The Effect of Various Concentrated Feed Mixtures on Certain Blood Parameters in Tuj Lambs

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SUMMARY

This study was performed to determine the effects of various protein sources on certain blood metabolites, added to the Tuj lamb concentrates used for 1, 30, 60, and 90 day periods. In the study, a number of 24 weaned Tuj lambs, male 3-3.5 months old and weighing meanly 19.5 kg, were randomly divided into four groups, and each animal was put in individual cages. Concentrates containing various protein sources were prepared, and hay was used as roughage. Blood samples were obtained through the jugular vein on the days of 1, 30, 60, and 90 after the morning feeding. The effects of the periods and various protein sources were determined using the plasma samples obtained on the first, 30th, 60th, and 90th days from the groups fed with the concentrates possessing four different phyto-protein sources. The parameters obtained hereby were at normal levels even though there were numerical differences in some of the blood parameters, thus nourishment may have certain effects on these parameters. Overall, this study has revealed that using corn gluten meal, cotton seed meal, soybean meal, and sunflower meal as protein sources has no negative effect on certain blood parameters.

Key Words   Blood parameters, Lamb, Protein sources, Tuj

Tuj Kuzularında Farklı Konsantre Yemlerin Bazı Kan Parametreleri Üzerine Etkisi

ÖZET

Bu çalışma, tuj kuzularının konsantre yemlerinde kullanılan farklı protein kaynaklarının 1, 30, 60 ve 90 günlük besi sürelerinde bazı kan metabolitleri üzerine etkisini belirlemek amacı ile yapıldı. Denemede, 3-3.5 aylık yaşta, ortalama 19.5 kg canlı ağırlıkta, 24 baş Tuj ırkı erkek kuzu kullanıldı. Rasgele seçilen ve 6 baş kuzu içeren 4 ayrı deneme grubu oluşturuldu. Her bir hayvan bireysel kafeslere konuldu. Gruplara farklı protein kaynakları içeren konsantre yem karmaları hazırlandı. Kaba yem olarak çayır kuru otu kullanıldı. Sabah yemlemesinden sonra *vena jugularis*'ten 1, 30, 60 ve 90. gün kan örnekleri alındı. Dört farklı bitkisel protein kaynağı içeren değişik konsantre yemlerle beslenen gruplarda 1, 30, 60, 90. günlerde alınan plazma örneklerinde yapılan analizlerde dönemlerin ve farklı protein kaynaklarının kan plazma parametreleri üzerine etkisi incelendi. Farklı protein kaynaklarıyla yapılan çalışmada bazı kan parametrelerinde farklılık saptansa da parametrelerin düzeylerinin normal sınırlarda olduğu, beslenmenin bazı kan parametreleri üzerine etkisi olabileceği saptandı. Bu araştırma sonucunda, rasyonlarda protein kaynağı olarak mısır gluten unu, pamuk tohumu küspesi, soya küspesi ya da ayçiçeği küspesi kullanılmasının bazı kan parametreleri üzerine olumsuz etkisinin olmadığı kanısına varıldı.

Anahtar Kelimeler   Kan parametreleri, Kuzu, Protein kaynakları, Tuj

INTRODUCTION

Feed stuff undergoes several metabolic changes in the body, which does have different effects in the blood circulatory system. This, in turn, gives essential feedback on the nourishment character and health status of the animal. Studies performed on the nourishment and its relation with blood parameters have documented that the levels of protein (Karabulut et al. 1999; Kaya and Yalcin 2000), urea and glucose (Karabulut et al. 1999), and total cholesterol and total lipid (Kaya and Yalcin 2000) are not affected harmfully by various concentrates. Yet, it has been reported that protein source did not influence serum protein (Schloesser et al.1993), urea nitrogen (Schloesser et al. 1993; Thomas et al. 1994), glucose (Hoaglund et

al.1992; Schloesser et al. 1993; Thomas et al. 1994) and nonesterified fatty acid concentrations (Schloesser et al. 1993). On the other hand, it has been documented that there were no differences in total protein, glucose (Tripathi et al. 2001), urea nitrogen (May et al. 1990) among lambs fed various concentrated feed mixtures.

The levels of blood urea and ammoniac nitrogen increase as feed consumption starts. Yet, these levels augment significantly in the case of adding urea and zeolite to the ration. Moreover, they are changeable broadly in relation to the several factors (Filya et al. 1999). On the other hand, adding dried rumen content to the concentrates of merinos' lambs affects metabolism particularly by lowering the level of serum protein, but does not change

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the levels of total lipid, ketone bodies and urea-N significantly (Yildiz 2000).

In the case of either deficiency-excessiveness or unbalance of any given nutrient in the ration, changes can be seen in blood parameters including plasma protein, glucose, lipid, non-esterified fatty acids and β-hydroxybutiric acid. These changes show that nourishment may have positive-negative effects on blood parameters. Meanwhile, certain blood parameters can be affected by the levels of roughage and concentrates, nutrient contents of feed and antinutritional factors.

Above all, significance of the interrelation between nourishment and blood metabolites in animal nutrition is obvious. This study has performed to reveal the effects of feeds containing various protein sources on the certain blood parameters in Tuj lambs.

MATERIALS and METHODS

A number of 24 weaned Tuj lambs, male 3-3.5 months old and weighing meanly 19.5 kg, were used in the study. Animals were randomly divided into four groups, and each animal was put in individual cages.

Table 1. The contents of the concentrates (%)

Item (%)	Groups			
	1	2	3	4
Corn	16	23	20	23
Barley	42.75	29.75	37.75	29.75
Sunflower meal	-	-	-	31
Soybean meal	-	-	17	-
Cotton seed meal	-	31	-	-
Corn gluten meal	12	-	-	-
Wheat bran	21	8	17	8
Molasses	6	6	6	6
Limestone	1	1	1	1
Salt	1	1	1	1
Vitamin and mineral mix	0.25	0.25	0.25	0.25
Dry matter	88.45	88.32	89.09	89.25
Organic matter	81.97	82.38	83.52	83.09
Crude protein	16.12	16.15	16.13	15.83

The trial was lasted for 105 days, the first 15 days being habituation period and the 90 days being treatment stage. Four different concentrates were prepared for the treatment groups. The levels of dry matter, organic matter and crude protein of the concentrates were determined by the method indicated in AOAC (1996). The contents of the concentrates are displayed in Table 1 (Aksu Elmali and Kaya 2009).

Rations were prepared in accordance with the daily nutrient requirements of the animals (NRC 1985). Daily feed consumption of each animal was determined in the habituation period, and, during the treatment stage. The animals were fed with the rations ad libitum which contained roughly 20% roughage (hay) and 80% concentrates, and daily amount was gradually increased everyday as much as the 10% of the amount consumed on previous day. Water was available at any time.

Blood samples were obtained by the use of EDTA comprising and vacuum-operated tubes through the jugular vein on the days of 1, 30, 60, and 90 right after the morning feeding. They were centrifuged at 3000 rpm for 15 min., the plasmas were acquired and stored in deep freezer at -40°C until analyzed. Spectrophotometer was used in determining the metabolite levels in the plasma samples.

Data were analyzed by one way analyses of variance (ANOVA) using SPSS 11.5 program. Significances of the differences among the groups were determined by Duncan test (Duzgunes et al. 1983), and the results were given as mean±standard deviation (x±Sx).

RESULTS

The levels of blood parameters and the differences obtained on the days of 1, 30, 60, and 90 are displayed in Table 2.

There were no significant differences on the levels of the total protein, BUN, glucose, cholesterol, NEFA, βHBA, AST and ALT, determined on the days of 1, 30, 60, and 90. However, the triglyceride level was high at the beginning, decrease on the 30th day (P<0.05).

The effects of the concentrates possessing various protein sources on the levels of the total protein, BUN, glucose, triglyceride, cholesterol, NEFA, βHBA, AST and ALT were shown in Table 3.

In all the periods, by comparison, the total protein (P<0.001) and BUN (1st day P<0.001; 30th, 60th and 90th day P<0.01) levels were higher in the 3rd group fed with the feed containing soybean meal, the glucose level in the 1st group fed with the feed comprising corn gluten meal, and the triglyceride and cholesterol levels in the 4th group fed with the feed having sunflower meal. The effects of the concentrates with various protein sources were insignificant on the levels of the NEFA, βHBA, AST and ALT in the four groups.

Table 2. Certain blood parameters in different periods (n=24)

Parameters	1st day	30th day	60th day	90th day
Total protein (g/dl)	7.29±0.04	7.37±0.05	7.33±0.04	7.32±0.04
BUN (mg/dl)	14.41±0.33	14.31±0.36	13.84±0.38	14.35±0.36
Glucose (mg/dl)	43.30±0.60	43.73±0.34	43.94±0.35	43.60±0.54
Triglyceride (mg/dl) *	18.06±0.38a	16.27±0.30b	16.78±0.43b	17.25±0.48ab
Cholesterol (mg/dl)	126.94±1.57	125.42±1.51	127.46±1.61	127.49±1.92
NEFA (mmol/l)	0.37±0.01	0.39±0.01	0.39±0.01	0.40±0.01
βHBA (mmol/l)	0.39±0.01	0.39±0.01	0.39±0.01	0.38±0.01
AST (U/l)	139.08±1.53	140.30±1.61	138.08±1.79	138.41±1.76
ALT (U/l)	24.26±0.44	24.18±0.34	24.53±0.30	24.57±0.31

*P<0.05 The difference between the periods comprising different letters in the same column is statistically significant.
BUN: blood urea nitrogen, NEFA: non-esterified fatty acids, βHBA: β-hydroxybutiric acid, AST: aspartate aminotransferase, ALT: alanine aminotransferase.

Table 3. Certain blood parameters of the groups fed with various protein sources (n=6)

Days	Parameters	Groups			
		1	2	3	4
1st day	Total protein (g/dl) *	7.18±0.06[b]	7.19±0.02[b]	7.58±0.02[a]	7.23±0.03[b]
	BUN (mg/dl) *	12.95±0.47[b]	14.25±0.54[b]	16.32±0.40[a]	14.13±0.32[b]
	Glucose (mg/dl) **	46.55±1.12[a]	41.92±0.84[b]	42.82±0.91[b]	41.93±0.99[b]
	Triglyceride (mg/dl) *	17.33±0.51[b]	16.92±0.34[b]	17.30±0.38[b]	20.70±0.56[a]
	Cholesterol (mg/dl) **	124.10±2.22[b]	121.92±2.71[b]	125.75±2.73[b]	135.98±1.61[a]
	NEFA (mmol/l)	0.35±0.02	0.38±0.01	0.41±0.01	0.36±0.03
	βHBA (mmol/l)	0.42±0.03	0.37±0.02	0.43±0.03	0.34±0.02
	AST (U/l)	141.03±3.23	141.35±3.36	140.00±3.09	133.95±2.18
	ALT (U/l)	23.33±1.14	23.53±0.60	25.07±0.73	25.10±0.86
30th day	Total protein (g/dl) *	7.27±0.03[b]	7.28±0.08[b]	7.67±0.07[a]	7.23±0.04[b]
	BUN (mg/dl) **	13.35±0.43[b]	15.57±0.58[a]	15.60±0.57[a]	12.73±0.41[b]
	Glucose (mg/dl)	45.15±0.67	43.40±0.52	43.68±0.66	42.70±0.56
	Triglyceride (mg/dl) ***	16.07±0.37[b]	15.33±0.51[b]	15.85±0.37[b]	17.82±0.68[a]
	Cholesterol (mg/dl)	122.45±2.75	127.95±2.32	121.50±2.76	129.78±3.39
	NEFA (mmol/l)	0.36±0.01	0.42±0.02	0.36±0.01	0.41±0.03
	βHBA (mmol/l)	0.41±0.01	0.39±0.02	0.42±0.03	0.35±0.01
	AST (U/l)	141.32±3.41	138.55±3.73	141.35±3.36	140.00±3.09
	ALT (U/l)	23.42±0.54	25.02±0.78	23.92±0.51	24.37±0.85
60th day	Total protein (g/dl) *	7.22±0.03[b]	7.23±0.03[b]	7.60±0.03[a]	7.28±0.03[b]
	BUN (mg/dl) **	13.70±0.65[b]	13.43±0.61[b]	15.88±0.68[a]	12.33±0.30[b]
	Glucose (mg/dl) **	46.02±0.50[a]	43.30±0.58[b]	43.10±0.43[b]	43.33±0.56[b]
	Triglyceride (mg/dl) ***	16.90±0.36[ab]	16.87±0.35[ab]	15.00±0.34[b]	18.35±1.36[a]
	Cholesterol (mg/dl)	126.35±2.19	123.38±3.20	126.35±2.19	133.77±4.00
	NEFA (mmol/l)	0.37±0.02	0.39±0.03	0.38±0.01	0.44±0.04
	βHBA (mmol/l)	0.38±0.02	0.39±0.02	0.41±0.03	0.37±0.01
	AST (U/l)	141.22±3.35	141.13±3.41	131.40±3.04	138.55±3.73
	ALT (U/l)	24.32±0.79	25.03±0.46	24.85±0.61	23.92±0.51
90th day	Total protein (g/dl) *	7.21±0.03[b]	7.27±0.06[b]	7.58±0.04[a]	7.24±0.03[b]
	BUN (mg/dl) **	13.48±0.46[bc]	14.78±0.73[ab]	16.27±0.38[a]	12.85±0.41[c]
	Glucose (mg/dl) ***	45.58±1.17[a]	44.45±0.89[a]	42.90±0.42[ab]	41.45±1.05[b]
	Triglyceride (mg/dl) *	14.92±0.34[c]	16.38±0.33[b]	16.93±0.34[b]	20.75±0.50[a]
	Cholesterol (mg/dl) **	126.52±2.21[b]	124.53±2.82[b]	120.73±3.01[b]	138.17±3.50[a]
	NEFA (mmol/l)	0.39±0.01	0.44±0.02	0.38±0.02	0.40±0.03
	βHBA (mmol/l)	0.37±0.02	0.38±0.01	0.41±0.03	0.35±0.01
	AST (U/l)	134.07±1.95	141.32±3.55	133.93±3.50	144.32±3.48
	ALT (U/l)	24.30±0.73	24.83±0.61	24.00±0.53	25.13±0.65

* P<0.001, ** P<0.01, *** P<0.05, The difference between the groups comprising different letters in the same column is statistically significant.

DISCUSSION and CONCLUSION

Protein level in the ration, as well as its quality, is extremely essential in animal nutrition. There are significant decreases in the levels of plasma proteins in the case of long term protein insufficiency in the ration (Rowlands 1980). This study determined that the levels of plasma proteins in all the periods were 7.29-7.37 g/dl (Table 2), indicating no difference. Likewise, the levels of the total protein in the blood plasma of the 3rd group fed with the feed possessing soybean meal were significantly higher (P<0.001) on the days of 1, 30, 60, and 90 even though the total proteins were documented to be at normal levels (Kaneko 1989) in all groups (Table 3). Kaneko (1989) have reported that the normal levels of plasma total proteins in sheep were 6.0-7.9 g/dl.

The levels of blood total proteins are in the limits of 6.74-7.83 g/dl in the case of adding cotton seed meal and sunflower meal to the concentrates as protein sources (Yalcin et al. 1995). Studies have reported that usage of various concentrates (Deniz and Tuncer 1995; Kucukersan

and Kucukersan 1995; Tripathi et al.2001; Yildiz et al. 1998), as well as the levels and types of the roughages used in the ration, and the season (Denek et al. 2006) does have no effect on the total protein concentrations.

Additionally, adding various common vetch seed *(Vicia sativa L.)* concentrations to the lamb concentrates has been revealed to have no negative effect on the levels of blood total proteins (Kaya and Yalcin 2000). The results of our study are also similar to the literature reports indicated above.

In a study where sunflower meal was used as protein source, the levels of blood total protein on the days of 1 and 30 were parallel to our results, but it was determined as 10.15 g/dl on the 90th day (Kucukersan et al. 1996; Yildiz et al. 1998) too found the level of plasma total protein in the sheep fed with various concentrates containing cotton seed meal to be 17.09 g/dl on the 60th day. On the other hand, the total protein level in our study is, at most, 7.28 g/dl in the groups consuming sunflower meal and cotton seed meal. This might be due to age and

breed of the animals and the levels of other nutrients in the concentrates, used in the study.

In our study, the BUN level was higher in the 3rd group fed with soybean meal containing concentrate on the days of 1, 30, 60 and 90. Plasma urea-N concentrations are affected by the protein consumption. There is a positive correlation between the low level of urea-protein in the ration and blood urea concentrations (Rowlands 1980). The results of our study are also similar to the literature reports indicated above. The blood urea-N level of the lambs fed with the concentrates containing 16.1% crude protein by the use of sunflower meal and cotton seed meal as the protein sources, is 14.64 mg/dl on the 30th day, and has increased up to 28.26 mg/dl on the 90th day (Yalcin et al. 1995). The BUN levels found in our study are lower than those of Yalcin et al. (1995), which may be because of the amount of the protein sources and variations in their digestibility. In the case of using sunflower meal and cotton seed meal as the protein sources in the concentrates, the urea-N level is measured as 19.21 mg/dl at the beginning, and has increased up to 29.75 mg/dl on the 56th day of the trial (Filya et al. 1999). The highest BUN levels found in this study are on the 60th day in the 3rd group fed with the soybean meal containing ration. Variations in the content of the crude protein and protein sources may be the cause of changes in the BUN levels. Protein consumption and variation in their digestibility may be the cause of decrease or increase in the BUN levels at the beginning or finishing.

A study done on using various concentrates has shown insignificant effect of the differences in glucose levels (Tripathi et al. 2001). Yet, higher fat level has also had no effect on the glucose levels (Khorshidi et al. 2008). In our study, blood glucose levels in the four periods were numerically higher in the 1st group fed with the concentrates containing corn gluten meal, but there was no negative effect of the various protein sources determined.

In ruminant rations, there are mainly triglycerides, galactolipid and phospholipids at lower levels (Lewis and Hill 1983). Even if the amount of fat is high in the ration, plasma triglyceride levels have been reported to be similar (Khorshidi et al. 2008). In our study, the highest level has been determined in the 4th group fed with sunflower meal containing ration, even though the plasma triglyceride levels are significantly different.

Concentration of ketone bodies in blood plasma is low at normal conditions. Glucose inadequacy in liver and excessiveness of free fatty acids are the key factors of their increase (Rook 1983). In our study, the level of the β-hydroxybutiric acid, one of the ketone bodies, was normal (Kaneko 1989), and there were no statistically significant differences among the periods and groups. The effect of the difference in protein source was also insignificant. The low level of the βHBA found in this study has suggested that the rations used have no negative effect on it.

NEFA levels were normal in our findings, and there were no significant differences among the periods and groups. There was also no negative effect of the different sources of phyto-protein on the levels of the plasma NEFA.

Cholesterol levels in our study are not significantly different among the periods, but show significant differences among the groups at the beginning and end of the treatment (P<0.01). It is a common sterol found in animal tissue, and is provided through any feedstuff from animal source in the ration (Church and Pond 1988). As seen in Table 1, the concentrates used in this study contain no feedstuff originated from animals. The differences among the groups may be due to the individual variations. Yet, as fat level in the ration gets higher, cholesterol level increases significantly as well (Khorshidi et al. 2008). In the case of using concentrates at different levels, possessing hazelnut skin, cholesterol levels have been determined to increase (Kucukersan and Kucukersan 1995). In our study, cholesterol level in all the periods and groups were found to be at normal levels. Kaya and Yalcin (2000) have reported that adding vetch seed *(Vicia sativa L.)* at different levels to the lamb concentrates has no negative effect on the levels of total blood cholesterol, which is in parallel with our findings.

Abou-Zeina et al. (2008) have reported significant increases in the AST and ALT levels in the case of malnutrition in sheep. Kaneko (1989) have reported that the normal levels of plasma AST in sheep were 60-280 U/l. These levels were normal (Kaneko 1989) in our study, and there were no significant differences among the periods and groups.

As far as the different periods applied in this study are concerned, various protein sources used including corn gluten meal, and cotton seed meal, soybean meal and sunflower meal have been determined to have no negative effects on the blood metabolite levels.

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