Livestock Studies, 2020; 60 (1): 16-24

ORIGINAL ARTICLE

LIVESTOCK STUDIES

Effect of diet protein and energy levels on serum biochemical profile of fatty tailed sheep

Necdet İlker İçil[1]*, Esad Sami Polat[2], Behiç Coşkun[3]

[1] Bornova Veterinary Control Institute, İzmir, Turkey
[2] Selçuk University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Disease, Konya, Turkey
[3] Konya Food and Agriculture University, Faculty of Agriculture and Natural Sciences, Konya, Turkey

Article History

Received: 13 January 2020
Accepted: 17 July 2020

***Corresponding Author**

necdetilkericil@gmail.com

Key words

Metabolic profile test, Akkaraman, serum biochemical parameters.

Abstract

The aim of this study is to determine relationships between metabolic profile with diet in fat tailed sheep. With this aim, one hundred twenty Akkaraman sheep coming twenty out of six flocks, which gave birth a year ago, clinically healthy, were chosen. The sheep chosen were bled from jugular vein and serums obtained were analyzed for; glucose, total protein, albumin, BUN, cholesterol, AST and GGT, as biochemical parameters. During the physiological states of late pregnancy 115th and 125thdays, and postpartum, 10 days after partition-early lactation, same procedures were followed and data collected was compared by using Z test and for concerned metabolite, unlike statistically averages values of different flocks were determined. While different flocks were coded as DA (different from average), the similar ones were coded as IA (inside in average). The relationships between diet composition and metabolic profile were determined through regression analysis. According to the adjusted determined coefficients (R-Sqadj) which was obtained from regression analysis and interactions between diet compounds in DA group and serum biochemical profile were detected more meaningful than the group IA which was created as a different group. While cholesterol has strongest relationship with diet energy, BUN was the best reflector of diet crude protein level.

Introduction

Livestock are involved in an effort to balance their feed consumption and physiological nutritional requirements. Therefore, they try to consume nutrients to meet the maintenance and yield needs. The establishment of this balance depends on the feed characteristics and the needs of the animal. The level of metabolite concentrations in the critical periods of the animal's lifetime affects yield temporarily or permanently. Obtaining data amongst the relationship between the diet nutrients and blood metabolites could enable to meet the needs of every physiological state of sheep with balanced diet. In studies conducted on this target, while a large number of consensuses were provided for diet-component relationships with some of the metabolites, while some are meaningless.

In studies on the effect of diet energy level on glucose, Lee et al. [12] reported that diet energy value was generally effective on blood glucose concentration in cattle, while Kida [11] reported that it was effective only in the middle and late lactation periods. In studies conducted in sheep, flushing has been reported to have an important effect on serum glucose concentration [15, 24]. Furthermore, in a study on non-pregnant sheep, it is stated that an increase in serum glucose value is observed in parallel with increasing diet energy value [5]. Most of the energy need in ruminants is met by the volatile fatty acids produced in the rumen. The increase in the production of ketone bodies in ruminants is a result of increased lipolysis as a result of decreased serum glucose level due to the inability of the diet to meet the energy needs. Therefore, it could be said that the relationship between BHBA (Beta Hydroxy Butyric Acid), another metabolite associated with energy metabolism, and the diet energy value is indirectly dependent on glucose. However, this relationship between two parameter is not always linear and unidirectional.Bani Ismail et al. [3] state that a significant negative relationship between BHBA and glucose is observed only in goats with a BHBA level greater than 0.86 mmol / l. Cholesterol is another metabolite which reflects diet energy level [11].

It is stated that the effect of diet protein content on serum BUN (Blood Urea Nitrogen) concentration is at significant levels within studies on both cattle [4, 8, 17] and sheep [5, 14, 23]. It has been shown in several studies that there is a direct relationship between serum albumin concentration and diet protein level, which is reported to include nutritional conditions [16], among the factors affecting its synthesis, in both sheep and cows [9,12, 21, 26]. However, it is argued that serum albumin level is significantly affected by dehydration, and therefore BUN values should be considered rather than albumin as a means of estimating diet protein level [11]. Dietary energy and insulin levels also have an effect on albumin synthesis, and the rate of decrease in energy deficiency could only be eliminated by glucose feeding [5, 13, 19].

In ruminants; activity of AST (Aspartate-Aminotransferase) and GGT (Gamma Glutamyl-transferase) is often contact with FCS (Fat Cow Syndrome), depression of feed intake and ketosis [17]. According to Meyer and Harwey [17], increasing serum AST activity is sensitive marker for liver damage even in subclinical case. It is reported that fatty liver syndrome is seen because of large amount of body fat mobilization resulting the hypoglycemia in pregnancy toxemia [22], and one of the reason of increasing AST activitiy is the reduction of the diet energy [1]. The increase of serum activities of aminotransferases, which is used for determining the size of the change of hepatocelluler membran permeability (e.g. death of cell), it is also described as increased synthesis rate [18]. While Şahinduran et al.[20] described that increasing GGT activity is resulted in cholestasis and subsequent hepatobiliar circulation defect, it is reported that GGT, which is stated to have an antioxidant effect outside the cell and an antioxidant effect inside the cell [6], could be used as an oxidative stress indicator also [10].

The studies about morphological features of Akkaraman ewes which is the experimental breed of this study, live weight is as 57.6 kg [2] and as 66.4 kg [25] reported. The metabolic energy (ME) and crude protein (CP) needs of sheep with 60 kg live weight, and over 1.5 years old ages are suggested as 3.11 Mcal, 137.00 g and 3.37 Mcal, 200 g, respectively during the late pregnancy and postpartum early lactation period [7].

Various studies have previously been conducted on the relationship between the diet components and the biochemical profile and relationships at different levels have been identified. The group design in the studies conducted in this direction generally takes the form of groups that consume different diet components and changes in biochemical parameter/parameters are monitored through these groups. Our investigation was carried out in field conditions and the feeding patterns of the animals were not interfered. For this reason, the groups were formed not according to the diet components they consume, but according to the degree of deviation of the biochemical parameters they have, and the numerical data about the role of the diet in differentiation of a biochemical parameter from the average was tried to be obtained. For this purpose, a third group to cover all animals, as well as two groups created by determining the flocks with positive or negative deviations from the averages for each of the measured biochemical parameters was created and the biochemical profile-diet relationship was examined for each group separately.

Although as far as we know, there is no direct study on the effects of diet components on the biochemical profile in the Akkaraman breed, which is a fat-tailed sheep race, similar studies have been conducted in other sheep breeds and cattles.The data obtained in these studies were discussed and evaluated on the basis of metabolite groups.

Materials and Methods

Animals and Nutrition

This study was carried out on the sheep herds involved in the National Countryside Small Ruminant Animal Project of Minisitry of Food Agriculture and Forestry, TAGEM on Sivas Kangal Akkaraman sheep. A sum of 120 prompt randomly selected sheep from 6 different flocks, clinically healthy and fertile animals that had given birth in the previous year, were used for this experimental research. The investigated animals were kept in their own flock that were monitored by giving numbers, identified by organic dyeing paint visually to be captured easily for monitoring and sampling. Due to the investigation periods took place within the indoor conditions as late pregnancy and post partum-early lactation, there was no pasture affect on flocks. The daily diet and nutritional values of the research material consumed during the sampling periods were performed as shown in Table 1.

Sampling and Analyses

From selected 20 animals, 7-10 ml of blood was taken into containing plain vacuum glass tubes with serum gel via *V. Jugularis* during the late pregnancy (between the 115th and 125thdays of pregnancy) and postpartum (in the first 10 days after delivery-early lactation) period. The sampling process was carried out before the morning or evening feeding. Following standing at room temperature for 20-30 min, the tubes were centrifuged at 3,000 rpm for 10 min and the serum samples stored at -20°C until biochemical analyses. Glucose, cholesterol, AST, GGT, BUN, total protein, albumin were determined within 1 month following sampling for each period with the use of commercial kits *(Shenzhen Mindray Bio-Medical Electronics Co. Ltd, Shenzhen, Chine)* at auto analyzer *(BS 200 chemistry analyzer, Shenzhen Mindray Bio-Medical Electronics Co. Ltd, Shenzhen, Chine)* which was located at University of Cumhuriyet, Veterinary Faculty, Departmant of Internal Diseases. BHBA was measured with a device *(Optium Xceed, Abbott Diabetes Care Ltd.)* which is run with strip *(Freestyle Optium, Abbott Diabetes Care Ltd.).*

Table 1. Nutritive value of feeds and diet fed to ewes during the study.

Flock number	Physiological Period	Diet fed (g)		Nutritive value		
				DM, kg	ME, Mcal	CP, g
1	LP	Barley	600	1,31	2,76	87,29
		Straw	850			
	PP	Barley	600	1,31	2,76	87,29
		Straw	850			
2	LP	Barley	350	1,30	3,08	116,64
		Wheat	350			
		Alfa alfa hay	125			
		Grass hay	125			
		Straw	500			
	PP	Barley	200	1,30	1,63	68,24
		Wheat	200			
		Alfa alfa hay	100			
		Straw	250			
3	LP	Concentrate feed*	500	1,34	3,12	158,56
		Milk concentrate**	200			
		Alfa alfa hay	300			
		Straw	300			
	PP	Concentrate feed *	500	1,38	3,21	166,12
		Concentrate feed (milk)**	200			
		Alfa alfa hay	450			
		Straw	400			
4	LP	Barley	200	1,30	2,81	114,52
		Wheat	200			
		Oat	100			
		Alfa alfa hay	150			
		Grass hay	200			
		Straw	600			
	PP	Barley	200	1,30	2,81	114,52
		Wheat	200			
		Oat	100			
		Alfa alfa hay	150			
		Grass hay	200			
		Straw	600			
5	LP	Barley	700	1,01	3,02	124,58
		Milk concentrate**	250			
		Grass hay	150			
		Wet sugar beet pulp	400			
	PP	Barley	700	0,97	2,79	109,68
		Grass hay	350			
		Wet sugar beet pulp	400			
6	LP	Rye	600	1,16	2,70	101,40
		Grass hay	300			
		Straw	400			
	PP	Concentrate feed (milk)**	600	1,16	2,77	122,45
		Grass hay	300			
		Straw	400			

LP: The late pregnancy period. **PP**: The postpartum period. **DM**: Dry matter. **ME**: Metabolic Energy. **CP**: Crude protein. * Concentrate feed %88 DM, 2750 Mca/kgl ME, %14g CP. ** Concentrate feed (milk)** %88 DM, 2481 Mca/kg ME, % 16 CP.

Groups and Statistical Analysis

The average value of the data from all 6 flocks was determined for each metabolite in both physiological periods (general average value). The average value of each flock in both physiological periods for each metabolite was compared with the general average value determined using the Z test. Thus, flocks that were statistically different from the general mean value for the relevant metabolite were determined. The direction of these flocks that differ from the general mean value was determined by the Z value getting a positive or negative sign. The symbol Z+ refers to flocks that have higher than average mean for the relevant metabolite, while Z- refers to flocks having lower mean than general mean. As a result of the comparison, all flocks of both periods different from the averages were considered different from the general averages and coded as **DA** (Different from Average), and those outside the **DA** group were accepted as inside the general averages and coded as **IA** (Inside the Average). The total of **DA** and **IA** were called total group **(TOT)**. Thus, there were 3 groups with different n counts for each metabolite. For example, for glucose metabolite, as stated in table 3, the **DA** group formed 4 flocks and the count of n of this group was (20x4) 80 (each flock consists of 20 sheep). These four flocks formed the following flocks; -*In the late pregnancy period*; **1-)** 1 flock as Z+ (number 5 flock), **2-)**1 flock as Z- (number 6 flock), - *in the postpartum period;* **3-)**1 flock as Z+ (number 3 flock), **4-)** 1 flock as Z- (number 2 flock) (Table 3). The count of TOT group n for the same metabolite was 240, (6x20) 120 in the late pregnancy period and (6x20) 120 in the postpartum period. Since the **TOT** group ncount was 240 and the **DA** group n count was 80 for the glucose metabolite, the difference gave the number of **IA** group n (160). Groups of other metabolites were also determined by this method.

The relationship between diet components and metabolite levels was determined on the basis of groups created. The relationship between the diet energy content and blood glucose level for the **DA** group, the blood glucose level of 80 animals performed as **DA** group. Same method was applied in diet nutrients and metabolite relationships in other metabolites. While the relationship between diet energy level and glucose, BHBA, cholesterol, AST and GGT concentrations were determined in the groups, the relationship between diet protein level and BUN, total protein and albumin were determined. Due to the role of insulin on albumin synthesis, the relationship between total protein and albumin with diet protein level, as well as their relationship with diet energy level were examined. The live weights of the sheep within in the flocks were around 60 kg during this period.

The ratio of nutrients of the diet to meet the needs in late pregnancy and postpartum periods was determined with the formulas *"Diet Energy Amount x 100 / Energy Requirement"* for diet energy and *"Diet Crude Protein Amount x 100 / Crude Protein Requirement"* for the diet crude protein. In the formulas, the energy and crude protein requirement of the sheep were accepted as 3.11 Mcal, 137.00 g and 3.37 Mcal, 200 g, respectively, in late pregnancy and postpartum period [7].

Relationship between diet components and serum biochemical parameters were determined by correlation and regression analysis. Adjusted determination coefficients (R-Sqadj) were determined by regression analysis of interactions that showed statistically significant ($p<0.05$) correlations. The significant of the determination coefficient was tested with variance analysis (ANOVA). The differences between the nutritional requirements of the diet nutrients were determined by chi-square (Chi-Sq) test. All statistical analysis was performed with the computer program (Minitab 17) for Windows, version 8.0.

Results

The diet energy and crude protein values of the herds to meet the specified needs of sheep during late pregnancy and postpartum periods are presented in Table 2. The rates of the crude protein and energy amounts of the diets given to the experimental flocks and sufficiencylevels to meet the daily needs of the animals were statistically important except for the energy content of the late pregnancy period Table 2. When compared with the diet energy values according to the obtained chi-square values, it was seen that the variation between crude protein ratios was wider.

The flock/flocks which statistically different from the general average value obtained by comparing the average values of each flock with the general mean value in both physiological periods for each metabolite occurred are shown in Table 3. Flock numbers differentiating from the values higher than the general average value performed are shown in column 'Z+' and, lower values as shown in the 'Z-' column. The number of flocks that differ from the general averages for both physiological periods as a result of the comparison was specified in the column "The count of flocks the DA group".

Adjusted determination coefficients (R-Sqadj) of regression analysis for interactions that show statistically significant correlations between diet components and metabolites concentrations were shown in Table 4. According to the value of determination coefficients, the relationships between diet components and metabolites were stronger in the **DA** group than other groups (Table 4).

Table 2. Diet nutritional factor and the rate of requirements.

	Metabolic energy				Crude protein			
	LP		PP		LP		PP	
Requirement*	3.11 Mcal		3.37 Mcal		137.00 gr		200.00 gr	
Flocks	diet	rr%	diet	rr%	diet	rr%	diet	rr%
1	2.76	89	2.76	82	87.29	64	87.29	44
2	3.08	99	1.63	48	116.64	91	68.24	34
3	3.12	103	3.21	95	158.56	121	166.12	83
4	2.81	90	2.81	83	114.53	84	114.53	57
5	3.02	97	2.79	83	124.58	91	109.68	55
6	2.7	87	2.77	75	101.4	74	122.45	58
p		0.824		0.06		0.001		0.000
Chi-Sq		2.18		16.27		21.64		24.63

LP: The late pregnancy period. **PP**: The postpartum period. **rr**: The rate of requirements.*According to diet program [7].

Table 3. The flocks, different from general average value and differences direction.

	Late pregnancy			Postpartum			
		Flock numbers			Flock numbers		Count of the DA group flocks
Metabolites	mean	Z+	Z-	mean	Z+	Z-	
Glucose(mg/dl)	64.92±9,40	5^{th}	6^{th}	55.16±10,75	3^{th}	2^{nd}	4
BHBA(mmol/l)	0,35±0,16	3^{th}	-	0,42±0,26	4^{th}	2^{nd}	3
Cholesterol(mg/dl)	60,62±13,48	2^{nd};3^{th}	4^{th};6^{th}	62,23±13,29	3^{th}	-	5
AST(IU/l)	10,24±5,16	-	1^{st}	14,72±5,67	3^{th}	1^{st}	3
GGT(IU/l)	6,28±0,70	6^{th}	3^{th}	6,04±0,64	-	6^{th}	3
BUN(mg/dl)	3,21±0,36	3^{th}	1^{st};4^{th};6^{th}	3,07±0,36	3^{th}	1^{st};2^{nd};6^{th}	8
T.Protein(mg/dl)	74,95±18,03	3^{th};5^{th}	1^{st};6^{th}	104,71±29,82	3^{th};5^{th}	1^{st};6^{th}	8
Albumin(mg/dl)	43,02±12,57	3^{th}	1^{st}	46,77±16,22	3^{th}	2^{nd}	4

Z+;The flocks, different from average with positive direction, **Z-**: The flocks, different from average with negative direction. **BHBA**: Beta Hydroxy Butyric Acid. **AST**:Aspartate-aminotransferase,**GGT**: Gamma glutamyl-transferase, **BUN**: Blood urea nitrogen, **T.protein**: Total protein.

Table 4. The relatiosnhip of diet compounds and metabolites.

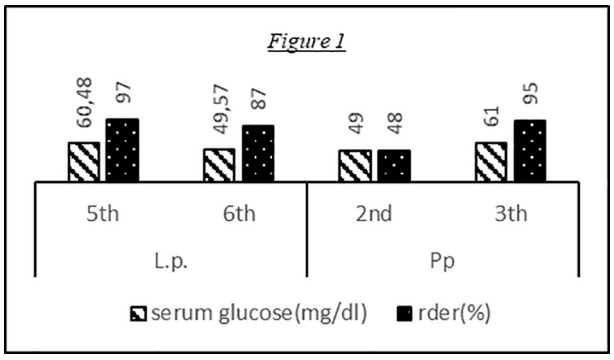
	Determination coefficient of variable(R-Sqadj)					
Groups	**DA**		**IA**		**TOT**	
DC	**Energy**	**Protein**	**Energy**	**Protein**	**Energy**	**Protein**
Metabolite						
Glucose	13,55 *** (n=80)		ns (n=160)		4,13 ** (n=240)	
BHBA	14,27 ** (n=60)		ns (n=180)		4,58 *** (n=240)	
Cholesterol	34,33 *** (n=100)		ns (n=140)		2,84 ** (n=240)	
AST	27,8 *** (n=60)		8,42 (-) *** (n=180)		1,40 (-) * (n=240)	
GGT	24,78 *** (n=60)		ns (n=180)		ns (n=240)	
BUN		58,7 *** (n=160)		21,71 *** (n=80)		37,9 *** (n=240)
T.Protein	31,63 *** (n=160)	27,11 *** (n=160)	ns (n=80)	ns (n=80)	8,05 *** (n=240)	15,83 *** (n=240)
Alb	43,2 *** (n=80)	52,91 *** (n=80)	ns (n=160)	2,13 * (n=160)	17,89 *** (n=240)	32,6 *** (n=240)

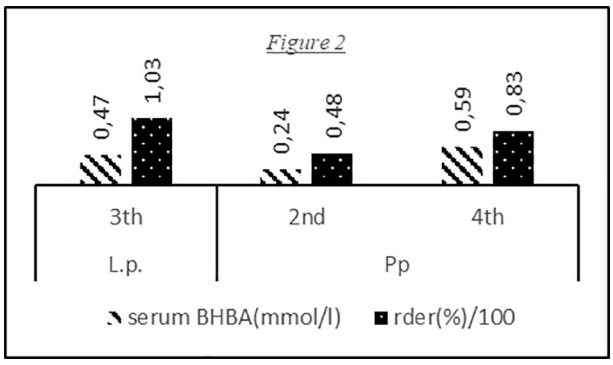
DC: diet compound. **DA**: flocks, different from the average. **IA**: flocks, inside the average. **TOT**: Total group. **ns**: not significant. **(-):** negative correlation. **BHB:** Beta Hydroxy Butyric Acid. **AST**: aspartate-aminotransferase .**GGT**: gamma glutamyl-transferase. **T.protein**: total protein. *p<0.05 **p<0.005 ***p=0.000 (according to variance analyze).

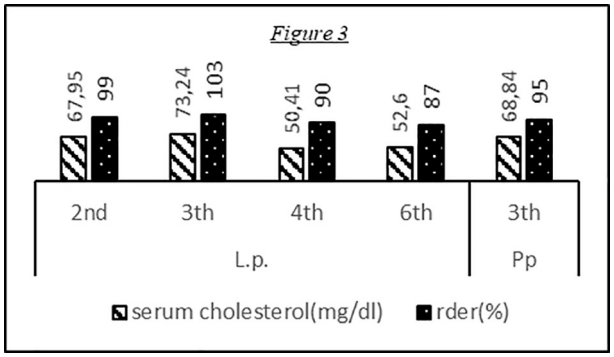
Discussion

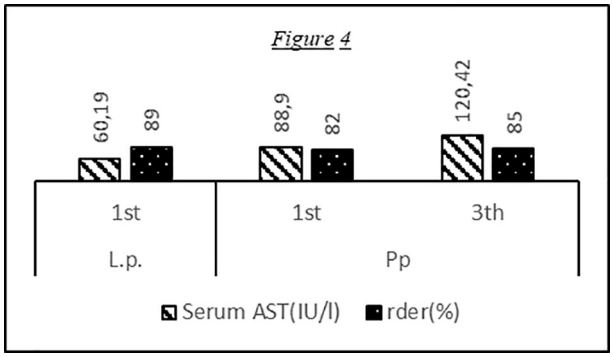
According to determination coefficients, the relationships between diet components and metabolites were generally stronger in the DA group compared to other groups. In other words, the flocks that differed in terms of a metabolite from the general averages, either positive or negative, were in a more significant relationship with the diet component than the flocks that did not differ. This difference could be interpreted that diet may also be a factor in the differentiation of the metabolite from the averages. It is also seen in the diagrams that this difference in meaning in the relationship degrees could be caused by diet (Figure 1-8). Because these diagrams were developed by using the flocks included in the DA group with differentiation directions as in Table 3 and the ratio of the diet used in these flocks to meet the requirement (Table 2). The diet components and the metabolite levels related are shown together in the flocks forming the DA group, the relationship between the two variables has become more visible in these diagrams (Figure 1-8),

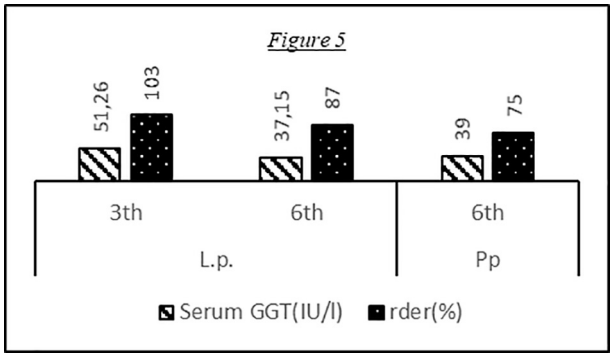
According to the values of the determination coefficient, the relationship between protein metabolism metabolites and the diet component was stronger than energy metabolism metabolites. This difference in relationship levels may have occurred due to the relatively wide spectrum observed in diet protein levels according to chi-square values (Table 3).In other words, it seems possible that the depth of variation between the diet protein ratios of the flocks may increase the significance of the relationship by causing high variation in the metabolites associated with the diet protein.This form of relationship could be seen as another proof of the relationship between diet and biochemical profile.

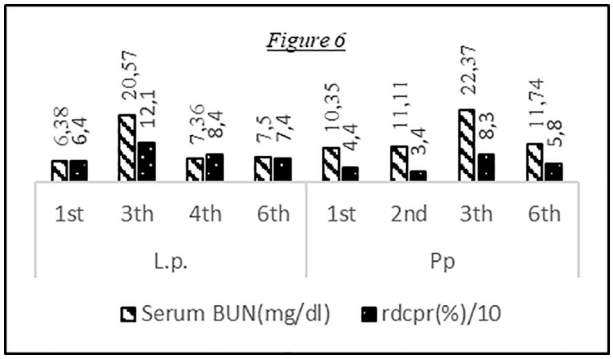


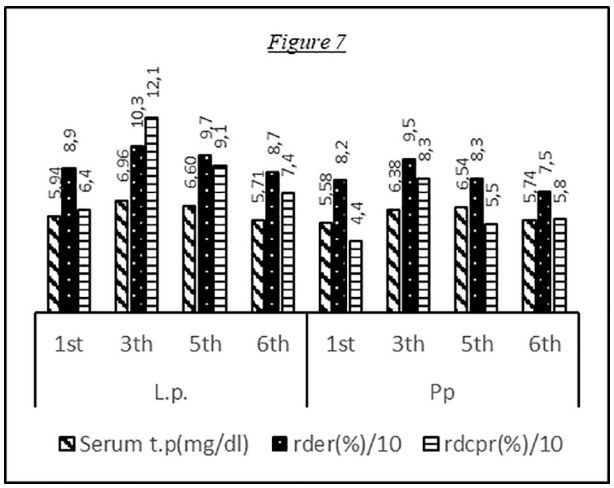












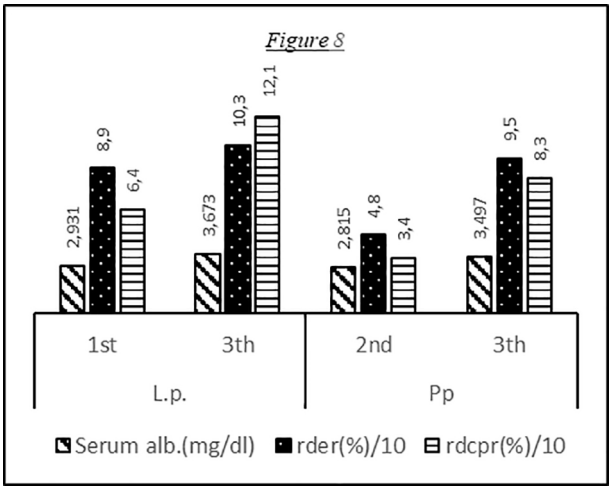


Figure 1-8. Serum mean metabolite concentrations and the ratios of the diet to meet the energy and protein requirements of the herds in the establishments that consist the **DA** group.

L.p.: Late pregnancy. **Pp:** Postpartum. **rder%:**The rate of diet energy requirement. **rdpcr%**:The rate of diet crude protein requirement. **BHBA:** Beta hydroxybutyric acid. **AST:** Aspartate amino transferase. **GGT:** Gama glutamyl transferase. **BUN:** Blood urea nitrogen. **t.p:** Total protein. **Alb**: Albumin. The y-axis shows physiological periods and flock numbers.
In order for the variables to form similar columns in the same diagram, some transformations were made in the (rder%) and (rdcpr%) ratios. These transformations: **1-**1% of (rder%) was used in figure 2 **2-** 1 in 10 of (rdpcr%) was used in Figure 6. **3-** In Figures 7 and 8, 1 of 10 (rder%) and (rdpcr%) was used

Energy Metabolism and Diet

It was observed that glucose, BHBA and cholesterol do not have a statistically significant relationship with diet energy value in the IA group. While the relationships between glucose and BHBA concentrations and diet energy level occur approximately in the TOT and DA groups, in the case of cholesterol, DA group interactions appear to be significantly stronger than the total group (Table 4).

In studies focusing on the effect of diet energy level on glucose, Lee et al. [12] reported that diet energy value was effective on glucose concentration in cattle, while Kida [11] reported that it was effective only in the middle and late lactation periods. In determining the energy balance and the amount of energy taken by diet, especially in early lactation, the blood glucose level [11] is significantly changed by flushing applications in sheep [15, 24]. On this study the relationship of glucose concantdiet and diet energy, which was found not significant on IA groups was found significant on DA group (Table 4). This difference in meaning shows the effect of diet energy level on blood glucose value deviating from the mean. This finding is supported with the result of Caldeira et al. [5] study on sheep for meeting energy needs of sheep at certain rates, reported that the serum glucose levels of sheep fed with a low energy content are low compared to other groups.

The increase in the production of ketone bodies in ruminants is a result of increased lipolysis due to the inability of the diet to meet its energy needs [22]. Ketone bodies occurred and raised in numbers from fats catabolism when glucose does not meet the body energy requirements. In this case, unlike glucose, which positively correlates with diet energy, the BHBA-diet energy relationship was expected to occur negatively. However the correlation between diet energy and BHBA levels were found to be positive (Table 4). The positive relationship in this study may be due to the levels of BHBA obtained in the studymay be explained as; Bani Ismail et al. [3] suggested that the relationship between glucose and BHBA is occurs when concentration of BHBA is greater than 0.86 mmol/l. The mean BHBA concentration obtained in the study presented was 0.35 and 0.42 mmol / l during late pregnancy and postpartum periods, respectively.

While cholesterol, which is the one of the major indicator of body energy index [11] has been least affected metabolite from diet energy level compared with other energy metabolism metabolite in total group, but has been most affected in DA group (Table 4). Because of these deep differencies, which have reexisted among the experimental groups, cholesterol may be the one of the most useful instrument for assessing energy level of diet.

Liver Enzymes and Diet

According to the determination coefficients in the presented study, it is seen that the effect of diet energy value on AST is more visible than the GGT, even if it is moderate (Table 4). In the study conducted by Abdalla et al. [1], which was providing 50% and 100% of energy requirements of sheep, mean AST concantdiet respectively 55.11 u/l and 82.34 u/l, GGT concentration 32.57

u/l and 38.23 u/l was found [1]. In this study, the negative relationship between the diet energy value and serum AST level in the IA group was compatible with the data of the study conducted by Abdalla et al. [1]. However, in the case of the DA group, there was a positive relationship between diet energy value and serum AST and GGT levels (Table 4). This incompatibility between the groups may be occurred by the participation of liver enzymes into the circulation not only due to hepatocellular damage but also from leakage from over production [18]. Because the diet energy is relatively high, the liver is one of the first organs to be affected in the accelerated metabolism and probably more transamination reactions. The increased production of transaminases into the circulation in the form of leakage will increase blood concentrations. When evaluated in terms of metabolic rate and increased enzyme synthesis, the positive correlation between diet energy and GGT activity could also be associated with oxidative stress. Because it is reported that GGT, which is stated to have an antioxidant effect outside the cell, and an antioxidant effect inside the cell [6], could be used as an oxidative stress indicator as well as evaluating liver functions [10].

Protein Metabolism and Diet

While the effect of diet protein level on BUN, total protein and albumin metabolites, which are reflecting protein metabolism [11], was significant on almost all group, this effect was more meaningful within the DA group then the other (Table 4).

The relationship between the diet protein level and BUN concentration found important in all of the groups in our study, supported by the data obtained by Torell et al. [23] and Lobley et al. [14] in their studies on sheep. It is reported that there is a linear relationship between NH_3, which is transferred from portal visceral organs, to the liver, and dietary nitrogen intake, and this correlation is up to $r^2 = 0.96$. [14]. Also a strong correlation between BUN and nitrogen intake was reported in the study conducted by Torell et al. [23] by giving pelleted alfa alfa hay under pasture conditions ($r^2 = 0.99$) as well. Similar results were taken from the researches which were realised on cattle about this matter and BUN is describe as the good indicator, which reflects of rumen ammonium concentration [11, 12].

Several previous studies have shown that there is a direct relationship between serum albumin concentration and nutritional status, particularly protein intake, in both sheep and cows [9, 12, 26]. Albumin, which was reported to be affected by nutritional conditions [16], had a relatively strong relationship with diet protein in our study (Table 4). Mazzaferro et al. [16] suggested that one of the synthesis factors of albumin is the diet and %6 of daily nitrogen intake is used for synthesis of albumin. Furthermore, it is stated that when diet and protein deficiency takes a long time, albumin synthesis decreases by 50-60% and diet with 0-4% protein does not slow down this decrease [21]. Furthermore, increasing serum albumin and total protein levels are parallel with increases of diet energy level on nonpregnant ewes [5]. It is stated that the relationship between albumin and diet protein could be explained by the fact that the amino acid requirement for the synthesis of the molecule can not be taken in the diet, but also plays a role as an amino acid provider due to an inadequate diet [5].

It is suggested that dietary energy also has an effect on albumin synthesis, and in energy deficiency, the reduced synthesis rate of albumin could only be eliminated by feeding with glucose [19]. Also, the insulin level must be sufficient to synthesize the appropriate amounts of albumin [13]. In our presented study, it was determined that albumin and total protein concentrations are related to the diet energy level as well as the relationship between the diet protein level (Table 4). This data support the data that described, increasing serum albumin and total protein levels are parallel with increases of diet level on nonpregnant ewes, by Caldeira et al. [5]. However, in the emergence of this relationship, the high dry matter of energy-intensive feeds and thus the formation of dehydration may also contribute. Because dehydration is reported to increase the albumin level proportionally [11].

Conclusion

In our study, it was determined that there are varying degrees of relationship between the diet components and the serum biochemical profile. It is very difficult to set up with a mathematical formula that provides certainty for these relationships. Because it is a known fact that the biochemical profile is affected by countless agents and metabolic pathways besides the diet factor. However, considering the difference in diet-biochemical relationships between the groups formed in our study, it seems possible to modify the serum biochemical profile with diet arrangements. Because the interactions between the diet components and serum biochemical profile in the DA group were found to be more significant than the other group, the IA group.In other words, the diet-biochemical profile relationship of the flocks that differed upwards or downwards in terms of a metabolite was stronger than those that did not differ. This difference in the degree of relationship of the groups could be interpreted that diet may also be a factor in metabolite deviations.

It is thought that serum cholesterol level is a good indicator for evaluating the diet energy level, but caution should be taken in use of transferases because the direction of interaction changes between groups to determine the diet energy level. The relationship between glucose and BHBA and diet energy level was not as strong as cholesterol.

It is one of the common outcomes of both this study and previous studies that there are very strong relation-

ship between the diet protein level and BUN. Therefore, it can be said that the BUN level is an indicator that can be used in diet protein arrangements. It is thought that total protein and especially albumin concent of diets can be used to evaluate both protein and energy levels of the diet considering the level of the diet.

This article is the part of 'Investigiation of Metabolic Profile Test Applicability in Sheep Herds' Project which was supported by General Directorate of Agricultural Research and Policy with the project number TAGEM/HAYSÜD/14/A07/P01/003.

Credit

This study was presented in the 1st International Livestock Science Congress, 31 Oct.-01 Nov. 2019, Antalya, Turkey.

References

1. Abdalla EB, El-Rayes MAH, Khalil FA, Abou El-Ezz SS, Ibrahim NH (2014). Effect of restrıcted feedıng on body weıght, some hematologıcal and bıochemıcal parameters ın sheep and goats raısed under semı-arıd condıtıons. In 2014 ADSA-ASAS-CSAS Joint Annual Meeting. Asas.
2. Aktaş AH, Doğan Ş (2014). Effect of live weight and age of Akkaraman ewes at mating on multiple birth rate, growth traits, and survival rate of lambs. Turkish Journal of Veterinary and Animal Sciences, 38(2): 176-182.
3. Bani Ismail ZA, Al-Majali AM, Amireh F, Al-Rawashdeh OF (2008). Metabolic profiles in goat does in late pregnancy with and without subclinical pregnancy toxemia. Veterinary clinical pathology, 37(4): 434-437.
4. Broderick GA. Clayton MK (1997). A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. Journal of dairy science, 80(11): 2964-2971.
5. Caldeira RM, Belo AT, Santos CC, VazquesMI, Portugal AV (2007). The effect of body condition score on blood metabolites and hormonal profiles in ewes. Small Ruminant Research, 68(3): 233-241.
6. Celi P (2010). The role of oxidative stress in small ruminants' health and production. Revista Brasileira de Zootecnia, 39: 348-363.
7. Diet program. In Selcuk University Faculty of Veterinary Medicine, Department of Zootechnics and Animal Feeding web page: Date of access: 10.01.2015. Address of acces: https://www.selcuk.edu.tr/veteriner-zootekni_ve_hayvan_besleme/Ayrinti/4402-rasyon-programi.
8. Hammond AC. (1983). Effect of dietary protein level, ruminal protein solubility and time after feeding on plasma urea nitrogen and the relationship of plasma urea nitrogen to other ruminal and plasma parameters. Journal of Animal Science, 57(suppl 1): 435.
9. Hoaglund CM, Thomas VM, Petersen MK, Kott RW (1992). Effects of supplemental protein source and metabolizable energy intake on nutritional status in pregnant ewes. Journal of animal science, 70(1),:273-280.
10. Kataria N, Kataria AK (2012). Use of serum gamma glutamyl transferase as a biomarker of stress and metabolic dysfunctions in Rathi cattle of arid tract in India. Journal of Stress Physiology & Biochemistry, 8(3).
11. Kida K (2003). Relationships of metabolic profiles to milk production and feeding in dairy cows. Journal of veterinary medical science, 65(6): 671-677.
12. Lee AJ, Twardock AR, Bubar RH, Hall JE, Davis CL (1978). Blood metabolic profiles: their use and relation to nutritional status of dairy cows. Journal of dairy science, 61(11): 1652-1670.
13. Lloyd CE, Kalinyak JE, Hutson SM, Jefferson LS (1987). Stimulation of albumin gene transcription by insulin in primary cultures of rat hepatocytes. American Journal of Physiology-Cell Physiology, 252(2): 205-214.
14. Lobley GE, Weijs PJM, Connell A, Calder AG, Brown DS, Milne E (1996). The fate of absorbed and exogenous ammonia as influenced by forage or forage–concentrate diets in growing sheep. British Journal of Nutrition, 76(2): 231-248.
15. Maurya VP, Naqvi SMK, Joshi A, Mittal JP, Singh VK (2011). Influence of thermal stress on estrus behaviour and fertility of native Malpura sheep under semi-arid region of India. Indian Journal of Animal Sciences, 81(1): 15-18.
16. Mazzaferro EM, Rudloff E, Kirby R (2002). The role of albumin replacement in the critically ill veterinary patient. Journal of Veterinary Emergency and Critical Care, 12(2): 113-124.
17. Meyer DJ, Harvey JW, (1998). Evaluation of hepatobiliary system and skeletal muscle and lipid disorders. p:157-187. In: Veterinary Laboratory Medicine. Interpretation and Diagnosis. 2ª Ed. ISBN:978-0721662220 WB Saunders Company Philadelphia, USA.
18. Pappas SC, Hoofnagle JH, Young N, Straus SE, Jones EA (1985). Treatment of chronic non-A, non-B hepatitis with acyclovir: Pilot study. Journal of medical virology, 15(1): 1-9.
19. Princen JMG, Mol-Backx GPBM, Yap SH (1983). Restodiet effects of glucose refeeding on reduced synthesis of albumin and total protein and on disaggregated polyribosomes in liver of starved rats: evidence of a post-transcriptional control mechanism. Annals of Nutrition and Metabolism, 27(3): 182-193.
20. Sahinduran S, Sezer K, Buyukoglu T, Albay MK, Karakurum MC (2010). Evaluation of some haematological and biochemical parameters before and after treatment in cows with ketosis and comparison of different treatment methods. J. Anim. Vet. Adv, 9(2): 266-271.
21. Sakuma K, Ohyama, Sogawa K, Fujii-Kuriyama Y, Matsumura Y (1987). Low protein—high energy diet induces repressed transcription of albumin mRNA in rat liver. The Journal of nutrition, 117(6): 1141-1148.
22. Sargison ND (2007). Pregnancy toxaemia. P: 359-362. In: Diseases of sheep. Edit: I. D. Aitken, 4th ed. ISBN-13: 978-14051-3414-9, Iowa, USA.
23. Torell DT, Hume ID, Weir WC (1974). Factors affecting blood urea nitrogen and its use as an index of the nutritional status of sheep. Journal of animal science, 39(2): 435-440.
24. Venter JL, Greyling JPC (1994). Effect of different periods of flushing and synchronized mating on body weight, blood glucose and reproductive performance in spring-mated ewes. Small Ruminant Research, 13(3): 257-261.
25. Yilmaz A, Tepeli C, Tekin M. E, Akmaz A, Garip M, Polat ES, Coşkun B, Çağlayan T (2011). Determination of live weights and body measurements of Kangal Type Akkaraman sheep in producers conditions. J. Food Agr. Environ, (9): 366-370.
26. Yokus B, Cakir DU, Kanay Z, Gulten T, Uysal E (2006). Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. Journal of Veterinary Medicine Series A, 53(6): 271-276.