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The Effect of Pea (*Pisum sativum* L.) Substitute on Some Blood Metabolites and Some Rumen Parameters in Lambs Consuming Sainfoin Hay (*Onobrychis viciifolia*)

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Abstract: The aim of this study was to investigate the way Pea Seeds (PS) can affect some blood metabolites and rumen parameters in lambs being fattened through consumption of sainfoin (*Onobrychis viciifolia*) hay (SH). The main nutrient source in the groups was SH. The SH was partially replaced by PS in two of three groups of animals. About 24 16 week-old Norduz female lambs (31.72±2.47 kg) were divided into three groups and fed their diets. SH and PS were available in the diets in the following SH:PS ratios (on a body weight basis): 3:0 in the first (control) group (C), 2:1 in the second group (PI) and 1:2 in the third group. The diets of the groups were of different nutritional content. The animals were split randomly between the three groups (C, PI and PII). The research was completed in 25 days and the animals were fed twice a day over this period. Blood and rumen liquid samples were taken twice: one pre-trial (B) and the other post-trial (A). Blood Triglyceride (TG), Cholesterol (CH), Creatinine (CR), Direct Bilirubin (DB) and potassium values and increases in Live Weight (LW) of the animals were not found to be significant due to the dietary treatment. However, Protozoa Numbers (PN), Total blood Protein (TP), Albumin (AL), Urea (UR), Globulin (GL), Chlorine (Cl) and sodium (N) values and the rumen NH level increased significantly by the end of the trial ($p<0.05$).

INTRODUCTION

Because of the debate on the use of genetically modified organisms (GM) and organic production which aims to ensure the optimal interaction between soil-plant-animal and to create sustainable production including local resources, legume seeds have been accepted as an alternative source to the soybean in animal feeds. In addition to their high content, leguminous grains are necessary for increasing soil fertility, reducing greenhouse gas emissions and sustainable livestock systems[1-4]. In recent years, the EU Common Agricultural Policy has significantly increased the production of plant proteins, while farmers are encouraging environmentally friendly farming practices. For this purpose, it aims to promote the purchase of legumes in concentrates for feeding purposes[5].

Peas are categorized under the name *Pisum sativum*. *Pisum sativum* L. is used in the production of forage and fodder[6-8]. Worldwide, PS is the second most important legume fodder after soybean and its low number of anti-nutritional factors ensures good palatability[4, 9]. PS for feed is an important raw material that is widely used as an alternative to soy and other protein sources in animal feeds, especially in Canada and Europe[7, 10, 11]. PS is characterized by a high Crude Protein (CP) content of 20-30% and is rich in sulfur-containing amino acids and tryptophan but not lysine[12-15]. PS contains a considerable amount of starch (>40% of the dry matter) which is resistant to destruction in the rumen[16]. PS crude oil content varies from 1.5-2.5% of dry matter. Also, PS contains good levels of unsaturated fatty acids: 12.7% oleic acid, 35.3% linoleic and 5.4% linolenic acid[4, 12]. The amount of cellulose in the structure of the shell surrounding the PS feed (3-12%) is high. However, this cellulose needs a lot of digesting. PS is rich in Ca, P, K, Fe, Cu, Mg and Zn but it is deficient in Na[8, 10, 11, 13].

Onobrychis viciifolia Scop., of the Onobrychis genus is one of the most common forage crops that is important to agriculture. *Onobrychis viciifolia* Scop. has been traditionally cultivated worldwide for a long time. It is common to North America, Europe and Middle Eastern countries. In recent years, its use has increased due to some positive features. It is a plant suitable for cultivation in soils with low plant nutrients in arid and cold regions[2, 17]. However, their value is also linked to environmental issues such as reducing nitrogen pollution from animals grazing lush pastures with a high nitrogen content and lessening methane emissions from rumen fermentation[17]. Recent studies suggest that it has several other highly beneficial properties due to its unique tannin and polyphenol composition. Condensed tannins present in the Onobrychis species have been shown to confer anthelmintic properties, increase protein utilization and prevent bloating. Condensed tannins (CT) have improved live weight gain, wool production and reproductive efficiency in sheep fed temperate forages[19]. In addition, the voluntary feed consumption of *Onobrychis viciifolia* by sheep and cattle is 20-24% higher than for grasses and 10-29% higher than for trifolium pratense or *medicago sativa*. *Onobrychis viciifolia* yields lower milk and lower fat milk than alfalfa during the milk yield period while in young animals it provides a higher yield for live weight than alfalfa. It is reported that goats and lambs fed with *Onobrychis viciifolia* gain up to 400 g of body weight per day. As a result, it supports high growth rates in young ruminants[2, 17, 20]. The aim of this study was to investigate the effect of PS on the alteration of some blood metabolites and rumen parameters in lambs being fattened through consumption of SH.

MATERIALS AND METHODS

Diets and procedures: Twenty-four female Norduz lambs (16 weeks of age) were obtained from the Van-Yuzuncu Yıl University farm located in eastern Turkey. The lambs were randomly split into three groups with animals of approximately the same weight in each group. The lambs that were weaned one month prior to the start of the experiment consumed sainfoin dry as roughage. Trial consumption was started after a period of familiarization with pea consumption for ten days. The animal's feed requirement for dry matter was determined to be 3% of their live weight. Pea Seeds (PS), a legume grain feed and Sainfoin Hay (SH) as rough fodder were used as the feeds in this study. Accordingly, a three-group feeding system (Table 1) was generated in a way that 0.00% PS and 3.00% SHSH was given to the first group (C), 1.00% PS and 2.00% SH was given to the second group (PI) and 2.00% PS and 1.00% SH was given to the third group (PII). Dry Matter (DM), Crude Protein (CP), Crude Fat (CF) and Ash (A) analyses of the study feeds were carried out according to analysis method[21]] and the Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) analyses were carried out according to Van Soest[22].

Blood analyses: Blood samples were taken twice, once at the beginning of the study and the other on day 25 of the study. For blood analyses, blood samples of 10 mL were collected twice from the jugular vein of each animal (before and after the study) using blood collection cannula. The blood plasma was held at a temperature of -20°C until analyzed. Blood samples were centrifuged at 4000 rev/min and after collecting the resulting serum, they were sent to the biochemistry and physiology laboratory of the Van Yüzüncü Yıl University's Faculty of Medicine. A modular-type device called a Hitachi Automatic Analyzer of Japanese origin and Roche-branded kits were used in the blood analyses. From the blood plasma, the concentrations of the following indicators of the metabolic profile were determined: Total Oroteins (TP), Albumin (AL), Globulin (GL), Urea (UR), Creatinine (CR), Cholesterol (CH), Triglyceride (TG), Total Bilirubin (TB), Direct Bilirubin (DB) and Indirect Bilirubin (IB). The mineral element concentrations of potassium (K), sodium (Na) and Chlorides (Cl) were measured.

Table 1: Nutrient ratios of the feed used in the experiment and the ration amounts (%)

Parameters	PS	SH	DM	CP	CF	A	ADF	NDF
PS			91.00	24.20	2.35	7.05	12.50	25.30
SH			93.00	13.09	1.08	14.80	38.50	49.80
C	00.00	100.00	93.00	13.09	1.10	14.80	38.50	49.80
PI	33.33	66.66	91.40	16.79	1.50	12.10	29.80	41.59
PII	66.66	33.33	90.80	20.49	1.90	9.50	21.15	33.43

Pisium sativum L. (PS); Sainfoin Hay (SH); Control grup (C); second group (PI); third group (PII); Dry Matter (DM); Crude Protein (CP); Crude Fat (CF); Ash (A); Acid Detergent Fiber (ADF); Neutral Detergent Fiber (NDF)

Rumen pH analyses: Rumen liquid samples were taken twice, once at the beginning of the study and the other on day 25 of the study. A pH measurement was immediately carried out using a digital pH meter on 50 mL of rumen content that was extracted twice from the mouth of every animal by means of a rumen probe before and after the study. Rumen ammonia (NH_3) analyses were performed using the Nessler method in a HACH DR 2010 model spectrophotometer.

Protozoa counting method: For protozoa counting, the rumen content was put into a plastic bottle using rumen contractions. Like in a blood sample collection, rumen fluid was collected at the same hour of the day, before and after the study. A 5 mL amount of the rumen content was separated to achieve stability of the collected sample. A 15 mL fixing solution (1 L ethyl alcohol, 5 g pure NaCl and 0.3 g methyl green) was added to the sample. The samples were kept in a cold and dark place until counting. For protozoa counting, following homogenization, 0.05 mL of the sample was placed in a Thoma lam chamber. Lamella was placed on the lam in such a way as to create no air bubbles. Counting was conducted using a standard microscope with 104°40 magnification using a camera and screen. The number of protozoa was calculated using the formula below: The density (mm^3) of counted protozoa, number of counted small squares <volume of one small square dilution ratio.

Rumen content was taken twice from the mouth, once at the beginning of the study and once at the end of the study via a rumen probe for counting. Rumen liquid was taken at the same hour of day before and after the study. Two-Way ANOVA with repeated measurement of factor levels was conducted to determine whether there was a difference between implementation groups (T, PI and PII) and times (pre-study, post-study) with regard to the properties exhibited. As a result of the analysis of variance conducted, Tukey's multiple range test was employed in order to determine the averages of different groups. Tukey's multiple range test was carried out at sub-group level as Group×Time interaction to find out whether properties were statistically significant and in which the inter-average differentiation is important. All statistical analyses were executed using the Statistica software package (StatSoft, 2011).

Table 2: Live weight of lambs before (B) and after (A) the experiment (data represent mean±SEM)

Parameters (kg)	Time	C	P I	P II
Initial live weight	B	31.93±2.33#	31.75±2.37#	31.45±2.72 #
Final live weight	A	36.15±2.25	36.70±2.22	36.93±2.39
	P	<0.05	<0.05	<0.05

#: on the same column indicate a difference between averages

RESULTS AND DISCUSSION

Chemical composition: The chemical characteristics of the experimental diets are shown in Table 1. It was seen that nutrient values for SH used as a primary feed in the study (DM 93.00, CP 13.09, CF 1.08, A 14.80, ADF 38.50, NDF 549.8 in %) and the nutrient values of PS used as the study feed (DM 91.00, CP 24.20, CF 2.35, A 7.05, ADF 12.50, NDF 25.30 in %) were within the limits specified in published literature[1, 23-25].

Live weight: The use of different sources of protein in ruminant feed makes it necessary to examine the structure of the source. Rouissi *et al*.[26]. It was seen in various previously conducted studies that the use of legume grain has yielded good results in ruminants and in many other aspects[7, 27, 28] noted that peas can be used successfully in lamb diets, providing satisfactory results in terms of growth performance and meat quality. Pea substitution instead of sainfoin had no effect on live weight gains (Table 2) but there was a statistical difference between the live weights at trial and the post-trial live weights. The main reason for this difference is the age factor. The values obtained are not consistent with the results reported by Facciolongo *et al*.[14]. The possible reason for the absence of live weight gains in the groups due to the consumption of PG is the high rate of degradation of ADF and NDF and the level of protein in the structure of PG to meet the dietary needs of the lambs. In a study conducted with pea feeds, it was reported that partial or full use of pea feeds instead of soy beans did not adversely affect live weight gain in lambs. In another study, it was reported that the use of peas instead of soya bean meal+corn or of soya bean meal and sunflower meal+corn had no effect on live weight gains in lambs[29]. The use of 18 and 39% PS instead of soy did not affect live weight gain in those ratios where clover was used as roughage[12].

Table 3: Some metabolites in blood plasma of lambs Before (B) and After (A) the experiment (data represent mean±SEM)

Parameters	Time	C	P I	P II
CH (mg dL^{-1})	B	52.50±4.42	57.25 ±9.02	50.13±2.91
	A	41.13±2.53	40.63±1.34	44.25±1.31
TG (mg dL^{-1})	B	26.45±2.77#	25.49±2.75	23.53±1.62
	A	18.16±1.71	21.58±2.68	21.39±2.09
UR (mg dL^{-1}) [mmol L^{-1}]	B	34.63±2.73#	30.46±2.62#	35.98±3.95#
	A	50.14±4.97	58.81±5.57	65.41±5.16
CR (mg dL^{-1})	B	0.78±0.04	0.73 ±0.03	0.73±0.04
	A	0.73±0.04	0.66±0.03	0.71±0.03
TB (g dL^{-1})	B	0.06±0.01	0.06±0.01#	0.06b±0.01#
	A	0.06±0.01a	0.01±0.02 b	0.02±0.01 b
DB (mg dL^{-1})	B	0.23±0.017	0.24±0.09 #	0.21±0.05 #
	A	0.23±0.03	0.03±0.02	0.02±0.02
IB (mg dL^{-1})	B	0.29±0.17	0.27±0.09 #	0.28±0.04 #
	A	0. 28±0.03	0.03±0.02	0.00±0.02
TP(g dL^{-1})	B	7.19±0.17ab	7.01±0.14 b	6.86±0.20b#
	A	7.16±0.13ab	7.61±0.12 a	7.45±0.18 a
AL(g dL^{-1})	B	3.31±0.08b	3.36±0.06 b	3.35±0.13 b#
	A	3.37 ±0.06b	3.50 ±0.06 ab	3.73±0.09 a
GL(gr dL^{-1})	B	3.71±0.13	3.65±0.016	3.51±0.16 #
	A	3.89±0.12	3.83±0.15	4.25±0.16
K (mmol L^{-1})	B	5.17 ±0.26	5.14 ±0.24	5.11±0.16
	A	5.16±0.08	5.02±0.18	5.00±0.16
Cl (mmol L^{-1})	B	105.00±1.20#	103.13±1.61	102.88±1.59
	A	100.13±0.93b	103.88±0.88 a	101.88±0.88ab
Na (mmol L^{-1})	B	142.88±1.43	140.25±1.62 #	139.50±2.61#
	A	138.13±1.39b	143.63±0.86 a	143.38±0.89a
	P	<0.05	<0.05	<0.05

a,b: different small letters on the same line indicate a difference between averages; #: on the same column indicate a difference between averages

Some studies show that pea use has a positive effect on the growth performance of lambs and calves[14]. High PS inclusion in mixed feed (32 and 56%, respectively) does not cause a palatability problem[1]. With its highly preserved starch content, pea seed can also be added to the feed to eliminate the energy deficiency of starchy foods easily destroyed in the rumen[9]. In this study conducted with developing lambs, the use of PS (respectively 33 and 66%) instead of SH did not have a negative effect on the live weight. On the other hand, there are studies reporting that the use of PS in lambs and calves leads to negative results. There have been[30] many studies on the use of peas as a source of protein rather than soya bean meal. It has been reported that the use of peas in part or completely instead of soy meal did not significantly affect the growth performance of the lambs[4, 12]. The probable cause of these results is the difference in feed used.

Blood metabolites: Different levels of PG consumption did not affect the total CH in blood ($p<0.05$). There was a decrease in the total CH concentrations in all groups (Table 3). PG has been shown to have beneficial effects on animals by reducing the total serum cholesterol. The data obtained from this study are similar to published literature[4, 14, 27, 31, 32]. However, in some studies, it has been reported that the total CH concentration in blood decreases after the use of PS in the feed and this decrease is due to the amino acid profile in the feed structure[4]. The use of PS instead of PG in this study had no adverse effect on the total CH level in the blood.

There was a statistically significant decrease in blood TG values in group C, regardless of PG consumption ($p<0.05$). In addition, depending on PS consumption, there was a decrease in blood TG values in the PI and PII groups. This situation is consistent with published literature indicating that the consumption of roughage with a low crude fat content reduces the TG level in the blood[14, 27]. The other reasons for the decrease in the total TG level in the blood are that the lambs get breast milk before the trial and that the lambs have limited nutrition during the trial period.

The increased use of PS in lamb's feed increased their blood urea levels (Table 3) in the PI and PII groups ($p<0.05$). The increase in the amount of blood urea due to PS consumption was consistent with previous studies[11, 12]. The increase in the amount of blood urea is due to the higher degradation of the PS protein compared to the PG protein in the rumen and the higher protein content in PS compared to PG (17). As the fermentation of proteins in the rumen increases, the blood urea value decreases accordingly[4, 33].

There was no difference in the blood CR values after PS consumption among the groups (Table 3). These results are consistent with current studies that show the blood CR level is not affected by PS consumption in lambs but changes according to age[4, 33]. However, the lack of difference between the age groups requires explanation. The reason for this is probably that the trial period is short at 25 days[33]. After PG consumption, a

Table 4: Concentrations of ammonia in rumen liquid, the pH, the protozoa numbers (number mL^{-1}) and live weight of the lambs before (B) and after (A) the experiment (data represents the mean±SEM)

Parameters	Time	C	P I	P II
Ruminal pH	B	6.15±0.16	6.12 ±0.08#	6.07±0.14#
	A	6.24±0.04 b	6.46±0.19a	6.52±0.07a
Protozoa nmber	B	277500±79860	252500 ±25340#	315000±47320#
	A	345000±33960b	1137500±222850b	3052500±464370a
Ruminal NH3-N,	B	14.75±1.70	13.40±2.05#	14.68±1.75#
mg dL^{-1}	A	13.98±0.40c	21.08±1.33 b	34.78±3.21a
	p	<0.05	<0.05	<0.05

a,b: different small letters on the same line indicate a difference between averages; #: on the same same column indicate a difference between averages

statistically significant decrease was observed in the amount of TB, DB and IB (p<0.05). Blood bilirubin values obtained from the animals in the experiment (Table 3) were within normal limits[34]. High bilirubin values compared to normal limits in the blood are observed when there is excess erythrocyte breakdown and structural destruction of liver cells or the blockage of bile ducts. One of the reasons for this problem is the anti-nutritional factors found in feed[34, 35]. The decrease in bilirubin values after the experiment can be considered as a positive effect of PG consumption. It is thought that the circulating bilirubin protects the tissues from the peroxidation of organic compounds which are made up of lipids. Mechanisms leading to this phenomenon are probably diverse and have not yet been well studied. There may be two reasons for the decrease in bilirubin values. The first one is the increase in bilirubin excretion, and the second is the prolongation of the lifetime of red blood cells. However, in some cases, the decrease in the value of bilirubin may be due to anemia[19]. The increase in total protein values after PG consumption shows that PG consumption does not lead to anemia (Table 3). In this case, it can be said that PS consumption does not cause anemia, possibly prolonging the life of blood cells.

The effect of PG on blood parameters is shown in Table 3. There was a statistically significant difference between the groups in terms of blood TP values after PG consumption (p<0.05). The data obtained from the experiment are consistent with the data for lambs in some studies[35]. The possible reason is that the protein deficiency, especially the amino acid deficiency of the SH roughage, is compensated for by the TP proteins. In a study related to the use of Lupine or PS instead of soy, it was reported that leptin consumption negatively affected the blood TP level and had a positive effect opposing the effect of PG consumption[6, 14, 35, 36].

The blood AL values obtained after the consumption of PG were found to be high in the PII group (p<0.05) and was in line with published literature (15, 40, 45, 46). In cases where the consumption of PG is 66% of the total feed the blood GL levels were increased (p<0.05). There are some anti-nutrient factors that increase the GL values in leguminous seeds and some studies have shown that feed exchange or roughage consumption causes high blood GL values in some animals under stress conditions[36]. The increase in blood GL levels after 66% PS consumption in the PII group was due to the anti-nutritional factors in the structure of the PS and it was observed that the consumption of SH which is roughage, had caused a lower level of blood GL.

In the case of SH supplementation for lambs consuming SP as their coarse feed, no statistically significant difference was observed between the groups in terms of potassium concentrations in the blood plasma (Table 3). However, a significant difference was observed in the Na and Cl blood plasma concentrations between the groups (p<0.05). In this study, the blood K, Cl and Na concentrations obtained were similar to those reported for sheep by Kaneko[37] and for lambs in organic production reported by Antunovic[3]. The Na, K and Cl concentrations of blood obtained in this study were found to be slightly lower than the Na, K and Cl concentrations of animals consuming alfalfa and starch-rich foods in a study by Lepherd[38]. In that study, the average blood Na, K and Cl concentration values obtained from 50 Merino lambs consuming a mixture of alfalfa, oats and molasses were 147±0.4, 5.1±0.054 and 107±0.5 mmol L^{-1}, respectively. One possible reason for this is the age of the animals. Studies have shown that age affects the blood plasma K, Na and Cl concentrations[3]. The other common cause of this condition is the low quantity of K, Na and Cl in the sainfoin hay used as roughage[3, 37].

The effect of PG on the rumen parameters are shown in Table 4. There is a statistically significant difference between the groups in terms of rumen pH values after PG consumption (p<0.05). In this study, the pH of the rumen fluid for the control and experimental group is seen to be at physiological norms[39]. According to Kudlinskiene[39] the rumen pH normally ranges from 6.5-6.8 and the physiologically normal rumen pH is between 5.5 and 7.0. The rumen pH values obtained after the consumption of PG in this study are consistent with the lamb's data obtained in a study by Bahri[40]. The pH values were higher than the values observed for consumption of low starchy feed[41]. The reason for the high pH level in this study is that the starch level of the PS is relatively high compared to SH which is roughage. Peas are a good balancing feed for rations containing low levels of protein and low energy as well as foods containing high levels of undegradable protein in the rumen[39, 42]. In this respect, consumption of PG reveals an advantage in terms of rumen acidity[43]. In a study in which different feeds were tested, it was reported that the use of legumes positively influenced rumen pH compared to soya beans[43].

There was a statistically significant difference between the groups in terms of rumen protozoa concentrations after PG consumption ($p<0.05$). The reason for the apparent difference between the results is thought to be due to the increase in PG consumption in which the carbohydrate-protein ratio which is present in the structure of PS feed-as it is in other legume grain feeds-is higher than the control feed. It was seen that the effect of the PS feed consumption with SH is positive in this study (Table 4). The concentration of protozoa per ml of rumen fluid in ruminants ranged from 8,000 to 39,800,000 in different geographical regions and because of other factors[44, 45]. Different feeding habits may affect the number of protozoa within the rumen and the degree of rumen fermentation. The most important reasons for increasing the number of rumen protozoa is the amount of starch, protein and fiber in the feed. The population of protozoa in the rumen usually increases when the amount of starch increases in the feed and increases up to 60% in concentrated feed[46]. Protozoa are very active in protein degradation in the rumen.

In this study, the concentration of ammonia in the groups increased and this increase was statistically significant ($p<0.05$). This increase in ammonia is due to the use of a protein-rich PS feed in addition to the protein-poor PG used as roughage. This increase lags far below the maximum ammonia level given in published literature. As the increase in ammonia level gets close to the optimal level, it is important that the PS depletion indicates that the ammonia level does not reach a critical level (230 mg^{-1} 100 mL,)[47]. The maximal fermentation activity in the rumen would occur when the concentrations of ammonia-nitrogen (N-NH3) are between 19 and 23 mg^{-1} 100 mL of ruminal fluid (45 mg dL^{-1})[48] and the pH levels are between 6.5-6.8[39, 49].

CONCLUSION

In this study, it was observed that adding peas to sainfoin hay in the feed consumed by the lambs caused various changes in some blood and rumen parameters but these changes, except for bilirubin values, remained within normal limits and had no significant effect on the live weight of the lambs. The decrease in blood bilirubin levels is worth consideration. In conclusion, in countries where the use of genetically modified protein sources like soya beans is not desirable, it is possible to use peas as a source of protein and of starch in the lamb's feed where sainfoin hay is used as roughage.

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