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Changes in some blood parameters in yearling rams fed diets with different protein and lipid levels

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Abstract. An experiment was conducted to evaluate the effect of three rations with different lipid and protein levels on some blood biochemical parameters in yearling rams. The experiment was performed with nine Blackhead Pleven yearling rams, with initial average body weight 45.2 kg. They were divided in three groups of three animals each and housed indoor, in individual boxes. The animals from the three groups were fed 3 rations, conditionally termed ration I, ration II and ration III were tested. Ration I contained 1.00 kg ground barley and 1.00 kg meadow hay. To the others, a different protein and lipid source was added. Ration II consisted of 1.00 kg meadow hay, 0.800 kg barley mash and 0.200 kg sunflower expeller. Rations differed with respect to their lipid and protein contents. They were offered twice daily – 8:00 AM and 1:00 PM. Blood samples were collected from vena jugularis externa during three consecutive days, twice daily – before and 2.5 hours after feeding. The following parameters were assayed: total protein, albumin, globulins, total lipids, total cholesterol and HDL cholesterol. The effect of the three rations on studied parameters and the changes before and after feeding were followed out. The results showed that feeding rations with different protein and lipid sources did not result in statistically significant changes in blood total protein and albumin in yearling rams. There were neither considerable changes in total lipids concentrations. In animals fed ration II (containing sunflower meal), a pre-prandial increase in blood globulins (p<0.05) was noted compared to animals fed sunflower expeller. The globulin concentrations were also higher than average values in rams fed ration I, but the differences were not significant. Total and HDL cholesterol were significantly higher (p<0.01) 2.5 hours after feeding ration III containing sunflower expeller compared to both rations I and II.

Keywords: sheep digestion, blood parameters in sheep

Introduction

The production potential of feeds is mainly expressed through their nutritive value which is determined by all organic nutrients. The systems for feed evaluation also include their protein value although all other ingredients (fat, crude fibre, vitamins, minerals etc.) are also important for animal nutrition and performance. Traditional feeds are of low fat content. The chemical composition of lipids of roughages and concentrates differ significantly. This results in a different contribution of these two groups of feeds for satisfying body needs of fatty acids in ruminants.

Since 1970, the degradation of lipids in the rumen of ruminants, its effect on rumen function and the subsequent effect on lipid metabolism in animals is extensively researched. In the fore stomachs of ruminants, lipids are hydrolysed to free fatty acids and glycerol by enzymes in feeds and especially those released by microorganisms (Mansbridge and Blake, 1997). Short-chain fatty acids are products of microbial lipase activity, which are absorbed through the walls of the rumen and enter the blood circulation. Medium- and long-chain fatty acids are absorbed in the small intestine. Most unsaturated fatty acids are hydrolysed in the rumen and often, this process is accompanied by double bonds isomerisation (from cis- to trans-position).

Feeding ordinary rations results in hydrolysis of about 85% of fat (Harfoot, 1981; Doreau et al., 1997). Some of the factors influencing the rate of hydrolysis have been identified. The hydrolysis rate decreases when the amount of dietary fat is higher (Beam et al., 2000), when pH is low and after intake of ionophore antibiotics which suppress the activity and growth of bacteria and ciliates (Enev, 1983; Van Nevel and Demeyer, 1995, 1996b; Demeyer and Doreau, 1999). Another important transformation of fat in ruminant fore stomachs is the breakdown of double bonds of unsaturated fatty acids and their saturation with hydrogen. This process is known as fatty acids bihydrogenation. It occurs under the influence of microflora of fore stomachs (Song, 2003; Lundy et al., 2004).

Proteins also undergo substantial changes in fore stomachs and subsequent alimentary tract compartments. Resulting metabolic products are polypeptides, amino acids and ammonia. Their involvement in metabolism consists in utilisation by rumen microflora and fauna or absorption through rumen wall into blood circulation. Ammonia is the end product of degradation of plant and animal protein in the rumen and a source for synthesis of amino acids by many ruminal bacterial species. Its rumen concentrations varied within a rather large range and depend mainly on the rate of breakdown of proteins, the metabolic activity of ruminal microflora, evacuation rate of nutrient masses to the abomasum, the activity of absorption through the ruminal wall and level of microbial protein synthesis (Hristov et al., 2003), which is largely dependent on the species, breed and age of the animal, the diet composition, concentrate to roughage ratio, time, feeding intervals etc.

Regardless of the blood buffering systems, the different dietary composition and level of nutrients, nutritional supplements, enzymatic preparations and biotechnological products could influence the metabolism of proteins and lipids in the animal body and subsequently, to alter blood parameters, animal health and performance (Knoultfn et al., 2002). Many investigations conducted worldwide have studied the effect of rations with different structure on fermentation in fore stomachs and the digestion in ruminants. At the same time, there are no studies directly aimed at evaluation of the effect of various dietary protein and lipid levels in ruminant...
rations on their blood parameters.

The purpose of the present study was to investigate the effect of three rations with different lipid and protein levels on some blood biochemical parameters in yearling rams.

Material and methods

An experiment was conducted to evaluate the effect of rations with different lipid and protein levels on some blood biochemical parameters in yearling rams. The trial was carried out in the experimental base of the Animal Physiology unit to the Faculty of Agriculture, Trakia University, Stara Zagora. Three rations, conditionally termed ration I, ration II and ration III were fed to animals. All they were based on ground barley (1.00 kg). Ration I contained also 1.00 kg meadow hay. The others were supplemented with a different protein and lipid source – sunflower meal or sunflower expeller. Ration II consisted of 1.00 kg meadow hay, 0.800 kg barley mash and 0.200 kg sunflower meal. Ration III contained 1.00 kg meadow hay, 0.800 kg barley mash and 0.200 kg sunflower expeller.

Rations differed with respect to their lipid and protein contents. They were offered twice daily – 8:00 AM and 1:00 PM. The chemical composition of rations is presented in Table 1.

Table 1. Chemical composition of feeds

<table>
<thead>
<tr>
<th>Feed</th>
<th>DM (%)</th>
<th>Crude protein</th>
<th>Crude fibre</th>
<th>Crude fat</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meadow hay</td>
<td>88.20</td>
<td>9.03</td>
<td>9.03</td>
<td>1.90</td>
<td>1.00</td>
</tr>
<tr>
<td>Barley mash</td>
<td>89.90</td>
<td>9.60</td>
<td>9.60</td>
<td>1.70</td>
<td>1.30</td>
</tr>
<tr>
<td>Sunflower meal</td>
<td>88.80</td>
<td>32.50</td>
<td>32.50</td>
<td>1.50</td>
<td>5.70</td>
</tr>
<tr>
<td>Sunflower expeller</td>
<td>89.70</td>
<td>31.10</td>
<td>31.10</td>
<td>8.80</td>
<td>6.20</td>
</tr>
</tbody>
</table>

The experiment was performed with nine Blackhead Pleven yearling rams, with initial average body weight 45.2 kg. They were divided in three groups of three animals each. The animals were housed indoor, in individual boxes.

Blood samples were collected from vena jugularis externa during three consecutive days, twice daily – before and 2.5 hours after feeding. The following parameters were assayed: total protein, albumin, globulins, total lipids, total cholesterol and HDL cholesterol. The effect of the three rations on studied parameters and the changes before and after feeding were followed out. The analyses of blood serum were performed in a licensed laboratory (Bodylab, Sliven) using analytical methods described by Sivkova (2007).

The results of experiments were processed by Statistica for Windows software (Stat. Soft. Inc., 1994) and Microsoft Excel 2007. Graphs were built using Microsoft Excel 2007.

Results and discussion

Blood plasma is a mix of various proteins. They are termed individual serum proteins. The total serum protein includes all blood proteins except those of blood cells, haemoglobin and fibrinogen. Total protein is the sum of albumin and globulins. Its blood levels are influenced by nutritional factors (type and structure of the ration, supplementation with exogenous enzyme preparations) as well as physiological factors. The blood total protein concentrations in experimental animals is presented on Figure 1. Feeding ration I resulted in pre-prandial total protein levels of 83.8 g/l and 2.5 hours after feeding, it decreased insignificantly to 80.29 g/l. In animals fed ration with sunflower meal (ration II), total protein levels in blood before feeding were 85.39 g/l and 2.5 hours after feeding decreased substantially (p<0.01) to 79.85 g/l. In rams fed ration III, fasting blood total protein was 82.36 g/l and 2.5 hours after feeding, its concentrations increased slightly up to 82.87 g/l. The tendency towards reduction of blood total protein in animals after feeding rations I and II should be emphasized. The feeding of the third ration did not change a lot this parameter 2.5 hours after feeding. The comparison of the effect of the three rations on blood total protein showed that this parameter did not change significantly before and after feeding.

Sivkova et al. (2006) have studied the effect of the enzymatic preparation Xibeten-Cel on haematological and blood protein parameters in rams. According to the authors, the preparation added to four different diets exerted an influence on total blood proteins before and after feeding. The highest levels were observed in rams fed a diet consisting of alfalfa hay and concentrate.

![Figure 1. Effect of different dietary lipid and protein levels on blood serum total protein](image-url)

The albumins are a group of proteins with common physical and chemical properties such as high solubility in water, low to moderate solubility in concentrated salt solutions and thermo coagulation ability. Serum albumin is a primary blood plasma constituent that makes up the major proportion of total plasma proteins. For example, human serum albumin attains up to 60% of total plasma...
albumins in yearling rams.

Figure 2. Effect of different dietary lipid and protein levels on blood serum albumin

proteins. In ruminants, its concentrations are within 42.4–62.5 g/l (Sivkova et al., 2006). The other plasma proteins are under the general name of globulins. They are further divided into alpha-, beta- and gamma-globulins. Gamma-globulins are mainly involved in systemic immune functions. In the present study we established that blood albumin concentrations (Figure 2) varied between 45.28 g/l before to 47.77 g/l (2.5 hours after feeding). After feeding ration I, the pre-prandial albumin level was 46.25 g/l. Two and a half hours after feeding, it increased statistically insignificantly to 47.77 g/l. The same tendency in postprandial hours was observed after feeding ration II – increase in albumin from 45.28 g/l before to 45.97 g/l after feeding. Unlike rations I and II, feeding ration III resulted in a trend towards reduction 2.5 hours after feeding. The concentrations ranged from 47.65 g/l before to 46.94 g/l 2.5 hours after feeding experimental animals. As seen from the graph, feeding different dietary protein and lipid levels did not result in considerable changes in blood serum albumins in yearling rams.

Blood serum globulin concentrations are shown on Figure 3. In animals fed ration I, pre-prandial blood globulins averaged 37.65 g/l whereas after feeding, decreased significantly (p<0.05) to 32.62 g/l. After feeding ration II, globulin level was 39.90 g/l before feeding and significantly lower 33.88 g/l (p<0.01) after feeding. In rams fed ration III, globulin levels were higher after feeding (p<0.05).

Serum albumin concentrations correlated negatively with globulin concentrations. The total blood protein is mainly determined by serum albumin and globulins. That is why, the reduction in blood globulins in experimental animals after feeding rations I and II was anticipated at the background of increased postprandial albumin concentrations. Feeding a diet containing sunflower meal (ration II) resulted in increased blood globulins (p<0.05) before feeding compared to the ration with sunflower expeller. The concentrations in group II were also higher compared to ration I, but not significantly.

Santra and Pathak (2000) investigated the effect of different rations on some haematological indices in calves. They did not find any differences in total protein, albumin and globulin levels in blood in experimental animals, fed different concentrate/roughage ratios. Also, blood glucose was higher after feeding the high-concentrate ration.

The blood concentrations of total lipids in experimental yearling rams (Figure 4) varied from 1.70 g/l before feeding to 2.67 g/l 2.5 hours after feeding for all tested rations. After feeding ration I, there were no significant changes in blood total lipids. The pre- and after feeding average values were 1.98 g/l and 1.75 g/l, respectively. The same tendency of variation in this blood parameter in postprandial hours was demonstrated after feeding rations II and III as well. In animals fed ration II, blood total lipids changes from 1.70 g/l before feeding to 1.77 g/l 2.5 hours after feeding. In rams fed ration III, fasting total lipids averaged 2.23 g/l. Two and a half hours after feeding they increased to 2.67 g/l. The results indicated that the different dietary protein and lipid levels did not influence significantly blood total lipids concentrations.

Serum lipid concentrations are shown on Figure 4. Total serum lipids concentrations in rams increased significantly (p<0.05) after feeding the high-concentrate ration. The total serum lipids concentrations in rams fed ration I and II did not change significantly after feeding rations I and II.

Figure 3. Effect of different dietary lipid and protein levels on blood serum globulins

Figure 4. Effect of different dietary lipid and protein levels on blood serum total lipids

alcohol), a lipid found in cell membranes in the body of mammals which is transported through blood circulation. The cholesterol participates in all essential systemic biochemical processes. It is insoluble in blood, but is transported bound to one of the multiple sphere-shaped lipoproteins, whose outer surface carries water-soluble particles. The larger part of cholesterol is synthesised in the body, a small part only comes from food. Blood cholesterol levels in experimental animals are presented on Figure 5. They did not change considerably after feeding ration I. The initial mean value with this ration was 1.63 mmol.l⁻¹. Two and a half hours after feeding, it decreased slightly to 1.3 mmol.l⁻¹. With ration II, a statistically significant reduction (p<0.05) in total cholesterol was established 2.5 hours after feeding. The total cholesterol values ranged between 1.49 mmol.l⁻¹ and 1.29 mmol.l⁻¹ before and 2.5 hours after feeding, respectively.

Conflicting results have been obtained after feeding ration III, containing sunflower expeller. In pre-feeding hours, total cholesterol
was 1.64 mmol l⁻¹. It then increased to 1.82 mmol l⁻¹ (2.5 hours after feeding). The comparison of blood total cholesterol concentrations showed that yearling rams fed ration III (with sunflower expeller) increased statistically significantly (p<0.01) blood total cholesterol 2.5 hours after feeding, compared to rations I and II. HDL cholesterol, also called "good" cholesterol cleans the walls of mammalian blood vessels removing the excess cholesterol, which forms vascular plaques and provokes cardiovascular diseases. This excess cholesterol is brought by HDL cholesterol to the liver where it is metabolised. The low blood HDL cholesterol level is associated with increased risk for cardiovascular disease, even when total cholesterol concentrations are normal. Thus, the higher the blood HDL cholesterol levels, the better the health. Blood HDL cholesterol concentrations are presented on Fig. 6. The data are similar to those for total cholesterol observed in this study. The observed tendencies were the same. With the first two rations, the studied blood parameter decreased (from insignificantly to p<0.05) in postprandial hours, whereas after feeding ration III, the HDL cholesterol concentrations after feeding were higher. In the group fed ration I, mean fasting HDL cholesterol was 0.88 mmol l⁻¹ and decreased slightly to 0.67 mmol l⁻¹ 2.5 hours after feeding. After feeding ration II, blood HDL cholesterol decreased significantly (p<0.05) 2.5 hours after feeding to 0.64 mmol l⁻¹ from 0.77 mmol l⁻¹ before feeding. Before feeding ration III, average HDL cholesterol was 0.89 mmol l⁻¹ and 2.5 hours after feeding, increased insignificantly to 0.98 mmol l⁻¹. The data obtained for HDL cholesterol levels were comparable to those for blood total cholesterol. Both parameters increased (p<0.05; p<0.01) 2.5 hours after feeding ration III as compared to rations I and II.

The dietary level and quality of proteins and lipids in roughages and concentrates differ significantly. These nutrients undergo major changes in the alimentary tract of ruminants. The changes begin in the fore stomachs and then, in small intestine. They reflect on the overall function of the digestive system, peripheral blood composition and the systemic homeostasis in general.

In the fore stomachs of ruminants, lipids are hydrolysed to free fatty acids and glycerol. These metabolites are absorbed through the ruminal wall into the bloodstream. The medium-chain and long-chain saturate fatty acids are absorbed in the small intestine. The dietary protein also undergoes considerable transformation in fore stomachs and distal alimentary tract compartments of ruminants. The degradation products of proteins are polypeptides, amino acids and ammonia. Their participation in metabolic pathways consists in utilisation by rumen microflora and fauna, absorption through the ruminal wall and transfer into blood circulation.

The blood buffering systems maintain the primary haematological parameters within the respective reference ranges. Their variations in postprandial hours are not important. The various dietary ratios of main nutrients could influence the metabolism of proteins and lipids in fore stomachs and distal digestive tract compartments of animals. As a consequence, the metabolites from protein and lipid degradation, absorbed in blood, could alter the main blood parameters in small ruminants.

Conclusions

Feeding rations with different protein and lipid sources did not result in statistically significant changes in blood total protein and albumin in yearling rams. There were neither considerable changes in total lipids concentrations.

In animals fed ration II (containing sunflower meal), a pre-prandial increase in blood globulins (p<0.05) was noted compared to animals fed sunflower expeller. The globulin concentrations were also higher than average values in rams fed ration I, but the differences were not significant.

Total cholesterol and HDL cholesterol were significantly higher (p<0.05; p<0.01) 2.5 hours after feeding ration III containing sunflower expeller compared to both rations I and II.

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