# EFFECT OF ENERGY LEVEL AND PROTEIN CONTENT OF THE RATION ON SOME ASPECTS OF LIPID METABOLISM IN FATTENING LAMBS

Banskalieva, V., Chindarska, Z., Dardjonov, T. and Dimov, V.,
Institute of Animal Science, Kostinbrod 2232, Bulgaria

## SUMMARY

The experiments were carried on with lambs, weaned on age of 40 days, being afterwards on rations with different energy level and protein content. The data obtained at parallel variation of energy and protein show that no significant changes in the quantity of body fat, fatty acid composition of adipose tissue triacylglycerols and plasma free fatty acids and activities of glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase were registered. At isoenergy diets, the protein level does not affect neither fatty acid composition nor activities of the enzymes investigated. At isoprotein rations the higher level of energy causes significant decrease of 18:0 and increase of 18:1 in adipose tissue triacylglycerols and free fatty acids, accompanied with formation of more unsaturated subcutaneous fat.

## INTRODUCTION

The synthesis of endogenous lipids in not lactating ruminants is carried out mainly in the adipose tissue. The content and composition of lipids not only in the stores but also in the muscles are affected by a number of factors: the genotype, the season,the conditions of animal rearing, the composition and physical form of the ration etc.

Smith et al. (1984), Ilian et al. (1986) have found that the elevation of concentrates in the ration of growing animals leads to an increase in the quantity of deposited fats and a decrease of the protein:fats ratio in the body. It has been pointed that the amount of concentrates in the diet is associated with the activity of dehydrogenases, related to lipogenesis in adipose tissue (Martin et al. 1973, Opstvedt et al. 1967).

According to Haugebak et al. (1974) the variation of proteins in the ration has not substantial effect on the activity of lipoprotein lipase in the adipose tissue and the amount of intramuscular lipid. Black et al.(1987) showed a low utilization of metabolic energy of diets with lowered protein content.

Diets with a higher proportion of concentrates led to the formation of a relatively softer reserved fats (L'Estrange and Mulvihill 1975, Ray et al. 1975, Takahashi and Oota 1985).

The recommended levels of nutrition at present vary considerably (Kalaissakis and Papadopoulos 1985, Miller 1974, Scholaud 1972). This study was undertaken in connection with search of an appropriate and effective system of differentiated feeding of intensively fattened lambs in our country.

## MATERIAL AND METHODS

Three experiments with merino breed lambs have been conducted. Lambs were weaned at an age of 40 days, with an average liveweight of 13,0æ1,6 kg and were fattened to a final live weight of 36-42 kg. During the experimental period the animals were reared in groups (of 12) and fed ad libitum. The composition of the ration for each group is presented on table 1. The food intake was under daily control, and the live weight was checked each month (tables 2, 3).

In the first experiment (groups 1, 2, 3) the lambs received rations with variation of energy and protein content. In the II (groups 4, 5, 6) and III (groups 7, 8) trials the rations were isoenergetic and isoprotein with a varying level of protein and energy respectively.

Samples for analysis were taken from 3 animals of each group from the I and II experiment, and from 6 animals from the III experiment. Blood samples were drown prior to slaughter of animals. Samples of subcutaneous adipose tissue were taken from the area close to the tail, and the "meat" samples from the grinded deboned carcass (average of muscle and adipose tissue). The fat in the latter was analysed by ether extraction according to Soxhlet, and the nitrogen (protein) was analysed according to Kjeldal.

The activity of glucose-6-phosphate dehydrogenase (G6PDH - E.C.1.1.1.49) was analysed according to Glock and McLean (1953) and isocitrate dehydrogenase activity (ICDH - E.C.1.1.1.42) as described by Ochoa (1955). The

Table 1

Diet composition

| | Groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ingredients (%) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Corncorbs | 53.5 | 36.0 | 17.0 | 42.0 | 38.0 | 35.0 | - | - |
| Prairie hay | - | - | - | - | - | - | 32.0 | 11.0 |
| Sunflower (oil) meal | 35.5 | 35.0 | 40.0 | 17.4 | 28.0 | 37.4 | 40.5 | 42.6 |
| Berley | - | 10.0 | 15.5 | 14.0 | 12.0 | 10.0 | 13.0 | 20.0 |
| Wheat | 8.5 | 16.5 | 25.0 | 24.0 | 19.4 | 15.0 | - | - |
| Corn | - | - | - | - | - | - | 12.0 | 23.6 |
| Salt | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.7 | 0.7 |
| Vitamins, ADE | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Trace element mixture | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 |
| Dicalcium phosphate | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | - | - |
| Limestone | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 0.9 | 1.2 |
| Crude protein (g/kg) | 160.0 | 180.0 | 210.0 | 130.0 | 161.0 | 189.0 | 196.0 | 200.0 |
| Net energy (MJ/kg)* | 4.1 | 4.9 | 5.6 | 4.9 | 4.9 | 4.9 | 5.1 | 6.0 |
| Roughage/concentrate | 55.0 | 37.0 | 17.0 | 43.0 | 39.0 | 36.0 | 33.0 | 11.0 |
| ratio, % | 45.0 | 63.0 | 83.0 | 57.0 | 61.0 | 64.0 | 67.0 | 89.0 |

* - Kellner, O.

Table 2

Daily feed intake

| Groups | Daily feed intake (kg) | Consumed energy (MJ) | Consumed protein (g) | Feed input/kg daily gain |
|---|---|---|---|---|
| 1 | 1.30 | 5.39 | 208.0 | 6.88 |
| 2 | 1.16 | 5.68 | 209.0 | 6.10 |
| 3 | 1.24 | 6.99 | 260.0 | 5.56 |
| 4 | 1.00 | 4.97 | 132.0 | 7.00 |
| 5 | 1.10 | 5.39 | 175.0 | 6.40 |
| 6 | 1.15 | 5.62 | 218.0 | 5.20 |
| 7 | 1.13 | 5.74 | 221.0 | 6.50 |
| 8 | 1.10 | 6.57 | 220.0 | 5.10 |

Table 3

Live weight (kg) and average daily gain (g)

| Groups | Live weight | Averagl daily gain |
|---|---|---|
| 1 | 40.0 ± 4.8 | 190.0 ± 40.0 |
| 2 | 40.0 ± 3.6 | 192.0 ± 30.0 |
| 3 | 40.0 ± 4.1 | 232.0 ± 30.0 |
| 4 | 36.0 ± 6.3 | 150.0 ± 40.0 |
| 5 | 36.0 ± 5.4 | 160.0 ± 40.0 |
| 6 | 42.0 ± 5.0 | 190.0 ± 30.0 |
| 7 | 36.0 ± 3.9 [a] | 174.0 ± 30.0 [a] |
| 8 | 40.0 ± 4.9 [b] | 210.0 ± 50.0 [b] |

Significant differences ($p < 0.01$) are indicated with different superscripts

Table 4

Meat, fat and protein content (kg) of deboned carcass

| Groups | Meat | Protein | Fat | Fat/Protein |
|---|---|---|---|---|
| 1 | 9.7 ± 0.8 | 2.4 ± 0.4 | 2.6 ± 0.4 | 1.07 |
| 2 | 9.3 ± 1.2 | 2.5 ± 0.2 | 2.7 ± 0.3 | 1.08 |
| 3 | 9.4 ± 0.7 | 2.1 ± 0.2 | 2.3 ± 0.6 | 1.09 |
| 4 | 8.0 ± 0.9 | 1.3 ± 0.4 | 1.8 ± 1.0 | 1.35 |
| 5 | 8.6 ± 0.8 | 1.5 ± 0.5 | 1.7 ± 0.8 | 1.15 |
| 6 | 8.7 ± 1.1 | 1.5 ± 0.3 | 2.8 ± 0.7 | 1.79 |
| 7 | 8.0 ± 0.3 [a] | 1.3 ± 0.1 | 1.8 ± 0.2 | 1.46 |
| 8 | 10.2 ± 0.8 [b] | 1.5 ± 0.2 | 2.2 ± 0.2 | 1.43 |

Significant differences ($p < 0.001$) are indicated with different superscripts

protein in the homogenates was analysed according to the procedure of Lowry et al. (1951).

The extraction of tissue and blood lipids were carried out as described by Bligh and Dyer (1959). Until being analysed the extracted lipids were stored in a solution of 0,1% (w/v) of butyl hydroxytoluene in chloroform at -30°C. Triacylglycerols (TG) and free fatty acids (FFA) were isolated by thin layer chromatography on silica gel G with a solvent system of hexane:diethyl ether:acetyc acid - 85:15:1 (v/v/v). The fatty acid composition was analysed by gas chromatography using a glass column (3 m x 2 mm) packed with 3% SP 2330 on Supelcoport (100-120 mesh) and internal standard arachidic acid. The Student test was used as a criterion for statistical evaluation of the results.

## RESULTS AND DISCUSSION

The daily feed intake and conversion rates are shown in table 2. The amounts of feed input/kg daily gain is in inverse dependence to the quantity of the consumed energy. A parallel is observed between the daily gain (table 3) and the quantity of the energy received (table 2). The highest average daily gain was noted in groups 3 and 8, in which the level of energy nutrition was highest. According to Blaxter (1962) the increase in the proportion of concentrates in the diet leads to an improvement of the coefficient of digestion and the animals absorb more dry matter and productive energy.

The parallel variations of energy and protein in the ration (I experiment) does not lead to essential changes in the quantity of deposited fat and protein (table 4). The different ratios between the energy and protein in isoenergetic diets (trial II) has a pronounced influence on the fat/protein index. At the III experiment the fat/protein ratio in the two isoprotein groups does not change with the elevation of energy. The index values in this trial are higher than in the other experiments. This could be explained by the effect of the components of the diet.

With a simultaneous variation of the energy and protein (I experiment) no substantial differences were noted in the activities of the dehydrogenases in the subcutaneous adipose tissue (table 5).

The increase of the protein level at a constant energy content of the rations (II experiment), with the exception of the higher activity of ICDH in group 4, does not influence the activities of analysed enzymes. Black et al. (1987) showed that the production of NADPH is not directly linked with the quantity of protein in the ration. In present experiments a tendency has been observed that the activities of the enzymes to be higher in animals fed diets with the lowest protein content. In this group the lowest daily gain , the maximum feed input/kg daily gain and the lowest utilisation of energy has been noted. The low protein level in the diet of intensively fattened lambs, at a comparatively high

Table 5

Activity* of glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase in adipose tissue

| Groups | Enzymes | |
|---|---|---|
| | G6PDH | ICDH |
| 1 | 71.0 ± 17.8 | 79.3 ± 6.9 |
| 2 | 92.2 ± 14.8 | 74.6 ± 8.6 |
| 3 | 74.9 ± 18.3 | 72.7 ± 21.6 |
| 4 | 68.8 ± 4.0 | 93.9 ± 8.7[a] |
| 5 | 52.8 ± 16.2 | 51.3 ± 9.2[b] |
| 6 | 56.6 ± 5.8 | 50.8 ± 3.1[b] |

* nmol/min/mg protein

Significant differences ($p < 0.01$) are indicated with different superscripts

Table 6

Fatty acid composition (M%) of triacylglycerols in adipose tissue

| | Groups | | |
|---|---|---|---|
| Fatty acids | 1 | 2 | 3 |
| 14:0 | 3.6 ± 0.2 | 4.8 ± 0.6 | 4.1 ± 0.6 |
| 16:0 | 25.2 ± 1.0 | 27.3 ± 0.3 | 22.8 ± 5.0 |
| 16:1 | 3.1 ± 0.1 | 2.9 ± 0.1 | 3.1 ± 0.1 |
| 18:0 | 23.5 ± 0.6 | 22.3 ± 0.7 | 22.2 ± 4.6 |
| 18:1 | 38.6 ± 1.0 | 38.4 ± 1.2 | 40.5 ± 0.3 |
| 18:2 | 6.0 ± 0.3 | 4.3 ± 0.4 | 7.2 ± 0.5 |
| | 4 | 5 | 6 |
| 14:0 | 2.5 ± 0.4 | 2.6 ± 0.1 | 2.5 ± 0.4 |
| 16:0 | 22.9 ± 1.1 | 23.1 ± 1.5 | 22.2 ± 0.6 |
| 16:1 | 3.4 ± 0.1 | 3.8 ± 0.3 | 3.0 ± 0.1 |
| 18:0 | 22.4 ± 1.2 | 21.6 ± 2.3 | 25.0 ± 0.6 |
| 18:1 | 43.8 ± 2.3 | 43.8 ± 0.6 | 43.8 ± 0.7 |
| 18:2 | 4.9 ± 1.2 | 5.1 ± 1.2 | 3.9 ± 0.1 |
| | 7 | 8 | |
| 14:0 | 2.6 ± 0.3 | 1.8 ± 0.4 | |
| 16:0 | 23.6 ± 1.1 | 22.9 ± 0.5 | |
| 16:1 | 2.4 ± 0.1 | 5.2 ± 1.5 | |
| 18:0 | 25.0 ± 2.1[a] | 14.1 ± 1.6[b] | |
| 18:1 | 42.2 ± 2.7 | 48.8 ± 1.6[b] | |
| 18:2 | 4.3 ± 0.7 | 7.2 ± 1.0 | |

Significant differences ($p < 0.01$) are indicated with different superscripts

level of energy, reasonably stimulates the lipid formation in adipose tissue.

The activities of G6PDH and ICDH reflect the participation of the pentosophosphate and isocitrate pathways in the generation of reduced cofactors for the lipid biosynthesis in adipose tissues of ruminants. In this aspect data in literature are controversial (Vernon 1981). Martin et al. (1973), Opstedt et al. (1967), Smith et al. (1984) indicate that with the increase of concentrates in the diet the activity of G6PDH increases. In experiments with cattle (Yang and Baldwin 1973) the high-concentrated diets has not affected NADPH-generating dehydrogenases. In our former investigations (Banskalieva et al. 1987) it was pointed out that in the stage of vigorous growth (liveweight 20-25 kg) the activities of G6PDH is influenced by the quantity of concentrates, but at the end of the fattening period such a relationship was not observed.

The simultaneous variations of energy and protein, and the changes of protein level in the isoenergy diets has no essential effect on the fatty acid composition of adipose tissue TG (table 6). The results of the plasma FFA (table 7) were similar.

The elevation of energy at a constant protein level of leads to a significant increase of 18:1 and 18:2 and a decrease of 18:0 (tables 6,8). These changes make the lipids (TG and FFA) less saturated Fortin et al. (1981), Ray et al. (1975), Takahashi and Oota (1985) have established that the feeding diet with more concentrates leads not only to faster deposition of lipids, but also to formation of more unsaturated fat. The exact mechanism of the observed changes with ruminants fed on diets of different structure or different energy levels is not fully understood. The obtained results of the three experiments (strongly manifested in the III experiment) show that, it is rather the energy in the diet and not the protein level, the decisive factor for the composition of adipose tissue TG.

According to Garton (1965), Tove and Matrone (1962) the composition of fat stories is influenced by the exogenous fatty acids and the changes occurring with them in the rumen. It is more likely that the fatty acid composition is influenced by the intensity and specificity of the endogenous synthesis. The formation of lipids during the period of intensive growth is accompanied with changes in the activity of some adipose tissue enzymes. It has been established that acyl-CoA carboxylase, and stearoyl-CoA desaturase are strongly influenced by the composition and structure of the diet, and probably have a common mechanism of regulation (Jeffcoat and James 1977).

The mechanisms controlling the desaturation of lipids are not fully understood. It could be admitted that the increased energy level of the ration induces the activation of stearoyl-CoA desaturase in the adipose tissue. In ruminants an intensive lipid synthesis in adipose tissue is accompanied with a considerable desaturation of 18:0 (Vernon 1981, Wahle 1973). Gellhorn and Benjamin (1964) point out that the higher plasma level of insulin stimulates the desaturation of the long-chain fatty acids. Trenkle (1970) found that the level of this hormone increases with the elevation of concentrates in the diet. These results were confirmed in our laboratory (studies

Table 7

Fatty acid composition (M %) of plasma free fatty acids

| | Groups | | |
|---|---|---|---|
| Fatty acids | 1 | 2 | 3 |
| 14:0 | 0.7 ± 0.1 | 1.3 ± 0.3 | 1.1 ± 0.2 |
| 16:0 | 17.1 ± 0.2 | 19.4 ± 0.6 | 18.1 ± 0,7 |
| 16:1 | 2.9 ± 0.2 | 3.2 ± 0.3 | 2.5 ± 0.1 |
| 18:0 | 33.1 ± 2.5[a] | 28.7 ± 0.7[a] | 24.4 ± 1.6[b] |
| 18:1 | 37.7 ± 2.5 | 39.5 ± 0.7 | 42.4 ± 0.9 |
| 18:2 | 8.5 ± 0.4[a] | 7.9 ± 0.5[a] | 11.6 ± 0.2[b] |

Significant differences ($p < 0.05$) are indicated with different superscripts

Table 8

Fatty acid composition (M %) of free fatty acids in adipose tissue

| | Groups | |
|---|---|---|
| Fatty acids | 7 | 8 |
| 14:0 | 7.1 ± 0.4 | 5.8 ± 0.4 |
| 16:0 | 32.5 ± 1.2 | 29.2 ± 1.2 |
| 16:1 | 8.1 ± 0.4 | 6.6 ± 0.5 |
| 18:0 | 19.5 ± 0.7[a] | 14.6 ± 1.1[b] |
| 18:1 | 29.6 ± 1.8[a] | 37.8 ± 2.2[b] |
| 18:2 | 3.3 ± 0.3[a] | 6.0 ± 1.0[b] |

Significant differences ($p < 0.05$) are indicated with different superscripts

in progress). It was found that the level of plasma insulin is highest in animals fed on diets with highest energy contents . The increase of desaturase activity of adipose tissue is probably biologically justified, with the view of the formation of lipids with definite melting point.

The obtained results indicate that an intensive fattening of lambs with the use of more concentrates leads to an growth, accompanied with deposition of more fats in the carcass. The amount and composition of deposited fat is an important criteria for the quality of the produced meat. In relation to taste quality and the market demand it is desirable to produce lamb and mutton with softer fat. Most favorable in this aspect proved to be a diet with protein and energy content: 200 g and 6.0 MJ/kg respectively. The feeding of greater amounts of concentrates is, to a certain extent, compensated by a lower average daily feed input/kg daily gain, with more effective utilization of energy and better nutritive and market value of the produced meat.

## REFERENCES


Banskalieva, V., Dardjonov, T. and Dimov, V. (1987). Proceedings 4th International Symposium on Physiology of Ruminant Nutrition, Strebske Pleso, Czechoslovakia p113.

Black, J.L., Gill, M., Beever, D.E., Thornley, J,H. and Oldman, J.D. (1987). *Journal of Nutrition* **117**:105.

Blaxter, K.L. (1962). *In:* Energy Metabolism of Ruminants (1st ed.) Hutchinson/ London.

Bligh, E. and Dyer ,W. (1959). *Canadian Journal of Biochemistry and Physiology* **37**:911.

Fortin, A., Reid, J.T., Maiga, A.M., Sim, D.W. and Wellington, G.H. (1981). *Journal of Animal Science* **53**:982.

Garton ,G. (1965). *In:* Physiology of Digestion in the Ruminants (Butterworth, Ind.) Washington D.C. p30.

Gellhorn, A. and Benjamin, W. (1964). *Biochimica et Biophysica Acta* **84**:167.

Glock, G. and McLean, P. (1953). *Biochemical Journal* **55**:40.

Haugebak, C.D., Hedrick, H.B. and Asplund, J.M. (1974). *Journal of Animal Science* **39**:1026.

Jeffcoat, R. and James, A. (1977). *Lipids* **12**:469.

Ilian, M.A., Razzaque, M.A., Bond, C., Salman, A,J. and Salman, A.R. (1986). *Nutrition Reports International* **33**:435.

Kalaissakis, P. and Papadopoulos, G. (1985). Animal Meeting of the Europian Association for Animal Production, Halkidiki, Greece 2:202.

L'Estrange, J. and Mulvihill, T. (1975). *Journal of Agricultural Science* **84**:281.

Lowry, O., Rosebrough, N., Farr. A. and Randall, R. (1951). *Journal of Biological Chemistry* **193**:265.

Martin, R., Wilson, L., Cowan, R. and Sink, J. (1973). *Journal of Animal Science* **36**:101.

Miller, T. (1974). *Sheepbreeder and Sheepman* **94**:32.

Ochoa, S. (1955). *Methods of Enzymology* **1**:699.

Opstvedt, J., Baldwin, R. and Ronning, M. (1967). *Journal of Dairy Science* **50**:108.

Ray, E., Kroman, R. and Cosma, E. (1975). *Journal of Animal Science* **41**:1767.

Scholaud, W. (1972). *Der Tierzuchter* **5**:92.

Smith, S., Prior, R., Ferrell, C. and Mersmann, H. (1984). *Journal of Nutrition* **114**:153.

Takahashi, T. and Oota, S. (1985). *Japanese Journal of Zootechnical Science* **56**:711.

Tove, S. and Matrone, G. (1962). *Journal of Nutrition* **76**:271.

Trenkle, A. (1970). *Journal of Nutrition* **100**:1323.

Vernon, R. (1981). *In:* Lipid Metabolism in Ruminant Animals (Christie, W. Ed.) Pergamon Press, Oxford p279.

Wahle, K. (1973). *Comparative Biochemistry and Physiology* **48B**:87.

Yang, Y.T. and Baldwin, R.L. (1973). *Journal of Dairy Science* **56**:366.