IJAS Iranian Journal of Applied Animal Science

The Effect of Grain Particle Size (Barley and Wheat) on Rumen Fermentation Parameters in Fattening Lambs

Research Article

S.M. Hosseini[1*], T. Ghoorchi[1] and A. Toghdory[1]

[1] Department of Animal and Poultry Nutrition, Faculty of Animal Science, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran

Received on: 9 Dec 2023
Revised on: 5 Feb 2024
Accepted on: 8 Feb 2024
Online Published on: Jun 2024

*Correspondence E-mail: hosseini.1984@yahoo.com

Online version is available on: www.ijas.ir

ABSTRACT

This study was conducted to investigate the effects of grain (barley and wheat) size on rumen fermentation characteristics in fattening Dalaq breed lambs of the study was (3×2) completely randomized design with 6 treatments including; milled barley grain with sieve number two, milled barley grain with sieve number eight, unmilled barley grain, milled wheat grain with sieve number two, milled wheat grain with sieve number eight and unmilled wheat grain were plotted with 5 repetitions. Animals were housed in individual pens for 84 days. Rumen pH was not affected by experimental treatments. There was not significant (P>0.05) effect of grain paricle size on NH_3-N ammonia nitrogen and microbial count although though, processing type it was affected by the type of processing and in the treatment of barley grain it was more than wheat. Also in the type of processing, sieve No. 2 produced more ammonia nitrogen. Counting of protozoa was significant in treatments containing barley and wheat and in barley treatment it was more than wheat (P<0.01). Although the *Coliforms* and bacteria of lactic acid were not affected by the type of grain (P>0.01). The total concentrations of volatile fatty acids (VFA) was not affected by the type of grain. Microbial nitrogen and microbial protein produced in the rumen were significantly affected by experimental treatments and both of them were more than wheat in the treatment of barley seeds (P<0.01). Allantoin and uric acid were not affected, and absorbed purine and xanthine and hypoxanthine were affected by the grain effect. The activity of carboxy methyl cellulase enzyme was not affected by the treatments although microcrystals cellulase enzyme in intracellular and total was affected by the treatments and it was more in barley.

KEY WORDS cereals, enzyme activity, lamb, microbial protein, rumen fermentation.

INTRODUCTION

Barley grain processing (pericarp and indigestible hull) is regarded as an essential method for the rumen microbial population to access the endosperm surrounded (Wang and McAllister, 2002). Milling is the most common method of grain processing in Iran. The main effect of grinding and flouring the feed is to increase the voluntary consumption of the feed, which leads to a decrease in the digestibility of fiber materials. The decrease in the digestibility of chopped feed is due to the increase in the rate of passage through the rumen (Shams Sharq and Prizadian Kavan, 2012).

Ruminants do not use absorbed purine bases of external origin for the synthesis of nucleic acids, therefore absorbed purine bases are not metabolized and are excreted. Urinary purine derivatives are used to estimate microbial protein in the rumen of ruminants because a correlation between the duodenal flow of nucleic acids and purine derivatives has been reported (Chen and Gomez, 1995). By increasing the level of dietary concentrate, the urinary excretion of allan-

toin, uric acid, total purines and milk allantoin also increases (Ghoorchi and Ghorbani, 2018).

Microbial protein also plays an important role in providing the nitrogen needs of ruminants and provides most of the amino acids needed for the growth, maintenance and production of the host animal. In sheep, allantoin has the largest share in the estimation of microbial protein and includes about 60-80% of all purine derivatives excreted in urine. Uric acid and xanthine + hypoxanthine also contain 10-30 and 5-10% of all purine derivatives, respectively. Microbial protein plays an important role in providing the nitrogen requirement of ruminants, which provides most of the amino acids required for the growth, maintenance and production of the host animal (Vaithiyanathan *et al*. 2006). In order to produce microbial protein in the rumen, two simultaneous sources of nitrogen and energy are necessary for the rumen bacteria (Ghoorchi and Ghorbani, 2018).

The production of microbial protein in the rumen depends on two main factors; the rate of fermentation, which regulates the amount of food available to microbes per unit of time, and the rate of passage, which causes slow-fermenting materials to be removed from the reach of microbes and older microbes to be replaced by a new microbial population (Ghoorchi and Ghorbani, 2018). The pH of the rumen depends on the time of feeding and the amount of volatile fatty acids. The amount of protein parts of the diet changes the microbial population, which leads to changes in rumen pH and ammonia concentration. Feeding large amounts of seeds or starch-based diets, as well as excessive processing, reduces the pH of the fluid rumen environment (Ghoorchi and Ghorbani, 2018).

MATERIALS AND METHODS

Animals and experimental treatments

This study was conducted in the research farm of Gorgan University of Agricultural Sciences and Natural Resources (5 km from Gorgan city, Golestan province, Iran) for a period of 84 days. 30 lambs of Dalaq breed (4-5 month) were used. This study was carried out using a completely randomized design, involving a factorial arrangement of 2 × 3. It included three particle size variations (fine and coarse grinding, and no grinding) and two types of grains (wheat and barley). The different grain (wheat and barley) particle size (with fine or coarse grinding, and without grinding). The experimental treatments comprised: 1- barley grain ground using sieve number two, 2- barley grain ground using sieve number eight, 3- unground barley grain, 4- wheat grain ground using sieve number two, 5- wheat grain ground using sieve number eight, and 6- unground wheat grain. Each treatment group consisted of five animals (replicates).

The animals were housed in individual pens with free access to clean drinking water and mineral blocks. Diet was formulated (Table 1) to meet requirements for growing lambs (NRC, 2007). Diets were offered twice daily at 9 am and 4 pm. To calculate the daily weight gain, the animals were weighed every 2 weeks, before the morning feeding. Feed conversion efficiency was calculated using the data related to daily dry matter consumption and daily weight gain.

Diets chemical composition

Samples of diet were analyzed for dry matter (DM), crude protein (CP) and calcium and phosphorus according to AOAC (2000) procedures. Also, the determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was measured by Van Soest *et al*. (1991) method.

Rumen samples collection

On day 70 of the experiment, samples of whole ruminal contents were collected from 5 animals of each group, 4 hours after morning feeding by stomach tube. The samples were filtered through four layers of muslin cloth and were immediately transported to the laboratory by insulating flask.

Ruminal pH, ammonia nitrogen and volatile fatty acids (VFA) analysis

The ruminal pH was measured immediately after sampling with a portable pH meter (menthion pH meter name and company, country). Ruminal ammonia nitrogen (NH_3-N) content was determined using a phenol-hypochlorite method described by Broderick and Kang (1980). The 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).

Extraction of enzymes

The method described by Agarwal (2000) was used to measure the effect of the material on the activity of carboxy methyl cellulase (CMC) and micro-crystalline cellulase (MCC) enzymes. Extraction of enzymes from three different parts of ruminal fluid including intracellular (C) enzymes (bacteria, fungi and protozoa), extracellular (EC) (floating and free microbes in ruminal fluid) and enzymes secreted from attached microbes particles (particulate material) (PM) were extracted and their activity was measured according to Hristov *et al*. (1999) method.

Table 1 Ingredients and chemical compositions of experimental diets (% of DM)

Treatments	Wheat			Barley		
Ingredients (% DM)	Sieve No.2	Sieve No.8	No Sieve	Sieve No.2	Sieve No.8	No Sieve
Barely grain	0.0	0.0	0.0	52.0	52.0	52.0
Wheat grain	52.0	52.0	52.0	0.0	0.0	0.0
Beet pulp	14.0	14.0	14.0	14.0	14.0	14.0
Soybean meal	14.5	14.5	14.5	15.5	15.5	15.5
Wheat bran	14.0	14	14	13	13	13
Calcium carbonate	2.0	2.0	2.0	2.0	2.0	2.0
baking soda	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Mineral vitamin supplement[1]	1.5	1.5	1.5	1.5	1.5	1.5
Final ration percentage						
Concentrate	70	70	70	70	70	70
Chopped straw	15	15	15	15	15	15
Alfalfa	15	15	15	15	15	15
Chemical compounds						
Metabolizable energy (Mcal/kgDM)	2.70	2.70	2.70	2.70	2.70	2.70
Crude protein (%)	14.14	14.14	14.14	14.14	14.14	14.14
Neutral detergent fiber (%)	32.00	32.00	32.00.	32.00	32.00	32.00
Acid detergent fiber (%)	17.00	17.00	17.00	17.00	17.00	17.00
Calcium (%)	0.72	0.72	0.72	0.72	0.72	0.72
Phosphorus (%)	0.50	0.50	0.50	0.50	0.50	0.50

[1] Each kg contained: vitamin A: 250000 IU; vitamin B_3: 250000 IU; vitamin E: 500 IU; Mg: 50000 mg; Mn: 2500 mg; Zn: 10000 mg; Cu: 300 mg; Se: 25 mg and Co: 60 mg.

The particulate material (2 g) was suspended in 10 mL phosphate buffer (0.1 M, pH 6.8), 2 mL of 0.4% lysozyme solution and 2 mL carbon tetrachloride were added to it. Glucose released by enzyme activity was estimated as described by Miller (1959) using the dinitrosalicylic acid (DNS) method. Adding 3 mL of DNS reagent prevents the reaction of rumen fluid enzymes with the substrate. Under experimental conditions, enzyme activity was expressed as µmole of sugars released per minute per mL.

Estimation of microbial protein

The measurement of microbial protein produced in the rumen was performed using the method of estimating excreted purine derivatives based on colorimetric method (Chen and Gomez, 1995). The calculation of total purine derivatives (TPD) excreted in the urine in mmol per day was obtained from the sum of allantoin, uric acid, xanthine and hypoxanthine. Daily urine volume was collected in a plastic bucket containing 100 mL of 10% sulfuric acid solution (10% solution or one molar to prevent nitrogen loss at pH of less than 3). Every morning, all the urine produced by the animal collected and to prevent sedimentation (especially uric acid) of the urine sample during storage, a 10 mL sample of the daily amount is diluted with 40 ml of distilled water and then to estimate PD is stored at 20 ˚C until the test.

Statistical analysis

A completely randomized design (with 2×3 factorial arrangement) were employed, including three levels type of processing (mill with sieve no 2, 8 and No mill) and two type of grain (barley and wheat). Data were analyzed using the MIXED procedure of SAS (2013), using model 1. The mean of treatments was compared by Tukey test at a significant level of 5%. The animal effect was assumed to be random in both models. The statistical models were as follows:

(1) $Y_{ijk}= \mu + T_i + S_j + TS_{ij} + E_{ijk}$
(2) $Y_{ijk}= \mu + T_i + S_j + TS_{ij} + b(w_k-w) + E_{ijk}$

In model 1,
Y_{ijk}: observation for dependent variable.
µ: overall mean.
T_i: effect type of processing.
S_j: effect type of grain.
TS_{ij}: effect of interaction between T_i and S_j.
E_{ijk}: residual error.

In model 2,
Y_{ijk}: observation for dependent variable.
µ: overall mean.
T_i: effect type of processing.

S_j: effect type of grain.
TS_{ij}: effect of interaction between T_i and S_j.
b: initial weight regression coefficient and performance traits.
w_k: initial weight k of animal.
w: verage initial weight of animal.
e_{ijk}: residual error.

RESULTS AND DISCUSSION

As the there was not a significant (P>0.05) effect on the activity of cellulolytic enzymes, although the activity of microcrystal cellulase enzyme in the particle dependent (PM), extracellular (EC) and intracellular (C) and total (total of all three parts) the rumen was affected (Table 2).

The activity of carboxy methyl cellulase and microcrystal cellulase enzymes in three separate parts of the rumen contents including small particles (microbes attached to the part of the rumen particles), intracellular part (cells that are freely suspended in the liquid part of the rumen) and extracellular fraction (enzymes in the liquid fraction) are measured. Among these three parts, the highest hydrolytic activity of enzymes is related to the part of microbes attached to small particles, followed by intracellular enzymes and finally extracellular enzymes. These results can be due to the speed of colonisation of food particles with microbes (Agarwal, 2000).

The amount of total activity obtained from the enzymes investigated in this experiment is similar to the amount of total activity reported by Mirmohammadi (2012) in fattening lambs, who found the total activity of carboxy methy lcellulase and microcrystalline cellulase in the range of 143-282 and 203-203, respectively. It has been found that the greater number of microbes associated with the solid part increase the activity of fibrolytic enzymes and are mainly involved in fiber digestion which is consistent with the results of the present study (Cheng and McAllister, 1997; Cheng *et al*. 1984; Raghuvansi *et al*. 2007).

Similarly, Ghoorchi and Ghorbani (2018) reported the total activity of carboxy methyl cellulase and microcrystalline cellulase comparing the rumen fluid of slaughtered animals with fistula animals in the range of 185-440, 311-537, It was related to particles 17-60, 55-268, extracellular 56-138, 84-173, intracellular 48-245, 164-249 (nmol per minute) (respectively from right to left). The range of activities of the three parts of the enzymes obtained from the enzymes examined in this experiment is different from the activities of carboxy methy lcellulase and microcrystalline cellulase reported by Ghoorchi and Ghorbani (2018). This difference and range can be seen as the reason for different food, storage and management.

In other words, the type of diet fed to the animals has caused a change in the microbial population and subsequently a change in the enzyme pattern (Kamra *et al*. 2010).

The less level of enzyme activity in the cellular part of the leachate is because the cellulolytic microbes are attached to the food particles and the population of free microbes is much less in the liquid part (Agarwal, 2000; Raghuvansi *et al*. 2007). The minimum concentration of fiber-decomposing enzymes in the extracellular part of the rumen juice was expected, because these enzymes are attached to the cell membrane and only a small amount of them is due to destruction or mechanical breakdown of the fiber-decomposing microbes into the free cell liquid part (Minato *et al*. 1966; Agarwal, 2000). The results of the present experiment are in accordance with the findings of Mirmohammadi (2012) in fattening lambs that the activity of hydrolytic enzymes (including carboxy methyl cellulase and microcrystalline cellulase) in the solid, intracellular and extracellular parts of the rumen was in the range of 72-81, respectively. They reported 14-21 and 6-9 percent of the total activity. Also, Minato *et al*. (1966) found that 50-70% of rumen bacteria are attached to food particles. The amount of carboxy methyl cellulase activity in the solid, extracellular, intracellular, and whole rumen juice parts was higher than the corresponding parts in the microcrystalline cellulase enzyme. Carboxy methyl cellulase enzymes act on the middle part of the cellulose chain and tear it through hydrolysis and produce two shorter chains. However, micro crystalline cellulase attacks the free end of the chain and produces cellobiose in successive stages (Danesh Mesgaran *et al*. 2017). Therefore, the increase in the activity of carboxy methyl cellulase compared to microcrystalline cellulase is probably due to the presence of more substrate for it and is in accordance with the findings of other researchers (Mirmohammadi, 2012; Raghuvansi *et al*. 2007). In relation to the effect of the physical form factor on the activity of rumen enzymes in the feeding of fattening lambs with flour and block concentrate, Mirmohammadi (2012) showed that physical form factor caused a significant decrease in the total activity of alpha-amylase and carboxy methyl cellulose. which was consistent with the results of the present study.

Volatile fatty acids were affected (P>0.05) by the type of grain or the type of processing (Table 3). The results of measuring volatile fatty acids showed that the type of grain and the type of processing did not show a significant difference. These results can be caused by the lack of change in the raw materials of the diet in different treatments. In this experiment, it seems that the type of processing will not show a significant difference in the production of acetate, propionate and butyrate in the rumen.

Table 2 The effects of using barley and wheat grain size on the content of enzymes in the rumen of fattening lambs[1]

Treatment	Carboxy methyl cellulase				Micro crystals cellulase			
	C	EC	PM	Total	C	EC	PM	Total
Barely grain	196.78	83.53	230.99	511.3	171.94^{a}	89.35	287.47^{a}	548.77^{a}
Wheat grain	196.67	83.51	231.303	511.48	159.25^{b}	87.35	275.80^{b}	522.40^{b}
SEM	1.092	1.763	2.269	3.005	1.17	0.703	0.972	1.651
P-value								
Grain type (G)	0.9449	0.9928	0.9237	0.9665	0.0001	0.0557	0.0001	0.0001
Processing type (P)	0.2201	0.9856	0.9919	0.7821	0.6007	0.0001	0.0001	0.0001
G × P	0.0076	0.7943	0.9936	0.6502	0.5093	0.6476	0.8449	0.4931
Grain effect								
Mill with a sieve No.2	197.39	83.71	231.32	512.42	166.79	92.37^{a}	285.75^{a}	544.90^{a}
Mill with a sieve No.8	197.97	83.63	230.86	512.48	164.92	88.73^{b}	282.34^{a}	535.99^{b}
No sieve	194.79	83.23	231.86	509.27	165.08	83.94^{c}	276.82^{b}	525.84^{c}
SEM	1.338	2.16	2.78	3.681	1.433	0.861	1.191	2.022
Type of processing								
Barley × sieve No.2	199.38^{a}	83.87	231.22	514.48	174.48^{a}	93.82^{a}	291.31^{a}	559.62^{a}
Barley × sieve No.8	199.38^{a}	82.53	230.46	512.89	170.85^{a}	89.92^{ab}	288.74^{ab}	549.51^{ab}
Barley × No sieve	191.05^{b}	84.19	231.30	506.54	170.51^{a}	84.29^{c}	282.35^{bc}	537.15^{bc}
Wheat × sieve No.2	195.40^{ab}	83.53	231.43	510.36	159.09^{b}	90.92^{ab}	280.18^{c}	530.19^{cd}
Wheat × sieve No.8	196.08^{ab}	84.73	231.26	512.08	159.00^{b}	87.54b^{c}	275.94^{cd}	522.48^{d}
Wheat × No sieve	198.53^{ab}	82.27	231.22	512.01	159.66^{b}	83.58^{c}	271.29^{d}	524.53^{d}
SEM	1.893	3.054	3.932	5.206	2.027	1.218	1.685	2.86

[1] The activity of all enzymes is in terms of nanomoles of glucose released per minute.
C: intracellular; EC: extracellular and PM: particle dependent.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 The effects of using barley and wheat grain size on the content of volatile fatty acids (VFA) in the rumen of fattening lambs (mol/100 mol)

Treatment	Acetic (A)	Butyric	Propionic (P)	Fatty acid total	A/P ratio
Barely grain	65.79	12.4	23.07	97.82	2.86
Wheat grain	64.74	12.32	23.28	97.92	2.78
SEM	0.377	0.074	0.307	0.132	0.042
P-value					
Grain type (G)	0.61	0.4319	0.6329	0.6023	0.2334
Processing type (P)	0.455	0.3597	0.8602	0.1795	0.6807
G×P	0.0133	0.3843	0.8428	0.365	0.4295
Grain effect					
Mill with a sieve No.2	65.85	12.37	23.11	98.03	2.85
Mill with a sieve No.8	65.67	12.46	23.35	97.62	2.82
No sieve	64.27	12.27	23.08	97.96	2.79
SEM	0.461	0.091	0.376	0.162	0.052
Type of processing					
Barley × sieve No.2	65.83^{a}	12.51	23.13	98.15	2.84
Barley × sieve No.8	65.52^{a}	12.47	23.09	97.41	2.85
Barley × No sieve	66.01^{a}	12.24	22.98	97.89	2.88
Wheat × sieve No.2	65.88^{a}	12.22	23.06	97.90	2.86
Wheat × sieve No.8	65.81^{a}	12.47	23.61	97.82	2.79
Wheat × No sieve	62.53b	12.30	23.19	98.02	2.70
SEM	0.653	0.028	0.531	0.228	0.073

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

Consuming fodder-based diets increases the concentration of acetic acid and concentrate-based diets also causes the production of propionic acid in the rumen of ruminant animals (McDonald *et al*. 2011). The main influencing factor in the absorption of volatile fatty acids is their concentration. Therefore, the order of their absorption is as acetate, propionate and butyrate. However, other factors such as pH, rumen surface and volume are also involved (Ghoorchi and Ghorbani, 2018). Since the pH was not affected by the diet in this experiment, it is a good reason that the amount of volatile fatty acids did not change.

Karimizadeh *et al*. (2017) regarding the use of three physical forms of concentrate (mesh, pellet and block) in the final ration of fattening lambs showed that flour (mesh) concentrate increases the amount of acetate compared to pellet, although there is a significant difference between the treatments receiving pellet and flour from they did not affect the amount of propionate and the total amount of volatile fatty acids which agreed with the results of the present study. The reason for the lack of significant difference in the amount of acetate and butyrate may be attributed to the same concentration of ammonia, which stimulates the activity of fibrolytics and ultimately causes the increase of acetate and butyrate (Michalet- Doreau *et al*. 2002). As well as it seems that the use of the same percentage of barley and wheat in the diets of the treatments is a reason for not expressing a significant difference.

Rumen pH was not affected by experimental treatments. The concentration of ammonia nitrogen (NH_3-N) in the rumen fluid of lambs fed with diets containing wheat showed a significant decrease compared to other treatments ($P<0.01$; Table 4). The amount of total forms of total bacteria and lactic acid bacteria was not affected by the type of grain and the type of processing. However, the amount of protozoa was dependent on the type of grain.

The pH of the rumen depends on the time of feeding and the amount of volatile fatty acids produced. In addition,, the amount of fibers and protein parts of food rations changed the microbial population and then the final fermentation products in the rumen, leading to changes in rumen pH and ammonia concentration. The normal range of average pH of rumen contents is reported between 6.8-6.1 by Van Soest (1994) and between 6-7 (Ghoorchi and Ghorbani, 2018).

The results of this research showed that the amount of barley and wheat grains used, as well as the type of their processing in the diets of the treatments, did not have a significant effect on the pH value of the rumen which is probably due to the ion balance between blood and rumen contents which is one of the important factors for balancing rumen pH and prevents its abnormal changes.

Protozoa are more sensitive to changes in rumen pH than the bacterial population and disappear if the pH reaches more than 7.8 or decreases to less than about 5. The most accurate method to measure rumen pH is to use an automatic pH meter whose electrodes are placed inside the rumen (McDonald *et al*. 2011).

One of the problems in optimizing rumen fermentation is the lack of synchronization between protein and energy fermentation in the rumen. Proteins and carbohydrates have components that are broken down in the rumen at slow, medium and fast rates, and each of these components feed a specific microbial population. In general, proteins are broken down much faster than carbohydrates in the rumen.

This means that the highest rumen energetic power occurs when the proteins have passed their degradability peak and as a result of this lack of synchronization of energy and nitrogen release, it leads to the production of compounds such as ammonia, methane and nitrogen (Banink *et al*. 2012).

It is expected that by feeding diets based on barley, the fermentation pattern will change and considering that reaching the energy peak occurs faster in barley to solve the problem of non-synchronization of protein and energy release for rumen microorganisms. This change in the fermentation pattern can improve energy efficiency and reduce the amount of ammonia production and urinary nitrogen excretion (Nikkhah *et al*. 2013). When grain is added to the diet of ruminants, the rumen protozoa adapt to this condition by increasing their numbers and adequately destroy the excess starch (Mackie *et al*. 1978).

The results of this research showed that the amount of protozoa was higher in treatments receiving wheat than barley. The decrease in the amount of protozoa in treatments receiving barley seeds is probably related to this point that if the grain feeding level exceeds the protozoa's ability to dispose of excess starch, the protozoa population will decrease.

Protozoa have a stabilizing effect on rumen pH due to collecting excess starch in the rumen and storing it in the form of amylopectin. Protozoa by ingesting starch granules, reduce the rate of starch degradation in the rumen. This reduces the process of rumen fermentation, which prevents a sharp drop in rumen pH (Hristov *et al*. 1999) which is consistent with the results of the present study in order to increase protozoa and not change pH.

Agreeing with the results of the present study, Karimizadeh *et al*. (2017) reported that the amount of rumen pH and ammonia nitrogen had no significant difference among the treatments receiving concentrate in the form of flour and pellets in the feeding of fattening lambs.

Table 4 The effects of using different sizes of barley and wheat grains on pH, NH_3 and the count of protozoa, *Coliforms*, lactic acid bacteria and total aerobic bacteria in the rumen of fattening lambs

Treatment	pH	NH_3 (me/dL)	Protozo	*Coliforms* (cfu mL^{-1})	Lactic acid bacteria (cfu mL^{-1})	Total aerobic bacteria (cfu mL^{-1})
Barely grain	7.09	19.2^{a}	4.20^{b}	3.37	6.45	9.31
Wheat grain	7.12	17.33^{b}	5.63^{a}	3.38	6.40	9.45
SEM	0.039	0.081	0.024	0.044	0.058	0.061
P-value						
Grain type (G)	0.6828	0.0001	0.0001	0.8512	0.5461	0.1063
Processing type (P)	0.1488	0.0012	0.0016	0.0019	0.0001	0.0001
G×P	0.9046	0.2122	0.4804	0.1599	0.6985	0.3182
Grain effect						
Mill with a sieve No.2	7.12	18.61^{a}	4.89^{b}	3.55^{a}	7.04^{a}	10.47^{a}
Mill with a sieve No.8	7.09	18.13^{b}	4.84^{b}	3.25^{b}	6.35^{b}	9.28^{b}
No sieve	7.11	18.07^{b}	5.01^{a}	3.333^{b}	5.89^{c}	8.39^{c}
SEM	0.461	0.099	0.029	0.055	0.072	0.075
Type of processing						
Barley × sieve No.2	7.09	19.50^{a}	4.20^{bc}	3.64^{a}	7.03^{a}	10.30^{a}
Barley × sieve No.8	7.07	18.96^{a}	4.10^{c}	3.20^{b}	6.36^{b}	9.27^{b}
Barley × No sieve	7.12	19.14^{a}	4.30^{b}	3.28^{b}	5.97^{bc}	8.35^{c}
Wheat × sieve No.2	7.14	17.71^{b}	5.59^{a}	3.47^{ab}	7.04^{a}	10.64^{a}
Wheat × sieve No.8	7.10	17.29b^{c}	5.58a	3.30^{ab}	6.35^{b}	9.29^{b}
Wheat × No sieve	7.11	16.98^{c}	5.71a	3.38^{ab}	5.82^{c}	8.43^{c}
SEM	0.069	0.14	0.041	0.077	0.101	0.106

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

Table 5 The effects of using different sizes of barley and wheat grains on purine derivatives (PD) and particulate material (MP) in fattening lambs

Treatment	Microbial nitrogen (g/day)	Microbial protein (g/day)	Allantoin (mmol/day)	Uric acid (mmol/day)	Xanthine + hypoxanthine (mmol/day)[1]	Excreted purine (mmol/day)	Absorbed purine (mmol/day)
Barely grain	5.59^{a}	52.15^{a}	4.80	0.68	0.74^{b}	6.06	6.91^{b}
Wheat grain	4.45^{b}	49.23^{b}	4.00	0.66	0.83^{a}	7.01	8.14^{a}
SEM	0.087	0.08	0.086	0.169	0.2	0.064	0.06
P-value							
Grain type (G)	0.0001	0.0001	0.5239	0.3686	0.0042	0.0001	0.0001
Processing type (P)	0.5229	0.0001	0.9879	0.3547	0.6319	0.0001	0.0001
P×G	0.3935	0.0001	0.3206	0.9204	0.9011	0.0029	0.0093
Grain effect							
Mill with a sieve No.2	4.99	49.81^{c}	4.05	0.645	0.783	6.26^{b}	7.13^{b}
Mill with a sieve No.8	5.16	50.73^{b}	4.04	0.675	0.775	6.48^{b}	7.35^{b}
No sieve	5.04	51.47^{a}	4.02	0.687	0.808	6.86^{a}	8.09^{a}
SEM	0.107	0.098	0.106	0.021	0.251	0.079	0.074
Type of processing							
Barley × sieve No.2	5.52^{a}	51.02^{b}	4.21	0.66	0.74	5.62^{b}	6.51^{d}
Barley × sieve No.8	5.79^{a}	52.47^{a}	4.08	0.68	0.72	5.92^{b}	6.72^{d}
Barley × No sieve	5.46^{a}	52.97^{a}	3.95	0.69	0.76	6.63^{a}	7.50^{c}
Wheat × sieve No.2	4.46^{b}	48.75^{d}	3.90	0.62	0.82	6.90^{a}	7.74^{bc}
Wheat × sieve No.8	4.54^{b}	49.00^{d}	4.00	0.67	0.83	7.04^{a}	7.99^{b}
Wheat × No sieve	4.63^{b}	49.97^{c}	4.10	0.68	0.86	7.09^{a}	8.69^{a}
SEM	0.151	0.139	0.15	0.029	0.035	0.111	0.105

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

Contrary to the present results, Karimizadeh *et al*. (2017) reported that there was no significant difference in the total rumen protozoan population in fattening lambs receiving pellet and flour concentrate, however, in the group receiving block concentrate, the total population of rumen protozoa increased significantly.

Normally, when grain is added to the diet of ruminants, the rumen protozoa adapt by increasing in number and adequately destroy the excess starch (McIntosh *et al*. 1978). Bacteria are sensitive to rumen pH changes, although they are less sensitive than protozoa (Ghoorchi and Ghorbani, 2018). According to the results of the present study, Samanta *et al*. (2003) stated that the total population of ruminal bacteria in Barbary goats receiving block concentrate and flour did not create a significant difference. In general, the lack of significant change in the rumen microbial population in ruminants and also in this study can be justified by the findings of Kudo *et al*. (1990), that the rumen microbial population is less sensitive to diet than other rumen microorganisms.

The results of measuring the excretion of purine derivatives (PD) and the amount of microbial protein (MP) produced are shown in Table 5. According to this table, the amount of excretion of each purine derivative (allantoin, uric acid, xanthine+hypoxanthine) and the total excretion and absorption of purine derivatives from urine and the amount of microbial protein produced in the rumen were significantly affected by experimental treatments ($P<0.0001$).

Urinary purine derivatives are used to estimate microbial protein in the rumen of ruminants because a correlation between the duodenal flow of nucleic acids and purine derivatives has been reported (Chen and Gomez, 1995). By increasing the level of dietary concentrate, the urinary excretion of allantoin, uric acid, total purines and milk allantoin also increases (Ghoorchi and Ghorbani, 2018). In sheep, allantoin has the largest contribution in the estimation of microbial protein and includes about 60-80% of all purine derivatives excreted in urine. Uric acid and xanthine + hypoxanthine also contain 10-30 and 5-10% of all purine derivatives, respectively. Microbial protein plays an important role in providing the nitrogen requirement of ruminants, which provides most of the amino acids required for the growth, maintenance and production of the host animal (Vaithiyanathan *et al*. 2006). In order to produce microbial protein in the rumen, two simultaneous sources of nitrogen and energy are necessary for the rumen bacteria (Ghoorchi and Ghorbani, 2018).

In addition, the rate of passage of digestible materials through the rumen is also an important factor in the production of microbial protein in the rumen, and with the increase in the rate of passage, the rate of microbial protein production increases.

Consumed diet with high concentration materials increases the rate of passage of materials through the rumen and as a result the production of microbial protein increases. Increasing the feed level causes an increase in the passage rate and, as a result, an increase in the amount of microbial protein production (Salter *et al*. 1979).

The production of microbial protein in the rumen depends on two main factors; the rate of fermentation, which regulates the amount of food available to microbes per unit of time, and the rate of passage, which causes slow-fermenting materials to be removed from the reach of microbes and older microbes to be replaced by a new microbial population. The interaction between the passage speed and the quality of the diet makes the microbial efficiency highly variable (Ghoorchi and Ghorbani, 2018).

Therefore, the use of processing on barley grain may have an effect on the production of microbial protein due to the difference in the rate of passage and fermentation in the rumen. From the hydrolysis and deamination of proteins in the rumen, ammonia and amino acids are produced, which is a source of nitrogen for microbial growth. The concentration of ammonia inside the rumen varies widely and its range is from 2 to 40 mmol/dL.

Therefore, under suitable nutritional conditions, ammonia concentration should almost always be at a level to provide optimal growth of rumen bacteria (Ghoorchi and Ghorbani, 2018).

Therefore, in general, it can be stated that increasing the amount of dense substances in the feed and consequently reducing rumen pH may reduce proteolysis in the rumen (Ghoorchi and Ghorbani, 2018). Mirmohammadi (2012) reported that the effect of the physical form factor on the excretion of purine derivatives in fattening lambs is significant only in the daily excretion of allantoin. In this experiment, the amount of indicators related to microbial protein production was affected by the physical form of the concentrate, which is consistent with the present results. In the rumen fluid, ammonia as a nitrogenous compound plays a key role in the breakdown and construction of microbial protein (McDonald *et al*. 2011).

However, the lack of energy supply for the rumen bacteria in the groups receiving flour concentrate may have caused a decrease in the production of microbial protein in the rumen and ultimately less excretion of allantoin (Davies *et al*. 2013; Kiran and Matswang, 2007). Also, the increase in ruminal digestion of starch as a result of grain processing improves the consumption of ammonia nitrogen in the rumen and subsequently reduces its excretion in the form of urea in the urine (Tothi *et al*. 2003).

Microbial protein synthesis in the rumen is significantly affected by the availability of carbohydrates and nitrogen in the rumen, and the synchronization of ruminal digestion of these two causes an increase in microbial protein synthesis.

CONCLUSION

In general, the results of this study showed that barley grain compared to wheat grain improves yield in terms of ammonia nitrogen. It also produces more microbial nitrogen and microbial protein. In general, according to the results obtained in the type of processing and mutual effects, the use of broken wheat and barley grain (mill with sieve No. 8) shows improvement in rumen parameters and Farmers and animal feed factories are advised to use broken barley or wheat grains in the production of concentrates.

ACKNOWLEDGEMENT

Thanks to the Faculty of Animal Sciences for the many cooperations and thanks to MinooSabah company and Mr. Ebrahimi for the valuable help to implement this project.

REFERENCES

Agarwal N. (2000). Estimation of fiber degrading enzyme. Pp. 278-291 in Feed Microbiology. L.C. Chaudhary, N. Agarwal, D.N. Kamra and D.K. Agarwal, Eds., CAS Animal Nutrition, Izatnagar, India.

AOAC. (2000). Official Methods of Analysis. 17th Ed. Association of Official Analytical Chemists, Arlington, Washington, DC., USA.

Banink A., Gerrits W.J., France J. and Dijkstra J. (2012). Variation in rumen fermentation and the rumen wall during the transition period in dairy cows. *J. Anim. Feed Sci. Technol.* **171,** 80-94.

Broderick G.A. and Kang J.H. (1980). Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. *J. Dairy Sci*. 64-75.

Chen X.B. and Gomes J.M. (1995). Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: An overview of the technical details. Occasional Publication of the International Feed Resources Unit, Rowett Research Institute, Bucksburn, Aberdeen, United Kingdom.

Cheng K.J. and McAllister T.A. (1997). Comport mentation in the rumen. Pp. 492-522 in The Rumen Microbial Ecosystem. P.N. Hobson and C.S. Stewart, Eds., Chapman and Hall, London, United Kingdom.

Cheng K.J., Stewart C.S., Dinsdate D. and Cosertor J.W. (1984). Electron microscopy of bacteria involved in the digestion of plant walls. *J. Anim. Feed Sci. Technol*. **10,** 93-120.

Danesh Mesgaran M., Tahmasabi A. and Vakili S.A. (2017). Digestion and Fuel Formation in Ruminants. Publications of Ferdowsi University of Mashhad, Mashhad, Iran.

Davies K.L., McKinnon J.J. and Mutsvangwa T. (2013). Effects of dietary ruminally degradable starch and ruminally degradable protein levels on urea recycling, microbial protein production, nitrogen balance, and duodenal nutrient flow in beef heifers fed low crude protein diets. *Canadian J. Anim. Sci*. **93,** 123-136.

Erwin E.S., Marco G.J. and Emery E.M. (1961). Volatile fatty acid analyses of blood rumen fluid by gas chromatography. *J. Dairy Sci*. **44,** 1768-1771.

Ghoorchi T. and Ghorbani B. (2018). Rumen microbiology. Publications of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Hristov A.N., McAllister T.A. and Cheng K.J. (1999). Effect of diet, digesta processing, freezing and extraction procedure on some polysaccharide degrading activities of ruminal contents. *Canadian J. Anim. Sci*. **79,** 73-81.

Kamra D.N., Agarwal N. and McAllister T.A. (2010). Screening for compounds enhancing fiber degradation. Pp. 85-107 in In vitro Screening of Plant Resources for Extranutritional Attributes in Ruminants: Nuclear and Related Methodologies. P.E. Vercoe, H.P.S. Makkar and A.C. Schlink, Eds., IAEA, Dordrecht, the Netherlands.

Karimizadeh E., Chaji M. and Mohammadabadi T. (2017). The effects of Journal physical form of diet on nutrient digestibility. Rumen fermentation, rumination, growth performance and protozoa population of finishing lambs. *J. Anim. Nutr*. **3(2),** 139-144.

Kiran D. and Mutsvangwa T. (2007). Effects of barley grain processing and dietary ruminally degradable protein on urea nitrogen recycling and nitrogen metabolism in growing lambs. *J. Anim. Sci*. **85,** 3391- 3399.

Kudo H., Cheng K.J., Imai S., Han S.S. and Costerton J.W. (1990). Effects of feed on the composition of the rumen ciliate protozoa. *J. Anim. Feed Sci. Technol.* **29,** 159-169.

Mackie R.I., Gilchrist F.M.C., Robberts A.M., Hannah P.E. and Schwartz H.M. (1978). Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. *J. Agric. Sci. Cambrige*. **90,** 241-254.

Makkar H.P.S. (2010). *In vitro* Screening of Feed Resources for Efficiency of Microbial Protein Synthesis. Pp. 106-144. in *In vitro* Screening of Plant Resources for Extra-Nutritional Attributes in Ruminants: Nuclear and Related Methodologies. P.E. Vercoe, H.P.S. Makkar and A.C. Schlink, Eds., Springer, the Netherlands, New York.

McDonald P., Edwards R.A., Greenhalgh J.F.D., Morgan C.A., Sinclair L.A. and Wilkinson R.G. (2011). Animal Nutrition. Longman, Harlow, United Kingdom.

McIntosh F.M., Williams P., Losa R., Wallace R.J., Beever D.A. and Newbold C.J. (2003). Effects of essential oils on ruminal microorganisms and their protein metabolism. *Appl. Environ. Microbiol*. **69,** 5011-5014.

Michalet-Doreau B., Fernandez I. and Fonty G. (2002). A comparison of enzymatic and molecular approaches to characterize the cellulolytic microbial ecosystems of the rumen and the cecum. *J. Anim. Sci*. **80,** 790-796.

Miller G.L. (1959). Modified DNS method for reducing sugars. *Anal. Chem*. **31,** 426-428.

Minato H., Endo A., Higuchi M., Ootomo Y. and Uemura T. (1966). Ecological treatise on the rumen fermentation.1.The fractionation of bacteria attached to the rumen digesta solids. *J. General Appl. Microbiol*. **12,** 39-53.

Mirmohammadi D. (2012). Investigating the effect of the physical form of feed in diets with and without litter of broiler chickens on the performance of fattening lambs. MS Thesis. Tarbiat Modares University, Tehran, Iran.

Nikkhah A., Alikhani M. and Amanlou H. (2013). Effects of feeding ground or steam-flaked broom sorghum and ground barley on performance of dairy cows in midlactation. *J. Dairy Sci*. **87(1),** 122-130.

NRC. (2007). Nutrient Requirements of Small Ruminants, Sheep, Goats, Cervids, and New World Camelids. National Academy Press, Washington, D.C., USA.

Raghuvansi S.K.S., Prasad R., Tripathi M.K., Mishra A.S., Chaturvedi O.H., Mishra A.K., Saraswat B.L. and Jakhmola R.C. (2007). Effect of complete feed blocks or grazing and supplementation of lambs on performance, nutrient utilization and rumen fermentation and rumen microbial enzymes. *Animal*. **1,** 221-226.

Salter D. N., Mesvar K. and Smith R.H. (1979). The origin incorporated into compounds in the rumen bacteria of steers given protein and urea containing diets. *British J .Nutr*. **41(1),** 197-209.

Samanta A.K., Singh K.K., Das M.M., Maity S.B. and Kundu S.S. (2003). Effect of complete feed block on nutrient utilisation and rumen fermentation in Barbari goats. *Small Rumin. Res*. **48,** 95-102.

SAS Institute. (2013). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

Shams Sharq M. and Parizadian Kavan B. (2012). Livestock and Poultry Feed and Feeding. Gorgan University of Agricultural Sciences and Natural Resources. Gorgan, Iran.

Tothi R., Lund P., Weisbjery M.R. and Hvelplund T. (2003). Effect of expander processing on fractional rate of maize and barley starch degradation in the rumen of dairy cows estimated using rumen evaluation and in situ techniques. *J. Anim. Feed Sci. Technol*. **104,** 71-94.

Vaithiyanathan S., Bhatta R., Mishra A.S., Prasad R., Verma D.L. and Singh N.P. (2006). Effect of feeding graded levels of prosopis cineraria leaves on rumen ciliate protozoa, nitrogen balance and microbial protein synthesis in lambs and kids. *J. Anim. Sci*. **133,** 177-191.

Van Soest P.J. (1994). Nutritional Ecology of the Ruminant. Cornell University Press, New York.

Van Soest P.J., Robinson J.B. and Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. **74,** 3583-3597.

Wang Y. and McAllister T.A. (2002). Rumen microbes, enzymes and feed digestion-A review. Asian-Australasian J. Anim. Sci. **15,** 1659-1676.