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Full Length Research Paper

Interaction between barley grain processing and source of dietary protein on nutrients digestibility, rumen pH and ammonia concentration in sheep

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The effects of barley grain processing (whole vs. cracked) combined with two different protein sources (urea vs. soybean meal) on nutrients digestibility, rumen pH and ammonia concentration in sheep were investigated. Four permanently rumen fistulated Mehraban sheep were allocated in a 4×4 Latin square design. Four different experimental diets were used: 1) whole barley grain with urea (WB-U), 2) cracked barley grain with urea (CB-U), 3) whole barley grain with soybean meal (WB-S), and 4) cracked barley grain with soybean meal (CB-S). The study lasted 56 days which consisted of four 14-day experimental periods. The results showed that DM, OM and CP digestibility were not affected by treatments. However, digestibility of neutral detergent fiber (NDF) was affected by treatments with the highest fiber digestibility obtained for soybean fed animals. Although, average rumen pH was not different among treatments, determined pH on 2 h after feeding differed among treatments (P < 0.05). The results for rumen ammonia concentration was clarified that urea supplemented diets increased ammonia concentration compared to soybean meal fed diets. The rumen ammonia concentration for treatments WB-U and CB-U increased dramatically for both 2 and 3 h after feeding. The results of the present study showed that supplementation of sheep diet with soybean meal has significant effect on fiber digestion compared to urea supplementation and the increased ammonia concentration which was caused by urea supplementation may not be a valuable factor affecting nutrients digestibility in sheep nutrition.

Key words: Barley grain processing, protein source, sheep.

INTRODUCTION

The synchronization of the ruminal degradation rate of carbohydrates (CHO) and crude protein (CP) has been proposed as a method of improving nitrogen use efficiency and improving animal production (Cole and Todd, 2008). A number of studies have been carried out to investigate the synchronization of CHO and CP degradation in dairy cows (Robinson and McQueen, 1994; Shabi et al., 1998; Rotger et al., 2006). It is revealed in dairy cows that dietary manipulation such as increasing the amounts of ruminally fermentable carbohydrate, or grain processing can potentially increase urea-N transfer to the rumen and enhance animal performance (Kyriazakis and Oldham, 1997). Feeding a diet that is balanced for optimal release of energy and nitrogen in the rumen may provide the ruminant with valuable end products (Russell et al., 1992). Utilization of carbohydrates and nitrogen source by rumen microorganisms has potential to be altered by

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Abbreviations: WB, Whole barley; **CB,** cracked barley; **S,** soybean meal; **U,** urea.

grain processing (Theurer, 1986; Mc Carthy et al., 1989; Milton et al., 1997). On the other hand, dietary protein source also play a fundamental role in rumen metabolism in ruminant nutrition which could alter rumen fermentation pattern (Hunber and Kung, 1981). Two main dietary protein sources in ruminant nutrition are non protein nitrogen (NPN) and true protein (Hunber and Kung, 1981). Brito and Broderick (2007) compared urea with soybean meal in dairy cow nutrition. They reported the lowest efficiency for urea supplementation compared with other protein sources. Matras et al. (1991) stated that lambs fed barley grain combined with urea showed the best performance compared with the combination of sorghum and urea or sorghum with gluten meal. Different end products of protein sources degradation could be the source of nitrogen for the different rumen microbes. The cellulolytic bacteria require nitrogen in the form of ammonia and do not utilize amino acids or peptides as well as the non-structural bacteria (Reynal et al., 2007). It is determined that addition of true protein sources which produce amino acids and peptides in rumen fluid can improve digestion of organic matter, dry matter and fiber (Griswold et al., 2003). By considering the optimum concentration of rumen ammonia, Reynal et al. (2007) clarified that the alteration of proteolysis and peptide formation in the rumen by dietary manipulation such as true protein feeding may have nutritional benefits for both ruminal microbes and the host animal. Previous studies evaluated the different processing effects of cereals with or without the combination of different sources of proteins in dairy cows nutrition (Hall, 1973; Yang et al., 2001). However, limited studies have been performed regarding these aims in sheep nutrition. Therefore, the effects of different grain processing with different protein sources need more investigations to be carried out. In the present study, the effects of cracked versus whole barley grain accompanied with two different protein sources (soybean meal versus urea) on some rumen parameters and nutrients digestibility in sheep nutrition, were evaluated.

MATERIALS AND METHODS

Animals, treatments and management

Four ruminally cannulated Mehraban male sheep averaging BW 36.5 kg (±0.5) were assigned to a 4 × 4 Latin square with 14-day periods, that the first 7 days is considered as adaptation period and the last 7 days considered as sample collection period. The experimental diet and composition are given in Table 1. Two different kinds of barley grain (whole vs. cracked) were accompanied with two different sources of protein (urea vs. soybean meal) to make four different treatments as follows: T1 = whole barley grain with urea (WB-U); T2 = cracked barley grain with urea (CB-U); T3 = whole barley grain with soybean meal (WB-S), and T4 = cracked barley grain with soybean meal (CB-S). The sheep were kept in individual stanchions and were fed twice daily at 0800 and 1700 h. The animals had free access to water and salt block. Orts were collected and weights recorded once daily at 0700 h and the feeding rate were adjusted daily to yield orts of about 5 to 10% intake.

Experimental procedures and chemical analyses

The dry matter (DM) was determined in weekly composites of corn silage and alfalfa by drying at 60°C for 48 h (AOAC, 1990). Intake of DM was computed based on the 60°C DM determinations for total mixed ration (TMR) and orts. After drying, ingredients and TMR were ground through a 1 mm screen (Wiley mill), and period composites were prepared by mixing equal DM. Composite samples were analyzed for total nitrogen, DM, ash and organic matter (AOAC, 1990), sequentially for NDF and ADF (Van Soest et al., 1991). Fecal samples were collected each day during the days of collection period in each experimental period. Samples were composite per sheep per period and then oven-dried at 55 for 72 h and then ground through a 1 mm sieve. After analyzing the fecal samples for nutrients, total tract apparent digestibility of nutrients was determined by using acid insoluble ash as an internal marker (Van Keulen and Young, 1977).

Samples of rumen fluid were collected at 0 (before morning feeding) and 1, 2, 3, 5 and 7 h after feeding. Samples of rumen fluid were strained through four layers of cheesecloth, and pH measured immediately. For determination of ammonia nitrogen (NH3-N) in rumen fluid, 50 ml subsamples of strained rumen fluid were preserved by addition of 1 ml sulfuric acid 97% and stored at –20°C. Just before analysis, samples were thawed and analyzed for ammonia (Crooke and Simpson, 1971).

Statistical analysis

Data were analyzed using Proc Mixed in SAS (version 8.1; SAS institute Inc., Cary, NC). The following model was fitted to all variables which did not have repeated measurements over time:

$$Y_{ijk} = \mu + P_i + S_j + T_k + \varepsilon_{ijk}$$

Where Y_{ijk} is the dependent variable, μ is the overall mean, P_i is the effect of period i, S_j is the effect of sheep j, T_k is the effect of treatment k, and ε_{ijk} is the residual error.

The following model was used for ruminal variables for which there were repeated measurements over time (pH, NH_3-N):

$$Y_{ijkl} = \mu + P_i + S_j + T_k + Z_l + ZT_{kl} + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the dependent variable, μ is the overall mean, P_i is the effect of period i, S_j is the effect of sheep j, T_k is the effect of treatment k, Z_l is the effect of time l, ZT_{kl} is the interaction between time l and treatment k and ε_{ijkl} is the residual error. All terms were considered fixed except for ε_{ijkl} which was considered random. Differences between least square means were considered significant at $P<0.05$, and differences were considered to indicate a trend toward significance at $0.05 < P < 0.10$ using PDIFF in the LSMEANS statement.

Table 1. Ingredients of experimental diets (% of DM).

Ingredients	Treatments			
	WB-U	CB-U	WB-S	CB-S
Alfalfa hay	30	30	30	30
Whole barley	69.17	-	62.65	-
Cracked barley	-	69.17	-	62.65
Urea	0.83	0.83	-	-
Soybean meal	-	-	7.35	7.35

* Treatments were: WB-U, whole barley grain with urea; CB-U, cracked barley grain with urea; WB-S, whole barley grain with soybean meal; CB-S, cracked barley grain with soybean meal.

RESULTS

Digestibility trial

The data for digestibility of nutrients are presented in Table 2. Digestibility of OM and CP did not differ among treatments. However, digestibility of DM tended to be increased ($P = 0.07$) and digestibility of NDF increased among treatments ($P < 0.05$). The results showed that fiber digestibility is affected by feeding soybean meal compared to urea supplementation.

Rumen pH and ammonia nitrogen concentration

The data for rumen pH (by hours and average) are given in Table 3. The results showed that rumen pH at 2 h after feeding was differed among treatments. The results of the present study indicated that regardless of protein sources, cracked barley decreased rumen pH compared to whole barley 2 h after feeding. Other sampling times did not show any significant difference or even any clear trend toward significance among treatments. The data for rumen ammonia concentration (by hours and average) are presented in Table 3. The results showed that the 2 and 3 h post feeding differed among treatments and all the other sampling times did not show different in this study.

DISCUSSION

Soybean meal increased fiber digestibility in comparison with urea supplementation in this study. Protein degradation by rumen microbes results in the formation of ammonia in rumen fluid and peptides are intermediates in this process (Reynal et al., 2007). It is clarified that soybean meal has potential to increase the peptide nitrogen concentration in rumen compared with non protein nitrogen sources (Reynal and Broderick, 2005). The reports by Yang (2002) clarified the increase in peptide nitrogen concentration in rumen fluid could directly affect fiber digestibility. Compared with non protein nitrogen source, using the peptide protein source increased branched chain volatile fatty acids (BCVFA) concentration that probably has potential to affect fiber digestibility. It is determined in some studies that the addition of true protein sources improved digestion of OM, DM and fiber compared to non protein nitrogen sources (Fu, 2000; Yang, 2002). The work by Gorosito et al. (1985) showed that fiber digestion by ruminal bacteria was improved by BCVFA supplementation. In addition, Griswold et al. (2003) reported that peptide addition in continuous culture increased BCVFA production that they could increase fiber digestion. In the present study, as the different methods of barley processing were compared, there was no significant effect of processing on NDF digestibility and the main differences were caused by different protein sources. The previous works revealed that a basal concentration of ammonia nitrogen in rumen fluid is necessary to prevent depression in rumen fermentation and fiber digestibility (Jones et al., 1998). Our results suggest that all treatments supplied the basal concentration of ammonia nitrogen concentration for fiber digestibility and feeding soybean meal caused to increased fiber digestion compared to urea supplementation.

Rumen pH was decreased by feeding processed barley in compared to the whole barley. Cracking the cereals may increase availability of nutrients to rumen microbes compared with whole grains (Callison et al., 2001). Consequently, microbes could digest the nutrients more easily in cracked grains and therefore rumen pH has potential to decrease in cracked vs. whole grains. Previous works identified that protein might not have significant effects on rumen pH if the protein intake is in the range of ordinary requirements of animal. Reynal and Broderick (2005), by feeding the wide ranges of rumen degradable protein (RDP) from 10.6 to 13.2% to dairy cows reported no significant affect of high degradable protein content in diet on ruminal pH. Comparing the results for rumen pH for WB-U (6.27) vs. CB-U (5.97) and for WB-S (6.23) vs. CB-S (5.84), it is found that the cereal processing method is the main reason for rumen pH changes and protein source may not be as important as the grain in ruminant nutrition to control the rumen pH.

Table 2. Effects of different barley processing accompanying with different protein sources on nutrients digestibility in sheep.

Item	Treatments				SE	*P*
	WB-U	CB-U	WB-S	CB-S		
Nutrients digestibility (%)						
DM	66.61	65.23	70.30	70.02	0.61	0.07
OM	68.47	67.77	72.34	72.36	0.72	0.11
CP	70.19	66.49	73.20	71.89	0.28	0.19
NDF	22.25[b]	20.72[c]	30.48[a]	30.95[a]	0.80	0.003

[a, b, c] Least squares means within the same row without a common superscript differ (P < 0.05). * Treatments were: WB-U, whole barley grain with urea; CB-U, cracked barley grain with urea; WB-S, whole barley grain with soybean meal; CB-S, cracked barley grain with soybean meal.

Table 3. Effects of different barley processing accompanied with different protein sources on rumen pH and ammonia concentration.

Item	Time (h)	Treatments				SE	*P*
		WB-U	CB-U	WB-S	CB-S		
Rumen pH	0	6.59	6.82	6.50	6.68	0.03	0.21
	1	6.44	6.50	6.39	6.22	0.10	0.34
	2	6.27[a]	5.97[b]	6.23[a]	5.84[b]	0.09	0.02
	3	6.06	5.75	6.08	5.60	0.05	0.28
	5	5.86	5.94	6.01	5.33	0.05	0.14
	7	5.75	6.25	6.14	5.89	0.04	0.19
	Average	6.16	6.21	6.23	5.92	0.08	0.25
Rumen NH_3 (mg/L)	0	100.49	112.01	168.62	177.80	9.23	0.08
	1	270.04	238.53	205.18	230.48	8.42	0.25
	2	219.18[c]	244.53[a]	174.69[d]	230.62[b]	6.17	0.03
	3	155.71[d]	179.49[a]	144.82[c]	168.0[b]	6.23	0.04
	5	102.20	112.47	151.36	134.87	7.84	0.19
	7	82.76	86.96	161	109.20	7.46	0.13
	Average	155.06	162.35	195.72	175.18	7.19	0.15

[a, b, c] Least squares means within the same row without a common superscript differ (P < 0.05). * Treatments were: WB-U, whole barley grain with urea; CB-U, cracked barley grain with urea; WB-S, whole barley grain with soybean meal; CB-S, cracked barley grain with soybean meal.

The ruminal ammonia nitrogen concentrations are differed significantly among treatments. The concentrations of ammonia nitrogen and peptide nitrogen in rumen fluid depend on the source and amount of the degradable protein in rumen (Reynal et al., 2007). Vanhatalo et al. (2003) found a numerical increase in NH_3-N concentration in diet supplemented with caseinate in comparison with control diet. Casein is more rapidly degradable protein than most other proteins in rumen (Broderick and Wallace, 1988), therefore increased ammonia nitrogen concentration with casein infusion could be the result of the greater amount of caseinate degradation. Like casein, the urea is rapidly degradable protein source (NPN) which has potential to increase rumen ammonia concentration dramatically (Windschitl and Stern, 1988). In the present study, the greatest amount of ammonia nitrogen concentration in rumen fluid was for urea treatments (244.53 and 179.49 mg/dl for CB-U treatment for 2 and 3 h, respectively). It may be concluded that increased ammonia concentration in rumen fluid which caused by urea supplementation, may not be a useful factor to improve rumen fermentation pattern, and extra ammonia concentration could consequently negatively affect animal performance (Hunber and Kung, 1981).

Conclusion

The results clarified that urea supplementation in sheep

diet caused to increase rumen ammonia concentration. Overall, the results showed that using soybean meal as true protein source in sheep ration could improve fiber digestibility compared with urea supplementation as the source of non protein nitrogen.

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