



Original Research Article

Performance and ruminal parameters of fattening Moghani lambs fed recycled poultry bedding

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ABSTRACT

This study investigated the effects of recycled poultry bedding (RPB) on performance and protozoa population, microbial enzyme activity and microbial protein synthesis (MPS) in rumen contents of fattening lambs. Thirty-six male Moghani lambs (31.4 ± 3.2 kg body weight) were fed iso-energetic and iso-nitrogenous diets containing 0, 70, 140 or 210 g/kg dry matter (DM) RPB in a balanced randomized design (9 lambs per treatment). Results showed that final body weight, DM intake, average daily gain and feed conversion ratio were unchanged ($P > 0.05$) by RPB inclusion. Total protozoa population and sub-family of *Entoniniinae* and *Diplodiniinae* were linearly decreased by RPB ($L, P < 0.05$). For rumen fibrolytic enzymes including carboxymethyl-cellulase, microcrystalline-cellulase and filter paper degrading activity, the extra cellular, cellular and total (extra cellular plus cellular fraction) activity were similar ($P > 0.05$) by feeding the experimental diets. Inclusion of RPB in the diet linearly decreased extra cellular and total α -amylase activity ($L, P < 0.05$), while cellular activity was unchanged ($P > 0.05$). The extra cellular activity of proteases tended to increase ($L, P = 0.07$) and their total and cellular activity increased ($P > 0.05$) in lambs fed RPB. Incorporation of RPB into the diet had no effect ($L, P > 0.05$) on urinary purine derivative excretion and MPS. In conclusion, inclusion of RPB up to 210 g/kg DM had no negative impact on performance, ruminal fibrolytic enzyme activity and MPS, while it increased rumen protease activity and decreased protozoa population in fattening Moghani lambs.

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1. Introduction

Recycled poultry bedding (RPB) is a solid waste consisting of poultry excreta (urine and feces), bedding material, feathers and spilled feed (Azizi-Shotorkhoft et al., 2013). In Iran, the production of dry RPB exceeds 1.5 million metric tons annually (Statistical Centre of Iran, 2013). The proper use of inexpensive agro-industrial by-products such as broiler litter (BL) is important to improve livestock production (Negesse et al., 2007). The fact that it

is cheaper, with high crude protein (CP) content (150 to 350 g/kg DM; Obeidat et al., 2011) and some required minerals (Rankins et al., 2002), suggests the potential value of RPB as a ruminant feed. Moreover, the use of RPB in ruminant feeding has led to decreased production costs and greater total production, and is a mean of disposing of waste in an environmentally-friendly way (Elemam et al., 2009). However, owing to the presence of pathogenic bacteria it should be processed before offering to ruminants (McCaskey and Anthony, 1979). Most of the nitrogen (N) in RPB is in the form of non-protein nitrogen (NPN) which can be easily and rapidly degraded in the rumen (Animut et al., 2002). Ruminal degradability of uric acid (the main component of NPN in RPB) has been estimated at 960 g/kg (Zinn et al., 1996). Uric acid is broken down in the rumen at a slower rate compared with urea and consequently most of the ammonia is captured by the rumen microorganisms (Oltjen et al., 1968).

Studies conducted on the use of poultry litter as a feed ingredient have mainly focused on the productive performance of livestock. Different processed RPB has been successfully used in

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ruminant diets (Negesse et al., 2007; Azizi-Shotorkhoft et al., 2013; Baluch-Gharaei et al., 2015). Recycled poultry bedding contains copper (Cu) which has an inhibitory effect on the activity of ruminal protozoa (Kisidayová et al., 2000). For example, Vardyova et al. (2006) found that long-term feeding of Cu-containing pasture to sheep significantly decreased the total population of rumen ciliate protozoa. Recently, Baluch-Gharaei et al. (2015) also reported that the total population of rumen protozoa and sub-family of *Entoni-niinae* were significantly reduced as the level of deep-stack RPB increased in the diet of sheep as compared with a diet free of poultry litter. Therefore, feeding high levels of RPB to ruminants may change the population of rumen microorganisms, particularly protozoa, and consequently their hydrolytic enzyme activity.

To our knowledge, no literature data is available on the effect of heat-processed RPB in pellet-form diets on rumen hydrolytic enzyme activity and microbial protein synthesis (MPS). Therefore, this experiment was conducted to evaluate the effect of feeding different levels of RPB in pellet-form on performance, ruminal protozoa population, rumen microbial activity (measured as the activities of rumen hydrolytic enzymes) and MPS in fattening Moghani lambs.

2. Materials and methods

2.1. Heat-processed recycled poultry bedding

The RPB (large-scale, commercially processed at 80 °C for 20 min) which was obtained from the manufacturer (Sabzevar, Khorasan Province, Iran), contained a mixture of bird excreta, feather, spilled feed, cardboard and buttonwood shavings. To remove pathogenic bacteria and improve poultry litter quality, the material was processed under an indirect thermal operation in a special hot tank (with a capacity of 5 tons) for 20 min. The tank was comprised of 2 walls between which a hot steam (80 °C) was directed. Finally, the produced RPB was ground to pass through a 6-mm sieve by the factory.

2.2. Animal study

Animal care and use were conducted in accordance with practices outlined in the Guide for the Care and Use of Agriculture Animals in Agriculture Research and Teaching. Thirty-six male fat-tailed Moghani lambs (135 ± 15 days of age and initial body weight of 31.4 ± 3.2 kg) were randomly assigned to 4 groups of 9 lambs each in a balanced randomized design. Animals were housed in concrete floor pens (1.2 m × 1.1 m) in a closed shed building. The feeding trial lasted 84 days preceded by a 14-day adaptation period to the pens, diets and experimental conditions (totally 98 days). At the beginning of the adaptation period, all animals were treated for external (1 mL of Azantole 10% per 7 L of water, as spraying method; Bayer, Germany) and internal (Triclabendazole + levamisole, 12 mL per lamb; Darou-Pakhsh Co., Iran) parasites and vaccinated against enterotoxaemia (3 mL per lamb; Razi Vaccine and Serum Research Institute, Iran). Additionally, over the adaptation period, the amount of dietary RPB was gradually elevated for each lamb to reach levels considered as the experimental levels in the dietary treatments.

Four iso-energetic and iso-nitrogenous diets (Table 1) with different levels of RPB (0, 70, 140 or 210 g/kg DM) were formulated to meet the nutrient requirements of growing lambs (NRC, 1985). Experimental diets were pelletized under heat (50 to 60 °C) and pressure (between rollers and flat die) in pellet-form and of cylindrical shape (diameter 15 mm; length 25 mm) using a pelleting machine (Pishgam Industrial Company, Iran). Pelleted complete diets were individually offered *ad libitum* 3 times a day (at 08:00, 14:00 and 20:00) to ensure a level of approximately 5% feed refusal.

Table 1
Ingredients and chemical composition (g/kg DM) of the experimental diets.

Item	Experimental diets ¹			
	RPB0	RPB70	RPB140	RPB210
Ingredients				
Alfalfa hay	150	140	140	120
Wheat straw	72.5	72.5	64.2	65.0
Wheat bran	157	110	75	35
Barley grain, ground, dry	260	280	285	270
Maize grain, ground, dry	70.0	75.0	75.0	100
Wheat grain, ground, dry	135	135	145	150
Sugar beet pulp, dry	100	80.0	50.0	40.0
Soybean meal	40.0	22.5	15.0	0.0
Processed recycled poultry bedding	0.0	70.0	140	210
Urea	2.9	2.5	0.30	0.0
Sodium bicarbonate	2.95	3.0	3.0	3.0
Limestone	4.9	4.5	2.5	2.0
Minerals and vitamins ²	2.5	2.5	2.5	2.5
NaCl	2.5	2.5	2.5	2.5
Chemical composition				
Dry matter, g/kg fresh weight	907	910	914	915
Crude protein	136	136	136	136
Metabolizable protein	90.5	89.6	89.4	88.7
Ash-free neutral detergent fiber	336	339	340	339
Ash-free acid detergent fiber	163	159	154	147
Non fiber carbohydrates	451	448	441	439
Starch	370	364	360	355
Ether extract	20.9	19.1	18.6	18.1
Ash	52	59	67	74
Ca	5.40	5.55	5.20	5.30
P	4.10	4.15	4.30	4.40
Cu, mg/kg DM	6.85	9.60	12.5	15.3
Metabolizable energy, MJ/kg DM	10.63	10.63	10.63	10.63

¹ Diets were: recycled poultry bedding (RPB) included in the diets at 0 (RPB0), 70 (RPB70), 140 (RPB140) or 210 (RPB210) g/kg dietary dry matter.

² Contained (per kg): 99.2 mg Mn, 50.0 mg Fe, 84.7 mg Zn, 1.0 mg Cu, 1.0 mg I, 0.2 mg Se, 9,000 IU vitamin A, 2,000 IU vitamin D, and 18.0 IU vitamin E (Roshd Daneh Co., Iran).

Animals had free access to fresh water. Samples of feed and orts were collected daily and bulked for further analyses. Representative samples were pooled to obtain a composite per lamb within the treatment. All animals were individually weighed every 21 day at 08:00 after a 16-h feed deprivation. For each lamb, average daily gain (ADG) was calculated by linear regression analysis of body weight vs. time. Feed conversion ratio (FCR) was calculated as g daily dry matter intake (DMI) per g ADG.

2.3. Rumen liquor sampling

On day 75 of the trial, rumen liquor (RL) samples (70 to 80 mL) were taken from 5 lambs in each treatment at 3 h after feeding using a flexible polyvinyl chloride stomach tube. The first 10 to 20 mL portion of collected samples was discarded to avoid saliva contamination (Jasmin et al., 2011).

2.4. Protozoa population

Total numbers and generic composition of rumen ciliate protozoa were determined according to the methods described by Dehority (2003). For this purpose, a sub-sample of RL (2 mL) was pipetted into screw-capped test tubes containing 5 mL of formalinized physiological saline solution (20 mL formaldehyde in 100 mL saline containing 0.85 g sodium chloride in 100 mL distilled water). Then, 2 drops of brilliant green dye (2 g brilliant green and 2 mL glacial acetic diluted to 100 mL with distilled water) were added to the test tube, mixed thoroughly and allowed to stand overnight at room temperature. Total and differential counts of protozoa were made in 30 microscopic fields at a magnification of × 20 in a haemocytometer (Neubauer improved, Marienfeld, Germany).

2.5. Fractionation of rumen liquor and enzymes extraction

For estimation of fiber and protein degrading enzyme activities, rumen contents were immediately transferred to the laboratory in a preheated (39 °C) insulated thermos flask under anaerobic condition. The microbial enzymes in different fractions of RL samples were extracted as described by Hristov et al. (1999). Approximately 50 mL of rumen contents were strained (SRL) through 4 layers of cheese cloth and divided into 2 fractions, extra-cellular (liquid portion) and cellular (cell suspended freely in the liquid portion of RL). About 10 mL of SRL was centrifuged at $450 \times g$ for 5 min at 37 °C and the pellet was considered as the protozoal fraction (PF). The supernatant was centrifuged at $27,000 \times g$ for 20 min at 4 °C and the pellet was considered to be the bacterial fraction (BF). After centrifuging, clear supernatant was used as the source of extra-cellular fraction. To extract enzymes from the cellular fraction, pellets containing microbial biomass (PF plus BF) were suspended in a volume of 0.1 mol/L phosphate buffer (pH 6.8) equal to extra-cellular liquid. Lysozyme solution (4 g/L) and carbon tetrachloride were separately added to the suspension at the rate of 5 mL/30 mL cell suspension. Lysozyme treatment was followed by sonication in an ice bath for 6 min with a 30 s pulse rate and power supply of 0.5. The suspension was then incubated for 3 h at 39 °C and centrifuged at $27,000 \times g$ for 30 min at 4 °C. Supernatant was collected and used as an enzyme source for the cellular fraction.

2.5.1. Assay of enzyme activity

Activity of microbial enzymes in the different fractions of rumen contents was determined as described in detail by Agarwal (2000). To estimate carboxymethyl-cellulase (CMCase) activity, the reaction mixture contained 1 mL phosphate buffer (0.1 mol/L, pH 6.8), 0.5 mL strained SRL, 0.5 mL carboxymethyl-cellulose (carboxymethyl cellulose; 10 mg/mL for CMCase) and was incubated for 60 min at 39 °C. To determine the activity of microcrystalline-cellulase (MCCase) the reaction mixture 1 mL of microcrystalline-cellulose (10 mg/mL) was used as the reaction substrate for 60 min at 39 °C. The reaction time was comparable for assay of enzymatic activity with the carboxymethyl-cellulase substrate. Reaction mixture to determine activity of α -amylase activity consisted of 1 mL of 0.1 mol/L phosphate buffer (pH 6.8), 0.5 mL of SRL, 0.5 mL of starch solution (1%, as the reaction substrate) which was incubated for 60 min at 39 °C. For filter paper degrading (FPD) activity, the assay mixture contained 1 mL phosphate buffer (0.1 mol/L, pH 6.8), 0.5 g Whatman No. 1 filter paper and 1 mL SRL which was incubated for 60 min at 39 °C. All reactions were stopped by the addition of dinitro-salicylic acid reagent. The glucose produced in all reactions was estimated according to the method described by Miller (1959) with glucose as standard. The activities of CMCase, MCCase, FPD activity and α -amylase were then calculated considering that one unit of enzyme produced 1 μ mol glucose per hour per mL from the degradation of respective substrates.

For estimation of the protease activity (μ g hydrolyzed protein/h per mL), the reaction mixture which contained 1 mL phosphate buffer (0.1 mol/L, pH 6.8), 0.25 mL SRL and 0.25 mL casein (2.5 mg/mL) was incubated for 2 h at 39 °C. The reaction was stopped by adding trichloroacetic acid (200 mL/L) and protein was determined according to the procedure of Lowry et al. (1951).

2.6. Ruminal synthesis of microbial nitrogen

To measure urinary purine derivatives (PD), on day 60 of the trial, 5 lambs in each treatment group were individually housed in metabolic cages. Lambs were allowed a period of 10 days for adaptation to the crates followed by a collection period of 7 days.

During the collection period, urine samples from each animal on each treatment were collected in a vessel containing 100 mL of sulfuric acid solution (containing 10 mL of concentrated sulfuric acid in 100 mL of distilled water) to maintain final pH below 3, which was placed below the urine outlet in the metabolic cages. Collected urine was weighed. To prevent the precipitation (particularly of uric acid) of PD in urine during storage, a sub-sample of 10% was diluted 3 times and then stored at -20 °C for the estimation of PD (Chen and Gomes, 1995). Representative samples collected from each lamb over 7 days were pooled to obtain one final composite sample for analysis for each animal. Urinary PD was estimated by the spectrophotometric methods as described by Chen and Gomes (1995). Allantoin was determined in urine by the colorimetric method at 522 nm after its conversion to phenyl hydrazone. The sum of xanthine and hypoxanthine were calculated by their conversion to uric acid with xanthine oxidase (Sigma; Catalog No. X-1875, 5 Units, Germany), with subsequent optical density at 293 nm. Uric acid was measured from the reduction in optical density at 293 nm following degradation of uric acid to allantoin with uricase (Sigma; Product No. U-9375, Germany). Based on the Chen and Gomes (1995) technique, the non-linear equation to describe the quantitative relationship between absorption of microbial purines and excretion of PD in urine is:

$$Y = 0.84X + (0.150W^{0.75}e^{-0.25X}).$$

where Y is the daily urinary PD excretion as mmol/d, X the daily absorbed exogenous purines as mmol/d, and $W^{0.75}$ the metabolic body weight (kg) of the animal.

The calculation of X from Y based on the above equation was performed by means of the Newton–Raphson iteration process as given below:

$$X_{(n+1)} = X_n - \frac{f(X_n)}{f'(X_n)}$$

where $f(X) = 0.84X + 0.150W^{0.75}e^{-0.25X} - Y$ and the derivatives of $f'(X) = (0.84 - 0.038W^{0.75}e^{-0.25X})$.

Produced microbial N was then estimated by the following equation:

$$\text{Microbial N(g/d)} = \frac{X \left(\frac{\text{mmol}}{\text{d}} \right) \times 70}{0.116 \times 0.83 \times 1,000} = 0.727X.$$

where 70 is the N content of purines (mg N/mmol), 0.116 is the mean ratio of purine-N to total-N in mixed rumen microbes and 0.83 is the assumed digestibility of microbial purines (Chen and Gomes, 1995).

2.7. Analytical methods

Samples of heat-processed RPB and experimental diets were oven-dried at 60 °C to reach a constant weight, and then ground to pass a 1 mm sieve (Wiley mill, Swedesboro, USA). Dry matter (#930.15), N (#984.13), ash (#924.05) and ether extract (EE, #920.39) were measured using standard methods as described in AOAC (1990). Starch content of the RPB was analysed according to AOAC (#996.11; 1990). Ash-free neutral detergent fiber (NDFom) and ash-free acid detergent fiber (ADFom) were determined and expressed exclusive of residual ash based on the methods of Van Soest et al. (1991). Lignin (sa) was determined by solubilization of cellulose with 720 g/kg sulfuric acid, according to the procedure

described by Robertson and Van Soest (1981). Non-fiber carbohydrate (NFC) was calculated as:

$$\text{NFC (g/kg DM)} = 1,000 - (\text{NDFom g/kg DM} + \text{CP g/kg DM} + \text{EE g/kg DM} + \text{ash g/kg DM}).$$

Calcium, Mg, Cu, Mn, Fe and Zn were measured by atomic absorption (Perkin Elmer, Model PinAALce 900z, USA), Na and K by flame emission spectrometer (Jenway, Model PFP7, UK; Temminghoff and Houba, 2004) and P by the vanadate/molybdate (yellow) method (Chapman and Pratt, 1961). Metabolizable protein content (MP) in the RPB was measured using the *in situ* degradability technique (AFRC, 1992). Metabolizable energy (ME) value of RPB was calculated using the equation described by Deshck et al. (1998). The *in vitro* digestible organic matter (DOM) (as g/kg DM), which was determined using rumen fluid and pepsin in 2 stages (Tilley and Terry, 1963), was used to calculate ME content of RPB (Deshck et al., 1998):

$$\text{ME (MJ/kg DM)} = \text{DOM (g/g DM)} \times 18.5 (\text{MJ/kg DOM}) \times 0.80.$$

2.8. Statistical analysis

Data were analyzed using the General Linear Model (GLM) procedures of SAS (SAS Institute, 2001), as a balanced randomized design. The statistical model was:

$$Y_{ij} = \mu + T_i + e_{ij},$$

where Y_{ij} is observation, μ is the general mean, T_i is the effect of RPB and e_{ij} is the standard error of term. All data (i.e., performance, rumen protozoa population, enzymatic activity and MPS) were analyzed for linear (L) or quadratic (Q) responses to RPB level using orthogonal contrasts of SAS. Significance was declared at $P \leq 0.05$.

3. Results

The chemical composition, ME value and mineral contents of RPB are shown in Table 2.

Table 2
Chemical composition, metabolizable energy (ME) and mineral content (g/kg DM) of recycled poultry bedding (RPB).

Item	Content
Dry matter, g/kg fresh weight	930
Crude protein	238
Non-protein nitrogen, g/kg total N	451
Metabolizable protein	145
Ether extract	22.3
Ash-free neutral detergent fiber	353
Ash-free acid detergent fiber	185
Lignin (sa)	75.0
Metabolizable energy, MJ/kg DM	9.3
Non-fiber carbohydrates	203
Starch	19.2
Ash	184
Ca	16.4
P	9.4
Mg	5.7
K	7.3
Fe	1.03
Na, mg/kg DM	430
Mn, mg/kg DM	324
Cu, mg/kg DM	52
Zn, mg/kg DM	345

3.1. Performance

Increasing the level of RPB in the diet had no effect ($P > 0.05$) on performance parameters including the final body weight, DMI, ADG and FCR (Table 3).

3.2. Ruminal protozoa population

Increasing amounts of RPB in the diet (Table 4) decreased ($L, P < 0.05$) the total protozoa population and the sub-family of *Entoniniinae* and *Diplodiniinae*, while *Isotricha*, *Dasytricha* and *Ophryoscoleciniinae* sub-families were unchanged ($P > 0.05$).

3.3. Rumen enzymatic activity

For fibrolytic enzymes including CMCase, MCCase and FPD, the extra cellular, cellular and total (extra cellular plus cellular fraction) activity were unchanged by feeding experimental diets ($P > 0.05$; Table 5). For α -amylase, extra cellular and total activity decreased ($P < 0.05$; Table 5) while cellular activity remained unchanged ($P > 0.05$) as dietary levels of RPB increased. However, by adding RPB to the diet, the extra cellular activity of proteases tended to increase ($L, P = 0.07$; Table 5) while their total and cellular activity increased significantly ($P < 0.05$).

3.4. Purine derivatives and MPS

Dietary inclusion of RPB had no effect ($L, P > 0.05$) on the urinary excretion of allantoin, uric acid and xanthine plus hypoxanthine, total PD excreted and total PD absorbed (Table 6). Additionally, MPS was unchanged ($L, P > 0.05$) by feeding the experimental diets.

4. Discussion

4.1. Feed intake and performance

The lack of DMI differences among the animals was related to the relatively similar chemical composition and physical form (all experimental diets were in pellet form) of the diets, because composition and physical characteristics of the feed could change feed intake (Baumont, 1996). Similar ADG among the treatments was parallel to comparable DMI of the lambs, since growth rate greatly depends on optimizing feed intake and feed conversion to gain (Olfaz et al., 2005). Experimental diets had no effect on FCR due to the relatively similar DMI and ADG among the lambs. Consistent with our results, Obeidat et al. (2011) reported no differences in FBW, DMI, ADG and FCR when autoclaved poultry litter was fed to Awassi lambs at levels of 0, 100 or 200 g/kg DM. However, in the study of Elemam et al. (2009), DMI increased in lambs fed a diet containing 450 g/kg sun-dried poultry litter compared with those fed 0, 150 or 300 g/kg of poultry litter. In another work,

Table 3
Effect of recycled poultry bedding (RPB) on performance parameters in fattening Moghani lambs.

Item	Levels of RPB in diet, g/kg DM				SEM	P-value	
	0	70	140	210		L ¹	Q ¹
Initial body weight, kg	30.7	31.7	31.1	32.0	1.16	0.89	0.87
Final body weight, kg	51.3	50.9	50.4	52.5	0.71	0.86	0.48
Dry matter intake, kg/day	1.70	1.78	1.62	1.70	0.024	0.12	0.18
Average daily gain, g/day	245	229	230	244	5.4	0.87	0.60
Feed conversion ratio	6.93	7.75	7.04	6.97	0.42	0.23	0.34

SEM = standard error of means.

¹ L, linear; Q, quadratic.

Table 4
Effect of recycled poultry bedding (RPB) on rumen protozoa concentration (\log_{10}/g digesta) in fattening Moghani lambs.

Item	Levels of RPB in diet, g/kg DM				SEM	P-value	
	0	70	140	210		L ¹	Q ¹
Total protozoa	5.66	5.53	5.40	5.32	0.046	<0.01	0.54
<i>Isotricha</i>	2.22	2.19	2.20	2.15	0.038	0.24	0.72
<i>Dasytricha</i>	1.68	1.64	1.59	1.56	0.058	0.15	0.88
<i>Entoniniinae</i>	4.80	4.63	4.54	4.43	0.048	<0.01	0.55
<i>Diplodiniinae</i>	2.76	2.66	2.56	2.40	0.050	<0.01	0.60
<i>Ophryoscoleciniinae</i>	1.12	1.11	1.04	1.06	0.038	0.14	0.79

SEM = standard error of means.

¹ L, linear; Q, quadratic.

Table 5
Effect of recycled poultry bedding (RPB) on the rumen hydrolytic enzyme activity (U/mL per min or as stated) in fattening Moghani lambs.

Item	Levels of RPB in diet, g/kg DM				SEM	P-value	
	0	70	140	210		L ¹	Q ¹
Carboxymethyl cellulase							
Extra cellular	5.22	4.70	4.44	4.56	0.779	0.52	0.69
Cellular	7.18	7.83	7.73	7.05	1.02	0.91	0.52
Total	12.4	12.5	12.2	11.6	1.19	0.61	0.78
Microcrystalline cellulase							
Extra cellular	3.08	3.32	4.17	3.31	0.798	0.67	0.49
Cellular	5.66	4.85	5.69	5.82	0.64	0.65	0.48
Total	8.74	8.17	8.85	9.12	1.18	0.74	0.72
Filter paper degrading activity							
Extra cellular	9.12	9.27	8.87	8.82	1.11	0.79	0.93
Cellular	11.0	10.8	10.5	10.4	1.01	0.65	0.94
Total	20.2	20.1	19.4	19.2	1.98	0.70	0.95
α -amylase							
Extra cellular	7.53	6.36	5.12	4.07	0.28	<0.01	0.85
Cellular	20.5	19.8	20.2	19.4	1.43	0.67	0.97
Total	28.0	26.2	25.3	23.5	1.49	0.04	0.88
Protease, μ g hydrolyzed protein/mL per min							
Extra cellular	105	115	109	117	3.32	0.07	0.76
Cellular	253	271	281	291	6.11	<0.01	0.49
Total	358	386	389	407	7.58	<0.01	0.49

SEM = standard error of means.

¹ L, linear; Q, quadratic.

Table 6
Effect of recycled poultry bedding (RPB) on urinary purine derivatives (mmol/d) excretion and ruminal microbial protein synthesis in fattening Moghani lambs.

Item	Levels of RPB in diet, g/kg DM				SEM	P-value	
	0	70	140	210		L ¹	Q ¹
Purine derivatives							
Allantoin	12.4	12.1	11.9	11.8	0.97	0.68	0.93
Uric acid	1.82	1.79	1.74	1.64	0.09	0.13	0.67
Xanthine + hypoxanthine	0.70	0.81	0.76	0.78	0.11	0.71	0.70
Total purine derivatives excreted	14.9	14.7	14.4	14.2	1.04	0.21	0.92
Total purine derivatives absorbed	16.9	16.8	16.4	16.2	1.25	0.23	0.91
Microbial protein							
MPS, g/day	76.9	76.2	74.5	73.6	5.66	0.25	0.92
MPS, g/kg DOMI	63.4	64.9	57.2	58.7	4.40	0.30	0.87
MPS, g/kg DOMR	41.9	42.5	37.2	38.2	2.85	0.31	0.86

MPS = microbial protein synthesis; DOMI = digestible organic matter intake; DOMR = digestible organic matter in the rumen; SEM = standard error of means.

¹ L, linear; Q, quadratic.

ADG was unchanged when sheep were fed diets containing sun-dried RPB at levels of 0, 280 or 560 g/kg (Mavimbela et al., 2000). Recently, Mirmohammadi et al. (2015) showed that feeding RPB up to 200 g/kg DM in the diet of fattening lambs was possible without compromising growth performance and animal health.

4.2. Protozoa population

As reported by Kišidayová et al. (2000) and Vardyova et al. (2006), the reduction of protozoa in the rumen of lambs fed the experimental diets containing RPB compared with control lambs might be associated with higher Cu content in the diets with increasing levels of RPB. They found that Cu has a toxic effect on ruminal protozoa population in sheep. Additionally, Baluch-Gharaei et al. (2015) reported that total population of rumen protozoa and sub-family of *Entoniniinae* reduced in sheep fed diets containing RPB compared with those fed control diet. It should be stated that in our work, Cu concentration of diets containing higher levels of RPB (i.e., 140 and 210 g/kg) were greater than recommended requirements (i.e., 7 to 11 mg/kg DM) by the NRC (1985) for sheep.

4.3. Enzymatic assay

Differences among diets in terms of rumen enzyme activity could reflect the change in microbial communities according to the type of ration offered to animal, and thus the change in enzyme profiles (Kamra et al., 2010).

In agreement with our findings, Agarwal et al. (2000) also showed that cellular activity of rumen microbial enzymes (CMCase, MCCase, FPD activity, α -amylase and proteases) in Murrah buffalo was higher compared with the extra cellular fraction. The lower fiber degrading enzyme values in the extra cellular fraction was expected, because these enzymes are bound to the cellular coat and only a minor quantity is released into the liquid fraction due to mechanical injury or disintegration of the fiber-degrading microbes (Forsberg and Lam, 1977; Agarwal et al., 2000).

In the present study, despite a decrease in protozoa population (Table 4) by increasing the level of RPB in the ration, fibrolytic activity of rumen microbes (i.e., CMCase, MCCase and FPD) was unchanged among animals. It should be noted that in all the rumen liquor fractions from our work, we measured fibrolytic enzyme activity excreted by a mixture of rumen microbes, not solely for rumen protozoa. Therefore, this makes it difficult to interpret the effect of Cu content of the diets on the fibrolytic enzymes secreted by protozoa.

The α -amylase plays an important role in the rumen because it hydrolyzes dietary starch (Engvall, 1980). The higher α -amylase activity in the control diet compared with other diets may be because of its higher available starch and non-fiber carbohydrates (Table 1) for amyolytic microorganisms. Nasr (1950) also showed a positive correlation between rumen α -amylase activity and amount of starch fed to sheep. On the other hand, the reduction in α -amylase activity by RPB supplementation could partly be associated to a decreased rumen protozoa population due to the toxic effect of Cu in the diets containing RPB (Baluch-Gharaei et al., 2015). It is shown that amyolytic and cellulolytic bacteria rapidly colonize soluble and easily degradable carbohydrates, and this facilitates the availability of specific substrates for the renewed growth of other fibrolytic bacteria (Azizi-Shotorkhoft et al., 2016). However, in our study the greater α -amylase activity in the diet free of RPB did not enhance fibrolytic enzyme activity by feeding the former diet.

Up to 70% of dietary protein is degraded by the combined action of microbial proteases and peptidases (Selinger et al., 1996). The increased proteolytic activity in lambs fed RPB diet compared with those fed control diet was probably because of high NPN supplied by RPB (Table 2), which is quickly degraded by rumen microorganisms (Animut et al., 2002).

4.4. Purine derivatives and MPS

As described by Chen and Gomes (1995), allantoin, uric acid and xanthine plus hypoxanthine were in the normal ranges of 60 to 80,

10 to 30 and 5 to 10, respectively (expressed as percent of total PD excretion in sheep). Similar ruminal MPS by feeding the experimental diets containing different levels of RPB was possibly due to the similar CP, MP and ME contents of diets as well as similar DMI among animals. Microbial production in the rumen is influenced by many dietary and animal factors such as N concentrations, N sources, rates of N and carbohydrate degradation, carbohydrate in the diets, DMI as well as synchronization of supplying N and energy (Karsli and Russell, 2002).

5. Conclusion

Feeding Moghani lamb with diet containing heat-processed RPB up to 210 g/kg of dietary DM had no negative effects on animal health, performance, rumen fibrolytic enzyme activity or MPS, while RPB increased rumen protease activity and decreased the protozoa population. Heat-processed RPB as a cheap and safe feedstuff could be used as a protein supplement in fattening lambs diets.

Conflict of interest

The authors declare that they have no conflict of interest.

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