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Interaction effects of degradable nitrogen sources and straw treatment on rumen parameters and microbial protein synthesis in sheep

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ABSTRACT

In this study, the effects of 2 rumen degradable nitrogen, N, source (urea vs. casein) were investigated on rumen degradability, ammonia concentration, dilution rate, microbial protein synthesis (MPS) and MPS efficiency (EMPS) in sheep fed diet based on untreated straw (US) or treated straw (TS). Four rumen fistulated sheep were assigned to 1 of the 4 treatments: (i) US with urea (USU); (ii) US with casein (USC); (iii) TS with urea (TSU); and (iv) TS with casein (TSC), according to a 4 × 4 Latin square design. Barley straw was treated with 4.1% sodium hydroxide (w/w DM basis; pH 11.5) followed by 2% hydrogen peroxide. The treated straw was packed in 220 l drums and kept for 6 weeks. Compared to US diets, feeding the TS diets enhanced ruminal fiber degradability (62.3 vs 46.3%), total tract organic matter (OM) digestibility (59.4 vs 53.8%), OM intake (768 vs 546 g/d), dilution rate (0.082 vs 0.056/h), and MPS (45.7 vs 29.7 g/d), but it had no effect on EMPS. Rumen fiber degradability but not the DM one of only TS enhanced with casein supplementation as compared to urea supplementation. No interaction was found between supplemented nitrogen sources and straw type. It is concluded that substitution of US with TS increased intake, digestibility and MPS when supplemented with either urea or casein as nitrogen source. However, fiber degradability was improved with casein supplementation.

Key words: Microbial protein synthesis, N supplementation, Rumen degradability, Sheep, Straw treatment

Large proportions of structural carbohydrate in low-quality crop residues limit the voluntary intake and digestion by ruminants because of slow rates of microbial fermentation. Strategies for ruminants on crop-residue-based diets should aim at establishing an efficient rumen ecosystem that maximizes not only fiber digestion but also the production rate of microbial protein synthesis (Leng 1990). Several researchers have been able to stimulate fiber digestion by adding rumen degradable protein or branched chain volatile fatty acids (Liu *et al*. 2009, Zhang *et al*. 2013). Microbial protein flowing to the small intestine depends on nutrient availability and efficiency of use of these nutrients by rumen bacteria (Bach *et al*. 2005). Little information is available on the effect of interaction of straw treatment (energy availability) with rumen degradable N sources (ammonia and peptide) on ruminal microbial protein synthesis. The objective of present study is to evaluate the effect of two rumen degradable N sources (ammonia and peptide) on rumen degradability and microbial protein synthesis in sheep fed either untreated or treated straw.

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MATERIALS AND METHODS

Animals and experimental diets: Barley straw (1.4 cm nominal chop length) was treated with AHP (4.1% NaOH and 2% H_2O_2, w/w DM basis; pH 11.5 as described by Ghasemi *et al*. 2013). The treated straw was packed in 220 l drums and kept for 6 weeks at room temperature. Four rams (average body weight 54 kg, 1–2 years) with rumen fistula were placed in individual metabolism crates equipped with faecal and urine collecting equipment for a 10 days adjustment period before beginning the 90 days experiment. Animals were assigned to each of the following treatments: (i) untreated straw with urea (USU); (ii) untreated straw with casein (USC); (iii) treated straw with urea (TSU); and (iv) treated straw with casein (TSC) in 18 days period of a 4 × 4 Latin square design. Each period consisted of 10 days for adaptation to diets and 8 days for sample collection. Sheep had free access to water and salt licks. Before urea or casein supplementation, the diets were formulated using the CNCPS for sheep (CNCPS-S, srns version 1.9.4468) to contain the similar concentration of CP and to provide a negative balance of peptide in the rumen (Table 1). Protein

Table 1. Ingredients, chemical composition and rumen N balance of experimental diets.

Items	Treatments[a]			
	USU	USC	TSU	TSC
Ingredient composition				
UT barley straw (% of DM)	65.0	64.1	-	-
AT barley straw (% of DM)	-	-	65.0	64.1
Alfalfa hay (% of DM)	13.5	13.3	13.5	13.3
Barley grain (% of DM)	13.1	12.9	13.1	12.9
Wheat bran (% of DM)	6.0	5.9	6.0	5.9
Dicalcium phosphate (% of DM)	1.00	0.99	1.00	0.99
Min-Vit premixes (% of DM)	0.75	0.74	0.75	0.74
Urea (% of DM)	0.63	-	0.63	-
Casein (% of DM)	-	2.09	-	2.09
Chemical composition				
ME (Mcal/kg)	1.92	1.96	1.92	1.96
CP (% of DM)	11.0	10.8	11.0	10.8
NDF (% of DM)	58.1	57.3	58.1	57.3
NFC (% of DM)	20.2	20.0	20.2	20.0
RDP (% of DM)	80.4	80.0	80.4	80.0
RUP (% of DM)	19.6	20.0	19.6	20.0
Ruminal balance[b]				
N balance (%)	+30.3	+25.4	+30.3	+25.4
Peptide balance (%)	–13.3	+11.8	–13.3	+11.8

[a] USU, diet based on untreated straw supplemented with urea; USC, diet based on untreated straw supplemented with casein; TSU, diet based on treated straw supplemented with urea; TSC, diet based on treated straw supplemented with casein; ME, metabolizable energy; CP, crude protein; NDF, neutral detergent fiber; NFC, non-fiber carbohydrate; RDP, rumen degradable protein; RUP, rumen undegradable protein. [b]intake was adjusted for 600 g/d.

fractions were determined (Table 2) as described by Licitra *et al*. (1996). Casein and urea were mixed completely into the diets and fed to animals as total mixed ration.

Intake, ruminal degradability, total tract digestibility, ruminal dilution rate and microbial protein synthesis: The diets were fed *ad lib.* (5% ort) twice a day at 08:00 and 18:00. Feed intake was recorded daily for 5 consecutive days. Ruminal degradability of straw was measured using nylon bags (8 cm × 15 cm; 50 µm pore size) in the rumen for 38 h (calculated forage passage rate for the sheep: 2.65%/h, CNCPS-S srns version 1.9.4468). The total tract digestibility coefficients for OM and CP were estimated by a 5 days total collection of faeces. Faecal output was weighed daily and representative sample was taken. Samples were dried in a forced air oven at 60 °C for 72 h to measure the total tract digestibility. Ruminal fluid kinetics was estimated using cobalt-EDTA by dosing 50 ml of the marker (20 g Co-EDTA/1000 mL of deionized water) immediately before feeding (07:45). Samples of rumen fluid were collected at 0, 1.5, 3, 4.5, 6, 9, 12, 16, and 24 h of administration. The samples were centrifuged at 3,000 × g for 15 min and diluted to 1:10 using deionized water. Cobalt concentration was determined atomic absorption spectrophotometer. Rumen fluid was sampled at 0, 1.5, 3, 4.5, 6, and 9 h post feeding and immediately acidified by 7.2 N sulfuric acid (pH<3). Ammonia concentration in ruminal fluid was measured by the phenol-hypochlorite method (Broderick and Kang, 1980). Blood samples (10 ml) from the coccygeal vein were collected in EDTA-treated vacuum tubes at 4 h post-feeding. The tubes were centrifuged (15 min at 3000 × g), and the supernatants were then frozen at –20°C until analysis for blood urea N (BUN) which was assayed by commercial kits. Estimation of microbial protein supply was accomplished using total urine excretion of purine derivatives (PD). Urine was collected daily for 6 d in buckets and a representative sample of 50 ml was diluted (1:1) in distilled water and frozen until analysis. Allantoin and uric acid were measured by Chen and Gomes (1995). The xanthine and hypoxanthine were also measured together as uric acid after treatment of urine sample with xanthine oxidase.

Samples were analysed for total N, DM and ash (AOAC 2000). NDF (using heat stable alpha amylase) and ADF were measured using a fiber analyser.

Calculations and statistical analysis: Purine derivatives were considered as total extracted allantoin, uric acid, xanthine and hypoxanthine. Microbial protein flow to the small intestine was calculated from urinary output of purine derivatives using the Newton's iteration process (SAS Institute 2001). Efficiency of microbial protein supply (EMPS) was expressed as grams of microbial protein per 100 g of digestible OM apparently digested in rumen, which is assumed to be 65% of the digestible OM intake (Fadel Elseed 2005). Ruminal fluid dilution rate was estimated using following equation:

$$Y(t) = Aexp–kt$$

where, Y is Co concentration in ruminal fluid, t is time in h relative to dosing, A is Co concentration at this time zero,

Table 2. Protein fractionation (% of CP) and content of CP and neutral detergent fiber (% of DM) of experimental feeds.

	A	B1	B2	B3	C	NDF	CP
Barley straw	54.8	11.1	17.6	10.5	6.0	75.0	6.9
Alfalfa hay	28.2	19.7	28.2	14.7	9.1	46.5	15.7
Barley grain	16.5	18.9	38.4	20.2	6.1	25.6	12.1
Wheat bran	23.6	29.1	27.0	17.5	2.8	45.6	17.2
Casein	6.5	84.7	8.8	-	-	-	82.3
Urea	100	-	-	-	-	-	281.0

A: borate-phosphate soluble protein; B1: soluble protein in borate-phosphate but insoluble in trichloroacetic acid; B2: (CP-A-B1-B3-C); B3: (Neutral detergent insoluble protein - acid detergent insoluble protein); C: Acid detergent insoluble protein.

and k designates the fractional dilution rate constant of Co in rumen. Intake, digestibility, microbial protein synthesis and ruminal kinetic data were analyzed using the GLM procedure of SAS (SAS Institute, 2001) as a 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments. The statistical model used is as follows:

$$Y_{ijkl} = ì + A_j + P_i + T_k + S_l + (T \times S)_{kl} + e_{ijkl}$$

where, Y, ì, P, A, T, S, T×S, and e, respectively, are independent variable, overall mean, effects of period, animal, straw treatment, supplementation, interaction between treatment and supplementation and random residual error.

RESULTS AND DISCUSSION

Ruminal degradability, total tract digestibility and intake: Fiber degradability was improved largely as a result of straw treatments (Fig. 1). Furthermore, casein

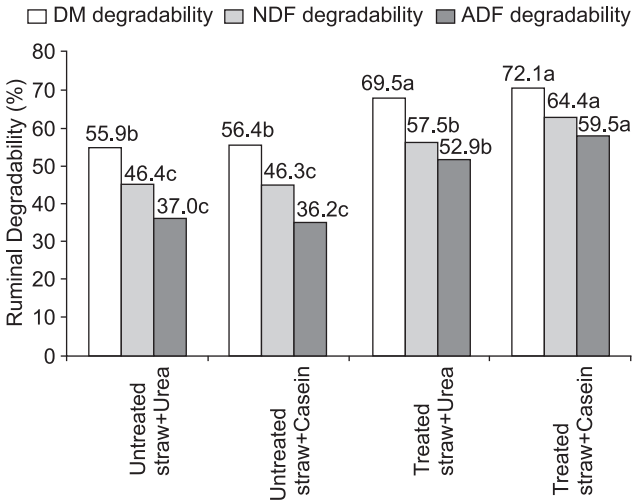

Fig. 1. Ruminal degradability of untreated and treated barley straw in sheep supplemented with urea or casein (SEM=3.74, 1.00 and 0.37 for DM, NDF and ADF degradability, respectively).

supplementation enhanced significantly (P <0.05) ruminal NDF and ADF degradability of treated straw than urea supplementation. It has been demonstrated that addition of branched chain amino acids (valine, leucine, and isoleucine) enhanced production of branched chain volatile fatty acids *in vitro* which are required for fiber degrading microorganisms (Kazemi-bonchenari *et al.* 2010, Zhang *et al*. 2013). Intake of OM and CP increased (*P*<0.05) as in case of treated straw feeding as also reported by Verma *et al.* (2011) which was owing to enhanced palatability and digestibility. Total tract CP digestibility in case of TS diets decreased as compared to US diets. This may be attributed to difference between metabolic faecal protein (MFP) secretion and DM intake. MFP is directly calculated from DM intake (NRC 2001). More faecal N was excreted by the sheep when untreated straw was replaced with treated straw. Verma *et al*. (2011) also observed reduced digestibility of CP in animals fed ammoniated wheat straw.

Dilution rate, and microbial protein synthesis (MPS) and microbial protein synthesis efficiency (EMPS): Treatment of straw led to increased ruminal dilution rate, urinary allantoin and uric acid excretion and MPS and decreased ruminal turnover (Table 3). Supplemented N sources did not affect PD excretion, MPS and EMPS. Similarly, there was not interaction (*P*>0.05) between straw type and N sources for these parameters. Efficiency of MPS in the rumen did not improve in substituting treated straw diets despite marked increase (P>0.05) in liquid dilution rate by 47%. Microbial protein synthesis in the rumen provides a major portion (50 to 80% of total absorbable protein) of the protein flowing to small intestine of ruminants (Stern *et al*. 2006). Treatment of straw improved MPS in the rumen (54%) as a result of increased digestible energy intake (intake and digestibility increased by 40% and 10%, respectively). The results of the present study showed no further improvements in MPS with casein supplementation in diet based on TS. However, Kazemi-Bonchenari *et al*. (2010) observed increased branched chain fatty acids concentrations, fiber digestibility and MPS when sodium caseinate infused into the rumen of dairy cows. Feeding treated straw diets lowered BUN value (P=0.13) compared to those fed untreated straw. Moreover, rumen ammonia concentration (9.0 vs. 12.1 mg/dl) was lower (P<0.01) in sheep fed treated straw (Fig. 2). Fermentable carbohydrate

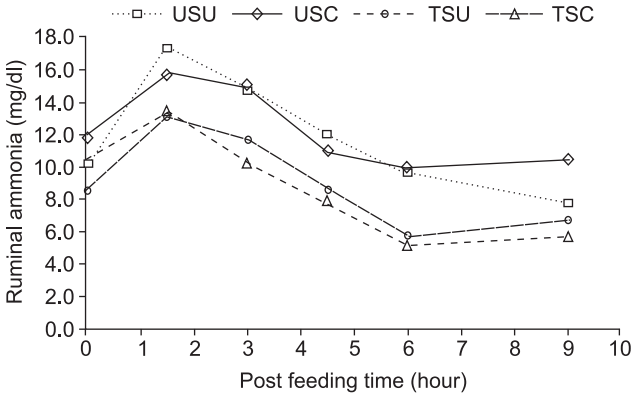

Fig. 2. Rumen ammonia concentration in sheep fed untreated straw-based diet supplemented with urea (□%, USU) or casein (◊%, USC); treated straw-based diet supplemented with urea (○%, TSU) or casein (Δ, TSC); SE:0.57.

supply in rumen can influence the amount of ruminal ammonia N assimilated into microbial protein (Hristov *et al*. 2005). The low fermentability of untreated straw based diets may explain the lower ammonia N conversion into microbial protein and the higher BUN value. It has been documented as rumen ammonia concentration decreases below 5 mg/dL, blood urea N transferred into the rumen and provides an increasing buffer against extremely low ammonia concentrations (Firkins *et al*. 2007). Lower BUN in sheep fed TS diet may reflect higher urea recycled in the rumen resulting higher MPS. Remond *et al.* (2002) reported significant net recycle of blood urea in rumen at rumen NH_3 concentrations below 9.5 mg/dl.

The results obtained from this study indicate that

Table 3. Intake, rumen degradability, total tract digestibility, MPS and BUN in sheep fed diet based on barley straw.

Items	Treatments[a]				SEM[b]	Probability[c]		
	USU	USC	TSU	TSC		Straw	RDN	S×RDN
Intake								
DM (g/d)	629	594	949	805	40.4	<0.01	0.10	0.05
OM (g/d)	532	561	803	681	34.7	<0.01	0.21	0.05
CP (g/d)	64.0	66.0	91.2	74.9	3.95	<0.01	0.10	0.04
DE (Mcal/d)	1.27	1.27	2.15	1.90	0.07	<0.01	0.33	0.32
RDP (g/d)	51.5	52.8	73.3	59.9	55.2	<0.01	0.05	0.04
RUP (g/d)	12.6	13.2	17.9	15.0	3.20	<0.01	0.28	0.05
Total tract digestibility of diet								
DM (g/d)	0.48	0.43	0.54	0.55	0.02	<0.01	0.63	0.17
OM (%)	55.2	52.4	59.1	61.0	1.78	<0.01	0.70	0.21
CP (%)	69.4	65.3	61.6	61.9	1.67	<0.01	<0.01	0.46
Faecal output								
CP (g/d)	19.5	22.5	35.0	28.3	2.57	<0.01	0.34	0.08
CP (%)	7.26	7.47	8.90	9.27	0.11	<0.01	0.08	0.60
Ruminal fluid kinetics[d]								
DR (h^{-1})	0.062	0.049	0.095	0.067	0.010	0.05	0.25	0.54
RV, (l)	9.58	9.88	8.84	10.61	0.870	0.99	0.33	0.47
RT, (h)	17.2	19.2	12.6	15.2	1.60	0.04	0.42	0.86
Purine derivatives (mmol/d)								
Allantoin	3.76	3.68	5.81	6.25	0.454	<0.01	0.32	0.57
Uric acid	1.77	1.92	2.36	2.35	0.179	<0.01	0.65	0.66
X+H[e]	0.51	0.57	0.63	0.81	0.095	0.08	0.41	0.55
MPS (g/d)[f]	29.4	30.0	46.1	49.8	2.67	<0.01	0.44	0.58
EMPS[g]	17.0	15.9	15.9	18.7	1.72	0.74	0.62	0.26
Blood								
[h]BUN, mg/dl	13.4	14.5	11.1	12.8	1.57	0.13	0.55	0.87

[a]USU, diet based on untreated straw supplemented with urea; USC, diet based on untreated straw supplemented with casein; TSU, diet based on treated straw supplemented with urea; TSC, diet based on treated straw supplemented with casein; [b]SEM, standard error of mean.[c]RDN, ruminal degradable nitrogen sources, S×RDN: interaction between straw and RDN; [d]DR, dilution rate; RV, rumen volume; RT, rumen turn over; [e]X+H, xanthine plus hypoxanthine; [f]MPS: microbial protein synthesis; [g]EMPS, efficiency of microbial protein synthesis (g/100 g digestible organic matter fermented in the rumen calculated as 0.65×digestible organic matter intake); [h]BUN, blood urea nitrogen.

microbial growth in sheep fed based on low quality forage can be enhanced as a result of increasing structural carbohydrates availability. No additional effects were observed in microbial growth and efficiency by altering rumen degradable N source. Casein supplementation only improved ruminal fiber degradability of treated straw.

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