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The Effects of Vetch (*Lathyrus sativus* L.) Seed Feed on Some Rumen and Blood Parameters and Rumen Protozoa of Lambs

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Abstract: The study investigates the effects of Common Vetch Seed (CVS) on some blood and rumen parameters and rumen protozoa of female, Sainfoin Hay (SH) fed, lambs. In the study, 24, female, SH-fed and weaned lambs that were 16 weeks old and weighed 36.25±2.49 kg on average were used. In the first group (control), the consumption of SH equal to 2% of the Live Weights (LW) of the lambs and (%) 0.00 CVS was allowed. Group rations were formed, so that, the second group was fed with SH equal to 1.25% of the LW and CVS equal to 0.75% of the LW and third group was fed with SH equal to %0.5 of the LW and CVS (equal to 1.50% of the LW). The blood and rumen fluid samples were collected on day 0 and day 30 of the experiment. Despite the statistical differences between some parameters investigated in the study, all parameters were within the physiological limits for lambs reported in the literature. The results of the study showed that feeding vetch at an amount equal to 75% of the total ration as a protein-rich feed to SH-fed lambs did not have any negative effect on the lambs and the total ration can be composed of legume hay and legume seeds.

INTRODUCTION

The increasing world population has created a great pressure on the limited soil resources to produce more food, feed, biomass and industrial raw materials. It has been reported that agricultural productivity should increase by 70% or 100% by 2050 and high-yield and stress-resistant plant varieties should be developed to meet this demand[1, 2]. In conformity with the "European Union Common Agricultural Policy", European Union countries encourage farmers to use environmentally friendly agricultural applications and legume as concentrate feed in animal breeding. In addition, in recent years, the use of local resources, widespread use of sustainable agriculture and increasing organic production are encouraged in many regions while the interest in the use of other legume seeds such as vetch is growing for optimal interaction between soil-plant-animal to avoid the use of genetically-modified products[3-5].

Vetch (*Latirus sativus* L.) is one of the oldest culture plants with a long domestication background[6, 7]. It is an important legume that can be used as a vegetable protein source with a low production cost, high potential for cheap and high-quality protein production and a series of

biological and agronomic advantages such as resistance to floods, cultivability in semi-arid regions, resistance to insects and pests, high disease- resistance, low toxin content, nitrogen fixation to soil, high seed yield and the high protein content of its seeds[8]. Vetch has become prominent with its great genetic diversity and shorter vegetation period compared to other legume feed plants[9]. In addition to being a food due to its protein-rich seeds, vetch has a great potential for use as feed and studies on vetch have increased in recent years[10]. In their study on the digestibility of important legume seeds, Gonzalez and Andres[11], reported that vetch had the highest rumen digestion (69.2%). Although vetch seeds contain antinutritional factors including trypsin inhibitors, chymotrypsin inhibitors, amylase inhibitors, lectins, tannins, phytate and oligosaccharides, reports have shown that they did not cause a serious health problem in animals but the effects of antinutritional factors on animal performance is not well understood[9, 12]. It has been reported that *Lathyrus sativus* L. with a low ODAP content can be added to the rations of poultry, pigs and sheep in the ratios of 40%, 30% and 70, respectively. In addition, to the positive results obtained in the studies on the use of vetch in animal nutrition, studies on different nutrition strategies in which vetch seeds are used are needed[8].

Moreover, within the scope of soil improvement studies, the interest in the production of lucerne, sainfoin and other similar legume as roughage has been growing in recent years[13, 14]. In regions where legume seeds are produced in addition to the roughage legumes such as lucerne, sainfoin and trefoil an important amount of roughage material is left over after the collection of the seeds. In the case where these wastes are used as feed, the concentrate feeds that mostly contain soy bean meal and corn are usually used to balance the ration[15, 16]. A study on sainfoin showed that the dry matter digestibility of sainfoin was higher than that of lucerne depending on the growth stages[17]. The study investigates the effects of using vetch as energy and protein source instead of soybean meal (protein) and corn (energy) on some blood and rumen parameters and rumen protozoa of SH-fed lambs.

MATERIALS AND METHODS

Animal and ration: In the study, 24 female, SH-fed and weaned lambs of about 16 weeks of age and weighing 36.25±2.49 kg on average were used. Sainfoin Hay (SH) was used as the roughage and vetch seed (CVS) was used as the replacement feed. The feeds were given in special feeders to avoid spillover and scattering and thus, fully achieve feeding. The blood and rumen fluid samples (the first sample collection) were collected after the study groups were formed and before the animals were fed in the morning. During feeding, prior to the actual feeding, exercise feeding was carried out for 5 days to accustom the animals to the rations; then, the actual feeding was carried out for 25 days. As in the case of the first blood and rumen fluid collection, the second samples were collected before the feeding in the morning on day 30 of the study. The mineral vitamin supplement contents of which are given in Table 3 was added to the rations at a level of 0.10% during the experiment. The feed dry matter requirement of the lambs was determined to be 2% of their live weights. According to the live weights and groups of the animals, CVS and SH were fed to the control group (C) in the amount of CVS 0.00%/SH2.00%, to Group 1 (CVSI) in the amount of CVS 0.75%/SH 1.25% (37.5% of the ration) and to Group2 (CVSII) in the amount of CVS 1.50%/SH 0.50% (50% of the ration) by dividing the total amount (Table 1, 2) into 2 and feeding at 08:00 in the morning and 18:00 in the evening.

Nutrient analysis: The nutrient contents of the feeds were analyzed at the beginning of the study. The Dry Matter (DM), Crude Protein (CP), Ether Extract (EE) and crude Ash (A) analyses of the feeds were carried out in accordance with the AOAC[18] while the Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) analyses of the feeds were carried out in accordance with Van Soest *et al*.[19] (Table 1).

Blood serum analyses: For the blood analyses, 10 mL blood samples were collected from each animal by blood drawing from vena jugularis with a cannula for twice a day (prior to feeding in the morning) at the beginning (day 1) and end (day 30) of the study. After removing the serum of the blood samples with centrifugation, the samples were kept in a cool and dark environment until analysis. In the serum, Blood Glucose (BG), Total Protein (TP), Triglyceride (TG), Blood Urea Nitrogen (BUN), Calcium (Ca), Phosphorus (P), potassium (K), Chloride (Cl) and sodium (Na) amounts were measured. In the blood analyses, a modular-type Hitachi Automatic Analyzer device, Tokyo/Japan and Roche kits were used.

Rumen analyses: As was the case for blood collection, before and after the study, 50 mL rumen fluid samples were collected twice from the mouth of each animal with a rumen probe on the same hours as blood collection (prior to feeding in the morning). The samples were slightly stirred to stabilize the rumen and then, the pH values of the samples were immediately measured with a digital pH meter.

Table 1: Nutrient contents of CVS and SH (%)

Factors	DM	CP	EE	A	ADF	NDF
CVS	95.45	27.28	0.68	10.30	11.29	45.09
SH	92.89	11.43	0.86	16.97	47.44	66.69

Table 2: Given feed amounts

Factors	CVS*	SH*	CVS**	SH**	CVS***	SH***
C	0.0	100	0.000	0.725	0.00	2.00
CVSI	37.5	62.5	0.272	0.453	0.75	1.25
CVSII	75.0	25.0	0.544	0.181	1.50	0.50

*CVS and SH Ratios in Ration (%);**CVS and SH amounts (grams/lamb);***Ratio of CVS and SH to Animal weight (%)

Table 3: Chemical composition of group feeds (%)*

Factors	DM	CP	EE	A	ADF	NDF
C	92.89	11.43	0.86	16.97	47.44	66.69
CVSI	93.85	17.37	0.79	14.47	33.88	58.89
CVSII	94.81	23.32	0.73	11.97	20.33	50.49

*Vitamin mineral (Fascovite) was added to the diet at a rate of 0,10%. 1 kg of Faskoviti; 1,000,000 IU of vitamin A, 200,000 IU of vitamin D3, 400 mg of vitamin E, 500 mg of vitamin B1, 500 mg of vitamin B2, 304 mg of vitamin B6, 5 000 mg of Fe, 1000 mg of Cu, 5 000 mg of Zn, 80 mg of Mn. , 20 mg Co, 21 mg Se, 9,180 mg Mg, 12 750 P, 18 750 mg Ca

Rumen ammoniac analysis was carried out by applying the Markham distillation method to the distillation unit of the Kjeldahl device. Rumen ammoniac amount was calculated in accordance with Markham[20]: NH_3-N (mg/100 mL) = (Spent N/70 H_2SO_4×0.2×100)/Sample amount (mL).

After a slight stirring to allow re-homogenization, 5 mL of the rumen fluid was transferred to a 25 mL container and 15 mL of the stabilization solution (1lt ethyl alcohol, 5 g pure NaCl, 0.3 g methyl green) was added to the fluid. The samples were kept in a cool and dark environment until counting. During the counting, after homogenization, 0.05 mL of the sample was placed on the Hydro Bios Kiel (0.5 mm) glass slide and covered with a cover glass while avoiding bubble formation. The counting was carried out at a magnification of ×20 using a standard microscope with a camera and screen. The protozoa count was calculated using the formula given below. The result was multiplied by two, since, the protozoa between two lines were counted by skipping one section among the sections divided by the lines on the glass slide. Then, the result was multiplied by four, since, the rumen content was diluted three-fold with the stabilization solution. Finally, the result was multiplied by 20 to find the result for 1 mL of rumen, since, the counting was carried out for a 0.05 mL rumen content. Total protozoa amount: Protozoa amount counted on the camera ×2×4×202.5 statistical analyses.

The descriptive statistics for the properties were given as means and standard errors. To determine whether the application groups (C, CVI and CVII) were different in terms of the properties and times (B and A), the two-way ANOVA with repeated measurement on one-factor levels was performed. The Tukey multiple comparison test was used to determine the different mean values of the groups. All statistical analyses were carried out using the STATISTICA package program[21].

RESULTS

The nutrient content of the Sainfoin Hay (SH) used in the study as the roughage source comprised DM 92.89%, CP 11.43%, CF 0.86%, A 16.97%, ADF 47.44%, NDF66.69%, respectively (Table 3). The nutrient Content of the Vetch (CVS) used as the grain feed comprised DM 95.45%, CP27.28%, CF0.68%, A10.30%, ADF11.29% and NDF 45.09%, respectively (Table 3). In the SH-fed lambs, there were no differences between the BG, TP, TG, Ca, P, Cl and Na amounts in the blood serum depending on time or CVS consumption ($p<0.05$). The time- dependent decrease in the Ca amounts of the SH-fed groups at the end of the experiment was independent of CVS consumption while the time-dependent increase in the K amounts was also independent of CVS consumption ($p<0.05$). As seen in Table 4, the blood serum BUN levels increased with increasing CVS consumption and were different from the control group ($p<0.05$). The rumen pH levels of the SH-fed lambs did not differ depending on time or CVS consumption while the rumen protozoa increased with time in all groups ($p<0.05$), however, there were no significant differences between the control group and CVS-consuming groups (Table 5). With the addition of CVS to the rations of the SH-fed groups, the rumen NH3-N amounts increased and were different from that of the control group ($p<0.05$).

DISCUSSION

The nutrient contents of the SH and CVS used in the study are close to those in other studies but different from those in some studies. The differences in the nutrient contents of the feeds are attributable to variety, breeding and harvesting conditions[12, 22-24].

Table 4: Pre (A)-and post (B)-research Blood Sugar (BS), Total Protein (TP), Triglycerides (TG), urea Blood Urea Nitrogen (BUN) blood Calcium (Ca), blood potassium (K), blood phosphorus (P), blood chlorine (Cl), blood sodium (Na) (data represents the mean±SEM)

Time	C	CVSI	CVSII	CVSIII
BG (mg dL^{-1})	B	66.00±3.95	66.88±12.35	67.88±4.25
	A	60.75±5.48	56.63±8.30	74.13± 5.93
TP (g dL^{-1})	B	7.26±0.13	6.79±1.38	7.69±0.16
	A	7.17±0.16	6.49±0.94	7.51±0.17
TG (mg dL^{-1})	B	23.93±2.31	23.30±4.42	19.11±2.01
	A	24.68±3.43	17.60±2.87	22.00±1.60
BUN (mg dL^{-1})	B	44.63±1.00Ba*	37.75±5.63bB#	41.13±1.63aB
	A	42.84±1.68Bc	50.68±7.35bA	62.39±2.23aA
KALSİYUM (mg dL^{-1})	B	10.23±0.10A	9.20±1.3A	10.17±0.21A
	A	9.52±0.14B	8.59±1.24B	8.91±0.34B
FOSFOR (mg dL^{-1})	B	6.59±0.39aB	5.53±0.87bB	5.47±0.45bB
	A	6.92±1.02cA	8.21±0.28bA	9.75±0.68aA
POTASİYUM (mmol dL^{-1})	B	4.98±0.18B	4.14±0.67B	4.97±0.21B
	A	5.63±0.20A	5.51±0.67A	5.19±0.19A
KLOR (mmol dL^{-1})	B	108.50±0.87	88.63±12.79	102.50±3.49
	A	103.75±0.45	91.13±13.04	100.50±0.98
SODİUM (mmol dL^{-1})	B	150.00±1.21	123.63±17.93	143.63±5.29
	A	145.63±0.87	128.13±18.31	143.00±1.36
	P	<0.05	<0.05	<0.05

*It shows different group means in the same time level (same line) (p<0.05); #: Different capital letters within the same group (in the same column) show the difference between pre-research and post-research (p>0.05)

Table 5: Pre (A)-and post (B)-research the amount of ammonia, the pH and the protozoa numbers in rumen liquid (data represents the mean±SEM)

Time	C	CVSI	CVSII	CVSIII
Ruminal pH	B	6.60±0.04	5.79±0.83	6.58±0.09
	A	6.68±0.02	5.83±0.83	6.55±.05
ProtozoaNumber (×10 mL^{-1})	B	1.07500±0.32084B	1.70220±0.39255B	1.50410±0.43363B
	A	3.70030±0.34604A	3.67580±0.66146A	5.18780±0.70838A
Ruminal NH_3-N (mg dL^{-1})	B	24.863±1.754B	19.238±2.976B	20.563±1.646B
	A	30.803±0.678cB	63.281±10.805bA	89.919±8.595aA
	p	<0.05	<0.05	<0.05

*It shows different group means in the same time level (same line) (p<0.05). #: Different capital letters within the same group (in the same column) show the difference between pre-research and post-research (p>0.05)

The blood parameter results given in Table 4 were obtained under the same growing conditions and thus, the differences in the parameters are attributable to feeding. Lestingi *et al*.[25] reported that the use of different legume seeds did not affect the BG levels and BG levels ranged from 65.40-70.90 mg dL^{-1}. The BG values in lambs varied from 144/100 mL to 70 mg^{-1} 100 mL depending on age[26, 27]. The BG levels in three and four-month-old lambs were in the range of 68.3-82.0 mg dL^{-1}[28, 29]. The BG levels found in this study were within the limits specified in the literature[26, 27, 29]. In a study carried out with different legume seeds, the BUN levels in lambs were reported to be between 21.24 and 25.58 mg dL^{-1}[25]. Ragni *et al*.[30], reported that the BUN levels in cattle were 35.16, 31.29 and 37.56 mg dL^{-1} when fava beans and lupin were used as an alternative to soy, respectively. Cannas *et al*.[30], reported that when the protein levels were 13.9, 16.3, 18.6 and 21.1% in parallel with the increase in the ration protein, the BUN levels increased to 12.9, 17.7, 23.4 and 26.7 mg dL^{-1}, respectively. The BUN levels in this study were higher than those reported by Ragni *et al*.[31]. This discrepancy between the results was attributed to the use of a starch-rich ration in the other study while the total ration in this study consisted of SH as the roughage feed and CVS as the concentrate feed. In this study, the lowest BUN value was observed in the control group. When the ration amino acid level is not balanced to allow protein production, the increased protein consumption can increase the BUN level[32]. In monogastric animals, legume seeds are known to decrease the blood TG level depending on their fiber contents[33]. In this study, the blood TG levels in lambs did not decrease after CVS consumption. Observing no decrease in the blood TG levels with the consumption of SH and CVS was associated with the fiber digestion in rumen[34]. This is also attributable to the low fat content of the SH and CVS that constitute the ration[35]. In this study, the TG levels after CVS consumption were higher than the blood TG levels found in the study carried out by Malekkhahi *et al*.[29] . On the other hand, the TG levels in this study were close to those found in the studies on certain legume types carried out by Facciolongo *et al*.[36]. The blood TP levels in the 0-3 months-old suckling lambs under different feeding conditions were reported to vary between 6.3/100 and 7.3 g/100 mL[27]. El-Barody *et al*.[28], reported that the blood TP levels of 4-months-old lambs were in the range of 6.90-7.60 g dL^{-1}. In their study, Lestingi *et al*.[25], found that the use of fava bean and sweet lupin did not affect the blood TP levels and the TP levels ranged from 6.06-6.58 g dL^{-1}. Another cause of the

increase in the blood TP level is the antinutritional factors in the rations[25]. In the study, the blood TP level did not increase after CVS consumption which indicated that the antinutritional factors did not create stress on the immune system.

The normal blood Ca levels in lambs were reported to be in the range of 8.30-11.43 mg dL^{-1}[37-40]. The blood Ca level is affected by the Ca content of the ration[35]. The decrease in the blood Ca levels in all groups was due to the drop in Ca levels after weaning despite the high blood levels of suckling lambs. The decrease in the blood Ca levels was attributed to the fact that the lambs were still suckling a month before the study. The blood Ca levels that were within the normal limits despite the decrease in the blood Ca level indicated that the use of CVS in rations in which SH is used as roughage will not negatively affect blood calcium level. In the study, the blood P levels of the lambs were close to the upper value determined by Wiener and Field[41] for blood P levels. In rations in which CVS was used as the roughage, the use of CVS increased the blood P level. It has been reported that a ration P level between 0.15 and 0.23% allowed a normal blood P level, which led to well-developed lambs[42]. The P levels of the SH and CVS used in the study were reported to be 0.84 and 0.42%, respectively in the literature. The increase in the P levels of the lambs was associated with the high P levels of both feeds used in the study[8, 43]. The blood serum P level is affected by the mineral content of the ration[35]. On the other hand, this can also be due to the balanced Ca/P level in the ration after the CVS consumption. The normal blood K levels in lambs were reported to be in the range of 4.53- 8.31 mg dL^{-1} in the literature[38, 39, 40, 44]. In the study, the blood K levels of the lambs were within the normal limits. The increase in the blood K levels of all groups at the end of the study was attributed to rumen development. The consumption of SH and CVS did not negatively affect the blood K levels of lambs. The normal Cl levels in lambs were reported to be in the range of 103.50-119.67 mg dL^{-1} in the literature[38-40, 44]. The blood Cl levels of the lambs in the study were within the normal limits. The addition of CVS to the ration did not affect the blood CL levels. The results showed that the consumption of SH and CVS did not negatively affect the blood Cl levels. The normal blood Na levels in lambs were reported to be in the range of 142.38-154.09 mg dL^{-1} in the literature[38, 40]. In the study, the blood Na levels of the lambs due to SH and CVS consumption were within the normal limits. The results indicated that the consumption of SH and CVS did not affect the blood Na levels of lambs.

Ruminal pH can range from 4.4-7.8 depending on the type of diet[45]. In rations comprising feeds with a high cellulose content, rumen pH should be around 6.5 to achieve high cellulose digestion (81%)[46]. In this study, the pH values of rumen were within the normal limits in the C and CVSII groups but the pH level in the CVSI group was below the optimum value (Table 4). The pH value of this groups was already low before CVS consumption. This is attributable to the individual effects of the animals in the group. One of the important findings of the study is that the SH and CVS used as the study feed did not negatively affect rumen pH. The negative pressure on pH caused by the easily digestible and carbohydrate-rich CVS was associated with its high nitrogenous compound content. As a consequence, rumen pH did not remain within ideal limits. The limits for the rumen ammoniac amount range from 2-190 mg dL^{-1}[47]. The mean rumen NH-N amount for the highest microbial fermentation was reported to be 45 mg mL^{-1}[48]. The rumen NH3-N amounts in the animals fed with starch-based rations were 10.12-10.47 mg mL^{-1} while they reached 110 mg mL^{-1} in the animals fed with protein-based feeds[49]. In the study, the rumen NH3-N amount caused by CVS consumption was above the value at which maximum microbial fermentation occurs[48]. This led to the conclusion that CVS should be fed together with a carbohydrate-rich feed to achieve maximum microbial activity. The initial rumen protozoa count increased in the CVSI and CVSII groups and the C group at the end of the study. The rumen protozoa count was reported to be 10-10 mL^{-1} in the literature[50-54] . The increased rumen protozoa count in all groups at the end of the study was due to the ongoing rumen development in lambs. The close number of rumen protozoa to the ideal limits after CVS consumption indicated that the use of CVS did not have a negative effect on protozoa.

CONCLUSION

In conclusion, among the parameters investigated in the study, blood serum glucose, total protein, triglyceride, Ca, P, Na, Cl and Na levels and rumen pH and rumen protozoa counts were within the normal limits reported in the literature while the blood serum BUN and rumen HH3-N levels were higher than the mean values reported in the literature. In light of the data, the daily rations of lambs can be prepared with SH and CVS as was in the study and CVS in an amount up to 75% of the total ration can be used in the rations of SH-fed lambs. On the other hand to reduce the levels of blood serum BUN and rumen NH3-N that increased due to the addition of CVS to the normal limits, a feed with a high carbohydrate content can be used in the ration and further studies regarding this issue are needed.

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