HEALTH AND NUTRITIVE PREVENTION OF HEALTH PROBLEMS ON DAIRY FARMS

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ABSTRACT

The aim of this study was to assess the practicality of the biological control of nutrition for feeding evaluation in dairy cows. Stepwise regression analyses were used to evaluate the relationship between feeding, nutrient composition and structure of the total mixed rations (TMR) and the metabolic markers (rumen and blood metabolism) in the transition period and at the peak of lactation in 540 cows from 30 commercial dairy herds. We observed a significant relationship between crude protein in the TMR, level of NH_3 in the rumen and urea nitrogen in blood ($P < 0.0001$; $r = 0.85$) in the transition and high production groups of cows. Non-esterified fatty acids (NEFA) was the parameter that most closely reflected the level of negative energy balance and lipomobilization in dairy cows in 33.4% and 14.8% of cows before and after parturition, respectively. The negative energy balance of the cows after parturition and high positive relationship regression coefficient ($P < 0.0001$) for bilirubin, AST and GGT confirmed 45% incidence of liver damage of the cows after parturition. It was concluded that the values of metabolic markers analyzed in the rumen and blood, which deviated from the reference values, are useful tools for assessment of the feeding system, homeorhetic adaptation of metabolism, and detection of metabolic problems of dairy cows in early lactation.

Key words: dairy cows; lipomobilization; NEFA; biological nutrition control

INTRODUCTION

To satisfy nutrient requirements of high-yield dairy cows consistent peripartal metabolic adaptation must be ensured with respect to following: rumen fermentation in the peripartal phase and at the peak of lactation (5); metabolic transformation of energy metabolism (lipomobilization and liver adaptation (1); endocrine and nutritional adaptation with shift from rumen to abomasum digestion of proteins and saccharides (4, 2); increased demands on energy, proteins, minerals, vitamins and supporting substances (3).

MATERIAL AND METHODS

In the years 2010–2012 we used biological methods to evaluate the nutrition level of 540 dairy cows from 30 herds during preparation for calving, after calving and at the peak of lactation. We focused on nutrition composition of rations and its effect on rumen fermentation and selected parameters of protein, energy and mineral metabolism and liver load markers in order to evaluate the relationship between manifestation of metabolic disorders and physiological adaptation in transition phase from the dry period to lactation.

RESULTS AND DISCUSSION

1. Nutrition composition of rations. Analysis of trough samples showed the following trends:

a) reduction of protein content after calving and at the peak of lactation with mean proportion of nitrogen substances (NS) 153.6 and 158.1 $g.kg^{-1}$ dry matter (DM), resp., in 59% and 51%, resp., of the examined samples of total mixed rations (TMR);
b) consistent proportion of starch and non-fibrous saccharides (NFS) with mean values after parturition 259 and 372 $g.kg^{-1}$ DM, resp., and at the peak of lactation 261 and 373 $g.kg^{-1}$ DM, resp. (in the recommended range);
c) increased proportion of neutral detergent fibre (NDF) with mean level at the upper limit (> 34%) and individually increased levels of NDF in 53% and 60%, resp., of analysed trough samples of TMR which indicates lower

quality of fodder harvested in late vegetation phase; d) the mean calculated net energy for lactation (NEL) reached 6.54 MJ.kg^{-1} DM after calving and 6.59 MJ.kg^{-1} DM at the peak of lactation and was decreased in two thirds of examined herds in comparison with the recommended level.

2. Level of rumen fermentation. Rumen fermentation was evaluated by analysis of samples of the rumen content collected by probe at 4–6 hours after feeding. Accurate quantification and determination of proportions of volatile fatty acids (VFA) in the rumen is important for the following evaluations:

a) level of peripartal adaptation of rumen fermentation;
b) fermentation in relation to ration composition;
c) structure of TMR for stabilisation of rumen function (stimulation of rumen contractions and rumination) in the peripartal phase.

Analysis of rumen fermentation in the examined cows showed the following:

- **In groups in preparation for calving** prevailed fermentation characteristic of bulk type of rations with: a) increased pH – mean level and individually in 56 % of cows; increased proportion of acetic acid, mean 67.25 % and individual increase in 80 % of samples; borderline proportion of propionic acid, mean 19.9 % and individual decrease in 60 % of cows. Such fermentation indicates low level of adaptation to intake of productive rations after calving.
- **In groups after calving** abrupt change to concentrate rations resulted in tendency to decreased pH (mean and individually in 44 % of cows) accompanied with decreased proportion of acetic acid and increased proportion of propionic acid and rumen fermentation characteristic of the concentrate type of rations.
- In groups at the peak of lactation we observed metabolic adaptation with compensated level of fermentation and not disturbed functionality of the rumen. Overall evaluation proved an alarming decrease in pH below 6.0 in 28 % of cows and decrease in propionic to acetic acid ratio below 2.5.

Mean NH_3 levels in the rumen of cows from the examined groups fluctuated around mid-reference values (18.5–20.0 mg.100 ml^{-1}). Decrease in individual values below 15 mg.100 ml^{-1} correlated with decrease in NS in TMR in lactating cows with individual decrease in NH_3 in the postpartum phase in 32 % and at the peak of lactation in 26 % of cows.

3. Evaluation of the level of intermediary metabolism. This evaluation includes processes of synthesis, splitting and biological transformation of substrates at the level of organs, tissues and cells with the aim to supply and distribute the nutrients necessary for maintenance, growth, production, reproduction and growth of animals.

Evaluation of energy balance through analysis of serum non-esterified fatty acids (NEFA), as the degree of lipomobilization in cows during preparation for calving, reached marginal values (0.35 ± 0.16 mmol.l^{-1}, on average). Increased level of lipomobilization (NEFA > 0.35 mmol.l^{-1}) was confirmed in 33.4 % cows (mean value of NEFA 0.53 ± 0.1 mmol.l^{-1}). These cows showed significantly higher levels of bilirubin ($P < 0.0001$) in comparison with cows with compensated lipomobilization and mean value of NEFA equal to 0.25 ± 0.05 mmol.l^{-1}. In cows after calving the increased lipomobilization (NEFA > 0.6 mmol.l^{-1}) was confirmed in 14.8 % of cows (mean value of NEFA 0.73 ± 0.1 mmol.l^{-1}) while in cows with compensated lipomobilization the mean NEFA values reached 0.35 ± 0.1 mmol.l^{-1}. In groups of cows with compensated and non-compensated lipomobilization all investigated indicators of load on the liver (bilirubin, AST, GGT) showed significant increase ($P < 0.0001$) which indicated metabolic load on the liver after parturition.

The results obtained in the study together with the evaluated relationships confirmed the suitability of biological control of nutrition level for evaluation and management of nutrition and production health and nutritive prevention of health problems in the current system of rearing of dairy cows.

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ANALYSIS OF PERIPARTAL PHASE IN RELATION TO LEVEL OF PRODUCTION AND HEALTH OF DAIRY COWS

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ABSTRACT

The objective of this study was to determine and evaluate occurrence of subclinical ketosis, protein to fat ratio and body condition score in Holstein dairy herd. For management of dairy cow herd is very important to recognize certain biological control points and their correct occurrence. We determined decreasing trend in prevalence of subclinical ketosis when comparing results obtained on day 5 and 15 of lactation, what is partially due to the treating of positive cows with monopropylene on day 5. The mean body condition score (BCS) of the herd was 3.66 while the optimum is 12, or 45%, as reported by various authors. The mean protein to fat ratio was 0.75, which, according to other studies is an indicator of higher prevalence of subclinical ketosis that was also acknowledged. On the basis of our results we advise to take more care about BCS and dry matter intake during the calving time. Our study detected also other control points and investigated their correlations in more detail.

Key words: body condition score; fat to protein ratio; monitoring of dairy herd; subclinical ketosis; transition period

INTRODUCTION

Management of dairy herd from the biological aspect is as important as management of the technical one (feeding, milking, bedding, etc.). Herdsmen or vets have in their hands tools for systemic and continuous management. Control points which we use should be measurable, objective, easily interpretable and should be related to herd economics. In practice there are methods for monitoring of production, health and reproduction of herd. On farm management should control basic biological principles of these monitoring methods and according to possibilities to use them in prophylactic programmes.

The aim of our study was to show the use of some of them under practical conditions in a high yield herd and to present their results.

MATERIALS AND METHODS

All data were collected from the farm Agrocontract mliečna farma, a.s. Cows were housed in free mattress stalls with chopped straw or sawdust bedding. They were milked three times per day and fed total mixed rations (TMR) once a day (twice in summer months).

Body condition score (BCS) was evaluated on the first day of dry period by 5 point system according to Wildman *et al.* (5).

Ketogenesis was evaluated from milk samples on days 5 and 15 of milking by means of KetoTest (Elanco US). All cows detected as positive were treated with monopropylene. The fat to protein ratio was determined from the first control examination of milk after calving.

RESULTS AND DISCUSSION

The mean herd BSC was 3.66, while 43% of cows had higher BCS on average (Table 1.). Optimum BCS after calving according to Roche *et al.* (4) is 3.0 to 3.25. In our herd it was determined in 12% of cows. Lower BCS is associated with decreasing production and reproduction and higher BCS is associated with reduction of dry mater intake and production in first days of milking and with increasing risk of incidence of metabolic disorders. Wildman *et al.* (5) reported that optimum levels of BCS at calving should be in the range of 3.25–3.75, during the first 100 days of milking 2.5–3.0, in the middle of lactation 2.75–3.25, at the end of lactation 3.0–3.5 and in the dry period 3.25–375.

Table 1. Results of BCS, content of fat and protein in milk

	n	Mean	STD	Min	max
BCS	589	3.66	0.32	3	5
Milk fat (%)	809	4.24	0.97	1.4	7.7
Milk protein (%)	809	3.04	0.34	1.4	4.4
Fat to protein ratio	809	1.4	0.3	0.62	2.52
Protein to fat ratio	809	0.75	0.16	0.40	1.61

n – number of examined animals

Table 2. Results of test on ketogenesis (year 2009)

Month	Day of lactation	n	Negatively tested (%)	Positively tested (%)
May	5	66	58	42
	15	46	76	24
June	5	62	55	45
	15	57	88	12
July	5	64	53	47
	15	63	73	27
August	5	44	48	52
	15	57	74	26
September	5	68	56	44
	15	59	85	15
October	5	74	70	30
	15	71	83	17

n – number of examined animals

All performed tests showed 31.7 % positivity for ketogenesis (Table 2.). It reached 42.6 % and 20. % on days 5 and 15 of lactation, respectively. The lower number of positive cows on day 15 can be partially ascribed to the positive effect of treatment with monopropylene. Duffield (2) reported 41 % occurrence of subclinical ketosis in the first two months after calving. In addition, the incidence of left displaced abomasum was positively correlated with high probability of subclinical ketosis (>20 % in the first two weeks after calving). Dohoo and Martin (1) described high occurrence of subclinical ketosis during first 65 days of milking (7–32 %), with peak in 4th week and decrease in the subsequent days. By contrast, Duffield *et al.* (3) reported the highest occurrence in the first 2 weeks. Our results were in accordance with the mentioned authors (30–52 % on day 5 and 12–27 % on day 15 of milking).

The mean fat to protein ratio in the herd was 0.75 (Table 1.). According to Duffield *et al.* (3) the critical ratio indicating risk of subclinical ketosis is ≤0.75 in more than 40 % of herd at first examination after calving. In our herd it was detected in 54 % of cows.

CONCLUSIONS

The detected incidence of ketogenesis, subclinical ketosis and BCS of cows were identical or slightly higher as those detected or recommended by other authors. Higher BCS determined in dry cows correlated with the reduction of dry mater intake after calving and with incidence of ketosis in the herd. This tendency was confirmed by measuring beta-hydroxybutyrate (BHB) in milk and also by evaluation of the fat to protein ratio. We recommend to pay more attention to increase in dry mater intake. When moving cows among groups one should evaluate not only production and days of lactation but also body condition score. Active monitoring of ketogenesis and consecutive feeding of glucoplastic substances can be recommended as a substantial tool in biological herd management.

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EFFECT OF RUMEN DEGRADABILITY AND ILEAL DIGESTIBILITY ON METABOLIC TRANSFORMATION OF PROTEINS IN DAIRY COWS

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ABSTRACT

The nitrogen use efficiency in dairy cows may vary between 16 and 36 %. The key factors in the rumen include the efficiency of N capture and the modification of protein degradation. Under typical feeding conditions there is a limited net recycling of N into the rumen, but understanding the factors controlling urea transport across the rumen wall may reverse the balance to take advantage of the recycling capabilities of ruminants. The aim of the study was to evaluate the level of crude protein (CP) in samples of total mixed rations (TMR) in relation to NH_3 level in the rumen content and serum urea in examined animals and to analyse degradability of TMR.

Key words: CP; microbial protein; NH_3; RDP; TMR; urea

INTRODUCTION

Effectiveness of transformation of proteins in rations on milk proteins does not exceed 30 % and thus more than 70 % nitrogen substances (NS) is lost in faeces (30 %) and urine (40 %), mostly in the form of urea. Nitrogen use efficiency (NUE) is the ratio between nitrogen in milk and N accepted through total mixed rations (TMR). Efficient use of N from TMR is limited by proportion and degradability of proteins, amino acid composition of digestible proteins at small intestine level (NUE at the level of 16 to 36 %) (4). Synchronization of energy and proteins for the metabolism increases yield of microbial proteins and decreases excess of N at rumen level which decreases serum urea and elimination of N. By metabolic transformation in dairy cows, 360 g N of the total 600 g of accepted N is transformed to urea and of that amount 225 g of N returns back to GIT (1).

The aim of the study was to determine rumen degradability and intestinal digestibility of proteins in investigated TMRs and to evaluate efficacy of utilization of proteins from TMR through analysis of the relationship between NS accepted in TMR and their influence on NH_3 rumen level and serum metabolic markers in cows in the 1st stage of lactation.

MATERIAL AND METHODS

In the experiment we used *in situ* method (2) to determine degradability of proteins in selected TMR in the rumen of fistulated cows (2). The feeds (productive rations) were incubated for 16 hours in the rumen of these cows. Analysis of the content of nutrients in TMRs was carried out according to Commission Regulation (EC) No. 152/2009 of January 2009, laying down the methods of sampling and analysis for the official control of feed. Ammonia level in the rumen was determined quantitatively by Kjeldahl method. Protein metabolism profile was evaluated at serum level by determining the markers urea and creatinine was determined photometrically, using respective kits and a biochemical analyser "Ellipse".

RESULTS AND DISCUSSION

Level of determined parameters of protein nutrition at the peak of lactation are summarised in Table 1. Level of crude protein (CP) in TMRs reached 146.2 ± 16.2 g.kg^{-1} dry matter (DM) with considerable individual variations in the observed herds. Standard levels of NS (16–18 %) for dairy cows in the 1st stage of lactation were detected in 3 samples of TMR, in 5 samples they were closely below the lower limit and in 7 samples the mean NS was 130.9 g.kg^{-1} DM, i. e. t the level recommended for preparation for calving. Determination (in situ) of rumen degradation of proteins (RDP) in TMRs showed that the degradability of proteins differed and reached mean level of 53.5 ± 8.1 %. Rumen degradation of

proteins depends on the level of NS, solubility and structure of proteins and microbial proteolytic activity. It is also related to interactions of nutrients in TMR, particularly sources of energy and N, through composition of structural and non-structural saccharides and the ratio of bulk feed (BF) and concentrate feed (CF) BF : CF in TMR.

Table 1. Protein nutrition parameters in rations and TMR of cows at the peak of lactation

Markers	min – max	x ± STD
Dry mater intake kg	19.0–25.1	21.96 ± 1.71
BF : CF	42 : 58–64:36	53 : 47
CP-TMR g.kg^{-1} DM	102.6–169.8	146.2 ± 16.2
Degradability CP-analysis %	41.4–67.8	53.5 ± 8.1
Bypass CP-TMR %	32.1–58.6	46.5 ± 8.1

Evaluation of NS intake, rumen degradability of NS and relationship to NH_3 rumen level and serum urea and creatinine is presented in Tables 2 and 3. Low and standard intake of NS affected intensity of rumen transformation of proteins, the rumen-hepatal N-cycle in dairy cows. Ammonia level in the rumen reflects transformation of proteins and is a suitable indicator of source of nitrogen available for microbial synthesis of proteins. At a decreased content of NS (121.6 ± 13.5 g.kg^{-1} DM) with degradability reaching only 54.9 ± 5.5 % in comparison with standard demands of lactating cows the mean NH_3 levels determined in the rumen were 11.3 ± 3.3 mg.100 ml^{-1} and serum urea reached 2.8 ± 0.4 mmol.l^{-1}. Decreased level of NH_3 in the rumen decreases microbial synthesis of proteins due to limited intake of NS for microbial growth.

Table 2. Relationship between decreased intake of NS and transformation of proteins in cows at the peak of lactation

Marker	CP g.kg^{-1} DM	Degrad. CP %	NH_3 mg.100 ml^{-1}	Urea mmol.l^{-1}	Creatinine µmol.l^{-1}
TMR 1	130.5	51.1	11.0	3.3	65.2
TMR 2	131.8	51.0	15.5	2.7	72.8
TMR 3	102.8	62.6	7.4	2.4	96.8
X ± STD	121.6 ± 13.5	54.9 ± 5.5	11.3 ± 3.3	2.8 ± 0.4	78.3 ± 13.5

At increased level of NS (153.6 ± 1.9 g.kg^{-1} DM) in TMR with degradability of 57.1 ± 4.8 % the mean level of NH_3 in the rumen was 28.6 ± 2.1 mg.100 ml^{-1} and of urea 5.8 ± 0.5 mmol.l^{-1}. Increased intake of degradable NS and increase in rumen NH_3 level and serum urea increase demand on energy and load on the liver and decrease reproductive parameters of the herd. Synthesis and concentration of urea and its recycling is a function of total intake of NS in TMR, ratio of fermentation of saccharides and degradation of feed proteins and rumen transformation for microbial protein synthesis. Urea synthesis varies within the range of 40–70 % of N intake. At mean level of N intake 588–618 g.day^{-1}, the accepted N supports urea synthesis within the range of 44–58 % and urea recycling at the level of 30–43 % (3).

Table 3. The influence of standard intake of NS on transformation of proteins in cows at the peak of lactation

Parameter	CP g.kg^{-1} DM	Degrad. CP %	NH_3 mg.100 ml^{-1}	Urea mmol.l^{-1}	Creatinine µmol.l^{-1}
TMR 1	154.9	50.3	29.6	6.3	70.8
TMR 2	150.9	60.8	25.7	5.9	75.1
TMR 3	155.1	60.2	30.6	5.1	93.7
X ± STD	153.6 ± 1.9	57.1 ± 4.8	28.6 ± 2.1	5.8 ± 0.5	79.9 ± 9.9

In conclusion, the results of examinations and analysis of the level of NS in rations evaluated in relation to NH_3 rumen level and markers of blood serum proteins metabolism in cows in the 1st stage of lactation constitute a model of diagnostic testing of the level of protein nutrition.

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COMPARISON OF DIGESTIBILITY OF NUTRIENTS IN FEEDSTUFFS AND TMR DETERMINED BY *IN VIVO* AND *IN VITRO* METHODS

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ABSTRACT

The study investigated the most frequently used concentrate protein and protein-energetic feeds and total mixed rations (TMR) for dairy cows in the 1st stage of lactation. The *in vivo* and *in vitro* methods used showed high variability of degradability of nitrogen substances (NS) and digestibility of neutral detergent fibres (NDF). Mean degradability of NS in concentrate feeds determined *in vivo* reached 58.44 ± 13.16 % while *in vitro* method provided higher results (mean = 51.84 ± 14.35 %). Mean digestibility of NDF in TMR determined *in vivo* reached 37.7 ± 6.57 % in comparison with NDF digestibility determined *in vitro* at the level of 24.9 ± 4.78 %. The mean degradability of NS in TMR determined *in vivo* was higher (53.3 ± 8.46 %) than that determined *in vitro* (49.5 ± 6.34 %).

Key words: ANKOM; SP degradability; daisy; *in vitro*; *in vivo*; NDF digestibility

INTRODUCTION

The quality of forage and total mixed rations (TMR), affected by proportion and digestibility of neutral detergent fibres (NDF), limits the usability of energy. Low digestibility of NDF at lower limit (25 %) of recommended range requires compensation of energy for production of milk in the form of increased proportion of concentrate feed. High digestibility of NDF of forage increases total dry matter (DM) intake in rations and voracity of cows which supports milk synthesis. Accurate determination of degradation of proteins in the rumen is used to formulate rations for cows in the 1st stage of lactation. Degradation of proteins is determined most frequently by *in vivo* method but also by *in vitro* method by means of an Ankom Daisy^{II} fermentor.

The aim of the study was to compare *in vitro* and *in vivo* methods for determination of degradability of NS and digestibility of NDF in protein-energy concentrated feeds and TMR for dairy cows in the first stage of lactation.

MATERIALS AND METHODS

Chemical analysis of forage and TMR

We analysed samples of protein-energy feeds (maize gluten, maize sprouts, maize pulp, malt culms), concentrate protein feeds (sunflower oil cake, extract soybean meal, extract rape meal), 15 samples of TMR and non-digested residue after incubation, by the methods according to Commission Regulation (EC) No. 152/2009 of January 2009, laying down the methods of sampling and analysis for the official control of feed, for the following parameters: DM, NS, crude fat, NDF and acid detergent fibre (ADF).

In vivo degradability of NS and digestibility of NDF

The samples of feeds and TMR were exposed to varying rumen incubation according to the type of feed and investigated nutrient by introducing them into the rumen of fistulated cows after morning feeding (the cows were offered rations containing 60 % bulk feed and 40 % concentrate feed in TMR DM). The time of incubation of concentrate feeds for determination of NS degradation was set to 12 h (1, 2). TMR samples were incubated for 16 h when determining degradability of NS and for 30 h when determining degradability of NDF. *In vitro* degradability of NS and digestibility of NDF

The *in vitro* method for determination of NS degradability and NDF digestibility in feed is based on the ANKOM Daisy^{II} fermentor, an incubator with rumen fluid and solid feed particles, i. e. partially digested feed from the rumen with adhered bacteria. This rumen fluid together with solid particles was taken from cows that were offered the same ration as with the *in vivo* method. The samples were incubated simultaneously with the *in vivo* tested samples and for the same time but with adequate time delay needed for final preparation of the incubation solution. Feeds and TMR were milled to the size of particles of 1 mm and approximately 5 g samples were weighed into bags of size 5 × 10 cm (AncomR510, pore size 50 µm), the same for both rumen *in vivo* incubation and *in vitro* incubation in Daisy^{II}.

Table 1. Degradability of CP and digestibility of NDF after *in vitro* and *in vivo* incubation (n = 15)

TMR	Degradability of CP (%) 16-hour incubation			Digestibility of NDF (%) 30-hour incubation		
	In vitro (A)	*In vivo* (B)	Diff. B–A	*In vitro* (A)	*In vivo* (B)	Diff. B-A
20/2012	46.2	65.5	19.3	18.8	27.3	8.5
48/2012	48.1	51.0	2.9	20.7	32.6	11.9
57/2012	41.2	55.7	14.5	26.2	34.9	8.7
87/2012	54.1	67.9	13.9	25.9	40.2	14.3
89/2012	55.4	66.0	10.6	31.5	47.1	15.6
5/2011	53.5	49.2	–4.4			
13/2011	44.8	51.1	6.4	31.9	39.3	7.5
24/2011	50.4	50.3	–0.1	19.5	33.1	13.6
40/2011	40.4	42.9	2.6	29.5	42.3	12.8
93/2011	44.6	42.9	–1.7	18.5	26.8	8.4
103/2011	63.2	60.9	–2.4	23.2	35.3	12.1
112/2011	53.6	54.6	1.0	26.7	42.1	15.3
124/2011	55.6	51.0	–4.6	25.0	42.8	17.8
176/2011	43.9	41.4	–2.5	21.4	36.1	14.7
179/2011	48.0	49.5	1.5	30.9	48.2	17.3
Mean ± STD	**49.51 ± 6.34**	**53.31 ± 8.46**	**3.80**	**24.97 ± 4.78**	**37.72 ± 6.57**	**12.75**

RESULTS AND DISCUSSION

The mean *in vivo* degradability of NS in investigated TMRs was 53.31 ± 8.46 % (41.4–67.9 %) while the mean *in vitro* degradability was 49.51 ± 6.34 % and the variability of *in vitro* values was lower (40.4–63.2 %). Higher *in vitro* decrease in NS compared to *in vivo* method was observed in 40 % of the investigated TMRs (Table 1).

With regard to NDF, all TMRs showed higher digestibility after *in vivo* incubation. The mean difference was 12.75 % and the difference between methods ranged from 7.5 % to 17.8 %. The mean *in vivo* digestibility of NDF was 37.72 ± 6.57 % with variations from 26.1 % to 48.2 %. The mean *in vitro* digestibility of NDF was low (24.97 ± 4.78 %; range 18.5–31.9 %). Of the examined feeds the highest degradability of NS was determined for malt culms by the *in vivo* method (77.42 %) and similar level of degradability was detected *in vitro* (72.52 %). The lowest degradability of NL was determined for maize gluten by both methods *in vivo* (40.37 %) and *in vitro* (34.60 %), although with higher difference between both methods. In our experiment we determined degradability of NS in three different samples of extract soybean meal. The mean degradability *in vivo* was 45.20 % (46.31 %, 42.82 % and 46.46 %) and *in vitro* 38.30 % (43.31 %, 34.38 % and 37.19 %).

The time of incubation of samples of concentrate feeds for determination of degradability of NS was set to 12 hours (1, 2). TMR samples were incubated for 16 hours for determination of degradability of NL and for 30 hours for determination of digestibility of NDF in the rumen. In lactating cows fed 3–4 times daily the feed is retained in the rumen for approximately 30 hours (3).

In conclusion, the differences in degradability of NS in individual concentrate feeds between the two methods ranged from 1.73 % (sunflower oil cake) to 12.57 % (DDGS II) with the mean of 6.6 %, which confirmed the difference between results of degradability of NS of concentrate feeds determined by the two compared methods. Higher level of degradation of NS in concentrate feeds was observed in all cases with the *in vivo* method. Congruence of results of degradability of NS in TMRs determined by both methods was observed in 73 % of the total of 15 TMR samples. Evaluation of digestibility of NDF in the rumen by the two methods showed considerable differences when comparing individual TMRs and also mean values of all investigated TMRs (24.97 ± 4.78 % *in vitro* compared to 37.72 ± 6.57 % *in vivo*).

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QUALITY ASSESSMENT OF MAIZE SILAGE ACCORDING TO FIBRE DIGESTIBILITY

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ABSTRACT

The aim of the study was to evaluate the effect of the content of neutral detergent fiber (NDF) and its digestibility (NDFD) and energy value (NEL) of selected forage. We confirmed the negative relationship between NDF content and energy value of corn silage ($r^2 = 0.76$). A positive effect on the energy value of feed was recorded in digestible fibre, described for silage ($r^2 = 0.40$) by coefficients of determination.

Key words: corn silage; digestibility; fibre; NDF

INTRODUCTION

At present, when the performance in dairy cattle breeding is increasing there is a need to deal with the content and composition of dietary fibre which is usually neglected as energy source. This can be resolved by evaluating the quality of the fibre (3). Two groups of feed were studied with maize silage as a crucial component of carbohydrate ration for cattle (2). NDF content in both maize silages varied over a wide range, from 200 to 550 $g.kg^{-1}$ DM (10), therefore it is necessary to evaluate NDF from both the quantitative and qualitative aspects (3). Many authors (1, 6, 7, 8) considered very important to evaluate the quality of roughage on the digestibility of NDF.

The aim of this study was to determine the level of NDF digestibility (NDFD) and how the NDFD affects the energy value of feed.

MATERIALS AND METHODS

We examined 21 samples of maize silage collected randomly from dairy farms. They were processed immediately and analysed for dry matter (DM), ash, organic matter (OM), fat, crude protein (CP), fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) nitrogen-free extract (NFE) and digestibility of dry matter (DMD), organic matter (OMD) and NDF (NDFD). The results obtained were used to predict the net energy of lactation (NEL) (6).

CF and its fractions, NDF and ADF, were determined using a unit Fibre Analyzer 220 and digestibility of DM, OM and NDF by means of Daisy II Incubator (9).

RESULTS AND DISCUSSION

Table 1 shows nutrient composition, *in vitro* digestibility and energy level of the examined feed. The mean DM in original mass of corn silage was 336 $g.kg^{-1}$. The obtained results were then used to predict the energy value of silage (NEL) according to (6), which reached on average 6.89 $MJ.kg^{-1}$ DM.

The composition of maize silage was compared with tabulated values (11). It was found that the mean content of fibre and fat was lower which naturally resulted in a higher content of NFE. The NEL corresponded to the tabulated value. The values of NDF, ADF and CP were lower than the ones recommended by NRC (5) while the NEL value was slightly higher.

It is know that fibre digestibility is negatively affected especially by ADF fractions. It was found that NDF level negatively affected the energy value of maize silage (Table 2).

Table 1. Chemical composition, *in vitro* digestibility and energy value of corn silage

Parameters	Maize silage (n = 21)		
	Mean	Range	STD
Chemical content (g.kg^{-1} DM)			
Dry matter in the original mass	336	195–432	1.28
Dry matter (DM)	1000	-	-
Organic matter (OM)	956	893–968	0.34
Crude protein (CP)	75	43–92	0.23
Ether extract – fat	31	27–37	0.06
Crude fibre (CF)	190	158–250	0.53
Neutral detergent fibre (NDF)	393	341–489	0.85
Acid detergent fibre (ADF)	220	179–297	0.59
Nitrogen-free extract (NFE)	659	588–711	0.70
Ash	44	32–108	0.34
***In vitro* digestibility (g.kg^{-1} DM)**			
DMD	755	627–837	1.02
OMD	718	567–817	1.36
***In vitro* digestibility (g.kg^{-1} NDF)**			
NDFD	379	300–500	1.33
NEL (MJ.kg^{-1} DM)	6.89	5.28–8.07	0.15

Digestible NDF affected positively the energy value of the analysed maize silage (Table 2).

Based on the results it is evident that in practice it is possible to find a great variability in the chemical composition of feed including NDF digestibility. Therefore we agree with Mertens (4) that the rations should be formulated first according to NDF content and NDFD should be used only for their tuning.

Table 2. Relationship between the content of NDF or NDF digestibility and the values of NEL (0.95 confidence interval)

NDF : NEL	NEL = 13.0228–0.0156x r = -0.869; p = 0.0000; r^2 = 0.7689
NDFD : NEL	NEL = 4.1247 + 0.007x r = 0.6388; p = 0.0018; r^2 = 0.4081

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EFFECT OF PARENTERAL APPLICATION OF SELENIUM AND VITAMIN E ON HEALTH OF MAMMARY GLAND IN DAIRY COWS DURING THE PERIPARTUM PERIOD

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ABSTRACT

From the herd of 120 Holstein cows we selected at random 36 cows and divided them to control (C) and two experimental groups (D1 and D2), 12 animals in each group. Blood samples were collected from *vena jugularis* approximately 14 days before calving (control sampling), and 3, 12 and 22 days after calving. On the day of control sampling, (14 days before calving) we administered subcutaneously to cows of D1 group Selevit *inj. a. u. v.* and Erevit 300 *sol. inj. a. u. v.* at a dose of 44 mg Se per cow and 1000 IU α-Toc per cow. Cows of group D2 were injected the same preparations at doses of 22 mg Se and 1000 IU α-Toc per cow. Changes in Se level and activity of glutathione peroxidise (GSH-Px) in blood of cows from group D1 were observed on days 3 and 12 after calving. In group D2 there were changes in the activity of GSH-Px on day 3 after calving. Clinical mastitis (CM) in D1 group was detected in 25% of cows and in D2 group in 33.3% of cows (the same as in control cows.

Key words: dairy cows; glutathione peroxidase; mastitis; selenium; vitamin E

INTRODUCTION

Some of the most effective antioxidant nutrients that are frequently deficient in mixed feed are selenium (Se) and vitamin E (α-Toc). Injection administration of synthetic forms of Se and α-Toc appears to be the most effective way how to satisfy acute requirements of dairy cows on both antioxidants, particularly during the peripartum period, when even the peroral supplementation cannot increase their reduced plasma level in dairy cows (3).

The aim of the present study was to observe the influence of a single parenteral administration of Se and α-Toc 14 days before parturition on occurrence of inflammation of the mammary gland and concentration of serum Se and activity of glutathione peroxidise (GSH-Px) in the blood of cows in the postpartum period.

MATERIALS AND METHODS

The study was conducted in a herd of Holstein cows that consisted approximately 120 animals. They were milked in a milking parlour Westfalia tandem 2×4 (Bönen, Germany), twice a day. All dairy cows obtained feed with the content of selenium up to 0.1 mg.kg^{-1} dry matter (DM). Fourteen days before calving we randomly selected 36 cows and divided them to 3 groups, 12 cows in each, one control group (C) and two experimental groups, (first group D1; second group D2). At 14 days before expected parturition, we collected feed samples (250 g) and 15 ml samples of blood from *vena jugularis* from all cows. Plasma and blood serum were obtained by the procedure of Kovacik *et al.* (2). Concentration of Se in rations and in blood serum was determined using procedure of Bax *et al.* (1). Activity of GSH-Px in the blood was determined by means of **Glutathione peroxidase assay kits (**Randox-Ransel, UK) using procedure according to Paglia and Valentine (4). After collection of blood samples (14 days before expected parturition) the first group of cows (D1) was administered subcutaneously a single dose of Selevit inj. a. u. v. (Biotika a. s., Slovakia), 20 ml per head, together with preparation Erevit 300 sol. inj. at a dose of 1.7 ml per head (Biotika a.s., Slovakia), i. e. 44 mg Se and 100 IU α-Toc per cow. Cows in the second group (D2) were administered subcutaneously a single dose of Selevit inj. a. u. v., 10 ml per head and Erevit 300 sol. inj. at a dose of 3 ml per head, i. e. 22 mg Se and 1000 IU α-Toc per cow. Control group (C) was not administered any preparations. The level of Se, activity of GSH-Px in the blood and health of the mammary gland was determined on three occasions: on day 3, 12 and 22 after calving and each cow was subjected to

complex examination for potential inflammation of the mammary gland according to Vasil (5).

One-way analyses of variance (ANOVA) with the *post hoc* Dunett's multiple comparison test was used to perform statistical analysis of all studied indices in rows (group relation). The results are presented as means ± SD.

RESULTS AND DISCUSSION

On day 22 after parturition we detected mastitis at the level of 33.3 % in the control (C) and 2nd experimental group (D2), but only at the level of 25 % in the 1st experimental group (D1).

Table 1. The effect of parenteral administration of Se and α-Toc on the reduction of clinical mastitis in dairy cows during 22 days after calving

Group	Doses Se/α Toc mg.cow^{-1}	Number of infected quarters	Occurrence of CM in group (%)
D1	44/1000	7	25
D2	22/1000	10	33.3
C		11	33.3

CM – clinical mastitis

In comparison with the C group, after parenteral administration of Se and α-Toc on day 14 before parturition, we recorded higher concentration of Se ($P < 0.01$) in blood serum of dairy cows from D1 group, namely in samples collected on days 3 and 12 after calving.

Table 2. The effect of parenteral administration of Se and α-Toc to dairy cows on mean level of Se (μmol.l^{-1}) in blood serum of cows before and after parturition

Group	C	D2	D1
Day 14 b. p.	0.777 ± 0.099	0.770 ± 0.071	0.820 ± 0.094
Day 3 a. p.	0.774 ± 0.077^{a}	0.891 ± 0.110	1.104 ± 0.123^{B}
Day 12 a. p.	0.764 ± 0.079^{a}	0.860 ± 0.051	1.081 ± 0.166^{B}
Day 22 a. p.	0.819 ± 0.057	0.781 ± 0.091	0.854 ± 0.165

b. p. – before parturition; a. p. – after parturition
$^{a\ B}$ – significant at $P < 0.01$

In comparison with the control group C, parenteral administration of Se and α-Toc resulted in increased mean activity of GSH-Px in the blood of cows in group D2 on day 3 ($P < 0.01$) and days 12–14 ($P < 0.001$) and in group D2 on day 3 after calving ($P < 0.01$).

Table 3. The effect of parenteral administration of Se and -Toc to dairy cows on mean concentrations of GSH-Px (μkat.l^{-1}) in blood before and after parturition

Group	C	D2	D1
Day 14 b. p.	771.6 ± 96.90	764.4 ± 66.55	769.0 ± 122.9
Day 3 a. p.	731.2 ± 105.6^{a}	899.7 ± 90.55^{b}	901.5 ± 88.93^{B}
Day 12 a. p.	696.0 ± 74.49^{a}	770.0 ± 66.10	845.7 ± 60.88^{b}
Day 22 a. p.	692.4 ± 66.62	766.7 ± 62.68	808.2 ± 90.59

b. p. – before parturition; a. p. – after parturition
$^{a\ B}$ – significant at $P < 0.01$; ^{ab} – significant at $P < 0.05$

Parenteral administration of synthetic forms of Se and α-Toc to dairy cows in the dry period is one of the ways how to prevent their deficit related to supplied feed rations and ensure adequate concentration of Se and activity of GSH-Px in the blood and thus increase the natural resistance of dairy cows to intramammary infections.

ACKNOWLEDGEMENT

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INFLUENCE OF UREA-FORTIFIED WHEAT FLAKES ON RUMEN FERMENTATION

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ABSTRACT

In feeding experiments wheat flakes enriched with urea were monitored in relation to productive indicators, level of fatty acids in the rumen, rumen fluid pH, ammonia level and representation of ciliates. Addition of wheat flakes did not affect significantly the total production of fatty acids or proportion of individual fatty acids. Yet it turned out that it supported proteosynthetic activity of micro-organisms due to higher intensity of fermentation processes and higher level of ammonia in the rumen fluid.

Key words: ammonium; dairy cows; fermentation; acids; proteosynthetic intensity; wheat flakes; urea

INTRODUCTION

Experiments were carried out investigating effect of wheat flakes fortified with urea on rumen carbohydrate metabolism and acids produced by fermentation. The production and proportion of acids gives a good picture of the course of fermentation, the activity of micro organisms and their species representation (1, 2). The populations of rumen micro-organisms are influenced mostly by foodstuffs and their nutrients that are the source of building material and energy for rumen micro-organisms which then provide the host animal the proteins and energy is contained in the resulting fermentation acids. The rumen microbes ferment carbohydrates to make volatile fatty acids which are the major source of energy for the animal.

The aim of this research was to determine the status and progress of the rumen metabolism of carbohydrates based on the composition of acids produced by fermentation and on pH of the rumen fluid.

MATERIALS AND METHODS

During the experiment we sampled and analysed rumen fluid obtained from two groups (control and experimental) of monitored cows. Rumen fluids were collected after the start of the second month. Each group consisted of 10 cows that formed a representative sample group in terms of milk yield and stage of lactation.

Rumen fluid samples were obtained by means of esophageal probe. The rumen fluid was examined for pH, total acids produced by fermentation, proportion of acetic acid, propionic acid and butyric acid, ammonia level and the number of ciliates using the common laboratory methods of analysis. The acids were determined by gas chromatography, ammonium by Kjeldahl and the number of ciliates by microbial monitoring in a Gerber chamber.

The cows were fed total mixed rations (TMR) with total amount calculated per cow equal to 47.44 kg and dry matter (DM) content 54.75 %. The content of crude protein (CP) calculated per DM was 18.63 % and non-degradable protein from CP reached 26.06 %. The energy level was 7.108 MJ net energy . kg DM^{-1}. The level of acid detergent fibre (ADF) was 17.56 % and of neutral detergent fibre (NDF) 29.53 %. In the experimental group part of the wheat meal and urea in a batch was replaced by the equivalent amount of urea-fortified wheat flakes (produced by the company ADW AGRO, Krahulov, CR) so that even after the treatment the experimental and control rations were balanced with regard to net energy and CP. The mean feed intake of cows was 3.08 % DM of body weight.

RESULTS AND DISCUSSION

The inspection showed that the raw milk was of high quality (milk intake Creamery Messwerte Romilch).

Comparison of parameters of the rumen fluid between both groups failed to show any significant differences. The values were within normal physiological range.

Nevertheless, it is possible to note some differences which may indicate differences in the use of feed nutrients by rumen micro-organisms. The levels of organic acids produced in the rumen indicate progressive fermentation processes in the rumen of dairy cows providing higher level of energy available for proteosynthetic activity for the production of microbial biomass. The same applies to the level of NH_3.

According to the proportion of acetic acid produced by

Table 1. Mean values of cows from the examined groups (n = 10)

Group	Acids g.100g^{-1}	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)	pH	NH_3 mmol	Ciliates 100.ml^{-1}
Exper.	0.802	57.1	24.5	18.4	6.10	11.06	355.2
Control	0.736	58.9	22.7	18.0	6.30	9.56	289.2

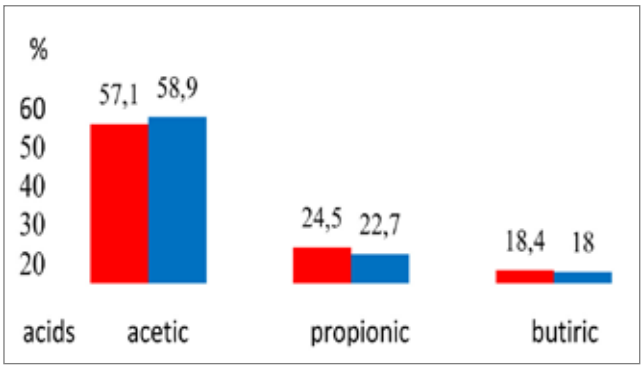

Fig. 1. Proportion of fatty acids in the rumen fluid in % of Σ acids

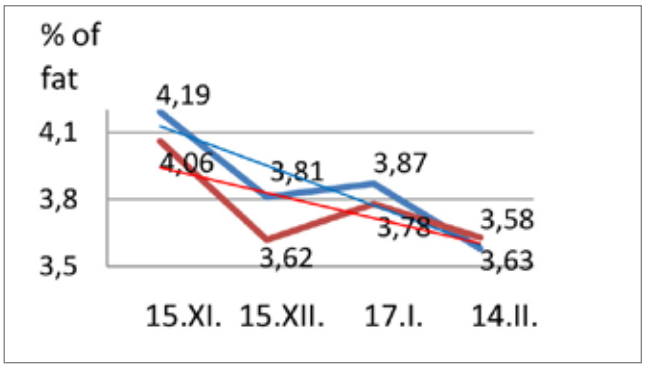

Fig. 2. Comparison of fat content of milk

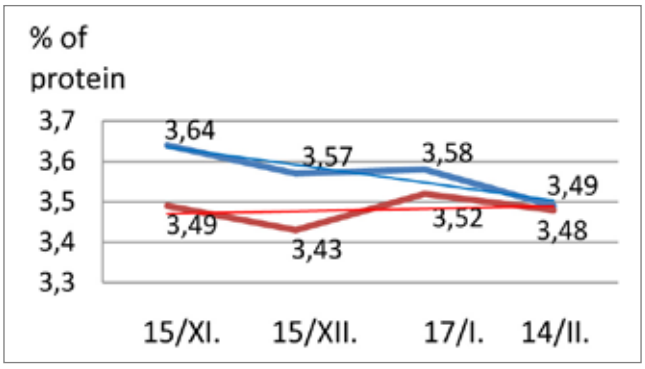

Fig. 3. Comparison of protein of milk

fermentation it was possible to evaluate the effect of the acid on formation of fat in mammary gland. Fig. 2. shows the differences in the fat content of milk between the compared groups of cows. Interestingly it shows declining trend in fat content. It is evident that the decrease in fat content in the experimental group was more gradual. The differences were insignificant and did not affect the quality of milk delivered to dairies.

Interesting is the content of propionic acid in the rumen fluid. Considering the fact that propionic acid is a precursor of milk proteins, the milk protein content in the experimental group should be higher. However, according to Fig. 2, the protein level in the experimental group, expressed in %, was lower. The difference was insignificant.

When evaluating the pH of the rumen fluid we observed a difference of about 0.1. Production of acetic acid in the experimental group was lower and production of propionic acid was higher. This may have some, although small, effect on the composition of rumen micro-organisms to the detriment of cellulolytic micro-organisms, because different levels of fatty acid could cause a decrease in pH of the rumen fluid. However, when taking into account the higher proportion of infusoria in rumen contents of cows from the experimental group, we can say that the environment of the rumen of dairy cows was not only in normal physiological range, but was more favourable than in the cows from the control group.

CONCLUSIONS

The results of our experiments allowed us to draw the following conclusions that are of general validity:

- the use of wheat flakes fortified with urea can be recommended for practical applications in the nutrition of dairy cows,
- wheat flakes enriched with urea promote fermentation in the rumen,
- more intensive fermentation will provide more free energy for microbial activity,
- wheat flakes regulate metabolic processes in the rumen of cows resulting in better conditions for proteosynthetic activity of rumen micro-organisms.

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DYNAMICS OF NUTRITIONAL VALUE OF GRAZING ON DRY LIMESTONE SLOPES IN MORAVIAN KARST

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ABSTRACT

Dynamics of nutritional value of grazing (crude protein, crude fibre, net energy NEL, PDI, Ca, P, Na, K and Mg) was evaluated on the dry limestone slopes of the Moravian Karst in 2009. There were differences in nutritional value between grass stand on the xerophytic and mezoxerophytic sites, with the content of energy and nutrients is able to meet the requirements of reactive ewes grazing here. Excess of minerals Ca and K and deficit of Na were observed.

Key words: grazing sheep; Moravian Karst; nutritional value; pasture stand; protected area

INTRODUCTION

Moravian Karst is the largest karst territory of Czech highlands. There are located approximately 1,000 caves. The mission of the protected area is to maintain all unique unanimated and animated nature and cultural and technical heritages to develop sustainable forms of economic exploitation of the region. An important role in the fulfilment of this mission plays grassland. The area in I, II, and III protected zone of the Moravian Karst is 144.9 ha, 274.5 ha and 226.2 ha, which is 68.7 %, 57.9 % and 7.5 %, resp., of agricultural land belonging to the appropriate zone. It seems that grazing is the optimal form of management of these areas (5). In contrast to the cultural pastures, there may be problems in evaluating their nutritional value. When the non-productive role of grasslands is preferred the grazing does not occur in the optimal time, higher production of biomass is usually accompanied by a sharp decline in its quality. The aim of the study was to observe the dynamics of the nutritional value of grassland in this unique biotope with regards to grazing animals.

MATERIALS AND METHODS

Several grassland were selected in the locality of the Moravian Karst in dry limestone slopes around the village Vilemovice. They were situated at an altitude of 300–500 m, exposure to E – W. Areas were derelict about 15 years and consequent succession could be seen. In 2005 project for restoring the grassland have started, which have been funded by the EU LIFE. The grassland were grazed at once in summer and autumn months by Romanov breed sheep (in 2009 for fourth year). For monitoring the following areas were selected:

- good covered grassland (Mezox LV – seminatural mezoxerophytic grassland),
- grassland with protruded bedrock (Xer V – seminatural xerophytic grassland).

Contents of dry matter (DM), crude fibre (CF), crude protein (CP), fat, ash, Ca, P, Na, K and Mg were determined (1). Gross energy (GE), metabolizable energy (ME), net energy of lactation (NEL), actually digestible crude protein in the small intestine (PDIN and PDIE) were calculated using regression equations (3, 4).

RESULTS AND DISCUSSION

There were differences between these areas in fibre content. They were exposed to a lack of moisture and therefore reliant on atmospheric precipitation (Tab. 1). Nevertheless, forage from xerophytic stands (Xer V) contained less fibre, with higher dynamics in its content than forage from mezoxerophytic stands (Mezox V), which were relatively better supplied with moisture. This was strongly influenced by the species composition of the stands – mainly grass dominated at stands Mezox V. The actual increase of the fibre content is not the primary problem at the sheep grazing. Sheep re-

Table 1. Content of dry matter, nutrients and energy in the pasture stand Vilémovice in 2009

The content of energy and nutrients in dry matter											
Month of the year	**DM %**	**CP %**	**CF %**	**NEL MJ.kg^{-1}**	**PDIN MJ.kg^{-1}**	**PDIE MJ.kg^{-1}**	**Ca g.kg^{-1}**	**P g.kg^{-1}**	**Na g.kg^{-1}**	**K g.kg^{-1}**	**Mg g.kg^{-1}**
Vilémovice – **Xer V**[1)]											
5	37.58	12.79	22.51	6.33	82.6	85.2	9.10	1.77	0.13	14.38	1.33
6	37.65	12.27	21.78	6.22	79.2	97.3	11.20	1.47	0.10	9.39	1.59
7	32.67	12.80	20.76	6.11	82.7	101.0	12.33	1.73	0.06	14.93	2.03
8	42.77	10.57	24.79	6.12	68.2	87.3	16.33	1.28	0.12	9.00	2.12
9	57.58	8.14	25.52	6.01	52.6	75.4	12.03	1.06	0.21	8.09	1.55
Vilémovice – **Mezox V**[2)]											
5	39.70	13.19	20.43	6.11	85.0	82.6	7.10	1.38	0.14	15.82	1.07
6	40.94	9.93	28.27	5.78	64.1	80.7	7.95	1.03	0.17	11.07	1.23
7	34.33	11.92	26.45	5.99	77.0	92.0	10.83	1.35	0.18	15.98	1.48
8	38.66	11.71	27.93	5.69	75.7	89.6	8.40	1.25	0.16	11.71	1.41
9	51.39	7.90	28.78	5.37	51.0	70.8	9.27	0.88	0.29	10.08	1.40

Xer V[1)] – semi-natural xerophytic grass stand in Vilémovice – once grazing
Mezox V[2)] – semi-natural mezoxerophytic grass stand in Vilémovice – once grazing

quire a relatively high content of fibre. Ewe at weight 60 kg needs 36.5 % of dry matter (2). The main problem is that with increasing content of fibre the nutrient digestibility and concentration of energy decrease. It is therefore necessary to use for grazing in these biotopes animals with lower requirements of these nutrients such are infertile ewes. Their requirements of the PDI, fibre and NEL on these grasslands were satisfied.

Very low profile of humus layer is characteristic for biotopes in Vilémovice and in many places the limestone bedrock come to the surface, which affects the species composition of vegetation and consequently the content of mineral nutrients in the species. Significantly higher levels of Ca were observed at the area Xer V and there was also significantly higher proportion of herbs (opposite the area Mezox V average of 3.79 g Ca per kg dry matter). For the other elements there were not so high differences between the evaluated areas. It is essential that the concentration of Ca, Na and K in dry forages did not meet the demand of the model animal – ewe 60 kg (2). The average content of Ca and K were higher from 3.6 to 5.1x (normalized over the need), while the average content of Na filled the need for only 0.1 to 0.16.

CONCLUSIONS

Protected biotopes must be evaluated not only in terms of non-production, but also in terms of production, taking into account all the characteristics of the biotope when grazing is used for their maintenance management.

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OCCURRENCE OF MYCOTOXINS IN FRESH AND CONSERVED ALFALFA

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ABSTRACT

Occurrence and concentration of some mycotoxins in fresh alfalfa and alfalfa silages as well as monitoring the effect of addition of biological additive on the presence and concentrations of mycotoxins was analyzed. We conserved wilted alfalfa harvested in the vegetation stage before flowering with biological additive (*Lactobacillus plantarum* 2×10^8 CFU.g^{-1}) in a granular form using application dose 0.5 kg.t^{-1}. The samples of fresh alfalfa and alfalfa silages were examined for total fumonisins, zearalenone, deoxynivalenol, T-2 toxin and total ochratoxins by immunoenzymatic analysis using ELISA Reader (NOACK, SR). In the fresh alfalfa silages we found the highest concentration of zearalenone followed by deoxynivalenol and T-2 toxin. Application of biological additives affected positively only the level of total fumonisins ($P < 0.05$).

Key words: alfalfa; biological additive; mycotoxins; silage

INTRODUCTION

Production and quality of milk is influenced by many endogenous and exogenous factors. One of the limiting factors is nutrition (1, 10). Quality of feed of dairy cows is most important not only in terms of nutrition but also from the hygiene point of view. Potential threat to health and production arises from feedstuffs contaminated with filamentous microscopic fungi and their low molecular secondary metabolites – mycotoxins (4, 11).

The aim of this study was to detect the occurrence and concentration of mycotoxins in fresh alfalfa and alfalfa silage as well as the impact of the addition of biological additive on the occurrence and concentration of the mycotoxins studied.

MATERIALS AND METHODS

Under experimental conditions we ensiled alfalfa (*Medicago sativa L.*) that was harvested before flowering and wilted for 48 h, without (control variant C) and with a biological additive (*Lactobacillus plantarum* 2×10^8 CFU.g^{-1}) (variant A) at a dose 0.5 kg.t^{-1}. We conserved the wilted alfalfa in an ensiling unit with the capacity of 4 dm^3. In mean samples of fresh alfalfa and alfalfa silages (8 weeks of fermentation) we determined the content of the following mycotoxins: FUM – total fumonisins; ZEA – zearalenone; DON – deoxynivalenol; T-2 – T-2 toxin; OTA – total ochratoxins. For the screening determination of content of monitored mycotoxins we used immunoenzymatic analysis using ELISA Reader (NOACK, SR).

RESULTS AND DISCUSSION

At harvest time, alfalfa will contain 205.9 g.kg^{-1} dry mater the content of which depends mainly on the phenological phase of harvesting and on climate conditions (8). Dry matter of investigated alfalfa silages was 470.1 (C) and 453.6 g.kg^{-1} (A). All samples of fresh alfalfa and alfalfa silages were contaminated with the analyzed mycotoxins with the highest concentration of ZEA, followed by DON and T-2 toxin. Silages of the variant A had higher concentration of ZEA in comparison with control ($P < 0.05$). Silages in our experiment had lower concentration of ZEA than those investigated by Nedělník and Moravcová (9). DON and ZEA are the major mycotoxins formed in silages (5). DON causes diarrhoea and vomiting (12), stagnant milk production and reproductive disorders (6). T-2 toxin reduces immunity and induces blood coagulation disorders and haemorrhages (13). Our results did not confirm previous findings according to

which selected strains of lactic acid bacteria are able to reduce of T-2 toxin and DON (3). Silages of the variant A exhibited higher level of OTA in comparison with control silages ($P < 0.05$). OTA damages kidneys and liver and has teratogenic and carcinogenic effects (7). According to Cabo *et al.* (2) several lactic acid bacteria exhibit potential antimycotic activity against *Penicillium*. The biological additive applied in our experiment positively affected the level of FUM ($P < 0.05$).

Table 1. Comparison of mycotoxin content in fresh alfalfa and alfalfa silage

µg.kg^{-1}	FUM	ZEA	T-2	DON	OTA
		Fresh Alfalfa			
$\overline{x}$	4.785^{c}	368.1^{de}	72.05	365	9.65^{fg}
S.D	0.021	0.707	2.616	16.688	0.212
v	0.443	0.192	3.631	4.572	2.198
		Alfalfa silage C			
$\overline{x}$	7.45^{ac}	389.7^{bd}	73.95	379.2	12.85^{f}
S.D	0.495	3.253	0.495	1.556	0.636
v	6.644	0.835	0.669	0.410	4.952
		Alfalfa silage A			
$\overline{x}$	4.335^{a}	412.15^{be}	73.95	377.25	14.2^{g}
S.D	0.045	0,778	3.606	9.405	0.707
v	0.011	0.002	4.877	2.493	4.980

C – control group; A – biological additive; FUM – total fumonisins ZEA – zearalenone; T-2 – T-2 toxin; DON – deoxynivalenol OTA – total ochratoxins; values with the same superscript in the column differed significantly at $P < 0.05$

CONCLUSIONS

All investigated mycotoxins were found in fresh alfalfa and alfalfa silages. ZEA was the secondary metabolite of microscopic fungi present in the highest concentration, followed by DON, T-2 toxin, total OTA and the lowest concentration was determined for total FUM. Application of the biological additive (*Lact. plantarum*) decreased only the concentration of FUM ($P < 0.05$).

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EFFECT OF GRASS SPECIES ON THE NEUTRAL DETERGENT FIBRE QUALITY OF SILAGES AND ITS PREDICTION

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ABSTRACT

The aim of this study was to compare the effect of grass species on chemical composition and quality parameters of neutral detergent fibre (NDF) of grass silages. The potentially degradable fraction of NDF (*b*), rate of NDF degradation (*c*), effective degradability of NDF (ED_{NDF}) and indigestible NDF (INDF) were evaluated as quality parameters of NDF. The data were used for testing of prediction options of ED_{NDF} and INDF. The highest crude protein (CP) content ($P < 0.05$) was detected for fescue grass. Rye grass had the lowest content of NDF and acid detergent lignin (ADL) ($P < 0.05$). The highest values for parameter *b*, *c*, ED_{NDF} and therewithal the lowest INDF were found for rye grass ($P < 0.05$). The ADL was the best single predictor using set of all tested silages (R^2-values of 0.433 (ED_{NDF}) and 0.636 (INDF)). Using two predictors, the accuracy level increased. The combination of CP and ADL gave R^2-values 0.597 and 0.808 for ED_{NDF} and INDF, respectively. However, better prediction equations were obtained using data for each grass species separately.

Key words: degradability; grass silages; indigestible fibre; prediction equations

INTRODUCTION

The cell wall carbohydrates can be quantified by determination of neutral detergent fibre (NDF) (7). Knowledge about digestibility of NDF in forage is critical for effective ruminant feeding (5). The *in situ* nylon bag technique is the most common method used to estimate NDF digestion. Indigestible NDF (INDF) content presents an important indicator of quality of grass cell wall carbohydrates and is a part of the forage cell wall unavailable to microbial digestion in ruminants, even if total tract residence time of fibre could be extended to infinite time (2). INDF is the non-digestible part of NDF. Methods for determination of effective degradability and INDF are time consuming and expensive. Prediction equations, based on basic parameters of chemical analysis, are cheaper and faster.

The aim of this study was to compare the effect of grass species on chemical composition and quality parameters of neutral detergent fibre (NDF) of grass silages.

MATERIALS AND METHODS

Thirty two silage samples made from four different grasses were evaluated in the present study (Table 1). Grasses were harvested, cut and ensiled without additives in hermetic glass vessels (3 litre capacity). At opening silages were analyzed for fermentation quality and for dry matter (DM), crude protein (CP), ash, ether extract (EE), NDF, acid detergent fibre (ADF) and acid detergent lignin (ADL) (1). *In situ* analyses were done and calculated as described in Jančík *et al.* (3, 4).

Results for chemical composition, rumen degradation parameters and INDF were analyzed using the MIXED procedure of SAS (6). Simple and multiple linear regressions were used for evaluation of relationships among parameters of chemical composition and utilization of NDF of grass silages.

RESULTS AND DISCUSSION

The chemical composition, NDF degradability parameters and INDF of tested grass silages are presented in Table 2. The highest CP content was found in fescue grass and the lowest ($P < 0.05$) in the hybrid Felina. The highest NDF content was detected in hybrid Felina ($P < 0.05$). The lowest *c* value and ED_{NDF} ($P < 0.05$) was found for hybrid Felina. The lowest ($P < 0.05$) content of INDF was found in rye grass. Differences among grass species might be related to differences in maturity of observed grasses (Table 1).

Table 1. Maturity stages[1] of used grasses for silages making in harvest dates

Year	Date	Grass species			
		Orchard grass	Rye grass	Fescue grass	Hybrid
2004	May 19	35	31	31	38
	May 26	51	32	32	50
2005	May 27	51	32	37	51
	June 10	61	51	55	59

[1]Based on decimal code described by Zadoks *et al.* (8) in which 30 to 39 refers to stem elongation, 50 to 59 to inflorescence emergence, and 60 to 69 to anthesis

Table 2. The effect of grass species on chemical composition and neutral detergent fibre quality parameters of silages

	Grass				
	Orchard grass	Rye grass	Fescue grass	Hybrid	se
Chemical composition (g.kg^{-1} DM)					
Ash	76.6^{a}	97.1^{b}	85.9^{ab}	87.9^{ab}	5.38
EE	30.8^{a}	39.3^{b}	27.6^{a}	29.2^{a}	1.92
CP	149^{b}	139^{ab}	178^{c}	119^{a}	15.4
NDF	541^{ab}	488^{a}	512^{a}	595^{b}	27.4
ADF	333	313	311	349	16.3
ADL	31.2^{b}	19.3^{a}	26.6^{ab}	25.1^{ab}	3.74
Degradability parameters of NDF					
b **(g.kg^{-1} DM)**	847^{a}	904^{c}	874^{b}	871^{b}	21.1
c **(h^{-1})**	0.0379^{b}	0.0427^{c}	0.0366^{b}	0.0325^{a}	0.0033
ED$_{NDF}$ (g.kg^{-1} DM)	550^{ab}	612^{c}	560^{b}	537^{a}	27.0
INDF (g.kg^{-1} NDF)	86.9^{c}	53.3^{a}	73.1^{b}	84.5^{c}	15.10

$^{a, b, c}$ – Different letters within a row indicate significant differences ($P < 0.05$)

Prediction equations of ED_{NDF} and INDF (based on parameters of chemical composition) were calculated for all tested grasses and separately for early and later maturing grasses and for each grass species (data not tabulated). The INDF was well predicted for all data sets ($R^2 = 0.636$ to 0.955), however equations based on two predictors gave every time higher R^2-values ($R^2 = 0.720$ to 0.995). Single linear regression didn't gave useful equations predicted ED_{NDF} of all grasses ($R^2 = 0.433$) and divided into early and later maturing grasses ($R^2 = 0.556$ and $R^2 = 0.441$, respectively). The better results were got for each grass separately excepting hybrid ($R^2 = 0.505$), especially using two predictors ($R^2 = 0.809$ to 0.984). Jančík *et al.* (3) predicted ED_{NDF} for dried grasses and found out that equations calculated for each species are more accurate. The specific correction equations for different forage types and species are needed also for prediction of organic matter digestibility (2, 4).

CONCLUSIONS

According to evaluation of NDF degradability parameters and INDF all estimated grasses are suitable forages to be ensiled for ruminant nutrition. However orchard grass and hybrid Felina should be harvested earlier for their rapid maturing. This study showed that the ED_{NDF} and the INDF content of grass silages could be effectively predicted from a combination of two chemical components, especially using data for each grass species separately.

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NUTRITIONAL VALUE OF ALFALFA IN RELATION TO VEGETATION STAGE AND HARVESTING

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ABSTRACT

We investigated the second alfalfa crop for nutrient content by collecting samples in three-day intervals and analyzing them for the content of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre ADF and ash. The DM of samples increased from 17.65% to 23.29% between the earliest vegetation stage of alfalfa up to the collection of plants in flower. Considering the age of plants, the concentration of nitrogen substances (NS) decreased from 23.84% at the time before the bud formation to 15.41% at flowering. The NDF increased from 32.17% to 48.1%. Comparison of nutrients content in alfalfa leaves and stems showed that the nitrogen content was 2.4-fold higher while NDF was 1.8-fold and ADF 3-fold lower in leaves at the end of the experiment.

Key words: alfalfa; content of nutrients; vegetation stage

INTRODUCTION

Successful rearing of farm animals demands sufficient basis of high quality forage in order to supply all nutrients necessary for their maintenance, growth and reproduction. Juicy bulk feed, either fresh or conserved, constitute an important portion of complete rations. The juicy bulk feeds supplied in the summer rations are defined in agriculture as "edible parts of plants excluding seeds that can serve as feed for grazing animals or be harvested for feeding purposes" (1). Leguminous plants, including clovers and alfalfa, are rich source of nutrients, particularly proteins. The costs of production of nitrogen substances (NS) through alfalfa are about 4-fold lower in comparison with extract soybean meal (3). Sunlight, temperature and moisture conditions differ from year to year and affect directly the development and maturation of plants. Thus the most consistent method for determination of harvest time is the vegetation stage and not the calendar date (9). A number of workplaces investigated the content of nutrients in leguminous plants in relation to vegetation stage and their efficiency (4, 6, 8).

The aim of our study was to observe the influence of productive and climatic conditions on nutritional value of the second alfalfa crop.

MATERIAL AND METHODS

We investigated alfalfa (*Medicago sativa*) stand grown in the Košice basin region, 200 m above sea level. Samples of whole plants, leaves and stems of the second alfalfa crop were collected in 3-day intervals between May 6 and 28, 2012, processed in a laboratory and analysed for dry matter (DM), NS, neutral detergent fibre (NDF), acid detergent fibre (ADF), fat and ash. During sampling we observed the height of the alfalfa stand, mass ratio of leaves and stems and compared the nutritional value of alfalfa in the same vegetation stage at the first, second and third mowing.

RESULTS AND DISCUSSION

The highest NS level was found at the first two samplings (238.37 and. 233.51 g.kg^{-1} DM) and the lowest at the last sampling (154.14 g.kg^{-1} DM.). Ash content ranged between 115.7 and 85.02 g.kg^{-1} DM and decreased with increasing age of the alfalfa stand. The levels of NDF and ADF increased from the first up to the last sampling. The lowest NDF value was 321.79 g.kg^{-1} and the highest 50435 g.kg^{-1}. The ADF range was 249.15–414.34 g.kg^{-1} DM (Fig. 1).

The ratio of leaf mass to stem mass was observed in relation to age. The mean leaf mass to stem mass was 48.14 : 51.82. The mean initial stem length of 39.4 cm increased to

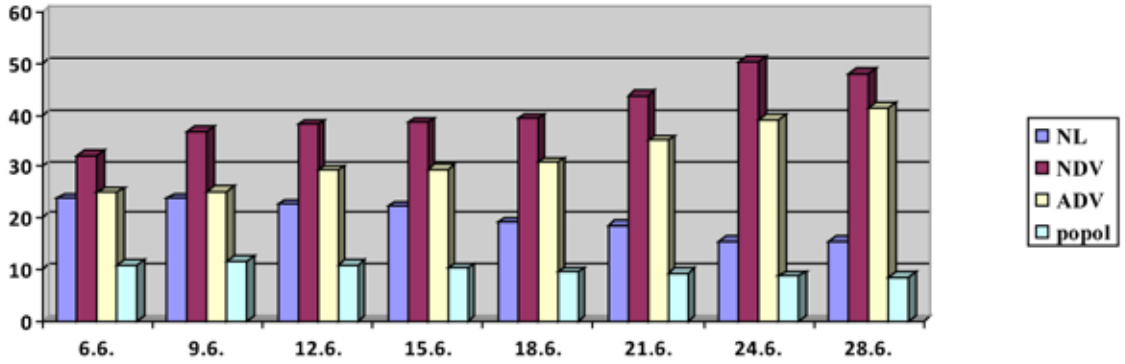

Fig. 1. Content of NS, NDF, ADF and ash at second alfalfa mowing (g.kg^{-1} DM)

the final 83.73 cm. At the last sampling (flowering stage) the height of some plants within the stand exceeded 100 cm.

Examination for the content of nutrients at individual samplings showed that the initial level of NS in leaves (30.5 %) decreased by the end of observation (25.4 %). The initial NS level in stems reached 14.5 % and the final was 9.8 %. NDF in leaves increased from the initial 17.84 % to 34.7 % at the final sampling. The highest level of NDF (63.04 %) in stems was reached at the last sampling. ADF in leaves increased from the initial 14.0 % to the final 17.12 % and in stems from 38.7 % to 51.98 %. The initial content of ash in samples of whole plants, leaves and stems was almost the same (11.5, 11.0 and 10.5 %, respectively).

The mean ratio of leaves to stems (48.18 %) corresponded to the age of the stand, i.e. to the 2-year old alfalfa stand. The ratio of leaves to stems in new stands may exceed 50 % and tends to decrease in the following years down to the level of 41 % (5).

The content of nutrient in alfalfa samples at individual vegetation stages was almost identical with values presented in tables on Nutrient needs and feed value of forage for ruminants. The first mowing occurred in the beginning of flowering in mid-May, at the mean stand height of 65.8 cm. The second mowing took place in full flowering of alfalfa at mean stand height of 83.7 cm, NS content 15 %, NDF almost 50 % and ADF 41 %. With regard to nutrient level, this term and vegetation stage was not optimal for mowing. Besides traditional harvesting of alfalfa considering the biological requirements – vegetation stage, the stands may be mowed according to their height, focusing most of all on nutritional value of this forage, but this usually means that the growing period of alfalfa is shortened to 2–3 years (2).

The second mowing took place 7 weeks after the first one, in the stage when the plant biomass did not comply with nutrition parameters of high quality forage. The probable intent of the latter harvest was to allow deposition of reserve substances in roots of this young alfalfa stand. The first mowing was done in the optimum vegetation stage of the stand with adequate nutrient levels (19.9 % DM; 20 % NS; 36 % NDF; 30 % ADF; 2.5 % fat; 9.8 % ash).

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THE INFLUENCE OF FERMENTATION PROCESS ON BUFFERING CAPACITY OF ENSILED RUMINANT FEED

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ABSTRACT

The goal of this study was to evaluate the buffering capacity of selected fermented feedstuffs. The buffering capacity of silages, defined as base-buffering capacity, was evaluated in five groups of silages (n = 145). The analysis of the base-buffering capacity of the silages confirmed significant differences in groups of maize silages and legume silages ($P < 0.001$), legume-grass silages ($P < 0.01$), grass silages ($P < 0.05$) and other silages ($P < 0.05$). Our results confirmed that the method used for determination of the base-buffering capacity appeared to be an adequate alternative and a more sensitive assessment marker particularly for maize silages in terms of evaluation of dry matter intake, but also of buffering capacity of rumen than the up-to-date used indicators.

Key words: buffering capacity; fermentation process; silage

INTRODUCTION

Silages are a dominant group of roughage in the dairy cows feeding system, thus assessment of course and successfulness of the fermentation process is a part of many systems of quality assessment and the opinions on the quality of silages (2, 4). The quality of the fermentation process depends on concentration of fermentable nutrients, buffering capacity of feedstuffs as well as on the state and activity of epiphytic microflora, system of conservation (4) and the rate of contamination of the conserved mass.

The aim of this study was to analyse the quality of fermentation process, determine the base-buffering capacity of selected types of silages and evaluate the silage samples in relation to the level of other indicators of the fermentation process.

MATERIALS AND METHODS

The quality of fermentation processes and the level of base-buffering capacity (bBC) were determined in the samples of corn silages_A (n = 69); legume silages_B (n = 22); grass silages_C (n = 17); legume – grass silages_D (n = 22) and in the group of silages labelled as other_E (n = 15). The buffering capacity was analysed by a modified method according to Moharrery (5). It was determined on wet samples at an amount equivalent to 5 g of dry mater (DM), mixed with 300 ml of distilled deionized water. The initial pH of silage was recorded after 3 minutes of equilibration. The base-buffering capacity of silages was defined as a milliequivalent 1 N NaOH necessary for titration of sample mixture to pH 7 and was calculated according to the following equation: (bBC) = [(millilitre 1 N NaOH) × 10^3] / 300. Silage pH was measured potentiometrically (pH meter Consort C830, Belgium) and quantitative determination of acids produced by fermentation was carried out after previous thinning (ratio 1 : 250) by the method of isotachophoresis in a two-column system using a capillary electrophoretic analyser EA100 (Villa Labeco, Slovak Republic). Acidity of water extract (AWE) was determined by alkalimetric titration to pH 8.5. Tukey's comparison test was used to compare the indicators of fermentative processes between the silage groups.

RESULTS AND DISCUSSION

The quality of fermentative process and the level of base-buffering capacity analysed in silage samples are presented in Table 1. Higher consumption of diluted 1 N NaOH and thus higher value of bBC in maize silages compared to leguminous silages results from faster acid production in maize silages and buffering ability of proteins or mineral elements in legume silages in which the fermentative process occurs mostly with slower acidification. Contrary to that our study showed that the mean sum of fermentative acids in maize

Table 1. Results of parameters of fermentative processes and bBC (means ± STD)

Group	bBC meq.l^{-1}	pH	AWE mg KOH.100g^{-1}	Sum of fermentative acids g.kg^{-1} DM
A	14.53 ± 3.37	3.83 ± 0.14	2119.43 ± 479.58	112.94 ± 27.00
B	9.91 ± 3.06	4.54 ± 0.27	2346.67 ± 538.63	128.85 ± 30.47
C	11.88 ± 3.98	4.35 ± 0.28	1885.25 ± 486.59	113.68 ± 38.42
D	11.56 ± 3.62	4.37 ± 0.21	2325.79 ± 695.05	139.30 ± 40.60
E	11.44 ± 3.46	4.28 ± 0.43	2367.15 ± 700.54	123.86 ± 34.97

Table 2. Statistical analysis and comparison of selected indicators of fermentative process within the groups of individual types of silages

	bBC meq.l^{-1}	pH	AWE mg KOH.100g^{-1}	Sum of fermentative acids g.kg^{-1} DM
A *vs.* B	***	***	ns	Ns
A *vs.* C	*	***	ns	Ns
A *vs.* D	**	***	ns	**
A *vs.* E	*	***	ns	Ns
B *vs.* C	ns	ns	ns	Ns
B *vs.* D	ns	ns	ns	Ns
B *vs.* E	ns	**	ns	Ns
C *vs.* D	ns	ns	ns	Ns
C *vs.* E	ns	ns	ns	Ns
D *vs.* E	ns	ns	ns	Ns

* – significant at P < 0.05; ** – significant at P < 0.01
*** – significant at P < 0.001; ns – insignificant

silages was lower than in leguminous silages or leguminous-grass silages (P<0.01). Bujňák *at al.* (1) reported that the recorded base-buffering capacity measured by consumption of NaOH in model samples of maize silages was 1.2–3.9 times higher than in alfalfa silages. Results of our examinations with respect to base-buffering capacity showed significant difference when comparing bBC of maize silages with other types of silages (Table 2). No significant differences in the acidity of water extracts (AWE) were found between the groups.

Because of the differences in experimental procedures and methods of expression of buffering capacity (3, 5, 6) it is sometimes difficult to compare the results of individual studies. pH of silage is traditionally used in quality evaluation of fermentative processes and depends on buffering capacity of plants, therefore it is true that two silages with the same pH have often different content of acids.

CONCLUSIONS

Analysis of outcomes of evaluation of the base-buffering capacity confirmed that the evaluated method of determination of buffering capacity is a suitable alternative that allows one to evaluate the fermentative processes of ensiling more precisely.

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LEVEL OF MINERALS IN FEEDSTUFFS FOR DAIRY CATTLE

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ABSTRACT

The study investigated the level of minerals (Ca, P, Mg, Cu, Zn, Mn) in cow rations according to production phases. Samples of feed: total mixed rations – TMR, corn silage, alfalfa silage (*Medicago sativa*) and grass silage, were collected from 10 farms with high-yield dairy cows located in various geographical regions of Slovakia. Samples of plant origin were examined for mean levels of calcium, phosphorus, magnesium, copper, zinc and manganese according to the methodology used by the List of the official methods of laboratory diagnostics of food and feed (Bulletin of the Ministry of Agriculture of the Slovak Republic, 2004). The levels of minerals in bulk feed for dairy cows in individual production phases corresponded to the range declared by the Nutrient requirements of dairy cattle (7th rev., 2001).

Key words: AAS; minerals/macro and trace elements; silage; TMR

INTRODUCTION

Livestock usually receive most of the minerals from feed and their intake is affected by the mineral content of plants and their seeds. The level of minerals in plants depends on four factors: the plant genotype; soil environment; climate; stage of maturity (7). The quality of food is a complex of several interrelated factors evaluated in several ways. An important indicator is the mineral content. Monitoring the mineral content is important in terms of the ecosystem (mineral cycles) but also in terms of feed quality (5). Bulk and concentrate foodstuffs are the natural source of minerals in farm animal nutrition. Utilization of minerals from feed can change by using different combinations of feed components, such as grasses, legumes and herbs (2, 3).

The aim of the present study was to analyze the level of minerals in feedstuffs intended for cows.

MATERIAL AND METHODS

We analysed feed samples from 10 selected dairy farms with high-yield dairy cows: total mixed rations (TMR), corn silage, alfalfa silage, grass silage and alfalfa for calcium, phosphorus, magnesium, copper, zinc, and manganese. The samples were processed by microwave digestion (MLS-1 200 MEGA, Milestone) and analysed for Ca, Mg, Cu, Zn, and Mn by flame atomic absorption spectrometry (AAS, Solar 939, Unicam). Inorganic phosphorus in the feed was determined spectrophotometrically.

RESULTS AND DISCUSSION

Quality of bulk feed depends on weather conditions, soil potential and the stage of maturity of the plant species. Total

Table 1. Mean content of macro-elements (Ca, P, Mg; g.kg^{-1}) and trace elements (Cu, Zn, Mn; mg.kg^{-1}) in TMR for dairy cows by production phases (mean ± STD)

	Before calving x ± STD	After calving x ± STD	Peak of lactation x ± STD
Ca	5.49 ± 1.97	6.52 ± 0.59	6.93 ± 1.16
P	4.03 ± 0.95	4.00 ± 0.92	4.66 ± 0.87
Mg	3.17 ± 0.76	3.62 ± 0.64	3.87 ± 1.17
Cu	18.02 ± 11.91	22.68 ± 17.56	22.49 ± 17.93
Zn	70.93 ± 27.43	95.10 ± 18.01	95.24 ± 23.05
Mn	84.81 ± 30.81	85.87 ± 23.44	91.06 ± 23.44

Table 2. Mean content of macro-elements (Ca, P, Mg; g.kg^{-1}) and trace elements (Cu, Zn, Mn; mg.kg^{-1}) in silage and bulk feed (mean ± STD)

	Corn silage	Alfalfa silage	Grass silage	Alfalfa
	x ± STD	x ± STD	x ± STD	x ± STD
Ca	2.06 ± 0.85	8.46 ± 2.31	4.10 ± 1.16	8.15 ± 1.34
P	2.58 ± 0.53	3.54 ± 0.94	2.65 ± 0.56	2.31 ± 0.69
Mg	1.84 ± 0.58	2.91 ± 0.46	1.86 ± 0.35	3.01 ± 0.40
Cu	9.80 ± 4.70	9.67 ± 4.39	8.10 ± 4.83	15.38 ± 3.13
Zn	55.99 ± 34.61	57.52 ± 27.51	53.60 ± 30.10	121.38 ± 33.01
Mn	4.44 ± 18.76	69.89 ± 23.98	84.03 ± 28.88	33.14 ± 6.56

mixed rations (TMR) ensure balance of nutrients in daily rations containing mixture of bulk and concentrate feed, minerals and vitamins. Analytical characteristics of nutritional value of TMR include presence of minerals. The mean level of Ca, inorganic P and Mg in the TMR for dairy cows before calving, after calving and at the peak of lactation determined in our study is presented in Table 1. Compared to values presented by Skalická *et al.* (11) the level of Ca was lower and mean level of Mg and P was almost the same. Table 1 presents also the levels of Cu, Zn, Mn and Fe in samples of TMR in different production stages.

Table 2 shows the mean mineral content in samples of bulk feed. Corn silage is an easily digestible carbohydrate food for dairy cows. Alfalfa is widely grown throughout the world as forage for cattle, and is most often harvested as hay, but can also be made into silage or grazed. In terms of the ratio of nutrients it belongs among protein feeds. Alfalfa silage is a major source of calcium compared with maize silage. Juráček and Bíro (4) reported higher levels of Ca in alfalfa silage with added chemical additives. Mean content of inorganic phosphorus in alfalfa was lower compared to alfalfa silage. Similar phosphorus content in alfalfa silage was reported by Petrikovič *et al.* (8). The content of trace elements in the feed is governed by the geochemical nature of soil and plant species. Several authors investigated level of trace elements in feed and blood serum of cattle from regions with different weather conditions (6, 9,10) and reported low Cu content in grass and grass hay.

In TMR, we noticed a slight increase in trace elements. Rearing of cattle is influenced by the quality of feed. Correctly adjusted feeding programme minimizes metabolic problems in dairy cows.

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CONCENTRATION OF CAROTENE IN ALFALFA *(Medicago sativa)* AS A FUNCTION OF VEGETATIONAL STAGE

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ABSTRACT

The aim of the study was to analyse quantitatively the changes in beta-carotene in the biomass of alfalfa (*Medicago sativa*) in the growth period of first harvest. The beta-carotene level was estimated in leaves and whole plants of alfalfa by the official method AOAC 970.64. The sampling was performed from May 10 till June 2, 2010. Alfalfa leaves contained more beta-carotene than the whole plants when averaged over the date of sampling; 188.11 ± 76.463 *vs.* 93.07 ± 63.105 mg kg^{-1} DM for whole plants (p = 0.0368). The concentration of beta-carotene decreased in the whole plants from 205.26 ± 16.461 to 54.08 ± 5.150 mg kg^{-1} DM (tendency equation y = –7.429x + 189.642) whereas in the leaves from 293.98 ± 7.570 to 167.75 ± 11 681 mg kg^{-1} DM (tendency equation y = –9.173x + 281.157). Values of the mentioned parameter were negatively correlated with dry matter in whole plants (correlation equation y = –21.751x + 552.047) and in leaves (correlation equation y = –24.149x + 700.139). Carotene content reached a maximum at or before first flowering and then declined as the blossoming progressed.

Key words: alfalfa; beta-carotene; clovers; green forages; spectrophotometric method

INTRODUCTION

Vegetation conditions can affect the vitamin content in the biomass of green forage crops. With the exception of high-quality green forage, the activity of vitamin A and its provitamin beta-carotene in feed for ruminants is unpredictable and often inadequate. Ruminants require supplementation with vitamin A or beta-carotene in the case of feed intake with low nutritional value or with a high proportion of feed grain. The inadequate utilization of beta-carotene from corn and its degradation in the rumen are the main reasons for supplementation of diets with a high proportion of concentrate feeds (2). The most suitable and often the most economical source of beta-carotene for ruminants is balanced ration on a continuous and daily basis. The most important components of this ration are green forages and within them the alfalfa (*Medicago sativa*) is one of the nutritionally finest.

The aim of the study was to quantitatively analyse the changes of beta-carotene content in the biomass of alfalfa (*Medicago sativa*) in the growth period of first harvest.

MATERIALS AND METHODS

The samples of alfalfa were taken from the production plot of a farm in Družstevná n/H., in the time of first cutting from May 10 till June 2, 2010. The alfalfa was cultivated on arable land at an altitude of 200 m above sea level. The plant matter was transported to a laboratory immediately after cutting. The dry matter was determined in whole plants and leaves the usual way after drying in a laboratory dryer. The quantitative analysis of beta-carotene was performed in the triplicate samples of whole plants and leaves according to the official AOAC method (1). The data are expressed as means ± standard deviation (STD) of single values (SAS, Version 8.2, 1999). The means of the results from the treatments were compared by one-way analysis of variance and the regression analysis was used for the evaluation of the time course of carotene concentration in the plant biomass.

RESULTS AND DISCUSSION

The results of determination of beta-carotene in whole plants of alfalfa and its leaves during the period of first cutting are expressed in the Table 1. From the point of view of the concentration of beta-carotene in whole plants, a decrease was observed since the sampling on the day 1 (May 10, 2010)

Table 1. The content of beta-carotene in whole plants and leaves of alfalfa (*Medicago sativa*) in the growth period of the first cutting

Sample	Date of sampling	DM in whole plants ($g.kg^{-1}$)	beta-carotene in whole plants ($mg.kg^{-1}$ DM)	DM in leaves ($g.kg^{-1}$)	beta-carotene in leaves ($mg.kg^{-1}$ DM)
1	day 0 (10/5/2010)	165.70	205.26 ± 16.461	188.80	293.98 ± 7.570
2	day 3 (13/5/2010)	nd	nd	184.40	267.05 ± 17.878
3	day 7 (17/5/2010)	181.40	172.75 ± 5.348	178.00	242.19 ± 6.823
4	day 10 (20/5/2010)	210.70	84.97 ± 11.938	225.90	161.20 ± 14.858
5	day 14 (24/5/2010)	221.70	29.62 ± 1.318	240.30	69.22 ± 10.075
6	day 17 (27/5/2010)	221.20	55.75 ± 2.936	219.50	115.40 ± 5.009
7	day 20 (30/5/2010)	251.40	49.07 ± 10.371	247.30	167.75 ± 11.681
8	day 23 (2/6/2010)	225.00	54.08 ± 5.150	nd	nd
	Average	**211.00 ± 26.660**	**93.07 ± 63.105**	**212.00 ± 2.606**	**188.11 ± 76.463**

DM – dry matter; nd – not determined

till day 14. Thereafter, the values were stabilized between days 17 and 23. As regards the leaves, the decrease of the mentioned parameter was observed after sampling between days 1 and 14 whereas the increase was observed between days 14 and 20. Similarly, Petrosyan and Abramyan (4) found that the concentration of carotene and vitamin E in fresh alfalfa was by 28 % higher in the stage of budding in comparison with the flowering stage. The analogical differences were observed between the vegetative stages of alfalfa that was ensiled with the different content of moisture, dried in hay in different conditions or treated with formic acid or combination of acids, as well. The leaves of alfalfa contained more beta-carotene (188.11 ± 76.463 $mg.kg^{-1}$ dry matter, $p = 0.0368$) than whole plants (93.07 ± 63.105 $mg.kg^{-1}$ dry matter). The tendency in course of the beta-carotene concentration in whole plants or leaves of alfalfa responds to the equations $y = -7.429x + 189.642$, and $y = -9.173x + 281.157$, respectively. Legumes are the better source of the beta-carotene than grasses in the later growth phase. The reason is the smaller ratio of stems to leaves in comparison with grasses. The content of beta-carotene negatively correlated with the content of dry matter in whole plants ($y = -21.751x + 552.047$) and leaves ($y = -24.149x + 700.139$). The changes of concentration of the beta-carotene in forages during the vegetation period influence the seasonal dynamics of vitamin A and beta-carotene content in the blood of dairy cows. The lowest concentrations of both parameters in the blood were observed in the spring period as a result of the insufficient saturation of cows with the beta-carotene in ration during the winter feeding period (3). The concentration of fat-soluble vitamins depends on the way of forage preservation. Drying is less protective towards this group of vitamins in comparison with ensiling.

In conclusion, according to the results of quantitative analyse of the content of beta-carotene in the biomass of alfalfa (*Medicago sativa*) it can be stated in the scope of vegetation changes during the period of first cutting as follows. The leaves of alfalfa contained the higher concentrations of beta-carotene than the whole plant. The content of beta-carotene in the whole plants and the leaves of alfalfa correlated negatively with the content of dry matter. The gradual decrease of concentration of the beta-carotene was observed in plant biomass in the course of growth phase during budding and flowering.

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EFFECT OF DIET WITH DECREASED CRUDE PROTEIN ON INTERMEDIARY METABOLISM AND FERMENTATION PROCESSES IN WEANED PIGLETS

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ABSTRACT

We investigated the influence of diets with different protein level on biochemical parameters in the blood, fermentation in the digestive system and performance of 12 weaned piglets divided to two groups (n = 6) and fed diets with 210.8 g.kg^{-1} crude protein (CP) (control) and 186.4 g.kg^{-1} CP supplemented with lysine, methionine and threonine (experimental). The decrease in the diet CP level was manifested by a significant ($P < 0.01$) decrease in blood urea (2.92 mmol.l^{-1} and 4.39 mmol.l^{-1}, resp.) which indicated increase in the biological value of the mixed feed. The decrease in the diet CP level was manifested by decreased level of volatile fatty acids (VFA) (39.67 g.kg^{-1} and 46.07 g.kg^{-1} dry matter DM, resp.) and a significant decrease ($P < 0.01$) in CP (203.1 g.kg^{-1} DM and 244.5 g.kg^{-1} DM, resp.) and NH_3 (355 mg.kg^{-1} and 398 mg.kg^{-1} DM, resp.) in the digestive system of piglets.

Key words: amino acids; ammonia excretion; piglets; urea; volatile fatty acids

INTRODUCTION

Correct determination of optimum needs for amino acids is important for full use of the genetic potential of pigs, maximum conversion of accepted proteins and minimum excretion of nitrogen into the environment (2, 6). Many experiments proved that balanced intake of essential amino acids at lower intake of nitrogen substances (NS) affected positively productive parameters of pigs and reduced excretion of nitrogen (2, 4).

The aim of the experiment was to investigate the influence of decreased NS level in the diet of weaned piglets with balanced content of amino acids on metabolic parameters in the blood and fermentation processes in the large intestine.

MATERIALS AND METHODS

The experiment was conducted during 4 weeks on 12 crossbred piglets (Slovakian White x Landrace) divided at weaning to two groups, 6 animals in each, with initial mean body weight (BW) 8.8 ± 0.6 (control) and 8.60 ± 0.7 kg experimental). Each group comprised equal number of females and castrated males. The diets were formulated to contain 2 levels of CP (210.8 g.kg^{-1} and 186.4 g. kg^{-1}, resp.). The low CP diet was supplemented with lysine (Lys), methionine (Met) and threonine (Thr). The experiment was carried out at the Institute of Animal Nutrition and Dietetics of the UVMP in Košice in compliance with the EU regulations concerning the protection of experimental animals. The diets were analyzed for dry mater (DM), CP, crude fibre (CF), neutral detergent fibre (NDF), ether extract (EE) and ash by the AOAC methods (1). Blood serum total proteins, albumin, urea and glucose were determined by commercial Bio-La-Tests (Pliva-LaChema Brno, Ltd, CR). Volatile fatty acids (VFA) were determined by isotachophoresis. Differences between the groups were evaluated by the paired *t*-test.

RESULTS AND DISCUSSION

Nutrition values of complete mixed feed used in the experiment are presented in Table 1.

No significant differences between groups in serum TP were observed throughout the trial and their levels (49.61 – 55.86 g.l^{-1}) were within the reference range (5). Similar applied to the level of albumin. Urea as an important indicator of protein nutrition showed marked changes. It was significantly higher ($P < 0.01$) in control piglets (2.92 – 4.39 mmol.l^{-1}) compared to experimental ones (1.69 – 3.06 mmol.l^{-1}). Lower serum urea indicated better retention of N and its lower release to the environment. No intergroup significant differ-

Table 1. Chemical composition of control and experimental diets (g.kg^{-1})

Parameters	Control diet		Experimental diet	
DM	903.70	1000	902.70	1000
CP	210.80	233.26	186.40	206.49
EE	13.30	14.72	12.90	14.29
CF	38.60	42.71	39.50	43.76
Ash	67.80	75.02	65.40	72.45
NDF	573.20	634.29	598.50	633.01
Lys	12.60	13.94	13.00	14.40
Thr	7.90	8.73	8.00	8.86
Met + Cys	6.70	7.41	6.90	7.64

Table 2. Effect of dietary CP on biochemical parameters of piglets

	Control diet (19. 5 % CP)				Experimental diet (16. 2 %)			
Week	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Total protein g.kg^{-1}	51.92 ± 2.55	52.94 ± 2.95	55.86 ± 2.78	54.70 ± 3.33	50.74 ± 2.99	53.74 ± 3.29	53.30 ± 2.74	49.61 ± 2.53
Urea mmol.l^{-1}	2.92^{a} ± 0.11	4.52^{a} ± 0.31	4.39^{a} ± 0.21	4.99^{a} ± 0.33	1.69^{b} ± 0.28	3.06^{b} ± 0.26	2.63^{b} ± 0.33	3.06^{b} ± 0.31
Albumin g.l^{-1}	30.28 ± 1.39	33.44 ± 2.99	34.82 ± 2.55	32.69 ± 2.17	29.07 ± 2.23	33.15 ± 2.38	32.56 ± 2.51	30.62 ± 1.98
Glucose mmol.l^{-1}	5.30 ± 0.39	4.92 ± 0.43	5.99 ± 0.31	3.77 ± 0.29	5.22 ± 0.51	5.03 ± 0.47	6.01 ± 0.33	3.81 ± 0.42

^{ab} – Significant differences at P < 0.01

Table 3. Parameters of the fermentation process in the digestive system

Parameters	Control diet	Experimental diet
Acetic acid g.kg^{-1}	21.70 ± 2.21	18.17 ± 2.27
Propionic acid g.kg^{-1}	15.12 ± 2.24	14.53 ± 1.83
Butyric acid g.kg^{-}	7.36 ± 0.49	5.83 ± 0.77
Total VFA g.kg^{-1}	44.18	38.53
pH	6.11 ± 0.33	6.64 ± 0.32
Crude protein g.kg^{-1}	238.90 ± 12.90^{a}	198.90 ± 18.60^{b}
NH_3 mg.kg^{-1}	436.00 ± 13.00^{a}	383.00 ± 15.00^{b}

^{ab} – Significant differences at P < 0.01

ences were detected for glucose. Glucose age dynamics values in both groups were in the range 3.77 – 6.01 mmol.l^{-1} which corresponded on average to physiological range (3.9 – 6.4 mmol.l^{-1} (5) or was slightly below the lower limit (3).

Evaluation of the fermentation process through determination of VFA in the faeces showed decreasing tendency in individual VFA in the group with lover level of NS in the diet but the differences between groups were insignificant. The difference in Total VFA reached 14 %. Intake of diet with decreased NS resulted in significant (P < 0.01) decrease in NH_3 (355 and 398 mg.kg^{-1} DM, resp.; difference 11 %) and NS (203.1 and 244.5 g.kg^{-1} DM, resp.; difference 17 %) in the faeces.

The decrease in NS in complete mixed feed for weaned piglets from 210.8 g.kg^{-1} to 186.4 g.kg^{-1} with supplementation of limiting amino acids Lyz, Met and Thr to the level of the control group resulted in significantly higher (P < 0.01) level of blood urea in the control piglets throughout the experi-

ment, insignificantly reduced level of VFA in the faeces of experimental piglets and significantly decreased ($P < 0.01$) NH_3 and NS in the faeces of experimental group.

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CHANGES IN SELECTED METABOLIC INDICES AFTER ADMINISTRATION OF PROBIOTICS

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ABSTRACT

There were 18 piglets included in the trial. The experiment lasted from the birth of piglets until the age of 35 days. Piglets were divided into the experimental group (n = 9) and the control group (n = 9). Each piglet from the experimental group received individually 3.2×10^7 of *Bacillus licheniformis* and *Bacillus subtilis* per day throughout the trial. Blood samples were collected on days 0, 7, 14, 21, 28 and 35. We observed a significant increase in haematocrit in the experimental group compared with the control on days 28 and 35. Haemoglobin was also higher in experimental group than in control piglets on day 28. Total serum proteins (TP) and serum albumin level (Alb) had an increasing tendency in the experimental group with significant differences between groups on days 14 and 28 (TP), and on day 35 (Alb).

Key words: metabolic indices; probiotics; suckling piglets

INTRODUCTION

One of the basic assumptions of a profitable rearing of farm animals is the proper care about the young ensuring their health. High direct and indirect losses are caused by diseases of the digestive tract. In relation to this it is clear that we should concentrate on finding means of prevention of the diarrhoeic syndrome. Great potential in this direction has been associated with probiotics. The following species of the genus *Bacillus* are most frequently used as probiotics: *coagulans, subtilis, clausii, cereus, toyoi* (4). In the agriculture, *Bacillus licheniformis* has also been used to improve the health status of pigs.

The aim of this study was to determine the effect of the preparation BioPlus 2B, containing probiotic bacteria *Bacillus subtilis* and *Bacillus licheniformis,* on selected parameters of haematological and protein profile in suckling piglets.

MATERIAL AND METHODS

Eighteen newborn piglets were included in the experiment. They came from two litters and were divided to the experimental group and control group.

The experiment lasted five weeks, from the birth until weaning. The experimental piglets (n = 9) were given the probiotic preparation BioPlus 2B which consisted of equal proportions of *Bacillus licheniformis* and *Bacillus subtilis.* Each experimental piglet received 0.01 g of the probiotic powder per day, i.e. 3.2×10^7 *Bacillus licheniformis* and *Bacillus subtilis*. No probiotic preparation was administered to the control piglets (n = 9).

During the trial, we determined selected parameters of haematological and protein profile in the serum of piglets. Blood samples were collected on days 0, 7, 14, 21, 28 and 35 of the experiment. Results were processed statistically by Student *t*-test.

RESULTS AND DISCUSSION

Haematocrit (Hc) values remained within the reference range in both groups throughout the experiment. Significant differences were observed between the groups at the 4th and 5th sampling. At the last two samplings, haemoglobin (Hb) level was higher in the experimental group and the difference between the groups at the 4th sampling was significant.

The number of leukocytes (Le) was within the standard range. None of the samplings showed significant differences in leukocytes between the groups. The level of bilirubin (Bi) remained stable in experimental group and fluctuated in control piglets (Table 1).

The level of total proteins in the experimental piglets showed a slight increase during the first two weeks. The level of total proteins in the control piglets showed a gradual de-

Table 1. Haematological profile and bilirubin in piglets´ blood

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Er–E ($10^{12}.l^{-1}$)	4.4 ± 0.66	4.8 ± 0.73	6.1 ± 1.36	6.4 ± 0.47	6.7 ± 0.52	7.2 ± 0.48
Er–C ($10^{12}.l^{-1}$)	4.7 ± 0.55	5.1 ± 0.96	5.9 ± 0.48	6.5 ± 0.27	6.7 ± 0.22	6.9 ± 0.22
Hc–E ($l.l^{-1}$)	0.28 ± 0.04	0.32 ± 0.04	0.36 ± 0.07	0.38 ± 0.03	0.38 ± 0.01^{b}	0.39 ± 0.03^{c}
Hc–C ($l.l^{-1}$)	0.29 ± 0.04	0.36 ± 0.07	0.37 ± 0.03	0.37 ± 0.03	0.35 ± 0.02^{b}	0.34 ± 0.03^{c}
Hb–E ($g.dl^{-1}$)	7.8 ± 1.35	9.1 ± 1.02	10.1 ± 2.13	10.6 ± 0.97	10.4 ± 0.6^{b}	10.7 ±1.07
Hb–C ($g.dl^{-1}$)	8. 8± 1.24	10.1 ± 2.13	10.6 ± 0.96	10.3 ± 0.88	9.6 ± 0.85^{b}	9.8 ± 1.05
Le–E ($10^{9}.l^{-1}$)	9.3 ± 1.05	13.6 ± 2.85	12.7 ± 3.72	10.9 ± 1.23	11.8 ± 1.12	14.7 ± 2.38
Le–C ($10^{9}.l^{-1}$)	12.3 ± 3.23	16.7 ± 3.36	10.1 ± 2.41	11.9 ± 2.38	15.0 ± 5.97	16.1 ±3.59
Bi–E ($\mu mol.l^{-1}$)	5.3 ± 0.49^{b}	5.2 ± 1.16	4.1 ± 3.24^{d}	4.9 ± 0.95^{c}	4.7 ± 0.8^{c}	5.5 ±1.5
Bi–C ($\mu mol.l^{-1}$)	4.5 ± 0.58^{b}	5.5 ± 1.42	9.6 ± 1.71^{d}	6.7 ± 1.38^{c}	6.9 ± 1.58^{c}	4.5 ± 0.99

E – experimental group; C – control group; Hc – haematocrit; Hb – haemoglobin; Bi – bilirubin
Er – erythrocytes; Le – leucocytes; ^{b} – $P < 0.05$; ^{c} –$P < 0.01$; ^{d} –$P < 0.001$

Table 2. Selected parameters of protein and lipid profile of piglet blood serum

	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
TP – E ($g.l^{-1}$)	72.1 ± 9.7	77.1 ± 6.5	78.6 ± 8.3^{b}	66.2 ± 4.6	62.5 ± 3.8^{c}	61.8 ± 5.7
TP – C ($g.l^{-1}$)	76.8 ± 6.2	71.9 ± 4.2	71.5 ± 2.9^{b}	65.4 ± 3.6	56.7 ± 4.4^{c}	63.7 ± 3.5
Alb – E ($g.l^{-1}$)	19.3 ± 3.95	28.4 ± 3.1	36.8 ± 3.51	38.2 ± 3.07	39.4 ± 2.93	40.7 ± 1.2^{b}
Alb – C ($g.l^{-1}$)	20.6 ± 0.88	26.9 ± 1.56	37.7 ± 2.79	40.6 ± 2.72	38.7 ± 2.61	38.4 ± 2.3^{b}
TIg – E (UZST)	20.6 ± 3.88	23.4 ± 3.5^{c}	21.8 ± 4.77	23.9 ± 2.23	23.5 ± 1.84	23.4 ± 1.69
TIg – C (UZST)	18.7 ± 3.02	16.0 ± 4.7^{c}	23.0 ± 1.18	25.1 ± 2.59	21.7 ± 1.58	21.1 ± 2.82

TP – total proteins; Alb – albumin; TIg – total immunoglobulins; E – experimental group
C – control group; UZST – units of zinc sulphate turbidity; ^{b} –$P < 0.05$; ^{c} –$P < 0.01$

crease throughout the experiment. Significant intergroup differences were recorded on days 14 and 28. Albumin levels in the experimental group increased gradually from the lowest level at 0-sampling to the highest at the final one. Significant intergroup difference in albumin level was observed at the final sampling. The level of TIg in the experimental group was steady throughout the experiment. Immunoglobulins in control piglets decreased at the 1st sampling which resulted in a significant difference between the groups (Table 2).

Our results resemble those obtained in the study by Herich *et al.* (1), which reported a significant increase in Hc and haemoglobin concentration in germ-free newborn piglets which were given *Lactobacillus casei* for ten days. Previous studies revealed an increase in the number of neutrophiles and decrease in the number of lymphocytes in the experimental group compared with the control after ten-day administration of *Lactobacillus casei* (1). These results suggest that the effect of probiotics on leukocyte counts depends on species and concentration of bacteria and can vary with different white cells.

Total proteins in our experimental group increased. *Bacillus licheniformis* and *Bacillus subtilis* produce many enzymes including the most important proteases, amylase and lipases. The enzymes help to digest proteins and saccharides in feed and facilitate their utilisation (3).

Stimulation of IgA and IgM production after probiotic

administration was described in a study by Lodinová-Žádniková *et al.* (2). It can explain why total serum immunoglobulins (TIg) in the experimental group were steady throughout the trial but varied considerably in the control piglets.

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PURIFIED β-GLUCAN FROM OYSTER MUSHROOMS (*Pleurotus ostreatus* L.) IN THE DIETS FOR CHICKENS: PERFORMANCE, MUCUS FORMATION AND FERMENTATION IN THE CAECUM

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ABSTRACT

The study investigated the effect of purified β-glucan from oyster mushrooms in concentrations of 20, 40 and 40 + 20 g.tonne^{-1} diet on the performance, mucus formation, and fermentation process in chicken caecum after 35 days of the trial. We observed a higher body weight (insignificantly) in B_{20} and B_{40} and lower in B_{40+20} groups in comparison with control group and significantly improved feed conversion ratio in all experimental groups ($P < 0.001$). Decrease in the mucus layer in the duodenum (significant in B_{20}, $P < 0.05$), jejunum and ileum (significant in all experimental groups, $P < 0.05$) and increase in the caecum (significant in B_{40+20}, $P < 0.05$), higher concentration of total short-chain fatty acids (SCFA), acetic, propionic (significant in B_{40+20} $P < 0.01$) and butyric acids and lower level of lactic acid were found in the supplemented groups. Caecal pH was not affected by the treatments.

Key words: caecum; chickens; intestinal mucus; mushroom β-glucan; performance

INTRODUCTION

β-glucans are principal structural components of cellular walls of grains, yeasts, algae and some bacteria. Their prebiotic properties are related to their positive influence on growth of beneficial micro-organisms in the digestive tract (8). An *et al.* (2) observed increased body weight and weight gains in broiler chickens receiving diet supplemented with β-glucan isolated from *Saccharomyces cerevisae*. The mechanism of effect of β-glucans has not yet been explained clearly. It has been assumed that their growth-stimulating effect could be ascribed partially to their immunomodulation properties or increased absorption surface in the small intestine and resultant increased absorption of nutrients (7).

The aim of the study was to observe body weight, feed conversion, production of mucus in the intestine and the level of fermentation in the caecum of chickens fed diets supplemented with purified β-glucan obtained from oyster mushroom.

MATERIALS AND METHODS

The experiment was carried out on 200 one-day-old chicks of Ross 308 hybrid, fed *ad libitum* complete mixed feed according to growth phases with balanced content of nutrients. The feed supplied to experimental groups was supplemented with purified β-glucan obtained from oyster mushrooms (*Pleurotus ostreatus*) (NATURES, s. r. o., SR), in groups B_{20} and B_{40} at a dose of 0.02 g.kg^{-1} and 0.04 g.kg^{-1} diet, resp., throughout the experiment, and in group B_{40+20} at a dose of 0,04 g.kg^{-1} and 0.02 g.kg^{-1} during the first and second growth phase, resp.. Control chickens did not receive β-glucan. The chickens were kept on deep litter at standard environmental conditions. Content of nutrients in mixed feed was determined by AOAC methods (1). Weight of chickens and feed consumption was recorded weekly. On day 35 of the trial 6 chickens from each group were killed by cervical dislocation and individual intestinal sections were sampled and examined for the content of mucus by alcian blue method modified by Faixová *et al.* (3). Caecal pH was measured potentiometrically and the level of fatty acids was determined by isotachophoresis (EA 100, SR). Results were evaluated by one-way ANOVA (GraphPad Prism 5) at levels $P < 0.05$; $P < 0.01$; $P < 0.001$.

RESULTS AND DISCUSSION

Evaluation of chickens on day 35 of the trial showed higher mean live b.w. in the groups B_{20} (1969.0 g) and B_{40}

Table 1. Mucus layer (μgAB.cm^{-2} intestine) in GIT of chickens (x ± SEM; n = 6)

	Control	B_{20}	B_{40}	B_{40+20}
Duodenum	14.80 ± 0.526[a]	8.41 ± 0.456[b]	11.58 ± 0.398	10.90 ± 0.693
Jejunum	15.61 ± 1.248[a]	7.67 ± 0.382[b]	8.50 ± 0.514[b]	9.81 ± 0.492[b]
Ileum	16.03 ± 0.702[a]	10.46 ± 1.029[b]	11.67 ± 1.328[b]	10.79 ± 0.406[b]
Caecum	11.29 ± 0.515a	11.37 ± 0.643	15.50 ± 1.300	19.79 ± 1.553[b]

[ab] – P < 0.05

Table 2. pH and SCFA content (mmol.l^{-1}) in caecum on day 35 of the trial (x ± SEM)

	Control	B_{20}	B_{40}	B_{40+20}
pH	5.98 ± 0.,17	6.43 ± 0.,13	6.,44 ± 0.10	6.44 ± 0.07
Lactic acid	16.75 ± 4.,95	13.,39 ± 2.83	14.86 ± 2.12	11.45 ± 0.80
Acetic acid	129.35 ± 13.23	134.34 ± 6.89	135.56 ± 7.65	136.70 ± 6.70
Propionic acid	23.48 ± 3.,26[a]	32.49 ± 2.54	30.48 ± 2.95	37.52 ± 1.40[b]
Butyric acid	15.89 ± 2.,77	16.15 ± 2.43	21.46 ± 2.36	18.26 ± 1.89

[ab] – P < 0.01

(1995.9 g) in comparison with the controls (1928.5 g). The lowest b.w. was observed in group B_{40+20} (1889.5 g). Differences between groups were insignificant. No marked influence of β-1,3/1,6-glucan from mushrooms on b.w. of chickens was recorded by other authors (5, 7). We observed in all experimental groups significantly improved conversion of feed ($P < 0.001$). Different doses of β-glucan in the diet resulted in decreased thickness of mucus in individual intestinal sections (Tab. 1), significant in group B_{20} in the duodenum, in all groups in the jejunum and ileum ($P < 005$), and increased thickness in group B_{40+20} in the caecum ($P < 005$). According to Jeurissen *et al.* (6), increased content of mucus can decrease absorption of nutrients or increase energy demands of the intestine. Beneficial effect of increased production of mucus depends on the thickness of mucus layer. Thinner layer may not suffice to prevent bacterial invasion but may have positive effect on production, as witnessed by better feed conversion observed in our study.

In the caecum in all experimental groups we detected lower level of lactic acid, higher levels of acetic, propionic (significant in B_{40+20} $P < 0.01$) and butyric acids and the sum of fatty acids but caecal pH was not affected significantly (Tab. 2). Similar results were recorded by Guo *et al.* (4) when investigating effects of extracts from mushrooms and plants.

In conclusion, addition of β-glucan to chicken diet in different concentrations failed to affect significantly the body weight of chickens but resulted in significant improvement of feed conversion, decreased thickness of mucus layer in the small intestine in all experimental groups (significant in the jejunum and ileum; $P < 0.05$), increased thickness of mucus in the caecum (B_{40+20}; $P < 0.05$) and increased production of volatile fatty acids (propionic acid in group B_{40+20}: $P < 0.01$). Caecal pH was not affected significantly.

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ALLOMETRIC GROWTH OF MAJOR BONE-FORMING MINERALS IN SLOW AND FAST GROWING CHICKENS DURING THE FIRST WEEKS OF LIFE

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ABSTRACT

Allometric growth of body macrominerals were examined in slow-growing laying-type cockerels (SG) and in fast-growing male broiler hybrids (FG) during the growing period from hatch to day 22. Allometric coefficients for dry matter, calcium, phosphorus and magnesium in relation to body weight were 1.091, 1.427, 1.383 and 1.284 for SG chickens and 1.075, 1.276, 1.233 and 1.216 for FG chickens, respectively. The deposition of Ca, P and Mg in SG chickens was faster than that of dry matter by 30.20%, 25.97% and 17.24%, and in FG cockerels by 18.45%, 14.27% and 12.71%, respectively. High allometric coefficients for Ca, P and Mg in both genotypes likely indicate a rapid growth of skeletal tissues which requires an adequate mineral nutrition during this period of growth. The deposition of Ca and P relative to dry matter was higher ($P < 0.05$) in SG chickens thus suggesting that the relative increase in these minerals may be affected by genotype.

Key words: age; Ca, Mg and P retention; chemical allometry; chickens; growth

INTRODUCTION

Requirements on concentration of available minerals in the diet for growing poultry depends on the inevitable mineral losses, the growth rate, the mineral concentration in gained body weight and the feed conversion ratio. Mineral concentration in body weight is quantitatively the most important, but it can be studied with reasonable effort by whole body analysis (4). It is generally accepted that from the prediction of body weight growth the growth of body constituents can be calculated by allometric equations.

The aim of the present experiment was to study relative growth rates of body Ca, Mg and P in broilers and laying type chickens during the first 22 days of post-embryonic life.

MATERIALS AND METHODS

Chickens of two contrasting genotypes were used: slow-growing male chicks of hybrid combination Isa Brown (SG) and fast-growing Ross 308 cockerels (FG). The diet contained 9.53 g.kg^{-1} diet Ca, 7.43 g.kg^{-1} diet P and 1.74 g.kg^{-1} diet Mg. Chickens were fed *ad libitum*.

In two-day intervals from hatch until the age of 22 days, samples of chickens were selected from each group so that their body weight was approximately the same as the mean body weight of the group. The selected chickens were euthanized and the content of the digestive tract was removed. The chickens were then autoclaved for 6 hours at 130 °C and 270 kPa pressure, freeze-dried and finely ground. The diet and whole body of chickens were analyzed for moisture, calcium, magnesium and phosphorus. Minerals were estimated after wet mineralization by sulphuric acid and hydrogen peroxide, Ca and Mg by flame atomic absorption spectrometry using an AAS1 Atomic Absorption Spectrometer (Carl Zeiss, Jena, Germany) at wavelengths of 422.7 nm (Ca) and 285.2 nm (Mg), and P spectrophotometrically as vanadate yellow using a Unicam 8625 UV/VIS Spectrophotometer (LabX, Midland, ON, Canada) at wavelength of 442 nm.

Allometric relationships were calculated using the power function (1): $Y = aX^b$, where Y – content of the body component in g; X – live body weight or dry matter weight of chicken in g; a – extrapolation of Y for X = 1; and b – allometric coefficient, ratio of percentage change in Y to the corresponding percentage change in X. The significance of differences between the data for the two genotypes were evaluated by a paired *t*-test. The statistical analyses were performed using Statgraphic Plus package (version 3.1, Statistical Graphic Corp., Rockville, MD, USA).

Table Table 1. Allometric functions $Y = aX^b$ for relations between macrominerals and live body weight or dry matter weight in two chicken genotypes

Y	Genotype[1]					
	SG			FG		
	a	b	I_{YX}	a	b	I_{YX}
Relations to live body weight (g)						
Dry matter	0.15947	1.0914[a]	0.996**	0.16403	1.0752[a]	0.999**
Ca	0.00062	1.4265[a]	0.998**	0.00091	1.2755[b]	0.998**
P	0.00065	1.3825[A]	0.996**	0.00101	1.2325[B]	0.998**
Mg	0.00007	1.2835[a]	0.995**	0.00008	1.2160[b]	0.997**
Relations to dry matter weight (g)						
Ca	0.00695	1.3020[a]	0.998**	0.00786	1.1845[b]	0.998**
P	0.00676	1.2597[a]	0.996**	0.00813	1.1427[b]	0.998**
Mg	0.00058	1.1724[a]	0.998**	0.00059	1.1271[a]	0.998**

[1]SG – slow-growing chickens; FG – fast-growing chickens; X – live body weight or dry matter weight (g); Y – analyte weight (g); b – allometric coefficient; I_{YX} – index of correlation; [a, b] – significant differences between genotypes at $P < 0.05$; [A, B] – significant differences between genotypes at $P < 0.01$; ** – Significant differences in I_{YX} at $P < 0.01$

RESULTS AND DISCUSSION

At the end of the experiment the body weight of FG chickens was about three times higher than that of SG chickens (782 g and 258 g, respectively).

Parameter estimates for the allometric relationships of dry matter and macrominerals weight with live body weight are summarized in Tab. 1. As indicated by the allometric coefficients, the proportion of dry matter weight in the chicken bodies increased with the increasing age of birds. Similar results were reported by Gous *et al.* (3). The deposition of Ca, P and Mg was higher than the rate of body weight growth. These differences were more pronounced in SG than in FG chickens ($P < 0.01$), significant for Ca and Mg ($P < 0.05$). All allometric coefficients differed significantly from one ($P < 0.05$).

Eits *et al.* (2) presumed that due to lower proportion of bones, the relative increase in ash in broilers is slower than that in laying-type chickens. The results of the present experiment supported this hypothesis as the allometric coefficient for skeleton-forming elements Ca, P and Mg in FG chickens was significantly lower than in SG chickens.

Allometric coefficients describing the relationships between weight of minerals and dry matter weight (Tab. 1) differed significantly ($P < 0.05$) from one. The deposition of Ca, P and Mg in SG chickens was faster than that of dry matter by 30.20 %, 25.97 % and 17.24 %, and in FG cockerels by 18.45 %, 14.27 % and 12.71%, respectively. In the case of Ca and P, the differences between genotypes were significant ($P < 0.05$).

The remarkably high allometric coefficients for Ca, P and Mg relative to body weight in both genotypes likely indicated the rapid growth of skeletal tissues, since the concentration of minerals in bones is higher than that in non-skeletal body components. Consequently, adequate mineral nutrition during this period of growth is of particular importance.

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SELECTED PROPERTIES OF EXTRACTS FROM *ORIGANUM VULGARE*, *SALVIA OFFICINALIS*, *ELEUTHEROCOCCUS SENTICOSUS* AND *STEVIA REBAUDIANA*

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ABSTRACT

Majority of plant polyphenols are easily oxidizable and able to reduce free radicals. Relevant effects may be expected depending on their bioavailability and the amount consumed. The study assessed the ability of plant extracts from siberian ginseng, stevia, oregano and sage to scavenge superoxide radicals in the range of concentrations where the highest biological activity was observed previously. Moreover, the extracts at different pH, roughly corresponding pH of cell and blood, were tested. We found that the extracts had better scavenging ability at lower pH. The expected higher ability of extracts from sage and oregano to scavenge superoxide radicals was not confirmed.

Key words: oregano; sage; Siberian ginseng; stevia; superoxide radical

INTRODUCTION

Various herbs and spices have been reported to produce significant amount of antioxidants (flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins) to prevent oxidative stress caused by free radicals and oxygen (1). Intracellular accumulation of reactive oxygen species (ROS), such as singlet oxygen, superoxide, hydroxyl, peroxyradicals and hydrogen peroxide, can arise from normal metabolic processes or toxic insults. ROS may perturb the cell's natural antioxidant defence systems, resulting in damage to all biological macromolecules. Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical. Due to high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive (5). Selected flavonoids can directly scavenge superoxides, whereas other flavonoids can scavenge the highly reactive oxygen-derived radical peroxynitrite (7). These properties as well as their ability to act as signalling molecules and modulate the activity of antioxidant enzymes may vary in a dose-dependent manner as well as internal conditions of the body.

The aim was to evaluate superoxide radical ($O_2^{\bullet -}$) scavenging activity of selected plant extracts in the concentration range of their most pronounced effects and pH conditions imitating those in the cell.

MATERIAL AND METHODS

The antioxidant properties of ethanolic extracts of the root of Siberian ginseng (*Eleutherococcus senticosus*), leaves of stevia (*Stevia rebaudiana*), oregano (*Origanum vulgare*) and sage (*Salvia officinalis*) at various concentrations (5–100 μg.ml^{-1}) were evaluated by the method of Bauchamp and Fridovich (2). By UV illumination under aerobic conditions, riboflavin is reduced by L-methionine and the reduced form reacts with oxygen forming a peroxide derivative which, after decomposition, provides $O_2^{\bullet -}$. The ions are captured by nitro-blue-tetrazolium (NBT). This compound changes colour upon the reduction. The transformation can be followed by spectrophotometry, measuring the absorbance at 450 and 560 nm. In the presence of antioxidant the $O_2^{\bullet -}$ is captured. Consequently, the photoreduction of NBT is inhibited (the solution is decolourized). The reaction mixture used contained riboflavin, NBT, phosphate buffer (pH 6.5 and 7.4) containing EDTA, L-methionine and tested plant extracts were added at different concentration ranging from 5–100 μg.ml^{-1} PBS. Reaction was initiated by placing vessels under an Hg lamp for 10 and 20 minutes. From the absorbance

Table 1. Percentage of inhibition of $O_2^{\bullet -}$ by selected plant extracts *in vitro*

		Concentration (μg.ml^{-1})				
		5	**25**	**50**	**75**	**100**
pH 6.5	*S. ginseng*	7.2	12.48	15.76	25.13	12.17
	Stevia	11.31	10.3	12.7	9.7	17.15
	Oregano	4.37	4.37	5.7	4.18	0
	Sage	1.53	0	6.12	12.32	6
pH 7.4	*S. ginseng*	6.66	11.81	14.83	17.58	17.16
	Stevia	13.54	14.14	13.32	10.7	5.58
	Oregano	10.95	3.44	4.78	10.2	10.33
	Sage	0	3.01	10.99	2.32	5.23

after UV illumination, the percentage of inhibiton of $O_2^{\bullet -}$ was calculated according to the following equation: (%) = $[(A_{control}-A_{sample})/A_{control}] \times 100$. All the measurements were made in triplicate.

RESULTS AND DISCUSSION

The most pronounced effect against $O_2^{\bullet -}$ was found at lower pH (6.5). Siberian ginseng extract showed the highest antioxidant activity, however, it reached a maximum of 25 % (Table. 1). It was found that an extract of Siberian ginseng root had antioxidant effect against $O_2^{\bullet -}$ (3). Other tested plant extracts showed lower ability to inhibit $O_2^{\bullet -}$ at the same pH. Higher inhibitory activities of the extracts at lower pH were at concentration of about 50–75 μg.ml^{-1}. At pH 7.4 (pH of blood plasma) the inhibitory effect was decreased by more than 20 %. Kosar *et al.* (6) analyzed the effect of pH (2 and 7.5) on the antioxidant activity of methanol extracts of basil, rosemary, thyme and sage, and found that alkalization decreased their antiradical activity, whereas acidic conditions increased that activity, which is consistent with our results. Low antioxidant effect (under 10 %) was found in sage and oregano extracts at both pH levels (6.5 and 7.4). In contrast, Yadav and Mukundan (8) reported more than 40 % inhibitory effect of sage against $O_2^{\bullet -}$. Cervato *et al.* (4) observed that the methanol extract from dried oregano leaves showed only 35 % scavenging effect on $O_2^{\bullet -}$ in comparison to water extract (70 %). Similar was demonstrated for sage. Thus, antioxidant activity may be affected by the method of determination and factors such as extraction method, location, climate, interaction with other ingredients and environmental conditions (pH). However, one should consider that $O_2^{\bullet -}$ is not highly reactive, is unable to penetrate lipid membranes and thus is restricted to the intracellular compartment. Unless it is not scavenged, it undergoes dismutation to hydrogen peroxide, with relative longer half-life than $O_2^{\bullet -}$ and ability to cross biological membranes, acting as well-regarded signalling molecule. On the other hand, reaction with water can generate a perhydroxyl radical implicated in lipid damage and protein oxidation. Which in practice means that the reduced uptake ability of extracts in certain conditions does not necessarily mean a total reduction of biological activity.

In conclusion, the generally higher antiradical activity against $O_2^{\bullet -}$ was observed at lower pH (6.5), more characteristic of the cells. Siberian ginseng showed the highest capacity. We did not confirm that oregano and sage extracts are excellent superoxide scavengers under these conditions. Our results are still promising because, depending on the method of extraction and other conditions such as pH, in relation with selected biological activities, we are able to estimate the effect of the extracts and the appropriate dose.

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SELECTED PROPERTIES OF *AESCULUS HIPPOCASTANUM* EXTRACT AND AESCIN

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ABSTRACT

The study investigated *in vitro* antioxidant activity of horse chestnut root extract (*Aesculus hippocastanum* L.) and its main active ingredient aescin against superoxide radicals. Several concentrations (5–100 μg.ml^{-1}) of of these substances were tested at different pH conditions (6.5 and 7.4). Superoxide scavenging activity of extracts was determined by the nitro-blue tetrazolium (NBT) reduction method. It was observed that superoxide radical was scavenged by tested compounds in a concentration and pH dependent manner. Higher antiradical activity was observed at lower pH (6.5). We found better antioxidant properties of horse chestnut extract compared to its principal active ingredient aescin.

Key words: aescin; horse chestnut; NBT reduction method; superoxide radical

INTRODUCTION

Recently, there is an increasing demand for finding natural antioxidants from plant materials to replace the synthetic ones. Therefore, there is a need to investigate their biological properties in order to develop new drugs (2). The antioxidant effect of plants is mainly due to the presence of phenolic compounds. They can act as free radical scavengers, singlet oxygen quenchers, chelators of transitional metals, such as iron, as well as reducing agents and activators of antioxidative defence enzyme systems suppressing radical damage in the body (9). In our study we focused on horse chestnut dry extract and its principal constituent aescin, which are mainly used to treat chronic venous insufficiency and oedemas. Several studies focused on their anti-inflammatory, antitumor, antiviral, antifungal and antioxidant effects (7, 10). It was demonstrated that extract of *A. hippocastanum* is a potent scavenger of active oxygen being almost 20 times more effective at absorbing superoxide radicals ($O_2^{\bullet -}$) than ascorbic acid (5).

We investigated the antioxidant effect of the mentioned extract and aescin against $O_2^{\bullet -}$ in the range of concentrations in which the highest biological activity was found, in relation to pH focusing on pH of blood and cells.

MATERIAL AND METHODS

The botanical name of horse chestnut is *Aesculus hippocastanum* L. Horse chestnut dry extract (HCE, 18 to 22 % aescin) and the saponin beta-aescin (E) were a gift from CALENDULA a. s. (Slovak Republic). The $O_2^{\bullet -}$ scavenging activity was determined using the method described by Beauchamp and Fridovich (1). The reaction mixture contained phosphate buffer (pH 6.5 and 7.4), EDTA, L-methionine, riboflavin, nitro-blue tetrazolium (NBT) and the tested substance in various concentrations (5–100 μg.ml^{-1}). It was exposed to UV light for 10 and 20 minutes before use. Absorbance of the solutions with and without the tested substance was determined at 450 nm and 560 nm before and after the UV illumination. This process was repeated with various concentrations of extracts and all the measurements were made in duplicate. The assay is based on the capacity of the antioxidants to inhibit blue formazan formation by scavenging the $O_2^{\bullet -}$ generated in riboflavin-light-NBT system. Decrease in absorbance indicates the consumption of superoxide anion in the reaction mixture. The percentage inhibition of $O_2^{\bullet -}$ generation was evaluated by comparing the absorbance values of the control

and experimental tubes and was calculated according to the following equation: % = [(Acontrol – Asample)/Acontrol] × 100.

RESULTS AND DISCUSSION

All aerobic organisms produce partially reduced metabolites of O_2 that have higher activities relative to O_2 (6). $O_2^{\bullet-}$ has an approximate half-life of 2–4 μs and undergoes fast, non-enzymatic, one-electron reduction to form hydroxyl radical. It has been noted that $O_2^{\bullet-}$ can undergo protonation to give up a strong oxidizing agent perhydroxyl radical which directly attacks the polyunsaturated fatty acids (PUFAs) in negatively charged membrane surfaces (3). All of the produced radicals have the potential to react with various biological substrates and have been implicated in several pathophysiological processes if not inactivated by antioxidant systems. It has been reported that antioxidant properties of some flavonoids are effective mainly *via* scavenging of $O_2^{\bullet-}$ (8). We observed maximum inhibition of $O_2^{\bullet-}$ (25 %) with horse chestnut extract at pH 6.5 and a concentration of 75 μg.ml^{-1} (Table 1.). Its principal active ingredient, aescin, showed maximum activity against $O_2^{\bullet-}$ (22 %) when used at higher concentration (100 μg.ml^{-1}). It has been found that the whole plants or mixtures of plants have greater *in vitro* or *in vivo* antioxidant activity than the isolated compounds. Potential synergistic interactions among the antioxidant compounds in plant extract may be responsible for this effect (11). We found maximum inhibitory effect against $O_2^{\bullet-}$ at lower pH with concentration peak at 50–75 μg.ml^{-1}. Higher pH of solution (7.4) halved the ability to scavenge the tested radical (12 %). Kosar *et al.* (4) observed that acidic conditions significantly increased radical-scavenging properties of selected plant extracts by increasing their reducing power. It is possible that the compounds responsible for the antiradical activity are not stable in the alkaline pH and loose their properties after alkalization.

Table 1. Percentage of inhibition of $O_2^{\bullet-}$ by horse chestnut extract and aescin *in vitro*

		Concentration (μg.ml^{-1})				
		5	**25**	**50**	**75**	**100**
pH 6.5	Aescin	9.28	10.82	16.41	7.21	22.79
	A. hippocastanum	10.65	19.31	17.33	25.91	13.29
pH 7.4	Aescin	6.83	7.07	12.26	13.21	13.68
	A. hippocastanum	0	1.21	12.04	8.89	11.8

Our observations allowed us to conclude that the horse chestnut extract had better antioxidant effect against $O_2^{\bullet-}$ than its principal ingredient aescin. The antioxidant activity of the investigated extracts depended on the concentration used as well as on pH level. Higher antiradical activity was observed at lower pH (6.5) which is more characteristic for the cells. Additional detailed studies on the isolation and characterization of the plant extract as well as *in vivo* assays will be necessary to confirm the antioxidant properties.

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USE OF MAIZE GLUTEN IN DIETS FOR PHEASANT CHICKENS

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ABSTRACT

The effect of maize gluten meal as a substitute for fish meal in the diet on body weight, weight gain, feed conversion ratio (FCR), protein and metabolizable energy conversion, health, and European Efficiency Index (EEI) was studied in 2200 pheasant chickens (*Phasianus colchicus*) in the first phase of rearing (0–28 days) under farm conditions. Performance variables were not significantly affected by dietary maize gluten meal inclusion. Tendency towards higher EEI values (32.1) and lower mortality rate (7.5%) were observed in experimental group in comparison to control group (31.8 and 8.36%). The results indicated that 6 % maize gluten meal can be included in the pheasant diet without negative effect on pheasant performance.

Key words: first phase of rearing; maize gluten; performance; pheasant chickens

INTRODUCTION

Currently, due to restricted use of high-quality sources of proteins of animal origin in mixed feed for poultry, with the exception of fish meal, there has been an effort to find corresponding feed of plant origin that in combination with extract soybean meal and balanced amino acids could satisfy the demands. Fish meal has besides excellent nutrition characteristics also some negative properties, particularly odour that is carried over to meat and eggs (6), when incorrectly stored it may be infected with salmonella (8) and last but not least one should consider its price when including it in mixed feed. When substituting feed of animal origin and fish meal we use most frequently extract soybean meal, brewer's yeast (8), full-fat soybeans (3) and others. Maize gluten meal, the side product of mill industry, with high content of nitrogen substances and energy can be used as fish meal substitute in mixed feed for poultry (5). After compensating for low level of lysine, it can be a suitable alternative of extract soybean meal (4, 6).

The aim of the study was to investigate under farm conditions how the substitution of fish meal with maize gluten may affect growth intensity, conversion of feed nitrogen substances and energy, effectiveness of rearing and mortality of pheasant chickens.

MATERIALS AND METHODS

The experiment was carried out on 2200 one-day-old pheasant chicks (*Phasianus colchicus* L.) hatched and reared in the Specialised establishment for rearing and diseases of game, fish and bees of UVMP in Rozhanovce. They were divided to 2 groups (1400 control, 800 experimental). Control pheasants (CK) were fed in the 1st stage (days 0–28) a diet containing fish meal (6%) *ad libitum* and the experimental birds (E) received the same proportion of maize gluten. The mixed feed supplied to the chickens complied with the requirements on nutrients and energy for this respective stage of growth. Weight of pheasant chicks (n = 50) and feed consumption were observed weekly and mortality was recorded throughout the period. Content of nutrients in feed was determined by AOAC methods (1). We calculated conversion of feed, nitrogen substances and metabolizable energy for the period of observation. European Efficiency Index (EEI) was calculated according to the formula: EEI = [weight in kg x (100 – % mortality)/(age in weeks × feed conversion)] × 100. The results were evaluated statistically by *t*-test (GraphPad Prism 5).

RESULTS AND DISCUSSION

Mean body weight of pheasant chicks and feed conversion in the 1st stage of rearing (days 0–28) are shown in Fig-

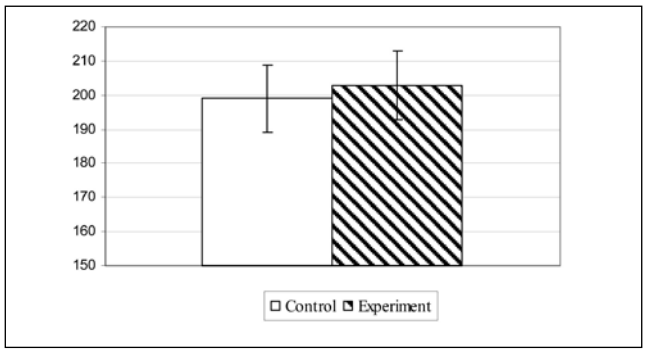

Fig. 1. Body weight of pheasant chickens (g)

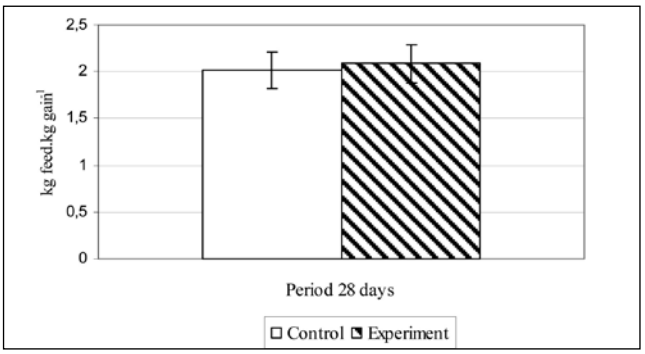

Fig. 2. Feed conversion ratio

ures 1–2. The *ad libitum* fed experimental chickens reached higher body weight in the 1st stage of the experiment (202.9 ± 3.35 g) in comparison with control group (199 ± 3.61 g) but the difference between groups (2 %) was insignificant. Substitution of fish meal with maize gluten caused increased total feed consumption per head in experimental group (386.1 g) in comparison with control (371.1 g) by 4 %, associated with higher feed conversion, nitrogen substances and metabolizable energy (by 3.5 %, 1.1 % and 2.9 %, resp.) compared to control but the differences were insignificant.

Supply of maize gluten to experimental chickens resulted in decrease in mortality by 11.5 % over the investigated period (7.5 %) in comparison with control group (8.36 %) which, at comparable conversion of feed, affected positively the efficiency index of rearing of pheasant chicks in experimental group (32.05) compared to the control (31.8). Differences between groups were insignificant.

Our results agree with those of Babidis *et al.* (2) who investigated substitution of fish meal with maize gluten in chickens and failed to observe significant differences in body weight, feed conversion and carcass characteristics. Similar results were obtained by other authors (4, 7) dealing with possibility of substituting fish meal with maize gluten. Neither body weight of chickens nor weight gain, consumption of feed, carcass yield and proportion of abdominal fat were significantly affected by supplying diets containing maize gluten. Also the study of Owings *et al.* (9) which focused on the influence of various proportions of maize gluten in rations supplied to growing turkeys found no differences in productive parameters in comparison with the control fed diet based on maize and extract soybean meal.

CONCLUSIONS

Our results showed that maize gluten in combination with extract soybean meal can be used as a substitute for fish meal in the diet for pheasant chickens in the first stage of rearing (days 0–28) with maintenance of productive parameters and decreased mortality.

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THE EFFECT OF OXYHUMOLITE ON NUTRIENTS UTILIZATION BY BROILER CHICKS AND *IN VITRO* DIGESTIBILITY OF DIETS

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ABSTRACT

The effect of oxyhumolite added to the diets (5 or 7 g.kg^{-1}) on nutrient utilization, dry matter and nitrogen excretion in broiler chicks was evaluated in the *in vivo* experiment, and the effect on ileal digestibility of diets was evaluated in the *in vitro* experiment. The feed, nitrogen and metabolizable energy conversion ratio, and dry matter and nitrogen excretion throughout the experimental period were lower in the test group compared to the control group (significantly in conversion ratio at P < 0.001). Both levels of oxyhumolite significantly increased digestibility coefficients of crude proteins (5 g.kg^{-1} P < 0.05; 7 g.kg^{-1} P < 0.01).

Key words: digestibility; excretion; feed conversion ratio; humic substances

INTRODUCTION

High excretion of nitrogen in intensive animal husbandry is one of the essential environmental problems. The excreted nitrogen is converted to ammonia through microbial fermentation in the litter. Higher level of ammonia in animal houses affects negatively the animal production and health of animals and staff (1). One possibility how to reduce nitrogen excretion by poultry is to reduce crude protein in the diets but its lower intake negatively influences animal growth and production (5). The effect of some additives might be reflected in better utilization of nitrogen and thus its lower excretion into the litter.

The objective of the present study was to investigate the effect of oxyhumolite on utilization of nutrients and excretion by broiler chicks and to study the effect of two different concentrations of oxyhumolite on ileal digestibility of diets *in vitro*.

MATERIALS AND METHODS

***In vivo* experiment.** One hundred unsexed 1-day-old broiler chicks (Ross 308 hybrid) were randomly divided to two groups (50 chicks per group) and housed on deep litter. The birds were fed corn-wheat-soybean meal based complete mixed diet in mash form according to growth phases (phase 1: 1st–2nd week; phase 2: 3rd–5th week; phase 3: 6th week) *ad libitum*. The test group diets were supplemented with oxyhumolite (total humic acids 68 %; free humic acids 48 %; minerals 18 %) in different amounts: 5 g.kg^{-1} diet during the 1st phase and 7 g.kg^{-1} diet during the 2nd and 3rd phases. The birds were individually weighed and their feed consumption was observed weekly. Dry matter and nitrogen excretion were calculated according to ASAE Standards (3).

***In vitro* experiment.** The mixed feed used in the *in vivo* experiment was analyzed *in vitro* using a Daisy II incubator for the determination of ileal digestability of dry matter and crude proteins. For all diets, 5 g samples were transferred to 15 nylon bags (5 × 10 cm, Ankom Technology). The analysis was performed according to Boisen and Eggum (4). Digestibility coefficients were calculated as follows: [(content of dry matter in the sample in g × content of nutrients in dry matter in %) – (content of dry matter in residuum of sample in g × content of nutrients in dry matter of residuum in %)/(content of dry matter in sample in g × content of nutrients in dry matter in %)] × 100.

The content of nutrients and metabolizable energy in diets and residues after digestion was determined according to AOAC (2). Statistical analysis was performed using *t*-test.

RESULTS AND DISCUSSION

***In vivo* experiment.** The feed, nitrogen and metabolizable energy conversion ratio values were significantly higher (P < 0.01) in the 1st phase and significantly lower (P < 0.01;

Table 1. The feed, nitrogen and metabolizable energy (ME) conversion ratio in respective trial phases (x ± SEM)

	Feed conversion ratio ($kg.kg^{-1}$)		Nitrogen conversion ratio ($g.kg^{-1}$)		ME conversion ratio ($MJ.kg^{-1}$)	
Week	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**
1–2	1.15 ± 0.02^{a}	1.24 ± 0.01^{b}	257 ± 3.77^{a}	279 ± 2.77^{b}	13.6 ± 0.23^{a}	14.7 ± 0.15^{b}
3–5	1.71 ± 0.01	1.72 ± 0.01	354 ± 2.71	346 ± 2.60	20.4 ± 0.16	20.4 ± 0.15
6	2.29 ± 0.03^{a}	1.85 ± 0.07^{b}	449 ± 6.37^{a}	356 ± 14.0^{c}	27.7 ± 0.39^{a}	22.4 ± 0.88^{b}
1–6	1.77 ± 0.01^{a}	1.69 ± 0.01^{c}	363 ± 2.15^{a}	340 ± 1.21^{c}	21.2 ± 0.13^{a}	20.2 ± 0.07^{c}

^{ab} – $P < 0.01$, ^{ac} – $P < 0.001$

Table 2. Dry matter and nitrogen excretion in respective trial phases

	Dry matter excretion ($g.head^{-1}$)		Nitrogen excretion ($g.head^{-1}$)	
Week	**Control**	**Test**	**Control**	**Test**
1–2	96.59	100.94	4.93	5.18
3–5	757.78	757.63	35.70	34.93
6	350.49	314.00	15.66	13.77
1–6	1204.85	1172.57	56.29	53.88

Table 3. Digestibility coefficients of dry matter (DC_{DM}) and crude protein (DC_{CP}) (x ± SEM)

	Control MF 1	MF 1 + Oxyhumolite ($5\ g.kg^{-1}$)	Control MF 2	MF 2 + Oxyhumolite ($7\ g.kg^{-1}$)
DC_{DM} (%)	67.75 ± 0.30	68.59 ± 0.45	74.67 ± 0.21	74.92 ± 0.22
DC_{CP} (%)	90.40 ± 0.09^{a}	90.82 ± 0.13^{b}	92.20 ± 0.06^{A}	92.49 ± 0.06^{C}

MF – mixed feed; ^{ab} – $P < 0.05$; ^{AC} – $P < 0.01$

P<001) in the 3rd phase of the trial in the test group compared to control (Table 1). The levels of these parameters monitored throughout the trial were significantly lower in the test group than in the control group (P<0.001). Similar to our study, better feed conversions were also observed by Kocabağli *et al.* (7) and El-Husseiny *et al.* (6).

Values of dry matter and nitrogen excretion in respective trial phases are shown in Table 2. Lower levels of dry matter and nitrogen excretion in the trial group in comparison to the control were found throughout the trial (3% and 4%, respectively).

In vitro experiment. The ileal digestibility of crude proteins was significantly increased by the addition of oxyhumolite to the diets in the amount of $5\ g.kg^{-1}$ (P<0.05) and $7\ g.kg^{-1}$ (P<0.01) (Table 3). Dry matter digestibility was not affected significantly. Increased digestibility coefficient of crude protein was also observed in *in vivo* experiment by El-Husseiny *et al.* (6).

CONCLUSIONS

The addition of oxyhumolite to the diets for broiler chicks resulted in better utilisation of nutrients but its positive effect was significant only from the 6th week of the trial. Lower dry matter and nitrogen excretion also indicates better utilization of nutrients from the diet supplemented with oxyhumolite. The results of *in vitro* experiment showed that the addition of oxyhumolite, especially at higher level, increased ileal digestibility of crude proteins. Our results need to by verified *in vivo*.

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