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Effect of reduced energy diet on some blood biochemical indicators in the late pregnant ewes

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Summary

The objective of the present study was to investigate effects of feeding pregnant ewes with a reduced-energy diet during the late gestation period by monitoring changes in blood levels of some biochemical parameters. A total of 30 5-6-year-old Chios ewes (20 pregnant, 10 non-pregnant) were used. On day 105 of their gestation the pregnant ewes were divided into 2 treatment groups of 10 animals in each: an energy restricted group (ER) and normal energy group (NE). The ewes that were not pregnant were assigned as a group of non-pregnant ewes (NP). Blood samples were taken from the ewes of all groups on days 120, 127, 134, 141, and 148 of gestation and analysed in order to determine levels of total protein, albumin, globulin, albumin/globulin (A/G) ratio, glucose, and total lipids. The results indicated that the levels of total proteins and globulin were found to be lower in the last 4 weeks of gestation in ER and NE groups as compared to those in the NP group. Levels of total protein and globulin in NE and ER groups persistently decreased between days 120 and 148 ($P < 0.05$). During the last 4 weeks of gestation glucose levels of pregnant ewes were found to be significantly lower ($P<0.05$) as compared to those of NP ewes. No difference was discovered between NE and ER groups in the levels of total protein, albumin, globulin, glucose and A/G rations. Levels of total lipids constantly increased in the last 5 weeks of gestation. The highest increase in total lipids was seen in the ER group ewes ($P<0.05$). These findings indicate that feeding with a reduced energy diet may not adversely affect maternal immunity and transfer of Igs to colostrum. However, significant increases in total lipids in the ER group indicate that increased energy needed for fast fetal growth was met by the mobilisation of reserve fats.

Keywords: late pregnancy, ewes

Energy needs of pregnant ewes significantly increase in the late gestation period. Growth and development of foetus is largely dependent on sufficient energy intake of mother ewes, especially in the last 6 weeks of gestation during which 70% to 80% of fetal growth occurs (10). In addition, uterus and placenta develop faster and mammary glands are prepared for lactation, all of which result in increased energy need (21). Daily basal nutritional requirement of sheep for living is 8,4 MJ ME (Metabolic Energy)/kg DM (Dry Matter) and 9.4% CP (Crude Protein). Based on energy and nutrient requirements, the nutrition of pregnant ewes can be scrutinised under two classes; as nutrition within the first 15 weeks and within the last 4 weeks of gestation. Basal nutritional requirement of a 55 kg body weight ewe is 8.8 MJ ME/kg DM and 10.6% CP. In the last 4 weeks of gestation, nutritional requirements increase to 9.6 MJ ME/kg DM-11% CP for ewes carrying single foetus and increase to 10 MJ ME/kg DM-12.8% CP for ewes with twin foetus (11, 22). In years when feeding ewes with concentrated feed is not feasible due to high feed costs, pasturing becomes a common alternative for sheep raisers. Under these conditions, the most common nutritional deficiency seen in the pregnant ewes is the energy deficiency (21). Feeding ewes with low-energy ration in the last stages of gestation periods results in poor body condition of ewes, loss of body weight, and low survival rate of lambs (1, 25, 26). Moreover, development of mammary gland before lambing slows down resulting in low milk yield, even if the ewes had high genetic potential for milk production (21). The energy deficiency in pregnant ewes also leads to decreases in the amount of colostrum, an essential source of nutrition for newborn lambs (23). There is a close correlation between levels of total serum proteins and serum immnunoglobulins (Igs). Cost of determining total protein level is lower than the cost of tests used for determining the level of Igs such as radial immundiffusion or Zing-sulphate turbidity tests (12). The levels of total plasma proteins and globulin can be used to monitor amount of passive diffusion and predicted amount of Igs in newborns (24). Reduced energy intake in pregnant ewes before lambing may cause occurrence of negative energy balance (8). Utilisation of proteins is dependent upon the level of energy intake (11). Catabolism of body-protein reserves increase when energy intake is limited (17). Levels of some body metabolites such as total protein and glucose are closely related to the energy and protein utilisation (26). As a result of negative energy balance, free fatty acids are mobilised from adipose tissue (20). Negative energy balance influences plasma insulin level as well (8). There is a close relationship between plasma insulin level and amount of free fatty acids in the blood (18). Determining levels of blood metabolites in ewes during a given period of gestation may help assessing status of anabolism or catabolism (2).

The objective of the study was to investigate influence of feeding pregnant Chios ewes, a breed with high twin rate, with reduced energy ration during the late pregnancy on the immunity and energy status.

Material and methods

Synchronisation of ewes for oestrus. In the present study, a total of 38 Chios ewes were synchronised for oestrus. Following a 15 day adaptation period, sponges containing 60 mg medroxyprogesterone (MAP) were applied intra vaginal to ewes using a special applicator. The sponges were removed after 5 days and 600 IU pregnant mare's serum gonadotropin (PMSG; folligon, Intervet, Netherlands) was administered to each animal intramuscularly. Forty eight hours after this application, 1 ram was added to each box of 10 ewes for random mating (15).

Experimental design and treatment groups. All ewes were fed with a ration of 9.14 MJ ME/kg DM and 10.23% CP (1400 g/day/head) within the first 3.5 months of gestation. All ewes were provided with *ad libitum* water during the entire study. On day 105 after mating, the ewes were examined by ultrasound for pregnancy and to compose treatment groups. The examination resulted in that 24 of 38 ewes were pregnant. The animals were divided into 3 groups as normal energy (NE) group, energy restricted (ER) group, and non-pregnant (NP) group. Ten randomly selected animals from appropriate ewes were assigned for each group and then placed in individual boxes and held under the same environmental conditions. As of day 105 of gestation, each group was began feeding with treatment rations as seen in Table 1 until lambing. All animals were fed twice a day (8:00 am, 04:00 pm) with the appropriate treatment ration as approximately 700 g for each animal (1400 g /day/head). Drinking water was provided *ad libitum*. The feeds given to each group were analysed for CP and ME (Tab. 2) (3). The ewes in NP, NE and ER were fed with rations containing 9.14 MJ ME/kg DM-10.23% CP, 10.20 MJ ME/kg DM-15.04% CP, and 8.82 MJ ME/kg DM--14.47% CP, respectively.

Sample collection. Blood samples were taken from jugular veins of the ewes on days 120, 127, 134, 141, and 148 of gestation. The samples were collected to vacuumed-tubes containing heparin. Plasma of the blood samples was separated by centrifuging at 3000 rpm for 10 min. The plasma samples were stored at –20°C until analysed.

Tab. 1. The content of diets fed to pregnant and non-pregnant ewes

Ingredient	NP[1] (%)	NE[2] (%)	ER[3] (%)
Pasture grass	75.00	34.00	65.00
Cracked barley	5.00	1.60	3.80
Cracked Corn	7.50	28.00	1.00
Sunflower meal	1.10	21.00	18.50
Wheat fine brain	8.00	3.00	4.30
Wheat brain	1.50	6.00	4.00
Cracked wheat	1.50	6.00	4.00
Vitamin-mineral premix[4]	0.20	0.20	0.20
Salt	0.10	0.10	0.10

Explanation: 1 – Non-pregnant group; 2 – Normal energy group; 3 – Energy restricted group; 4 – Vitamin-mineral premix provides (per Kg of concenrate): SiO_2 – 48.58%, Al_2O_3 – 14.72%, CaO – 11.11%, MgO – 11.65%, F_2O_3 – 9.19%, LO – 2.50%, Na_2O – 1.28%, K_2O – 0.44%, TiO_2 – 0.38%, MnO – 0.16%, CrO_3 – 0.06%, P_2O_5 – 0,03%, vitamin A – 10 000 000 IU, vitamin D_3 – 1 500 000 IU, vitamin E – 25 000 mg, niacin – 20 000 mg, pantotenik acid – 7000 mg, vitamin B_2 – 2500 mg, vitamin B_1 – 1500 mg, vitamin B_6 – 1500 mg, vitamin B_{12} – 15 mg

Tab. 2. The chemical composition of diets fed to pregnant and non-pregnant ewes

Nutrient	NP[1]	NE[2]	ER[3]
Dry matter (%)	88.02	88.13	87.58
Crude protein (%)	10.23	15.04	14.47
Crude fat (%)	3.90	5.21	3.29
Crude ash (%)	6.54	5.41	6.91
Crude fibre (%)	26.90	16.83	26.24
Ca (%)	0.89	0.73	0.70
P (%)	0.49	0.51	0.52
Metabolisable energy (MJ/kg DM)	9.14	10.20	8.82

Explanation: 1 – Non-pregnant group; 2 – Normal energy group; 3 – Energy restricted group

Biochemical analysis. Plasma total proteins, albumin, glucose, and total lipids concentrations were analysed by commercial test kits (Spinreact, SA-ctra sahre coloma, 7-E-17176 sant esteve de bas-Spain) and SEAC ch 100 photometer. Plasma globulin level and Albumin/globulin (A/G) ratio was calculated for each sampling interval.

Statistical analysis. Data were subjected to variance analysis using one-way ANOVA for comparing means between groups and between various days of gestation. A significance level of $p < 0.05$ was used. The SPSS statistical software package (Version 11.5) was used for all statistical analysis. All results were expressed as mean ± SE.

Result and discussion

Total plasma proteins in both NE and ER groups decreased between days 120 and 148 of gestation as pregnancy progressed (tab. 3). This decrease was more

Tab. 3. Plasma total protein and albumin levels of pregnant and non-pregnant ewes

Days	Total protein (g/dl)			Albumin (g/dl)		
	NE[1]	ER[2]	NP[3]	NE	ER	NP
120	6.79 ± 0.40^{Ba}	8.61 ± 0.55^{Aa}	7.42 ± 2.13^{AB}	3.19 ± 0.07	3.12 ± 0.09	3.63 ± 0.29
127	6.48 ± 0.39^{Bab}	7.32 ± 0.35^{Bab}	8.52 ± 0.48^{A}	3.39 ± 0.14	3.16 ± 0.19	2.99 ± 0.11
134	7.45 ± 0.32^{Aba}	7.01 ± 0.36^{Bb}	8.13 ± 0.20^{A}	3.28 ± 0.29	3.12 ± 0.19	3.70 ± 0.08
141	6.51 ± 0.44^{Abab}	5.04 ± 0.47^{Bc}	8.08 ± 0.74^{A}	3.41 ± 0.14	3.31 ± 0.26	2.92 ± 0.99
148	5.50 ± 0.81^{Bb}	5.05 ± 0.72^{Bc}	7.00 ± 0.36^{A}	3.09 ± 0.26	2.93 ± 0.78	3.67 ± 0.24

Explanation: 1 – Normal energy group (n = 10); 2 – Energy restricted group (n = 10); 3 – Non-Pregnant group (n = 10); a, b, c – Means within the same line with different letters differ ($p < 0.05$); A, B – Means within the same column with different letters differ ($p < 0.05$)

Tab. 4. Plasma globulin levels and A/G ratio of pregnant and non-pregnant ewes

Days	Globulin (g/dl)			A/G ratio		
	NE[1]	ER[2]	NP[3]	NE	ER	NP
120	3.41 ± 0.45^{Bab}	5.29 ± 0.50^{Aa}	4.69 ± 0.75^{AB}	1.09 ± 0.17^{b}	0.63 ± 0.06^{c}	1.18 ± 0.47
127	3.10 ± 0.39^{Bab}	4.11 ± 0.46^{Ba}	5.50 ± 0.55^{A}	1.16 ± 0.14^{b}	0.86 ± 0.13^{bc}	0.90 ± 0.27
134	4.70 ± 0.30^{a}	3.89 ± 0.48^{a}	4.45 ± 0.24^{Bb}	0.87 ± 0.13^{b}	0.86 ± 0.20^{bc}	0.85 ± 0.06
141	3.10 ± 0.39^{Bab}	1.73 ± 0.52^{Bb}	5.23 ± 0.63^{A}	1.27 ± 0.24^{Abab}	2.09 ± 0.64^{Aa}	0.70 ± 0.20
148	2.38 ± 0.49^{Bb}	2.22 ± 0.37^{Bb}	3.98 ± 0.35^{A}	2.24 ± 0.60^{a}	1.67 ± 0.36^{ab}	1.00 ± 0.12

Explanation: 1, 2, 3, a, b, c, A, B – as in tab. 3

Tab. 5. Plasma glucose and total lipids levels of pregnant and non-pregnant ewes

Days	Glucose (mg/dl)			Total Lipids (mg/dl)		
	NE[1]	ER[2]	NP[3]	NE	ER	NP
120	45.74 ± 4.02	50.61 ± 6.06	48.35 ± 2.53	118.5 ± 10.49^{b}	112.8 ± 7.87^{c}	119.7 ± 9.71
127	42.69 ± 5.41^{AB}	38.11 ± 3.18^{B}	51.86 ± 3.43^{A}	137.5 ± 10.22	141.2 ± 19.85^{bc}	121.8 ± 7.92
134	42.62 ± 4.12^{B}	37.36 ± 2.27^{B}	59.26 ± 4.23^{A}	133.7 ± 9.91^{AB}	158.4 ± 13.29^{Ab}	105.8 ± 10.36^{B}
141	43.28 ± 5.01^{B}	41.05 ± 4.31^{B}	59.49 ± 4.23^{A}	149.4 ± 9.93^{A}	168.0 ± 12.75^{Aab}	98.3 ± 6.19^{B}
148	32.95 ± 5.53^{B}	46.60 ± 7.93^{AB}	56.32 ± 4.21^{A}	135.0 ± 9.53^{B}	207.0 ± 9.72^{Aa}	120.8 ± 7.58^{B}

Explanation: 1, 2, 3, a, b, c, A, B – as in tab. 3

evident in ER group ($p < 0.05$). Total plasma proteins decreased to the lowest level on day 148 in NE and ER groups. Therefore, total plasma proteins of pregnant ewes were found lower than those of NP ewes, especially on day 148 of pregnancy ($p < 0.05$). Plasma albumin levels of the ewes were not significantly different between groups or between sampling days of the gestation (tab. 3). Globulin level constantly decreased in both NE and ER groups between days 120 and 148. This decrease was more consistent in ER group ewes compared to NE group ewes. When the groups were compared, plasma globulin levels were found significantly lower ($p < 0.05$) in NE and ER groups than those in NP group (tab. 4). Decrease seen in total plasma protein might be considered as a reflection of decrease seen in plasma globulin level. Likewise, changes in A/G ratio were parallel to changes in globulin and total protein (tab. 4). Globulin level is the limiting factor in A/G ratio. There was no significant difference in total protein, globulin, and A/G ratio between RE and NE groups. El Sherif and Assad (14) reported that plasma protein levels in pregnant ewes decreased significantly starting as of 16 weeks of gestation. The authors speculated that this decrease was caused by the fact that rapid growing foetus in the last month of gestation utilise maternal amino acids for muscle growth. Bayoumi et al. (4) found that total protein and globulin levels decrease in the last periods of pregnancy, especially in alpha and gamma fractions. They explained that this decrease was the result of synthesis of globulin-rich colostrum that would be secreted immediately after lambing. Chen et al. (9) reported that goat and sheep colostrum was quiet rich in globulin content in the following hours of lambing. They further stated that there was a negative relationship between time (h) and globulin content in the colostrum. Finding of low level of total proteins and low globulin level in the present study is consistent with the finding reported in the those studies. We believe that decrease in total plasma proteins are resulted from decrease in globulin level as Bayoumi et al (4) reported previously. Another finding of the present study that the albumin level did not significantly vary between groups also supports this opinion.

There was no significant difference in plasma glucose levels among NE, ER and NP groups between days 120 and 148. However, glucose levels of pregnant ewes (NE, ER) were found significantly lower ($p < 0.05$) than those of NP ewes. Glucose levels in NE and ER groups were not different (tab. 5). Level of total lipids of ER group ewes significantly increased between days 120 and 148 compared to other groups of ewes ($p < 0.05$). This increase reached to a maximum level on day 148. Although total lipid levels of ewes in NE group were relatively higher between days 120 and 148 than they were in NP ewes, this difference was not significant (tab. 5). Some researchers reported that glucose production increase during pregnancy. However, a great majority of this glucose was absorbed by uterus as a source of maternal glucose resulting in lower glucose level in the circulation (6, 19). In contrary, other researchers stated that the glucose level increased during gestation (7, 14). The reason why glucose levels in the NE and ER groups were lower than they were in the NP group might be that the foetus meets its energy need from maternal sources during rapid growth. Researchers reported that glucose level and insulin level decreased in ruminants that were under negative energy balance conditions (5, 8). Decrease of insulin level results in increase in the plasma level of free fatty acids (13, 16). Symonds et al. (27) found that the pregnant ewes fed with energy deficient ration in the last 4 weeks of pregnancy had significantly higher blood level of free fatty acid compared to those fed with sufficient energy ration. Increase in total lipids seen in ER group ewes that were under negative energy balance might be caused by mobilisation of reserve fats to meet energy requirement which was increasing parallel to rapid growth of the foetus (20).

In conclusion, one of the findings of the present study that there was no difference in levels of plasma total proteins, globulin and A/G ratio between pregnant (NE, RE) ewes and non-pregnant ewes indicate that hypocaloric stress did not adversely affect immunity. In addition, it can be claimed that no serious restriction related to maternal globulin should be expected in Igs transfer for colostrum. Mobilisation of reserve fats in ER group may render ewes predisposed for occurrence of energy-metabolism related diseases such as pregnancy toxaemia.

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GASTHUYS F.M., VAN HEERDEN M., VERCRUYSSE J.: Hebronemiaza spojówek u konia w Belgii. (Conjunctival hebronemiasis in a horse in Belgium). Vet. Rec. 154, 757-758, 2004 (24)

U ogiera araba w wieku 4 lat wystąpiło nagromadzenie śluzu w środkowej części lewego oka utrzymujące się przez okres 4 tyg. Tylko nieznaczne polepszenie stanu zdrowia uzyskano stosując miejscowo deksametazon i parenteralnie amoksycylinę. Badanie oka przeprowadzone w narkozie wykazało obecność licznych śródskórnych guzków w powiece i w spojówce powieki dolnej oraz złogów znekrotyzowanych komórek, eozynofilów i neutrofilów. Badanie mikroskopowe wyciśniętej zawartości guzków oraz złogów wykazało obecność larw nicieni *Hebronema*. Były one usytuowane w centralnej części guzka i miały wymiar 25-40 µm. Całkowite wyleczenie uzyskano po zastosowaniu flumiksyny i deksametazonu *per os* przez 5 kolejnych dni oraz iwermektyny.

G.