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The Effect of Replacing Wheat Straw with Soybean Straw on Feed Intake, Rumen Fermentation, Correlation between Neutral Detergent Fiber (NDF) Digestibility and Cellulose Activity Enzymes in Atabai Ewes

Research Article

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ABSTRACT

After the harvesting of agricultural products, including soybeans, waste remains that is not used for human consumption and can be used to feed ruminants. Therefore, the impact of replacing soybean straw with wheat straw on feed intake, ruminal fermentation, and cellulase enzyme activity in Atabai ewes was investigated im the current study. Eighteen ewes with an initial weight of kg were used in a compeletly randomized design in three groups, each of which included 6 ewes, for 42- day feeding period. The experiment treatment were: 1) control diet (without soybean straw), 2) diet with 20% soybean straw, and 3) diet with 40% soybean straw. The daily feed intake was calculated from the difference between the given feed and the post-manger feed. In order to measure rumen fermentation and the rumen protozoan population, volatile fatty acids (VFA) and cellulase enzyme activity, rumen fluid samples were taken from the animals in the last days of the experiment. The results showed that dry matter intake in ewes fed with 20 and 40% soybean straw was increased. No significant difference was observed between the different treatments in terms of rumen pH, protozoa and ammonia nitrogen ($P<0.05$). The particulate material and total activity of carboxy methyl- cellulose (CMM) and the microcrystalline-cellulase activity (MCC) were affected by inclusion of soybean straw in feed ($P<0.05$). There was no significant difference in the amount of VFA, acetate, butyrate, isobutyrae, valeret, butyrate among the experimental groups ($P>0.05$), whereas propionate increased with increasing amount of soybean straw in the diet ($P<0.05$). Also, the ratio of acetate to propionate decreased with increasing dietary level of soybean straw ($P<0.05$). These results suggest that soybean straw can be used as a forage source to completely replace wheat straw in ewes' diets.

KEY WORDS Atabai ewes, feeding behavior, ruminal fermentation, soybean straw, wheat straw.

INTRODUCTION

Straw is one of the most important sources of fiber production in the world, and is used as the main source of animal feed in many regions of the world, especially in arid regions, because in such regions, the problem of fodder shortage, which is the most important factor inhibiting animal husbandry, is considered (Dogan and Denek, 2021; Fekadie *et al.* 2022). Like most regions of the world, in Iran, the majority of straw produced comes from cereal crops, which have low digestibility and are poor in terms of protein, minerals, and vitamins needed by animals (Ghoorchi *et al.* 2023). Given the shortage of fodder in the country, identifying local food sources that can be used in animal nutrition is of great importance (Toghdory *et al.* 2020). Today, the production of crop residues has increased due to sharp rise in crop cultivated area to meet the growing demands of human population (Toghdory *et al.* 2018).

Broadly, there are two types of crop residues including field and industrial residues. The residues most commonly used in diets of small ruminants are field residues (Akram and Firincioglu, 2019). Crop residues are fibrous by-products that are left in the field after harvesting and include stem, leaves, stover and pods. These residues are comprised mainly of straw and stover (Majeed *et al.* 2002). Soybean (Max Glycine (L.)) is a dicotyledonous, annual plant from the leguminous family. After the harvesting of agricultural products, including soybeans, waste remains that is not used for human consumption and can be used to feed ruminants.Wheat straw, a by-product obtained after the removal of grain and chaff, is composed mainly of 32% cellulose, 19% hemicellulose and 5% lignin, and is used in livestock feeding and bedding, medicine and fermentation industry. Furthermore, wheat straw has higher neutral detergent fiber contents (NDF), ultimately leading to stabilized ruminal environment. Additionally, constraint in proper utilization of wheat straw by sheep and goats is the interlocking of polysaccharides of cell wall with lignin contents (Singh *et al.* 2005). The polysaccharides of feed offered to the ruminants are degraded by the combined activities of bacteria, protozoa and fungi with the help of different hydrolytic enzymes The fibrous feeds require a firm adherence of microbes before their degradation is initiated .These adherent microbes are retained in the rumen for a much longer time than the non adherent microbes (Hobson and Stewart, 1997; He *et al.* 2019). To assess the microbial activity under a particular set of conditions, the status of hydrolytic enzymes in the ecosystem is one of the most important indirect parameters, as these enzymes reflect quantitatively the presence of fibre degrading microbes (Silva *et al.* 1987; Asadi *et al.* 2018). The rumen contents have been separated into three spatially distinct fractions i.e. particulate material, cellular fraction (cells suspended freely in the liquid portion of rumen liquor) and extracellular fraction (the liquid portion) (Ishaq *et al.* 2017). Carboxymethylcellulase enzymes act on the middle part of the cellulose chain and tear it through hydrolysis and produce two shorter chains. But microcrystalline cellulase attacks the free end of the chain and produces cellobiose in successive stages. Fiber-degrading enzymes include total cellulase activity, carboxymethylcellulase and microcrystalline cellulase. The activity of these enzymes in three separate parts of the rumen contents, including small particles (microbes attached to the part of the rumen particles), the intracellular part (cells that are freely suspended in the liquid part of the rumen fluid) and the extracellular part (Enzymes in the liquid part) are measured (Silanikove *et al.* 2013; Asadi *et al.* 2024).

Among these three parts, the highest hydrolytic activity of enzymes was related to the part of microbes attached to small particles, followed by intracellular enzymes and finally extracellular enzymes (Agarwal *et al.* 2004). Silva *et al.* (1987) also reported that carboxymethylcellulase can be used as an indicator of the total population colonized on feed particles and can also be used to evaluate the difference in the rumen environment that affects fiber degradability. be used; Therefore, measuring the carboxymethylcellulase activity of bacteria attached to feed particles in the rumen is probably a suitable method for estimating the relative accumulation of cellulitic bacteria on feed particles (Guder and Krishna, 2019; Astuti *et al.* 2022).

Despite the increased production of crop residues in Iran, there are certain limitations regarding its use in small ruminants diet including lack of awareness among farmers and insufficient technical knowledge regarding the composition and nutritional value of these residues. Moreover, there is limited literature available regarding the replacement of Soybean straw instead of wheat straw in the diet of small ruminants. A study was conducted, therefore, to determine the effects of replacing Soybean straw instead of wheat straw as crop residues on Feed intake, Ruminal Fermentation, VFA and Cellulose Activity Enzymes in Atabai Ewes.

MATERIALS AND METHODS

Diets, animals and experimental design

This experiment was conducted at Gorgan University of Agricultural Sciences and Natural Resources Gorgan, Iran, from January until February of 2024 in accordance with the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Soybean crop waste plants required for this project were obtained from the agricultural fields of Gorgan University of Agricultural Sciences and Natural Resources located in Golestan province, and then the soybean plants were transferred to the feed store and after crushing, they were prepared for consumption by sheep. A total of eighteen ewes were assingned randomly to one of three treatment groups, having six animals each, for 6 weeks' experimental period. The dietary treatments included 0, 50% and 100%, substitution of soybean straw instead of wheat straw. The basic ration was adjusted according to the tables of the National Research Council (NRC, 2007; Table 1). Also chemical composition analysis of Soybean straw was presented in Table 2. The feed given and the feed of each animal are recorded daily to check and the amount of dry matter consumed.The total experimental period in this research was 42 days, which consisted of seven days of habituation to the ration and the conditions.

Table 1 Ingredients and chemical composition of the experimental diets used for Atabai ewes (% of diet DM)

Ingredient	of soybean straw Different levels		
	Control (0%)	20%	40%
Soybean straw	0.00	20.00	40.00
Wheat Straw	40.00	20.00	0.00
Barley	16.80	16.80	16.80
Corn	14.60	14.60	14.60
Wheat bran	7.30	7.30	7.30
Soybean meal	11.20	11.20	11.20
Beet pulp	6.60	6.60	6.60
Limestone	1.20	1.20	1.20
Salt	0.50	0.50	0.50
Mineral supplement[1]	1.00	1.00	1.00
Sodium bicarbonate	0.80	0.80	0.80
Chemical composition (% of diet DM)			
Dry matter (%)	88.64	88.27	87.94
Metabolizable energy (Mcal/kg)	2.30	2.31	2.33
Crude protein (%)	13.24	13.32	13.47
Acid detergent fiber (%)	27.48	27.86	28.08
Neutral detergent fiber(%)	41.49	44.82	47.24
Crude fat (%)	1.78	1.82	1.84
Ash (%)	4.88	4.92	4.97

[1] Vitamin and mineral premix provided per kilogram of diet: vitamin A: 1000000 U; vitamin D_3: 250000 U; vitamin E: 3000 U; Mn: 10000 mg; Zn: 10000 mg; Cu: 300 mg; Se: 100 mg; Ca: 100 mg; Fe: 3000 mg; Co: 100 mg; P: 30000 mg; Monensin: 1500 mg and antioxidant: 100 mg.

Table 2 Chemical and nutrient compounds of soybean straw

Chemical composition	Amount
Dry matter (%)	90.44
Ash (% DM)	8.09
Crude protein (% DM)	5.78
Neutral detergent fiber (% DM)	57.14
Ether extract (% DM)	5.32

Extraction of enzymes

In order to measure the activity of rumen cell enzymes including carboxymethylcellulase, microcrystalline cells from rumen samples were collected by esophageal tube three hours after morning feeding from the animals on the last day of the experiment period, and the rumen samples was collected by an insulated flask with The temperature of 32 degrees Celsius was transferred to the nutrition laboratory.The effects of dietary treatments on the activity of carboxymethyl-cellulase (CMM) enzyme and microcrystallinecellulase (MCC) were measured in different fractions of rumen fluid particulate material (PM), extracellular enzymes (EC) and cellular (C). The enzyme activity in three fractions of rumen content was determined according to the procedure described by Agarwal (2000) (Figure 1). The extraction of enzymes from the various fractions of rumen contents was performed according to Hristov *et al*. (1999). Glucose released by the activity of enzymes was estimated by dinitrosalicylic acid (DNS) method as by Miller described (1959). The enzyme activity was expressed as mol of released sugars produced per minute per ml under assay conditions.

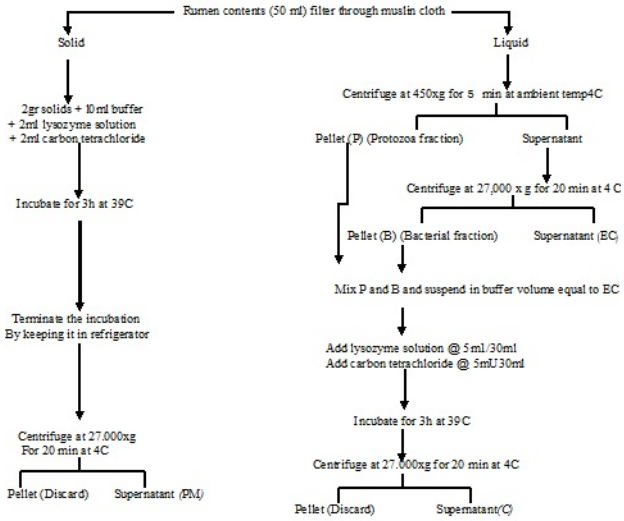

Figure 1 Fractionation of rumen contents and extraction of hydrolytic enzymes, Agarwal (2000)
PM: particulate material; EC: extracellular and C: cellular

Glucose released due to the activity of each of the tested enzymes was estimated according to the method of Miller (1959). The enzyme activity of the samples was determined in terms of nanomoles per minute per milliliter of released sugars (glucose) using a standard curve.

Ruminal pH, Ammonia Nitrogen and VFA Analysis

In last week of the experiment, rumen fluid samples were taken from each animal at 3 h post feeding. Approximately 100 mL of rumen liquor was collected from the rumen with

a stomach tube using light suction. Rumen liquor pH was recorded immediately after collection using a digital pH meter (Metrohm model 691. Switzerland). The rumen liquor was then strained through four layers of muslin cloth. The strained samples were stored after acidifying with 0.2 mol/L HCl solution and kept in labelled polypropylene bottles at -20 °C till further analysis. The NH_3-N concentration was determined for rumen fluid samples according to Broderick and Kang (1980). The volatile fatty acids (VFA) (acetic acid, propionic acid, butyric acid, isovaleric acid and valeric acid) were analyzed using the method of Makkar (2010). Samples for VFA analysis were prepared as described by Erwin *et al.* (1961) and analyzed by GLC (Sigma-Aldrich, USA) using a polyethylene glycol nitroterephthalic acid-treated capillary column (1.65 M×4.6 Mm) at 200 °C in the injector and 1.2 mL/min gas flow rate (24 mL/sec gas velocity). Eight lambs from each group were selected to determine rumen fermentation. Ruminal ammonia N was determined using a phenol-hypochlorite method according to the procedure of Broderick and Kang (1980). Analysis of volatile fatty acid (VFA) in the ruminal fluid was performed by gas chromatography using ethyl butyricacid as the internal standard as described by Cottyn and Boucque (1968). Dehority and Males (1984) method was used to count protozoa. First, after straining the rumen fluid with a cloth, 4 ml of rumen fluid was poured into a test tube wrapped in foil, then 1 mL of 18.5% formalin, 5 drops of methylene blue dye (2 grams of methylene blue) It was brought up to volume with 100 mL of distilled water) and finally 3 mL of glycerol was added to the contents of the test tube. Counting of protozoa was done by a microscope and a lens with 40 X magnification by a neobar slide. Counting was done 4 times for each sample and if there was a big difference between the counted protozoa, the counting was repeated. Finally, the number of protozoa per millimeter of rumen fluid was calculated (Ghoorchi *et al.* 2023).

Statistical analysis

The data enzyme activity, and ruminal fermentation were analyzed by General Linear Models (GLM) procedures of SAS (SAS, 2001), based on the statistical model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where:
Y_{ij}: observation.
T_i: effect of treatment.
ε_{ij}: residual error.

Differences among means with $P < 0.05$ were accepted as representing statistically significant differences.

RESULTS AND DISCUSSION

The results of the effect of replacing wheat straw with soybean straw on dry matter, crude protein, and NDF intake of Atabai ewes are shown in Figures 2, 3, and 4, respectively.

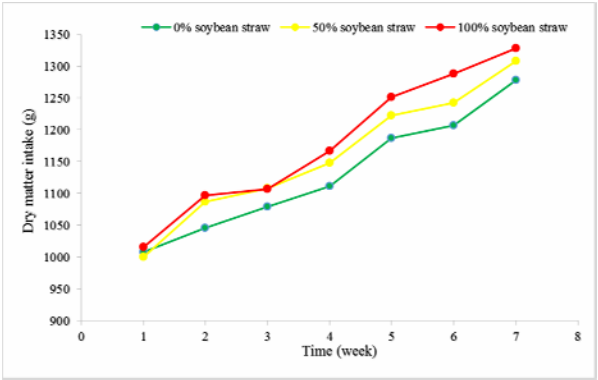

Figure 2 Dry matter intake (g/d) of *Atabai* ewes fed with diffent levels of soybean straw

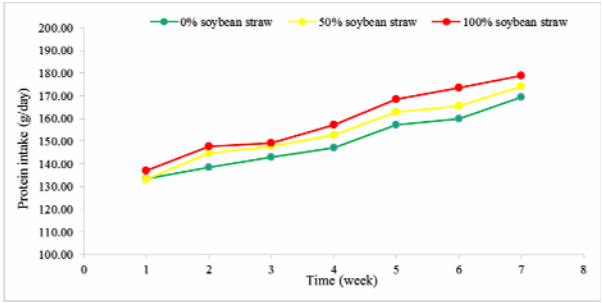

Figure 3 Crude protien intake (g/d) of Atabai ewes fed with diffent levels of soybean straw

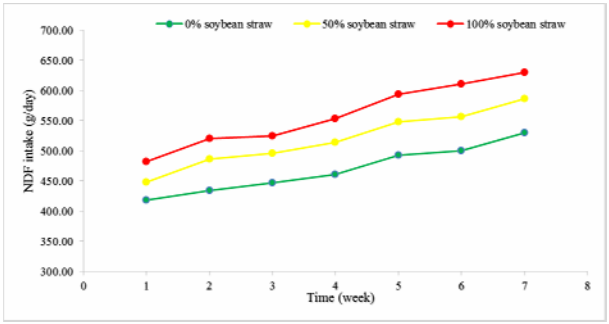

Figure 4 NDF intake (g/d) of Atabai ewes fed with diffent levels of soybean straw

In the present study, substituting soybean residues with wheat straw did not have a significant effect on dry matter, crude protein, and NDF intake. Based on the current study, Asadi *et al.* (2021) demonstrated that cotton plants can serve as a viable alternative to wheat straw in ewe diets at 100% inclusion. Their findings indicated that when 50% of

the diet consisted of cotton plants, ewes exhibited higher dry matter intake and weight gain, along with a lower feed conversion ratio compared to the other treatment groups. However, statistical analysis revealed no significant differences among the treatments in terms of yield and material. This study is agree with our findings. Pasandi *et al.* (2011) reported that soybean straw, similar to wheat straw, which has a low coefficient and is a lignocellulosic feed, can be incorporated into the diet of experimental groups at a level of up to 14% of the total ration. Mokhtarpour and Jahantigh (2009) found in their research that the inclusion of reed fodder as an economical supplement in the diet of Sistani heifers did not result in improved protein and feed digestibility and did not yield significant effects. Mehramiri *et al.* (2018) conducted an experiment and found that substituting mung bean straw for wheat straw in the diet of Arabian ewes is a viable option due to its cost-effectiveness and availability in certain regions. Soybean straw composition had no negative effect on dry matter consumption and milk production in crossbred lactating cows, and soybean straw can be used up to 75% instead of wheat straw (Mudgal *et al.* 2010). Mudgal *et al.* (2010) showed that soybean plant is superior to wheat because it is richer in terms of nitrogen content, so we can use soybean straw instead of wheat straw. Due to low palatability, soybean straw should be limited in the diet of cows, because soybean straw has a woody and hard stem. Due to its low palatability, soybean straw should be limited in the diet of cattle, because soybean straw has a woody and hard stem (Sruamsiri and Silman, 2008). Nutrient digestibility is a key factor that affects feed intake in ruminants (Majeed *et al.* 2021). In a study, researchers utilized soybean straw as feed and found no significant difference in dry matter intake between fattening calves fed soybean straw and those fed wheat straw (Pasandi *et al.* 2011). A study on Jersey breed heifers reported that replacing fresh forage with agricultural residues such as wheat straw, rice straw, and coconut branches increased dry matter intake (Kulathunga *et al.* 2015). Similarly, in another study, replacing palm branches with barley straw had no effect on feed intake in Holstein cows (Bahman *et al.* 1997). Additionally, a study by Bagherinasab *et al.* (2012) revealed that feeding male Holstein calves straw and canola stubble resulted in reduced feed consumption.

The activity results of carboxymethyl-cellulase and microcrystalline-cellulase enzymes in rumen fluid of tested sheep (nanomol per minute) are presented in Table 3. The highest amount of carboxymethyl-cellulase and microcrystalline-cellulase enzyme activity in Particulate material and their total activity was observed in ewes fed with 40% soybean straw diet ($P<0.05$). The activity of rumen enzymes reflects the microbes that are active in digesting food particles (Raghuvansi *et al.* 2007).

Fiber-degrading enzymes include carboxymethyl-cellulase and microcrystalline-cellulase. The activity of these enzymes in three separate parts of the rumen contents including small particles (microbes attached to the rumen particle part), intracellular part (cells that are freely suspended in the liquid part of the rumen fluid) and extracellular part (enzymes) existing in the liquid part) are measured. Among these three parts, the highest hydrolytic enzyme activity is associated with the microbes attached to small particles, followed by intracellular enzymes, and finally extracellular enzymes (Agarwal, 2000). In this experiment, the activity of particle-dependent enzymes exceeded that of both intracellular and extracellular enzymes. This could be attributed to the rapid accumulation of microbes on feed particles (Asadi *et al.* 2018). The variation in enzyme activity among the experimental treatments may be a result of changes in microbial population due to the animals' diet, leading to alterations in enzyme composition (Agarwal *et al.* 2004).The total activity of the enzymes analyzed in this study falls within a similar range to that reported by Asadi *et al.* (2018), ranging from 397 to 525 and 331 to 477 nmole per minute, respectively. Among the microbial enzymes investigated, the activity level in the solid fraction (particle-dependent) of rumen fluid was significantly higher than in the extracellular and intracellular fractions. Conversely, the extracellular fraction exhibited the lowest enzyme activity. Relationship between NDF digestibilty (% of DM; and Microcrystalline cellulose (µmol glucose released/min/mL) for the ewes data set ($P<0.01$) in Figure 5. The reduced enzyme activity in the extracellular fraction of rumen fluid can be attributed to the fact that cellulose-degrading microbes predominantly adhere to feed particles, resulting in a lower population of free-floating microbes in the liquid phase (Cao *et al.* 1987). It was anticipated that the extracellular fraction would have minimal concentrations of fiber-degrading enzymes, as these enzymes are typically associated with cell membranes, with only a small portion released through the destruction or mechanical disruption of fiber-degrading microbes into the free liquid phase (Chen and Gomes, 1995).

The carboxymethyl-cellulase activity in the solid, extracellular, intracellular, and total fractions of ruminal juice was observed to be higher than that of the microcrystalline-cellulase enzyme. Carboxymethylcellulose enzymes function on the middle section of the cellulose chain, breaking it down through hydrolysis to generate two shorter chains. In contrast, microcrystalline cellulose targets the free end of the chain, leading to the production of cellobiose in sequential steps.

Table 3 Effect of wheat straw replacement with soybean straw on activities of enzymes in various fractions of rumen contents (µmol glucose released /min/ml) in Atabai ewes

Item	Different levels of soybean straw			SEM	P-value
	Control (0%)	20%	40%		
Carboxylmethylcellulase					
Cellular fraction	66.23	71.04	76.19	3.417	0.1571
Extracellular fraction	137.87	142.55	146.23	6.718	0.3011
Particulate material	214.00^{b}	238.16^{ab}	246.75^{a}	3.765	0.0001
Total	418.10^{b}	451.75^{a}	469.17^{a}	13.576	0.0001
Microcrystalline cellulose					
Cellular fraction	102.63	109.11	115.63	4.205	0.4317
Extracellular fraction	127.50	130.36	124.12	6.417	0.5157
Particulate material	191.22^{b}	202.17^{ab}	216.58^{a}	6.544	0.0361
Total	421.35^{b}	441.64^{ab}	456.33^{a}	7.611	0.0001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Consequently, the higher total activity of carboxymethyl-cellulose compared to microcrystalline cellulose can likely be attributed to the availability of more substrate for the former enzyme, consistent with the findings of previous studies (Munasik *et al.* 2013). Relationship between NDF digestibilty (% of DM; and Carboxylmethylcellulase (µmol glucose released/min/mL) for the ewes data set (P<0.01) in Figure 6.

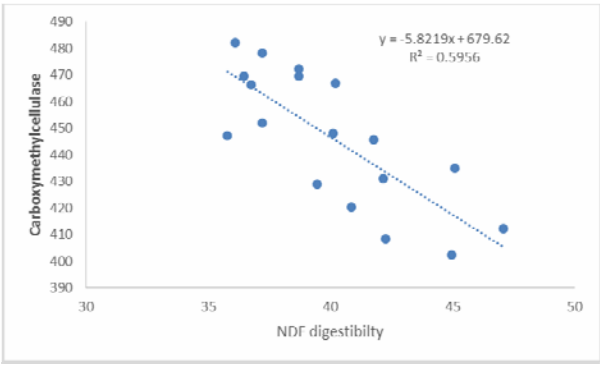

Figure 5 Relationship between NDF digestibilty (% of DM; and carboxylmethylcellulase (µmol glucose released/min/mL) for the ewes data set (P<0.01)

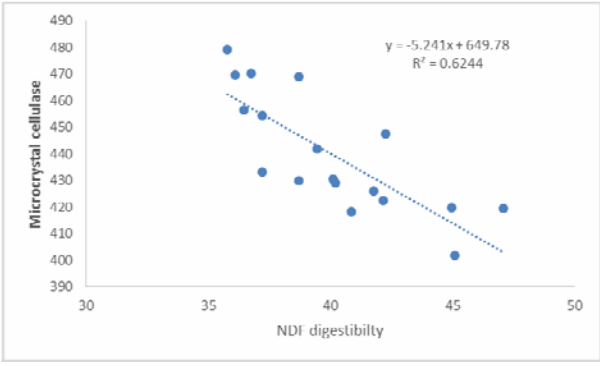

Figure 6 Relationship between NDF digestibilty (% of DM; and microcrystalline cellulose (µmol glucose released/min/mL) for the ewes data set (P<0.01)

Carboxymethyl cellulase can be used as an indicator of cloned total population of bacteria on feed particles also a good way to evaluate rumen environment that affects fibre degradation in the rumen (Silva *et al.* 1987). Microbes freely suspended in the rumen liquid may be due to the lesser enzyme activity in the cellular fraction. The extracellular fraction had lowest the enzymatic activity of among three rumen portion, because this enzymes are bound to the cell membrane and due to mechanical injury or disintegration of the fibre degrading microbes, Only a fewer amount is released into the liquid portion (Minato *et al.* 1966; Agarwal, 2000). Minato *et al.* (1966) finding 50-70 percent of rumen bacteria are attached to the particle feed. High activity of carboxymethyl cellulase to than Microcrystalline cellulase activity probably due to more their substrates and is consistent with the findings of other researchers (Raghuvansi *et al.* 2007).

The data in Table 4 shows the pH and ammonia nitrogen concentration of rumen liquid, as well as the rumen protozoan population of sheep. As the amount of soybean straw in the diet increased, there was a decrease in both the pH and liquid ammonia nitrogen of the rumen (P<0.05). Conversely, an increase in soybean straw levels in the diet led to an increase in the rumen protozoan population (P<0.05). The pH value of the rumen depends on the feeding time (Hindrichsen *et al.* 2002) and the amount of volatile fatty acids produced (Synder *et al.* 2006). The average rumen pH value of the ewes receiving experimental diets was almost in the normal range of 5.8-6.8 as reported by Van Soest (1994), so based on the results obtained, it can be said that the use of different levels of the cotton plant the grain in diets had no negative effect on rumen pH.

Harvatine *et al.* (2002) stated that the consumption of cottonseed in dairy cows increases the duration of food ingredients in the rumen, and this causes the action of rumination and the secretion of saliva to increase and prevent the drop in liquid pH.

Table 3 Effect of wheat straw replacement with soybean straw on pH, NH_3-N and protozoa (10^4 mL) *Atabai* ewes

Item	Different levels of soybean straw			SEM	P-value
	Control	20%	40%		
pH	6.91[a]	6.25[b]	6.28[b]	0.125	0.0001
NH_3-N (mg/dL)	19.77[a]	17.81[ab]	16.00[b]	0.896	0.0121
Protozoa	7.01[b]	8.56[a]	9.13[a]	0.673	0.0001

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of wheat straw replacement with soybean straw on ruminal fermentation in Atabai ewes

Ruminal fermentation (Mmol/L)	Different levels of soybean straw			SEM	P-value
	Control	20%	40%		
Acetate	71.64	73.01	76.81	2.567	0.3210
Propionate	28.06[b]	32.11[ab]	37.02[a]	1.870	0.0001
Butyrate	6.63	6.65	6.59	0.421	0.7811
Isobutyrate	1.73	1.39	1.40	0.018	0.8009
Valrat	1.78	1.73	1.75	0.097	0.4133
Isovalerate	1.17	1.30	1.21	0.103	0.6779
VFA	106.55	104.32	103.19	3.815	0.6235
Acetate to propionate ratio	2.55[a]	2.27[ab]	2.07[b]	0.051	0.0311

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

It is ruminated, also in line with the present experiment, they reported that the treatments receiving cottonseed produce more ammonia nitrogen than the control group. In line with the present results, Pires *et al.* (1996) also reported that there was no significant difference in rumen pH between the treatments receiving cottonseed and the control treatment in the diet of dairy cows. However, contrary to the results of the present experiment, they reported that rumen ammonia nitrogen was not affected by the consumption of cottonseed. Although the protein levels of the diets were the same in the present study, the reason for the increase in the concentration of ammonia nitrogen in the group receiving 40% of the cotton seed plant can be attributed to the higher ruminal degradability of the cotton seed plant compared to straw (Ghoorchi *et al.* 2023).

According to several reports, the rumen protozoan population increases significantly (about 27% on average) in 2 to 6 hours after consuming food (Van Soest *et al.* 1991). Today, it is known that the composition of the rumen protozoan population changes with the nutritional and physiological conditions of the host animal. One of the reasons for the increase of protozoa up to three hours after consuming feed is the availability of soluble sugars (Hobson and Stewart, 1997). Considering that the pH of the rumen at different times after feeding was not different among the treatments receiving different levels of cottonseed in the diet, therefore, attributing the increase in the protozoan population to the pH of the rumen is not convincing (Hobson and Stewart, 1997).

Contrary to the present results, Jenkins and McGuire (2006) showed that the use of wood materials with high lignin in the diet leads to the reduction of bacterial and protozoan population due to the physical coating on the fibers. Also, contrary to the results of this experiment, it is stated in a report that the replacement of canola straw in the diet of dairy cows reduces the rumen protozoan population (Bagherinasab *et al.* 2012). Probably, the reason for this difference in the results can be the presence of more glucosinolates, saponins and tannins in canola straw than in cotton plants (Misra *et al.* 2007) because when these substances are in the environment, the population of protozoa decreases (Mishra *et al.* 2007). An increase in the number of protozoa is usually associated with an increase in nitrogen recycling in the rumen (Franzolin and Dehority, 2010). It seems that the increase of protozoa causes more destruction of bacteria and as a result the production of ammonia also increases.

Table 5 demanstrates the influence of increasing different levels of dietary soybean on ruminal VFA in ewes. No significant differences were observed in the concentration of acetate, butyrate, isobutyrate , isovalerate and VFA, as well as the ratio of acetate to propionate among the experimental treatment (P>0.05). Howerer, only soybean straw demonstrated a significant increase in ruminanl propionate (P<0.001). We hypothesized that volatile fatty acids are feedback signals that condition food preferences in ruminants, low doses of propionate condition preferences for low-quality foods. Volatile fatty acids provide up to 80% of

the energy required by the animal. The concentration of total VFA depends mostly on DMI and also the effective degradability of thenutrients. Therefore, the lack of changes inthe total amount of VFA between the treatments herein may be due to the same digestibility of OM.

CONCLUSION

Overall results suggest that inclusion of elevated levels of soybean straw in ewes' diets had no negative effect on feed intake. However, based on economical point of view and availability of soybean straw and also the lack of adverse effect on ewes' health, it is suggested to incorporate up to 40% of diet DM in ewes' maintenance ration.

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