YYÜ TAR BİL DERG
(YYU J AGR SCI)
2016, 26(3): 341-347

Geliş Tarihi (Received): 01.02.2016
Kabul Tarihi (Accepted): 24.05.2016

Araştırma Makalesi/*Research Article (Original Paper)*

The Effect of Replacement with Different Levels of Sulfur and Sulfur-Free Broad Bean in Soybean Meal and Barley Based Rations on Some Rumen Fermentation Parameters

Mehtap GUNEY[1*], Murat DEMİREL[2]

[1] Department of Animal Nutrition, Faculty of Veterinary Medicine, Yuzuncu Yil University, Van, TURKEY
[2] Department of Animal Nutrition, Faculty of Agriculture, Yuzuncu Yil University, Van, TURKEY
*e-posta: mguney@yyu.edu.tr; Tel: +90 (432) 2251701/ 21558

Abstract: In this study, a total of 7 different isocaloric and isonitrogenic rations with and without sulfur (0.5% $CaSO_4$) were constituted so as to meet 25, 50 and 75% of the protein and energy obtained from soybean meal (SBM) and barley from the control ration (C) with broad bean (BB). Wheat straw (WS) and hay grass (HG) were used as roughage sources in the rations. The effects on rumen parameters of the rations prepared were analyzed in 3 yearling sheep fitted with rumen fistula and duodenal cannula. No difference was observed between groups in terms of pH, NH_3-N, propionic (PA) and butyric acid (BA) concentration of the rumen fluid ($P<0.05$). While rumen fluid pH was high before feeding, it decreased after feeding. Rumen NH_3-N, acetic acid, PA and BA concentrations also increased ($P<0.05$). As a result, it was concluded that 75% of protein and energy obtained from soybean and barley used in sheep rations can be met with broad bean, but addition of sulfur to rations with broad bean additive has no effect on rumen fermentation parameters.

Keywords: Rumen pH, Ruminant, Rumen fluid ammonia nitrogen, VFA (Volatile Fatty Acids)

Soya Küspesi ve Arpaya Dayalı Rasyonlara Kükürt Katkılı ve Katkısız Farklı Düzeylerde Bakla İkamesinin Kimi Rumen Fermantasyon Parametreleri Üzerine Etkisi

Özet: Bu çalışmada, soya küspesi ve arpadan gelen protein ve enerjinin %25, 50 ve 75'i bakladan karşılanarak kükürt katkılı ve katkısız 7 farklı izokalorik ve izonitrojenik rasyon oluşturulmuştur. Rasyonda kaba yem kaynağı olarak buğday samanı (BS) ve kuru çayır otu (KÇO) kullanılmıştır. Hazırlanan rasyonların kimi rumen parametreleri üzerine etkileri rumen fistüllü ve duodenum kanüllü 3 yaşlı koyunlarda araştırılmıştır. Kontrol rasyonu ile diğer rasyon grupları arasında rumen sıvısı pH, NH_3-N'u, propiyonik (PA) ve bütirik asit (BA) konsantrasyonu bakımından farklılık gözlenmemiştir ($P<0.05$). Sabah yemlemeden önce rumen sıvısı pH düzeyi yüksek iken yemlemeden sonra azalmıştır. Rumen sıvısı NH_3-N, asetik, PA ve BA konsantrasyonu ise artmıştır ($P<0.05$). Sonuç olarak, koyun rasyonlarına soya küspesi ve arpadan gelen enerji ve proteinin %75'inin bakla ile karşılanabileceği ancak bakla ilave edilen rasyonlara kükürt katkısının rumen fermantasyon parametrelerini değiştirmediği sonucuna varılmıştır.

Anahtar kelimeler: Rumen pH, Ruminant, Rumen sıvısı amonyak azotu, UYA (Uçucu Yağ Asitleri)

Introduction

Soybean meal has high protein content, and the biologic value of this protein is high due to its lysine content. However, the feed industry spends large amounts of money to import feeds due to insufficiency in Turkey (Karslı and Tasal 2003). Therefore, in recent years, use of alternative feeds, like legume seed feeds, as protein and energy sources for ruminants and poultry has been proposed (Dixon and Hosking 1992). As an alternative feed source broad bean is poor in sulfur amino acids. For this reason, the addition of amino acid including sulfur to the diet tends to increase microbial population linearly and thus increase digestion of roughage (Bal and Ozturk 2006).

Besides the feed sources consumed by animals, a suitable environment is required in the rumen to ensure development and reproduction of rumen microorganisms. Development of bacterial strains which ferment plant cell walls down to simple sugars and form VFA (Volatile Fatty Acids) as end products is enabled by ensuring an oxygen-free environment in the rumen. The VFA formed are used as energy source for microorganisms synthesizing amino acids for microbial protein production (Doig 2007).

In this study, considering the principle that alternative feed plants to soybean meal need research for ruminant rations, it was aimed to examine rumen fermentation characteristics of the addition of sulfur to sheep rations in which soybean meal was replaced with different proportions of broad bean.

Material and Methods

Animal material for the study constituted 3 yearling sheep with rumens fitted with fistula and T cannula. The basic protein source in the ration was soybean meal and broad bean, energy source was barley seeds, and roughage source was wheat straw and grass hay. Rations given to animals were prepared to be isocaloric and isonitrogenic with approximately 13.47% CP and 2.24 Mcal energy. In preparing the rations used in the study, energy and protein of the control ration was met by barley and soybean meal. Rations of other groups were prepared by using broad bean to provide 25%, 50% and 75% of the energy and protein obtained from barley and soybean meal (control).

Calcium sulfate ($CaSO_4$), as a source of 0.5% sulfur, was also added to the groups in which broad bean was used as replacement feed. In each group of rations, quantities of wheat straw and grass hay, the roughage sources, were kept identical. In the first group of control (C) ration, soybean and barley were used as concentrated feed source. In the second ration sample (25% BB), broad bean was used to meet 25% of the protein and energy obtained from soybean meal and barley in the control ration. In the third ration sample (50% BB), broad bean was used to meet 50% of the protein and energy obtained from soybean meal and barley in control ration. The fourth ration sample (75% BB) was prepared by using broad bean as replacement so as to meet 75% of the protein and energy obtained from soybean meal and barley in control ration. In the fifth (25% BB+0.5$CaSO_4$), sixth (50% BB+0.5$CaSO_4$) and seventh (75% BB+0.5$CaSO_4$) ration samples, similar proportions to the second, third and fourth rations were prepared, and 0.5% calcium sulfate ($CaSO_4$) was added as sulfur source.

In the adaptation period, feeds were given *ad-libitum* to the animals. In each period, pH of rumen fluids taken from the animals was measured. Kjeldahl distillation bulb was used to determine ammonia concentration in rumen fluid (Markham 1942). VFA levels were determined using a gas chromatography instrument (Santos et al. 1984).

DM, ash, CP, and ether extract analyses of the feeds and rations used in the study were conducted according to the Weende analysis method (AOAC 1990), and ADF and NDF analyses were conducted according to Van Soest and Robertson (1979).
Statistical analyses were conducted according to following mathematical model.

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

Y_{ijk} : Observation values

μ : Overall mean

a_i : Effect of the feeds

b_j : Effect of the samling time

$(ab)_{ij}$: Effect of the interaction between rumen parameters and sampling time

e_{ijk} : Random error

In the mathematical model, Least Square Means and Analysis of Variance results pertaining to the factors were obtained by using SAS (2007) package program. The differences between the averages obtained were compared with the Duncan Multiple Comparison test.

Results and Discussion

Nutrient matter contents of feed used in the study are given in Table 1. Formulations of diets used in the experiment are given in Table 2. Least square means and standard error results pertaining to the rumen fluid parameters of ration groups are given in Table 3.

Table 1. The nutrient content of feed materials used in the study (%)

Nutrition matter	Feeds				
	BB	SBM	Barley	WS	HG
DM	90.47	89.15	91.16	93.04	92.85
CP	21.06	45.49	9.20	2.31	9.50
Ether extract	0.70	0.46	1.74	0.40	1.34
Ash	3.46	6.14	2.39	10.10	7.62
NDF	26.00	12.82	35.55	76.42	52.47
ADF	12.22	6.55	8.64	50.25	32.85
CF	9.55	4.55	5.82	46.69	30.50
ME, Mcal/kg	2.82	2.73	2.89	1.59	2.33

DM: Dry matter, CP: Crude protein, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, CF: Crude fibre, ME: Metabolic energy, BB: Broad bean, SBM: Soy bean meal, WS: Wheat straw, HG: Hay grass

Table 2. Formulation of diets used in the experiment

Feeds	1.group	2.group	3.group	4.group	5.group	6.group	7.group
	C	%25 BB	%50 BB	%75 BB	%25BB+ $CaSO_4$	%50BB+ $CaSO_4$	%75BB+ $CaSO_4$
BB	0	15.86	31.65	47.51	15.86	31.65	47.51
SBM	20.61	15.36	10.10	4.84	15.36	10.10	4.84
Barley	44.21	33.60	23.07	12.47	33.60	23.07	12.47
WS	24.30	24.30	24.30	24.30	24.30	24.30	24.30
HG	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Vit+min	0.16	0.16	0.16	0.16	0.16	0.16	0.16
DCP	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Salt	0.6	0.6	0.6	0.6	0.6	0.6	0.6
ME, Mcal	2.24	2.24	2.23	2.23	2.24	2.23	2.23
CP, %	13.47	13.47	13.46	13.46	13.47	13.46	13.46

Table 3. Average and standard errors belonging to rumen parameters of ration groups

Ration groups	pH	NH_3-N, mg/100mL	AA, mmol/100mL	PA, mmol/100mL	BA, mmol/100mL
Control	6.29±0.04 ab	20.59±1.11b	3.99±1.16abc	1.07±0.07b	0.99±0.06ab
%25 BB	6.25±0.05ab	21.89±1.47ab	3.87±0.21bc	1.09±0.09b	0.95±0.08ab
%50 BB	6.17±0.05abc	22.69±1.47ab	4.59±0.21a	1.54±0.09a	1.14±0.08a
%75 BB	6.31±0.05ab	21.49±1.47ab	3.66±0.21c	1.13±0.09b	1.09±0.08a
%25 BB+$CaSO_4$	6.32±0.06a	21.83±1.70ab	4.41±0.24ab	1.17±0.10b	0.91±0.09ab
%50 BB+$CaSO_4$	6.16±0.05bc	20.68±1.47b	4.08±0.21abc	1.08±0.09b	0.94±0.08ab
%75 BB+$CaSO_4$	6.09±0.05c	25.38±1.47a	4.12±0.21abc	1.12±0.09b	0.77±0.08b
Hours					
0.hour	6.70±0.05a	22.62±1.35bc	3.58±0.19b	0.95±0.08b	0.88±0.08
2. hour	6.28±0.05bc	26.56±1.37a	3.94±0.19ab	1.19±0.08a	0.95±0.08
4. hour	5.90±0.05d	24.18±1.35ab	4.29±0.19a	1.24±0.08a	1.02±0.08
6. hour	5.97±0.05d	19.63±1.35c	4.42±0.19a	1.26±0.08a	1.00±0.08
8. hour	6.19±0.05c	19.39±1.35c	4.16±0.19a	1.18±0.08a	0.97±0.08
10. hour	6.33±0.05b	19.26±1.35c	4.10±0.19ab	1.16±0.08ab	1.02±0.08

NH_3-N: ammonia nitrogen, AA:Acetic acid, PA: propionic acid, BA: butyric acid
a,b,c,d: The values of each parameter in the same column with different numbers are importantly different ($p<0.05$)

Table 3. Average and standard errors belonging to rumen parameters of ration groups (continue)

Feed xHour		pH	NH_3-N, mg/100mL	AA, mmol/100mL	PA, mmol/100mL	BA, mmol/100mL
Control	0.hour	6.78±0.09	23.50±2.72	3.62±0.39	0.95±0.16	1.01±0.15
	2. hour	6.30±0.09	23.19±2.72	3.85±0.39	1.14±0.16	1.00±0.15
	4. hour	5.85±0.09	21.20±2.72	4.40±0.39	1.16±0.16	1.02±0.15
	6. hour	5.99±0.09	18.84±2.72	4.21±0.39	1.08±0.16	0.88±0.15
	8. hour	6.32±0.09	18.82±2.72	3.95±0.39	1.02±0.16	1.03±0.15
	10. hour	6.47±0.09	17.99±2.72	3.93±0.39	1.07±0.16	1.01±0.15
%25 BB	0. hour	6.71±1.13	24.44±3.60	3.17±0.51	0.82±0.21	0.86±0.20
	2. hour	6.39±1.13	26.98±3.60	3.75±0.51	1.16±0.21	1.03±0.20
	4. hour	5.95±1.13	21.75±3.60	4.05±0.51	1.16±0.21	0.97±0.20
	6. hour	6.01±1.13	18.52±3.60	4.23±0.51	1.19±0.21	0.93±0.20
	8. hour	6.22±1.13	19.81±3.60	3.87±0.51	1.11±0.21	0.94±0.20
	10. hour	6.26±1.13	19.82±3.60	4.16±0.51	1.14±0.21	0.97±0.20
%50 BB	0. hour	6.66±0.13	26.72±3.60	4.34±0.51	1.34±0.21	0.95±0.20
	2. hour	6.18±0.13	30.58±3.60	5.23±0.51	1.91±0.21	1.32±0.20
	4. hour	5.94±0.13	24.07±3.60	4.59±0.51	1.62±0.21	1.10±0.20
	6. hour	5.93±0.13	18.61±3.60	5.00±0.51	1.63±0.21	1.25±0.20
	8. hour	6.07±0.13	17.65±3.60	4.03±0.51	1.42±0.21	1.03±0.20
	10. hour	6.26±0.13	18.54±3.60	4.35±0.51	1.33±0.21	1.17±0.20
%75 BB	0. hour	6.82±0.13	18.52±3.60	3.39±0.51	0.89±0.21	0.98±0.20
	2. hour	6.37±0.13	27.74±3.60	3.80±0.51	1.20±0.21	1.14±0.20
	4. hour	6.04±0.13	26.96±3.60	3.66±0.51	1.21±0.21	1.24±0.20
	6. hour	5.99±0.13	21.19±3.60	3.94±0.51	1.35±0.21	1.20±0.20
	8. hour	6.22±0.13	17.45±3.60	3.25±0.51	0.95±0.21	0.85±0.20
	10. hour	6.43±0.13	17.07±3.60	3.94±0.51	1.21±0.21	1.18±0.20
%25 BB+$CaSO_4$	0.hour	6.74±0.14	17.65±4.16	3.95±0.59	1.05±0.25	0.81±0.23
	2.hour	6.28±0.14	21.95±4.16	3.77±0.59	0.97±0.25	0.74±0.23
	4.hour	5.77±0.14	24.63±4.16	4.72±0.59	1.23±0.25	0.96±0.23
	6.hour	6.17±0.14	18.32±4.16	4.86±0.59	1.23±0.25	0.96±0.23
	8.hour	6.40±0.14	24.11±4.16	5.06±0.59	1.40±0.25	1.10±0.23
	10.hour	6.56±0.14	24.33±4.16	4.09±0.59	1.16±0.25	0.90±0.23
%50 BB+$CaSO_4$	0.hour	6.68±0.13	20.68±3.60	2.98±0.51	0.72±0.21	0.69±0.20
	2. hour	6.30±0.13	25.63±3.60	3.32±0.51	0.88±0.21	0.61±0.20
	4. hour	5.92±0.13	25.04±3.60	4.29±0.51	1.23±0.21	1.18±0.20
	6. hour	5.89±0.13	19.42±3.60	4.46±0.51	1.18±0.21	1.01±0.20
	8. hour	6.00±0.13	17.74±3.60	5.00±0.51	1.32±0.21	1.11±0.20
	10. hour	6.15±0.13	15.59±3.60	4.44±0.51	1.18±0.21	1.03±0.20
%75BB+$CaSO_4$	0.hour	6.44±0.13	24.90±3.60	3.68±0.51	0.88±0.21	0.73±0.20
	2. hour	6.16±0.13	31.27±3.60	3.90±0.51	1.05±0.21	0.74±0.20
	4. hour	5.83±0.13	27.94±3.60	4.33±0.51	1.12±0.21	0.71±0.20
	6. hour	5.87±0.13	22.75±3.60	4.54±0.51	1.33±0.21	0.86±0.20
	8. hour	6.07±0.13	21.75±3.60	4.35±0.51	1.22±0.21	0.75±0.20
	10. hour	6.16±0.13	23.68±3.60	3.93±0.51	1.12±0.21	0.86±0.20

NH_3-N: ammonia nitrogen, AA:Acetic acid, PA: propionic acid, BA: butyric acid

While pH (6.29) of rumen fluid in animals consuming the control ration was found to be higher than pH (6.09) of rumen fluid of animals in ration group 75% BB+$CaSO_4$, it was found to be similar in other ration groups. Rumen pH of 25% BB+$CaSO_4$ ration group was higher found than that of ration groups given 50% BB+$CaSO_4$ and 75% BB+$CaSO_4$. On the basis of all ration samples, when sampling time was examined, it was determined that rumen pH of 6.70 before morning feeding (hour 0) decreased in subsequent hours, to 5.90 and 5.97 at hours 4 and 6, respectively, while rumen pH increased at hours 8 and 10 and reached the pH value of hour 2 following feeding ($p<0.05$; Table 3).

NH_3-N levels of rumen fluid varied between 20.59 mg/100 mL and 25.38 mg/100 mL. While NH_3-N concentration of rumen fluid in control group (20.59 mg/100 mL) and 50% BB+$CaSO_4$ (20.68 mg/100 mL) group was lower than that of the ration group given 75% BB+$CaSO_4$ (25.38 mg/100mL), the values were found to be similar in other ration groups. The low (22.62 mg/100mL) NH_3-N value before feeding (hour 0) increased 2 hours after feeding (26.56 mg/100mL), and decreased again in the following hours (Table 3). In each ration group, the fact that NH_3-N concentration of rumen fluid at hours 2 and 4 after feeding is higher than other hours is thought to be due to the CP degradation rate of the feeds and high level of degradable proteins in rumen.

pH and NH_3-N concentrations of rumen fluid of the ration groups with sulfur (25% BB, 50% BB, 75% BB) and without sulfur (25% BB+ $CaSO_4$, 50% BB+ 75% BB+ $CaSO_4$) BB were 6.25-6.18 and 22.02-22.70 mg/100 mL, respectively, and the difference between the groups was found to be insignificant (Table 3). The fact that pH levels are not different between the groups is thought to be associated with similarity of structures of the feeds constituting the rations, and variation in said parameters depending on time elapsed after feeding is thought to be associated with the nutrients emerging as a result of digestion and absorption of ammonia. Effect of feed x time interaction on pH and NH_3-N levels was found to be insignificant across all groups.

In the study, pH levels of rumen fluid decreased at hours 2 and 4, and NH_3-N levels reached peak level at hour 2 following feeding. Similar results were reported by Casper et al. (1999), where cows consuming barley and soybean meal had pH 6.4 to 6.6 before feeding, which decreased at hours 2 and 4 down to 5.5, increased to 5.8 at hour 6, and concentrations of NH_3-N of rumen fluid reached peak level at hour 2 after feeding. It was reported that mixtures composed of barley and soybean meal decreased pH after feeding, while NH_3-N levels increased after feeding, but decreased following hour 6 (Aksu 1999).

Similarly, as reported by Akca (2006), in mixtures with vicia sativa given as protein additive, addition of $CaSO_4$ had an important effect on pH and NH_3-N levels of rumen fluid. In a study on goats, it was reported by Qi et al. (1992) that addition of sulfur at different doses to the mixtures increased pH and NH_3-N levels of rumen fluid, and pH and ammonia levels of rumen fluid periodically increased at hours 2, 4 and 6 following feeding.

Acetic acid concentration of rumen fluid of control ration group was 3.99 mmol/100 mL, which was found to be similar with acetic acid concentrations of other ration groups. However, acetic acid concentration (4.59 mmol/100 mL) of rumen fluid of the ration group 50% BB was found to be higher than ration groups 25% BB and 75% BB, and similar with the other ration groups. Acetic acid concentration of rumen fluid was 3.58 mmol/100 mL before feeding (hour 0), started to increase in the hours following feeding, and increases at hours 4, 6, and 8 particularly were found to be significant. The increase in acetic acid concentration at hours 2, 4, 6 and 8 following hour 0 is thought to be due to the positive effect between the time elapsing following feeding and structural and non-structural carbohydrate digestion.

In terms of propionic acid concentration of rumen fluid; the value for control ration (1.07 mmol/100 mL) was found to be lower than that of 50% BB ration (1.54 mmol/100 mL) ($p<0.05$), and similar to that of other rations. The propionic acid concentration of rumen fluid that was 0.95 mmol/100 mL before feeding, and rapidly increased at hours 2, 4, 6, and 8 after feeding, slightly decreased at hour 10, and was found to be similar with that of control ration (Table 3).

The fact that propionic acid concentration in the group given broad bean is higher than the control group can be explained by the fact that as it is rich in starch broad bean leads to an increase in the concentration of propionic acid, an easily fermentable product in rumen.
No difference was observed between the control ration (0.99 mmol/100mL) and other rations for butyric acid concentration in rumen fluid. However, the value obtained from the ration 75% BB+$CaSO_4$ was found to be lower than the value for 50% BB and 75% BB. Sampling time was observed not to have an effect on butyric acid concentration. Table 3 shows that feed x time interactions are not significant for acetic, propionic and butyric acid concentrations of rumen fluid.

In rams consuming mixtures of corn and barley as carbohydrate source and soybean meal and fish meal as protein additive, as a result of consumption of soybean meal+barley the acetic acid level was 6.56

mmol/100 mL, propionic acid level was 1.92 mmol/100 mL, and butyric acid level was 1.12 mmol/100 mL, and VFA levels were found to be similar to those in rams consuming corn+soybean meal and corn+fish meal (Hussein et al. 1991).

In a study where pea seeds were added to mixtures as protein additive, it was reported that in the group given peas, pH of rumen fluid was 6.32, acetic acid level of rumen fluid was 6.78 mmol/100 mL, propionic acid level was 1.79 mmol/100 mL, and butyric acid level was 1.05 mmol/100 mL, and pea addition did not change pH, NH_3-N and VFA levels of rumen fluid (Poncet and Remond 2002).

It was reported that in animals consuming beans instead of soybean meal, VFA concentration reached the peak level at hour 2 following feeding, and decreased gradually thereafter, and in animals consuming soybean meal, peak level was reached later compared to animals consuming beans (Chikagwa et al. 2009).

Aksu (1999) examined VFA concentrations of rumen at different hours in sheep consuming mixtures containing barley and soybean meal, and reported that acetic, propionic and butyric acid concentrations increased at hour 3 following feeding, and tended to decrease again following hour 6.

As a result, it is thought that up to 75% of the protein and energy obtained from soybean meal and barley can be met with broad beans in sheep rations.

Acknowledge

This research paper was supported by the University Scientific Research Projects Directorate No. 2006-FB-096 and is taken from a doctoral thesis. In addition, it was presented as an oral presentation at the 7th National Animal Science Congress 14 to 16 September 2011.

References

Akca I (2006). The Effect of different level of sulfur on ruminal microbial protein synthesis in sheep fed grain wetch as (Vicia sativa L.) as protein source. (Master's thesis). Yuzuncu Yil University Healt Sciences Institue. Van.

Aksu T (1999). The Effects of different ration combinations on ruminal dry matten degrability, rumen fluid and blood paremeters, and crude nutrients digestibility and nitrogen retention (PhD thesis). Yuzuncu Yıl University Healt Sciences Institue. Van.

AOAC (1990). Association of Official Analtical Chemists. Official Methods of Analysis, 15th ed., Washington, DC. 1: 69-79.

Bal MA, Ozturk D (2006). Effects of sulfur containing supplements on ruminal fermentation and microbial protein synthesis. Research Journal of Animal Veterinary Sci. 1(1): 33-36.

Casper DP, Maiga HA, Brouk MJ, Schıngoethe DJ (1999). Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. J Dairy Sci. 82 (8): 1779-90.

Chikagwa SK, Adesogan AT, Szabo NJ, Littell RC, Phatak SC, Kim SC, Ariola KG, Huisden CM, Dean DB, Krueger NA (2009). Nutritional characterization of *Mucuna pruriens* 3. Effect of replacing soybean meal with Mucuna on intake, digestibility, N balance and microbial protein synthesis in sheep. Animal Feed Science and Technology, 148: 107-123.

Dixon RM and Hosking BJ (1992): Nutritional value of grain legumes for ruminants. Nutrition Research Reviewes. 5: 19-43.

Doig B (2007). Basics of beef cattle nutrition. Saskatchewan Agricultural and Food North Battleford. pp. 446.

Hussein HS, Jordan RM, Stern MD (1991). Rumınal proteın metabolism and intestinal amıno acid utilization as affected by dietary proteın and carbohydrate sources in sheep. J. Anim. Sci. 69: 2134-2146.

Karslı MA, Taşal T (2003). Effect of dietary inclusion of hazelnut and soybean meals on microbial protein synthesis. II. National Congress of Animal Feeding, 397-402, 8-10 Eylül, Isparta.

Markham P (1942). A steam distilation apparatus suitable for micro-kjeldahl analyses. Journal Biochem. 36: 790-797.

Poncet C, Remond D (2002). Rumen digestion and intestinal nutrient flows in sheep consuming pea seeds: the effect of extrusion or chestnut tannin addition. Anim. Res. 51: 201–216.

Qi K, Lu CD, Owens, FN, Lupton CJ (1992). Sulfate supplementation of Angora goats: Metabolic and mohair responses. J. Anim. Sci. 70: 2828-2837.

Santos KA, Stern MD, Satter LD (1984). Protein degradation in the rumen and amino acid absorption in the small intestine of lactating dairy cattle fed various protein sources. J. Anim. Sci. 58 (1): 244-255.

SAS (2007). SAS ® User's Guide: Statistics, Version 8. SAS Institute Inc., Cary, NC.

Van Soest PJ, Robertson JB (1979). Systems of analyses for evaluation of fibrous feed. In "Proc. Int. Workshop on standardization of analytical methodology for feeds". Ed, W. J. Pigden, C.C. Balch, and M. Graham. Int. Dev. Res. Center. Ottowa.