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INFLUENCE OF DIFFERENT LEVELS OF DIETARY VITAMIN A ON PROTEIN STATUS IN BLOOD SERUM OF LAMBS

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Three groups of 10 weaned female lambs of average body weight 20-30 kg, were fed with hay and concentrates for a 99 day trial. The feed for groups I, II and III containd 10.000, 20.000 and 30.000 IU vitamin A/kg, respectively. Blood samples were taken on days 1,52 and 99 of the trial and the concentration of total proteins and their fractions were measured.

At the beginning of the trial there no significant differences in the concentration of total proteins and their fractions among the groups. The increased quantity of vitamin A in the feed (20.000/U/kg) positively influenced the total protein concentration, but the established increase was limited and further increase of dietary vitamin A was withaut effect.

The increase of total protein occurred through an increase of α-and β-globulin at the expense of albumin with stable b-globulin fractions. The decline of the A/G ratio was proportional to the increase of dietary vitamin A content.

Key words: probeins, blood, vitamin A, lambs.

INTRODUCTION

Proteins have great physiological importance due to numerous functions in the organism. The concentration of blood plasma proteins is 5-6 times greater than the sum of all other organic and inorganic compounds. In healthy animals the total amount of blood proteins is mostly uniform, but even in normal physiological conditions significant variations of certain protein fractions can be noticed.

Ewe blood serum contains an average amount of 53.8 g/l of total proteins (cit. acc.: Dukes, 1976), in which 30,7 g/l is albumin and 23.1 g/l is globulin. Protein concentration depends on the age of the animals, especially γ-globulin levels

which, due to its physical and chemical properties, can not penetrate the placenta, and lambs have to acquire them via colostrum.

Jovanović et al. (1983) described the metabolical profile of ewes from three different areas during all four seasons. Among the other results, the authors cited that total blood protein level is highest during springtime (average 85.2; 91.9 and 94.4 g/l), and lowest during the winter (average 75.2; 76.1 and 74.4 g/l). Similar investigations (Jovanović et al., 1986) were made on 1.5-5 month-old lambs. The authors state that total blood protein level is the highest during springtime (average 73.22 g/l), and the lowest during the winter (average 65.93 g/l). Similar results were reported by Terek (1980), Udalova et al.(1982) and Adewuyi and Adu (1984).

Villette and Levieux (1982) investigated plasma immunoglobulin levels in 56 two-day-old lambs, in relation to maternal age. A moderate increase was noted for ewes aged 6 years (43.4 g/l), with a sharp decrease when aged 8-10 years (11.2 g/l). Terek (1980a) reported that blood protein level increases from the 3rd month, achieving the normal adult physiological level at 6 months. Sova et al. (1986) observed that male lambs had blood protein concentrations of 42.7-94.1 g/l, while female lambs had 85.8-88.9 g/l.

Numerous authors investigated the effects of diets and certain nutrients on the protein system. Thus Dahlborn (1988) showed that during starvation water metabolism is decreased, and total protein level is increased, which indicates hypovolemia. Thomas and Chiboka (1984) and Kazanovski et al. (1981) reported similar results.

Bruns and Webb (1985, 1986) demonstrated the positive influence of dietary vitamin A on immunoglobulin levels in lamb blood serum. When the dietary level of vitamin A was below requirements, a significant alteration in immunoglobulin concentration was noticed. Significant increases of total protein concentration accompanied increased amounts of vitamin B_6, inorganic sulphur and methionine (Vislnyakov et al., 1982), proteins (Akhulin and Plekhanov, 1981) and plant oils in the diet (Maksinov and Datsun, 1982), while feeding diets poor in some minerals led to decreased blood serum protein concentration.

Taking into account the numerous data in the literature and the fact that proteinaemia and the ratios among serum protein fractions is under the influence of many different factors, the aim of this study was to examine the influence of various levels of dietary vitamin A on the total protein and protein fractions in the blood serum of lambs.

MATERIALS AND METHODS

The 99 day long experiment was initiated with 3 groups of 10 female lambs (Pramenka x Würtemberg), of average live weight 20-30 kg, immediately after weaning. All lambs were in good health and condition. The technology of management and nutrition usual for the farm was used in the trial with minimal modification. The lambs were fed with concentrate (Table 1) and meadow hay (NRC, 1977). Feeding with mash was ad libitum, while hay was given maximally

up to 400 g/day per animal. The feed for groups I, II and III contained 10.000, 20.000 and 30.000 IU vitamin A/kg feed, respectively.

Table 1. Composition of complete feed mix and meadow hay for lambs (%)

Composition, %		Chemical composition, %		
Ingredient	Mash	Ingredient	Mash	Hay
Maize	53.0			
Sorghum	10.0	Moisture	87.48	90.69
Wheat	5.0	Ash	5.22	7.77
Soyabean meal, solvent	7.0	Crude protein	14.70	9.70
Rapeseed meal, solvent	4.0	Ether extract	0.51	1.26
Sunflower meal, solvent	8.0	Crude fiber	6.40	25.22
Sugar beet pulp, dehy.	5.0	N-free extract	60.65	46.44
Alfalfa meal,dehy.	5.0	Ca	0.58	0.52
Dicalcium-phosphate	0.5	P	0.51	0.20
Limestone, ground	1.3	NaCl	0.71	—
Iodised salt	0.7	NE, SJ/kg	0.64	0.34
Vitamin-mineral premix	0.5			
Group		I	II	III
Vit. A, 000 IU/kg		11.2	21.6	31.4

Blood samples were taken from v. jugulars with a sterile needle over previously disinfected skin, immediately after weaning (1st day), in the middle of the trial (52nd day) and at the end of the trial (99th day). Samples were taken from 8 lambs from each of the three groups. After blood coagulation and separation of blood serum, the concentration of total proteins was determined by a colorimetric method and protein fractions by paper electrophoresis (cit. acc.: Majkić-Singh, 1982). At the same intervals the feed was sampled and the amount of vitamin A was determined using the Carr-Price method (cit. acc.: Majkić-Singh, 1982).

All data were statistically processed and an appraisal was made of the significance of differences in mean values between the groups of lams.

RESULTS AND DISCUSSION

The examination of the total protein concentration in blood serum of lambs (Table 2) did not reveal significant differences among the groups at the beginning of the experiment. The mean protein concentrations were similar and ranged from 67.6-69.5 g/l. Physiological limits range from 65-70 g/l, according to Kochetov 1986. These results lead to the conclusion that the serum protein concentration in weaned lambs is very similar to the concentration in adult animals.

Evaluation of the influence of vitamin A on the protein concentration, showed only minor differences. Considering the obtained results, it can be concluded that the increased quantity of vitamin A in the feed (20 000 IU/kg)

positively influenced the total protein concentration, but the increase was limited and further increase of dietary vitamin A was without effect.

Table 2. Concentration of total protein and A/G ratio* in blood serum.

Group	Total proteins (g/l)			A/G ratio		
day	1st	52nd	99th	1st	52nd	99th
I	69.50±4.84	66.37±4.24^{x}	70.87±3.36^{y}	0.76±0.08^{x}	0.52±0.04^{a,y}	0.50±0.04^{a,y}
II	67.62±3.07	65.87±4.15^{x}	71.50±4.54^{y}	0.76±0.10^{x}	0.46±0.03^{b,y}	0.44±0.04^{b,y}
III	67.62±2.61	65.25±4.53	68.37±4.69	0.78±0.07^{x}	0.34±0.03^{c,y}	0.38±0.06^{c,y}

*Values expressed as X SD
a, b, c Mean values within column with unlike superscripts letters were significantly different ($p<0.05$, Student's t test)
x, y, z Mean values within row within row unlike superscripts leters were significantly different ($p<0,05$, Student's t test)

The relative serum albumin concentration in lambs at the beginning of the experiment was similar (Table 3) and ranged between 43.0-43.87%. The difference within the groups was also minimal and considered to be the result of individual variations. The albumin ratio obtained in this experiment was within the physiological limits (Kochetov, 1986). This author claimed that the albumin-total protein ratio ranges between 35-50% or less, while some data in the literature (Keay and Doxey, 1984; cit.: Dukes, 1976) claim the albumin/total protein ratio to be around 60%. The absolute concentration of albumin in the first group increased at the end of the experiment, following a decrease in the middle, but the values obtained were lower than those at the beginning of the experiment. The same phenomenon was observed in all experimental groups. The extent of the decrease in albumin concentration correlated with the increase of dietary vitamin A content.

The mean α-globulin concentration (Table 3) at the beginning of the experiment ranged from 12.78-13.19 g/l in the three groups. The α-globulin concentrations showed an increasing trend that was obvious in all groups. It may be concluded that the constant increase of α-globulin concentration in the experimental period is physiological and that the increased content of vitamin A in the diet only helps this mechanism without an essential influence on it.

The β-globulin concentration in each group of lambs was similar at the beginning of the experiment. Analysing the absolute index of β-globulin concentration a very slow increase in the first and the second phase of the experiment was noticed. This moderate elevation was insignificant and it can be concluded that the concentration of β-globulin slightly varies depending on age, and that it achieves adult levels after weaning. Critical and strict analyses revealed a slight negative correlation with the vitamin A quantity in the diet. On the other hand, the quantity of vitamin A in the feed cannot influence the physiological mechanisms that maintain β globulin concentration.

The mean concentration of γ-globulin in the lambs was similar and ranged between 19.0-19.66 g/l at the beginning of the experiment. During the first phase

of the experiment an obvious trend for γ-globulin concentration to increase was established. These results correspond to the data of other authors (Burns and Webb, 1985, 1986).

Table 3. Relative and absolute concentrations of protein fractions in blood serum.

Group / day	Protein fractions (%)			Protein fractions (g/l)		
	1st	52nd	99th	1st	52nd	99th
			Albumin			
I	43.12 ± 2.47^{x}	$34.12\pm1.96^{a,y}$	$33.37\pm2.00^{a,y}$	29.94 ± 2.26^{x}	$22.60\pm1.01^{a,y}$	23.65 ± 1.84^{y}
II	43.00 ± 3.21^{x}	$31.37\pm1.41^{b,y}$	$30.50\pm1.69^{b,y}$	29.06 ± 2.25^{x}	$20.65\pm1.22^{b,y}$	$21.82\pm1.99^{b,y}$
III	43.87 ± 2.17^{x}	$25.50\pm1.60^{c,y}$	$27.50\pm2.93^{c,y}$	28.91 ± 2.23^{x}	$16.60\pm1.05^{c,y}$	$18.84\pm2.82^{c,y}$
			α-globulins			
I	19.00 ± 2.73^{x}	24.62 ± 1.30^{y}	25.12 ± 2.36^{y}	13.19 ± 2.01^{x}	16.31 ± 0.80^{y}	17.71 ± 1.47^{z}
II	19.25 ± 2.82^{x}	24.62 ± 2.13^{y}	25.62 ± 1.41^{y}	13.02 ± 2.04^{x}	16.23 ± 1.79^{y}	18.32 ± 1.70^{z}
II	18.62 ± 2.26^{x}	25.50 ± 1.60^{y}	25.75 ± 2.91^{y}	12.78 ± 1.64^{x}	16.64 ± 1.59^{y}	17.71 ± 3.11^{y}
			β-globulins			
I	9.62 ± 1.60	9.75 ± 1.03^{a}	10.00 ± 2.07	6.70 ± 1.22	6.48 ± 0.92	7.17 ± 1.64
II	9.62 ± 1.60	11.25 ± 1.58^{b}	11.00 ± 1.41^{b}	6.50 ± 1.03^{x}	7.43 ± 1.31	$7.86\pm1.12^{b,y}$
III	9.37 ± 1.68	10.25 ± 1.39	9.12 ± 1.96^{c}	6.33 ± 1.12	6.69 ± 1.06	6.28 ± 1.56^{c}
			γ-globulins			
I	28.25 ± 2.25^{x}	$31.50\pm2.27^{a,y}$	$31.50\pm1.77^{a,y}$	19.66 ± 2.43^{x}	20.98 ± 2.84^{a}	$22.34\pm1.89^{a,y}$
II	28.12 ± 1.25^{x}	$32.75\pm2.49^{a,y}$	$32.87\pm1.73^{a,y}$	19.04 ± 1.58^{x}	21.57 ± 2.02^{y}	$23.49\pm1.69^{b,y}$
III	28.12 ± 1.81^{x}	$38.75\pm2.87^{b,y}$	$38.87\pm3.75^{b,y}$	19.00 ± 1.16^{x}	$25.32\pm2.92^{b,y}$	$25.24\pm2.95^{b,y}$

*Values expressed as X SD
a, b, c Mean values within column with unlike superscripts letters were significantly different ($p<0.05$, Student's t test)
x, y, z Mean values within row with unlike superscripts letters were significantly different ($p<0.05$, Student's test)

The A/G ratio summarised all the results obtained and confirmed the literature data indicating that there is a larger share of globulin fractions in the total proteins in lamb sera. An obvious decrease of the A/G ratio was noted when the content of vitamin A in the diet (r_A = -0,74**) was increased.

Pavlović and Vitić (1979) consider that the normal total protein content in sheep blood is 66 g/l with 40-48 g/l albumin depending on the sex. Male lambs have a lower concentration of albumin, but higher concentrations of β-and especially γ-fractions.

Ninkov (1987) defines 63-67 g/l as the average protein content in males and 73-75 g/l in female lambs. In the total protein content, the average amount of albumin in male lambs was 46-48%, while α-, β- and γ-globulins were 15-18%, 10-13% and 21-23%, respectively. The participation of albumin and globulin (α-, β- and γ- fractions) in female lambs was 49-52%, 15-16%, 10-12% and 22-23%, respectively.

Regarding the cited literature, our data are similar to those of Pavlović and Vitić (1979) and Ninkov (1987), due to the large variation limits cited by these authors. Generally concluding, it could be said that in normal physiological conditions a greater concentration of globulin than albumin can be found in sheep blood serum.

Data in the available literature suggest that even under normal physiological conditions significant variation of protein fractions can be observed in a specifed animal species. Different authors pointed out the importance of numerous factors than can influence total protein content and relations among protein fractions. The influence of age, breed, atmospheric conditions and season, sex, nutrition, etc., should be especially emphasised. Analysing the seasonal influences, Jovanović et al. (1983) found that sheep from three regions had the highest protein concentration during the spring (average 85.2; 91.1 and 94.4 g/l), with a significant decrease and the lowest values during the winter. Blood protein concentration in the summer was 75.1; 90.8 and 91.4 g/l, in the autumn 73.3; 80.8 and 86.6 g/l, and during the winter 75.2; 76.1 and 74.4 g/l. Similar results were reported by Terek (1980) and Udalovaet al. (1982), while Adewuyi and Adu (1984) suggested that blood protein concentration dependence on seasonal changes is more influenced by atmospehirc precipitation rate and relative humidity than by temperature changes. Terek (1980a) reported that blood protein level decreases during the first two months of life, increasing from the 3rd month to achieve normal physiological levels (as in adult sheep) in the 6th month. This finding could be an explanation for the mentioned differences. Sova et al. (1986) investigated the influences of age sex. Malelambs at 3 - 12 months old had blood protein concentrations of 42.7 - 94.1 g/l. In female lambs, 5 - 8 months old, blood protein concentration was 85.8 - 88.9 g/l, and in adult sheep 85.5 - 88.6 g/l. Regarding the age of the lambs in our experimentow, values are below those reported by Sova et al. (1980) for female lambs, but are higher than the values for male lambs at 3 months old.

Vitamin A is necessary to support growth and health of animals, but its metabolic function, explained in biochemical terms, is still incompletely known. On the other hand, it is well known that vitamin A is involved in the biosynthesis of proteins, and especially globulins. Higher dietary amounts provide a higher blood concentration (Sinovec and Ševković, 1991) which is necessary for optimal biosynthesis of proteins and their fractions in correlation with age.

Comparing our data with literature citations, we can conclude that the values of total protein concentration and protein fractiions found in weaned lambs, were inside the physiological limits, given both for lambs and adult sheep by numerous authors. All results were very uniform because the influence of many factors mentioned in this paper (breed, sex, age, season and nutrition) was avoided.

The increased quantity of vitamin A in the feed (20.000 IU/kg) positively influenced the total protein concentration, but the established increase was

limited and further increase of dietary vitamin A was without effect. The total protein increase resulted from an increase of α-and β-globulins at the expense of albumin with stable β-globulin fractions. The decrease in A/G ratio was associated with thee increase of dietary vitamin A content.

These results can be used as a valuable step in further investigations of total protein and protein fraction concentrations, both in weaned female lambs and adult sheep, but, of course, with full care and attention pait to possibilities of variations influenced by physiological and any other known reason.

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UTICAJ RAZLIČITIH KOLIČINA VITAMINA A NA STATUS PROTEINA U KRVNOM SERUMU JAGNJADI

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SADRŽAJ

Ogled je izveden na tri grupe po 10 ženske jagnjadi, telesne mase 20-30 kg, a trajao je 90 dana. Jagnjad su hranjena livadskim senom i smešama standardnog sirovinskog i hemijskog sastava, a razlika je bila u količini vitamina A. Smeše za I, II i III grupu sadržavale su 10.000, 20.000 i 30.000 IU vitamin A/kg. Uzorci krvi za ispitivanje uzimani su 1, 52. i 99. dana ogleda.

Na početku ogleda nisu utvrđene signifikantne razlike u koncentraciji ukupnih proteina i njihovih frakcija između grupa. Povećanje sadržaja vitamina A (20.000 IU/kg) pozitivno je uticalo na koncentraciju ukupnih proteina, dok isti efekat nije uočen daljim povećanjem sadržaja vitamina A u hrani.

Povećanje koncentracije ukupnih proteina zasniva se na povećanju β-globulina. Smanjivanje A/G odnosa proporcionalno je sadržaju vitamina A u hrani.