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THE EFFECT OF PROTEIN SOURCE ON PARAMETERS OF RUMEN CONTENT AND DIGESTIBILITY OF NUTRIENTS IN FATTENING LAMBS

D. RUZIC-MUSLICH[1]*, M. P. PETROVICH[1], M. M. PETROVICH[1], P. PERISICH[2], Z. BIJELICH[1], V. PANTELICH[1] and V. CARO-PETROVICH[1]
[1]*Institute for Animal Husbandry, Belgrade-Zemun, 11080, Serbia*
[2]*University of Belgrade, Faculty of Agriculture, Belgrade, Serbia*

Abstract

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In this paper, results of the study of the effect of different protein sources in concentrate mixtures on fluid rumen content and digestibility of nutrients in fattening lambs are presented.

Physiological study included 18 male lambs of MIS population, average body mass of approx, 35.0 kg, divided into three groups. Lambs were fed isoprotein concentrate mixtures (14% CP) which differed about protein source: sunflower meal (I), soybean meal (II) and fishmeal (III), resulting in different ratio of protein non-degradable in rumen: 43% (I), 51% (II) and 58% (III), respectively.

Content of ammonia nitrogen (NH_3-N) in rumen fluid of fattening lambs in said treatments was 42.46: 33.86: 31.35 mg/100 ml. pH rumen value in treatments I: II: III were 6.56: 6.35: 6.15.

Trial results show that the level of protein intake was not under significant influence of studied treatment, considering that digestibility coefficients were 52.58%: 51.30%: 55.12%. With the increase of the share of non-degradable protein in concentrate mixtures, the tendency of fat digestibility increase was observed: 76.13: 77.98: 87.17%, also of decrease of cellulose intake: 67.40: 45.87: 22.39% and NFE: 83.87: 76.05: 82.96%.

Key words: lambs, non-degradable protein, rumen fluid content, digestibility

Introduction

Standardization of proteins in diets for lambs aims to ensure adequate amounts of amino acids, which will satisfy their requirements. Amino acids available for absorption are provided from three sources: protein synthesized in rumen by microorganisms (microbial protein), dietary protein which was not degraded by rumen microorganisms (non-degradable protein) and protein of endogenous origin (Grubic et al., 1997). Orskov (1989) states that endogenous protein is negligible and that in lambs weighing 25 kg daily amount of endogenous protein reaching small intestines is 8.8g.

Rumen microorganisms degrade dietary protein to peptides, amino acids and ammonia, and subsequently these substances are used for synthesis of own proteins. In these processes of degradation and synthesis certain losses occur, diminished quantity of amino acids reaches the place of absorption (Grubic et al., 1992; Mekic et al., 1997).

Therefore, it is important, in intensive lamb fattening, to provide optimum level of non-degradable protein, considering that with the increase of genetic capacities of lamb also their requirements increase, especially in this protein fraction which passes through rumen undegraded and together with microbial protein reaches duodenum (Ružic-Muslic, 2006).

Zeremski et al. (1989) point out the significance of protein non-degradable in rumen, emphasizing that, if we want to maximize the utilization of genetic potential of high-yielding meat breeds, it is necessary to take into consideration the share of non-degradable protein, since microbiological synthesis from usual protein and energy sources is not enough to satisfy protein requirements of such animals.

The simplest way to achieve the optimal ratio of degradable and non-degradable proteins is to combine adequate protein sources. Level of protein degradation in rumen and reticulum is different in different feeds. Proteins of animal origin (fish, meat, meat-bone, blood meal) are less degraded

* *Corresponding author: e-mail:* muslic.ruzic@.gmail.com

than plant proteins, whereas non-protein nitrogen compounds (urea) are fully degraded.

Objective of this paper is to investigate the effect of different protein sources in diets on parameters of rumen content and digestibility of nutrients in fattening lambs.

Material and Methods

Study of the parameters of rumen fluid content, depending on the nutrition treatment, was carried out on the experimental sheep farm of the Institute for Animal Husbandry, Belgrade-Zemun, on 18 male lambs of MIS population, divided into three groups, of average body mass of approx. 35.0 kg, average age of approx. 100 days. Animals were fed concentrate mixtures with different source of protein and share of rumen non-degradable protein: 43% (I), 51% (II), 58% (III) which is presented in Table 1. Samples of rumen content were collected by using probe 4 hours before and 4 hours after feeding, and subsequently frozen in acetone and dry ice, after determining of pH value. Frozen samples were stored at temperature of–20^{0}C. 5 ml of each sample was treated with 1 ml of 25% metaphosphoric acid and centrifuged at 10.000 g/min, 15 min at 4^{0}C, and analyzed for presence of ammonia nitrogen, by using standard method (Official Journal of SFRY, No.15/1987).

Digestibility of nutrients was investigated by indirect method on same animals. Investigation lasted 13 days and it included preparation period (in duration of 7 days) and collection period (in duration of 6 days). During entire period, lambs received twice a day measured amount of concentrate mixture (400 g each) supplemented with Cr_2O_3 as indicator, in concentration of 0.5% (Tables 1 and 2).

During collection period, after every feeding, faeces was collected (for 150 g), for each animal, into special dish bearing identification of the animal, group, date and time of faeces collection. Upon completion of the trial period, samples from each dish were homogenized and representative sample was prepared for analysis. Samples and food and faeces remains were analyzed by standard method of chemical analysis (WENDE). Data obtained analytically were used for calculation of nutrients in indirect way, according to following formula:

$$\text{Digestibility, \%} = 100 - \left(\frac{\%\ \text{indic. in food}}{\%\ \text{indic. in faeces}} \times \frac{\%\ \text{nutrients in faeces}}{\%\ \text{nutrients in faeces}} \times 100\right)$$

Statistical processing of data was done by program Stat. Soft, Inc. (2003). STATISTICA (data analysis software system), version 6, by applying standard mathematical-statistical methods which included variance analysis and evaluation of significance of obtained differences with for this purpose appropriate test (Tukey honest significant difference test).

Results

Results of the chemical analysis of rumen fluid and adequate pH values are presented in Table 3.

Table 1
Structure of the concentrate mixture for fattening of weaned lambs, %

Feed	Concentrate mixture		
	I	**II**	**III**
Corn	73	79	82
Sunflower meal	23	5	7
Soy bean meal	0	12	0
Fish meal	0	0	7
Lime	2	2	2
Salt	1	1	1
Premix	1	1	1

Table 2
Nutritional value of concentrate mixtures

Nutritional indicators	Concentrate mixtures		
	I	II	III
Dry matter	87.1	86.5	86.8
OFU	1.2	1.2	1.2
NEM;MJ/kg(*)	7.51	7.98	7.91
Total protein	142	137	141
NP in CP(**)	43	**51**	**58**
**PDIN,g/animal/day	102	103	107
**PDIE,g/ animal/day	102	112	118
Ashes	25	23	27

The content of ammonia nitrogen (NH_3-N) in rumen fluid of fattening lambs, in treatments I:II:III was: 42.46: 33.86: 31.35 mg/100 ml. Established difference between lambs in the treatments I and II, as well as difference between lambs in the treatments II and III, was not statistically significant (P>0.05). At the same time, content of NH_3-N in rumen of lambs in treatments I and III differed by 11.11 mg/100ml, which was statistically confirmed (P=0.02). Rumen fluid pH values in lambs in treatments I: II: III were 6.56: 6.35: 6.15. Established differences varied within limits of random deviations (P>0.05).

Results presented in Table 4 represent average nutrient digestibility coefficients depending on the feeding treatment. Digestibility of total proteins: Average coefficients of total protein digestibility in lambs in treatments I: II: III were 52.58%: 51.30%: 55.12%. Established differences ranged within limits of random deviations (P>0.05).

In calculation of digestibility of these matters the endogenous protein fraction was not excluded, so values obtained in this way represented apparent digestibility of total proteins, which partly explained lower coefficient, values of protein digestibility.

Digestibility of fat: Significant effect of protein source on the degree of fat absorption was registered in analysis of obtained digestibility coefficients. The lowest digestibility of 76.13% was recorded in lambs in treatment I, and the highest (87.17%) in treatment III, and the fat digestibility in treatment II was 77.98%. Determined difference between treatments I and III was statistically significant (P=0.01). In addition, the difference in fat digestibility between animals in treatments II and III was statistically confirmed (P=0.02). So, with the increase of share of non-degradable proteins (43:51:58%) tendency of increase of fat digestibility was noticed.

Table 3
Nutritional value of concentrate mixtures

Treatment	NH3-N (mg/100 ml)	pH
I	$42.46_a \pm 7.22$	6.0 ± 0.32
II	33.86 ± 7.42	6.35 ± 0.82
III	$31.35_b \pm 5.01$	6.15 ± 0.29

Digestibility of crude fibre/cellulose**:** Average crude fibre/cellulose digestibility coefficients in lambs in treatments I: II: III were 67.40%: 45.87%: 22.39%. Established difference in the degree of adoption of fibre between lambs in treatments I and II was statistically very significant (P=0.006) as well as difference between lambs in treatments II and III (P=0.004). At the same time, recorded difference between animals in treatments I and III was statistically highly significant (P=0.003).

Digestibility of nitrogen free extractives (NFE): The degree of adoption of NFE in lambs in treatments I: II: III was 83.87%, 76.05% and 82.96%, indicating the presence of certain tendency of decrease in digestibility with the increase of share of NP in total protein. Difference in NFE digestibility between lambs of groups II and I was statistically confirmed (P=0.01).

Discussion

It is known that rumen bacteria use NH3 generated as product of protein degradation, in presence of adequate amounts of soluble carbon hydrates, in synthesis of amino acids necessary for obtaining of microbial protein. It is evident that different protein sources have induced different concentrations of ammonia nitrogen in rumen fluid. Proteins of animal origin, such as fishmeal, used as protein source in treatment III, are less degraded and therefore release less NH3, compared to proteins of plant origin (soybean meal in treatment II and sunflower meal in treatment I). This is in accordance to results of Seoane and Moore (1969) who state lower level of ammonia in rumen as consequence of relatively low solubility of fishmeal proteins. Saeki et al. (2004) report of similar observations in their study of the effect of diet I containing 12% of soy bean meal and diet II containing 8.5% of fish meal on nutrient digestibility and rumen content in lambs, and significantly lower (P<0.01) content of NH3-N in treatment with fish meal which is source with high content of non-degradable protein.

In their study of the effect of fishmeal on rumen parameters, Walz et al. (1998) report of the trial on lambs of average body mass of 25.0 kg. Feeding/nutrition treatment included

Table 4
Parameters of the fluid content of rumen

Nutrient	I	II	III
Total protein	52.58± 5.98	51.30± 3.51	55.12±1.95
Crude fat	76.13a± 3.49	77.98a± 1.95	87.17b± 1.30
Crude fibre/cellulose	67.40ad± 6.19	45.87c± 4.32	22.39ae± 1.91
NFE	83.87a± 3.21	76.05b±3.13	82.96± 1.52

use of diet with and without 3% of fishmeal. The concentration of ammonia nitrogen in rumen of lambs, which consumed diet without this protein component, is 5.9 mM, and in treatment with fishmeal 4.5mM. Rumen pH values are 6.69 and 6.52.

Santos et al. (1984) state that the content of NH3-N is by 40% higher in rumen of cows fed diet containing soy bean flour as main protein component, compared to cows fed diets containing corn gluten (as feed with high share of non-degradable protein). Also, Keery et al. (1993) in the study of the effect of different protein sources (and level of degradability) on rumen fermentation show that the concentration of NH3-N in rumen of young cattle fed diets containing soy bean flour, is by 38% higher compared to other animals in treatment with heat treated soy bean and corn gluten (representing sources of rumen non-degradable protein).

Results of our research show that in regard to pH value of rumen content no significant differences between studied treatments were observed ($P>0.05$), which is in accordance with results of Viswanathana and Fontenota (2009). In the study of the effects of protein supplements (soybean, hydrolyzed protein supplement, commercial supplement based on animal proteins, crab meal, urea and bio product of plant remains), which differed in the share of non-degradable protein, they establish that rumen pH shows no significant difference between treatments ($P>0.05$). At the same time, our data are in accordance with results presented by Titgemeyer et al. (1989), Seymour et al. (1992), Schloesser et al. (1993) and Cozzi and Poland (1994) in the examinations of feeds different in regard to protein source, i.e. share of rumen non-degradable protein.

About protein digestibility, by comparing our results with data of other authors obtained in similar researches, slightly lower values of protein digestibility can be observed compared to literature results. Difference between established protein digestibility coefficients in our research and literature data can be explained by fact that the degree of adoption of substances of the nitrogen complex is influenced by several factors: nature and structure of the diet, degree of protein solubility and protein and non-protein nitrogen ratio in diet.

Matras et al. (2000), in their study of nutrient digestibility in lambs fed diet I containing ground barley and urea (high level of rumen degradable protein) and diet II containing corn cracked and corn gluten flour (high level of rumen non-degradable protein), establish average protein digestibility coefficients: 60.2% (I) and 63.5% (II). Also, Dabiri and Thonney (2001) report that the degree of adoption of protein in lambs in treatment with soybean and fishmeal is: 75.3% and 74.2%. Our results are in accordance with data obtained by Christensen et al., (1993) who report no difference ($P>0.05$) in digestibility of protein with low degradability (55%) and highly degradable (70%) proteins. In study by Tiwari et al. (2000) there is no difference in protein digestibility in calves fed diets containing protein components different in regard to degradability (untreated walnut meal, walnut meal treated with formaldehyde and fish meal). Similar conclusion is reached by Viswanathana and Fontenota (2009), Keery et al. (1993), Ludden and Cecava (1995), who state that the degree of protein adoption is not significantly different between protein sources ($P>0.05$). Stock et al. (1981) state that protein digestibility is lower ($P<0.05$) in lambs fed diets containing urea, compared to diet containing combination of blood meal and urea. This confirms that for the optimal activity of rumen micro flora and maximum performance it is necessary to ensure optimal ratio between proteins which are degraded in pre-stomachs under the action of microorganisms (degradable protein) and by-pass proteins (non-degradable protein). The degree of degradation in rumen is different for different feeds, but in principle proteins of animal origin (fish, meat, meat-bone, and blood meal) are less degraded than plant proteins, whereas the non-protein nitrogen compounds (urea) is fully degraded. Therefore, choice of source of protein whose degradability in rumen and reticulum will satisfy the requirements for expected production is basis of proper diet formulation for lambs.

With the increase of share of non-degradable protein in mixtures (43:51:58%), a tendency of increase of digestibility of fat was observed. Apparently optimal conditions about degradable: non-degradable protein ratio and quantity of available energy were realized, which is optimal pre-condition for activity of rumen microorganisms. Our results are in accordance with results of the study of the effect of different protein sources (alfalfa meal and sunflower meal) in diets for lambs on digestibility of nutrients by Ljumovic et al. (1967) who report following values: 74.99% : 62.60%. In addition, our results are in accordance with results of Jovanovic et al. (1972). Negovanovic et al. (1983) and Ružic (1997). Pejic et al. (1986) point out that fat digestibility depends on its triglyceride structure, length of the fatty acid chain, as well as diameter of fat particles/granules, since only fat granules of specific size have sufficient energy to enter the digestive system and resorption. This is explained by Km/e, which represents the ratio between the particles of micellar and emulsion phase, and it increases significantly with the shortening of fatty acid chains.

Digestibility of crude fibre/cellulose shows tendency of decrease with the increase of level of non-degradable protein in mixtures for lambs. This trend is in accordance with research results of Husein et al. (1991) who report considerable improvement in fibre digestion in rumen in treatments with highly degradable protein.

Explanation is in the fact that growth of microorganisms and their activity depend on the quantity of degradable protein and available energy. Considering that the level of degradable protein in our research decreased from treatment I to III, the trend of decrease of fibre digestion degree in analogue treatments is understandable. This was especially observed in case of nutrition III. At the same time, in case of this type of nutrition with lower fibre content, and higher content of easy digestible carbon hydrates, higher quantities of propionic, butyric and acetic acid are formed resulting in decrease of rumen pH value. Common mechanism of pH buffering in rumen was not sufficient in this case, so lower pH value inhibits growth of cellulolytic bacteria, which depresses fibre digestion. Our results are contrary to data obtained by Milisa et al. (2004) who report following fibre digestion coefficients: 65%:66%:64%:73%, respectively, in treatments with 35%:34%:43%:42% of non-degradable protein in diets for sheep, which is associated with diet structure and associative effect of used feeds (cottonseed meal, wheat bran, corn gluten, combination of corn gluten and gluten feed).

About NFE digestibility, the declining trend was observed with the increase of the level of non-degradable protein in mixtures, especially in treatment II compared to treatment I (P=0.01). This is probably consequence of the diet structure causing depression in digestion of nitrogen free extractives, considering that conditions for the activity of the rumen micro flora were not optimal.

Values of results obtained in our research are slightly lower than data reported by Ružić (1997) who establishes NFE digestibility coefficient in trial with lambs fed diets containing different energy content of: 88 : 87 : 86%, going from the highest to the lowest energy level. Negovanović et al. *(*1983) report that increase of the energy level in diet is accompanied by the decrease in the degree of NFE adoption, with following values: 89.4% :, 88.00% : 86.20% . In general, we can conclude that obtained digestibility coefficients were high, which is understandable considering that this type of nutrients is chemically very heterogeneous and characterized with high solubility in water and easy adoption by the animal organism.

Conclusion

Based on obtained results of the investigation of the effect of different protein sources in mixtures on parameters of rumen fluid and nutrient digestibility, in lambs in intensive fattening, the following can be concluded:

Content of ammonia nitrogen (NH_3-N) in lamb rumen fluid, in treatments with sunflower meal, soy bean meal and fish meal, was 42.46: 33.86: 31.35 mg/100ml. Statistically significant difference (P=0.02) was established between III and I nutrition treatments.

Values of rumen pH in analogue treatments were 6.56: 6.35: 6.15.

Different protein sources in mixtures for lamb nutrition in intensive fattening had no significant effect on degree of total protein adoption.

With the increase of share of non-degradable protein, a tendency of increase in fat digestion was observed.

The degree of fibre and NFE adoption had declining trend with the increase of level of non-degradable protein.

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