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Effects of synchronizing starch and protein degradation in rumen on fermentation, nutrient utilization and total tract digestibility in sheep

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ABSTRACT

The objective of this experiment was to investigate the effects of synchronizing the dietary starch and crude protein (CP) degradation in the rumen on nutrient utilization, fermentation, and total tract digestibility in sheep. The four diets were formulated with different rates of starch and CP release in the rumen but with similar metabolic energy, starch, and CP. The diets were slowly degradable starch, slowly degradable protein; slowly degradable starch, rapidly degradable protein; rapidly degradable starch, rapidly degradable protein; and rapidly degradable starch, slowly degradable protein. The diets were fed to four cannulated sheep *ad libitum* in two equal portions, using a 4x4 Latin square design.

Dry matter intake (DM) was not influenced by either the rate of starch or protein degradation. There was no significant effect of dietary treatment on the digestibility of DM, organic matter, starch, CP, neutral detergent fiber or acid detergent fiber in the rumen and total tract. Ruminal pH was greater for sheep fed slowly degradable starch diets than rapidly degradable starch ($P< 0.05$). Ruminal total volatile fatty acid concentrations were not affected by treatments but the molar proportions of propionic acid were greater for sheep fed rapidly degradable starch diets than slowly degradable starch diets ($P< 0.05$). The ratios of acetic acid (A) to propionic acid (P) were higher for sheep fed slowly degradable starch diets than rapidly degradable starch diets ($P< 0.05$). Ruminal ammonia-N concentrations were not affected from the degradability characteristics of protein. Rumen pH and A:P were higher in diets containing slowly degradable starch than in diets rapidly degradable starch. Propionic acid was higher in diets containing rapidly degradable starch than in diets containing slowly degradable starch. Rumen fermentation and utilization of nutrients in the rumen affected starch degradability more than protein degradability. Synchronizing starch and protein degradation in rumen had no effect on the intake, digestibility of nutrients in sheep.

Key Words: Starch, Protein, Degradability, Synchrony, Sheep.

RIASSUNTO

EFFETTI DELLA DEGRADAZIONE RUMINALE SINCRONIZZATA DELLE PROTEINE E DELL'AMIDO SU FERMENTAZIONE, UTILIZZAZIONE DELLE SOSTANZE NUTRITIVE E DIGERIBILITÀ TOTALE NEGLI OVINI.

Lo scopo di questa indagine è stato quello di valutare gli effetti della degradazione sincronizzata nel rumine dell'amido e della proteina grezza (PG) sull'utilizzazione delle sostanze nutritive, sulla fermentazione e sulla digeribilità totale negli ovini. Sono stati utilizzati quattro tipi di dieta caratterizzati da un rilascio diverso nel rumine di amido e proteina grezza, ma con uguali contenuti di tali sostanze nutritive e di energia metaboliz-

zabile: dieta a degradazione lenta sia dell'amido che della PG, a degradazione lenta dell'amido e rapida della PG, a degradazione rapida sia dell'amido che della PG, a degradazione rapida dell'amido e lenta della PG. Le varie diete sono state fornite ad libitum a quattro pecore incannulate, in due razioni giornaliere di uguale entità, usando uno schema fattoriale 4x4.
L'assunzione di sostanza secca non è stata influenzata dal livello di degradazione né dell'amido né delle proteine. Non è stato registrato alcun effetto significativo del trattamento alimentare sulla digeribilità di sostanza secca, sostanza organica, amido, PG, NDF e ADF nel rumine e nell'intero tratto digestivo. Il pH ruminale è risultato più alto nelle pecore alimentate con amido a degradabilità lenta rispetto a quelle alimentate con amido a degradabilità rapida (P< 0,05). Le concentrazioni ruminali degli acidi grassi volatili non sono state influenzate dai trattamenti dietetici, mentre la concentrazione molare di acido propionico è risultata maggiore con le diete a rapida degradabilità dell'amido rispetto a quelle a lenta degradabilità (P< 0,05). Il rapporto fra acido acetico (A) e acido propionico (P) è apparso più elevato negli ovini alimentati con amido a degradabilità lenta rispetto a quelli alimentati con amido a degradabilità rapida (P< 0,05). La concentrazione di ammoniaca ruminale non è stata influenzata dalla degradabilità delle proteine. Il pH ruminale ed il rapporto A:P sono risultati più elevati con le diete ad amido degradabile lentamente piuttosto che rapidamente. L'acido propionico è apparso più elevato con le diete contenenti amido degradabile rapidamente rispetto a quelle con amido a lenta degradabilità . La fermentazione ruminale e l'utilizzazione dei principi nutritivi nel rumine hanno influenzato la degradabilità dell'amido più di quella delle proteine.
La sincronizzazione della degradazione ruminale di amido e proteine non risulta avere influenzato l'ingestione alimentare e la digeribilità delle sostanze nutritive negli ovini.

Parole chiave: *Amido, Proteina, Degradabilità, Sincronizzazione, Pecore.*

Introduction

Complex interrelationships exist between dietary starch and protein and the amount of protein and starch that will be utilized by ruminants, and these interrelationships can have important ramifications for overall efficiency of use of nitrogen (N) and energy. High-energy diets stimulate microbial protein synthesis, thereby increasing the supply of microbial protein (Cadorniga and Satter, 1993). However, it is uneconomical to overfeed protein and energy. The excretion of excess dietary protein as urine N represents lost energy. In addition, there are compelling environmental reasons for reducing urinary N excretion by ruminants.

Some researchers have shown that bacterial growth and fermentation in the rumen are optimized when the rates of fermentation of starch and protein are synchronized (Nocek and Russell, 1988; Hoover and Stokes, 1991; Broderick, 2003). However, attempts at optimizing synchronization have produced conflicting results. Some studies indicated that synchronization of dietary starch and protein supply to the rumen increased nitrogen retention and reduced absorption of ammonia (Matras *et al.*, 1991; Taniguchi *et al.*, 1995), but improved microbial efficiency and yield (Aldrich *et al.*, 1993; Sinclair *et al.*, 1995). By contrast, other researchers have reported no significant effects (Khorasani, *et al.*, 1994; Shabi *et al.*, 1998). Nocek and Russell (1988) suggested a process to synchronize the rate of supply of crude protein (CP) and energy yielding substrates with ruminal microbes as a means to improve the capture of rumen degradable protein. The synchronization of supplemental energy and CP could theoretically improve the utilization of N from in diets (Broderick, 2003), but this effect has not been well documented. In fact, there is little experimental evidence to support the benefits of close synchrony of energy and nitrogen release; indeed, owing to a compounding of effects of changes in dietary ingredients with effects of synchrony itself.

The objective of this experiment was to examine ruminal digestion, ruminal fermentation, and total tract digestibility as influenced by the different rate of degradable starch and protein in the ration.

Table 1. Composition and chemical analysis of diets (Dry matter basis)

		Treatment			
Ingredients (g/d)		SSSP	SSRP	RSSP	RSRP
Alfalfa hay		300	300	300	300
Wheat		-	-	780	495
Corn grain		639	589	-	209
Sunflower meal		280	55	159	-
Corn gluten feed		-	225	-	175
Hazelnut meal		50	100	30	90
Limestone		20	20	20	20
Salt		5	5	5	5
Dicalcium Phosphate		5	5	5	5
Vitamin-mineral mix*		1	1	1	1
Chemical composition:					
Dry matter	%	89.40	89.28	89.26	89.12
Crude protein	″	17.70	17.32	17.63	17.28
Starch	″	33.36	33.64	34.09	33.53
Organic matter	″	92.50	92.24	92.16	92.22
Neutral Detergent Fiber	″	26.87	27.81	29.89	28.33
Acid Detergent Fiber	″	18.14	18.16	18.31	17.02
Acid Detergent Lignin	″	4.50	4.33	4.84	4.14

SSSP: Slowly degradable starch, slowly degradable protein; SSRP: Slowly degradable starch, rapidly degradable protein; RSRP: Rapidly degradable starch, rapidly degradable protein; RSSP: Rapidly degradable starch, slowly degradable protein.

**Vit.A: 15,000,000 U/kg; vit D_3: 3,000,000 U/kg; vit E: 30,000 mg/kg; Mn: 50,000 mg/kg; Fe: 50,000 mg/kg; Zn: 50,000 mg/kg; Cu: 10,000 mg/kg; Co: 150 mg/kg; I: 800 mg/kg; Se: 150 mg/kg.*

Material and methods

Four ruminally cannulated adult Kivircik male sheep, approximately 3 years of age (50 kg ± 5 kg body weight) were allotted to four treatments in a 4x4 Latin square (with a 2x2 factorial arrangement of treatments). Sheep were housed in individual metabolism crates with continuous lighting and free access to water. Four diets were formulated using raw material proximate analysis and the determined degradability coefficients (Unpublished data); 1) Slowly degradable starch, slowly degradable protein (SSSP), 2) Slowly degradable starch, rapidly degradable protein (SSRP), 3) Rapidly degradable starch, rapidly degradable protein (RSRP), and 4) Rapidly degradable starch, slowly degradable protein (RSSP) (Table 1). These diets were calculated to have similar concentrations of metabolizable energy, starch, and CP based on published values National Research Council (1985). The four diets contained approximately 77% concentrate and 23% forage, which was alfalfa hay. The diets were prepared as total mix ration, divided to into equal portions, and fed intake twice daily at 0800 h and 1600 h. Feed refusals were measured before each morning feeding and represented about 10% of given feed.

Table 2. Intakes, rumen and total tract digestibility of nutrients in response to diet.

		SSSP	SSRP	RSSP	RSRP	SEM	S	CP	SxCP
Dry matter									
Intake	g/d	1241	1261	1227	1262	11.4	ns	ns	ns
Total tract digestibility	″	986	989	952	995	17.2	ns	ns	ns
Organic matter									
Intake	g/d	1148	1162	1131	1163	12.65	ns	ns	ns
Effective degradability	%	62.7	68.7	72.5	70.7	5.76	ns	ns	ns
Potential degradability	″	85.0	84.8	82.0	83.5	2.65	ns	ns	ns
Total tract digestibility	g/d	945	949	911	952	11.66	ns	ns	ns
Starch									
Intake	g/d	413	424	418	423	14.05	ns	ns	ns
Effective degradability	%	66.5	70.3	93.5	89.3	6.07	ns	ns	ns
Potential degradability	″	82.1	86.2	97.4	97.9	4.53	ns	ns	ns
Total tract digestibility	g/d	382	384	394	386	12.30	ns	ns	ns
Crude Protein									
Intake	g/d	220	218	216	218	5.44	ns	ns	ns
Effective degradability	%	72.4	74.1	74.9	77.7	2.11	ns	ns	ns
Potential degradability	″	81.8	83.3	84.9	85.7	3.90	ns	ns	ns
Total tract digestibility	g/d	183	174	176	175	10.02	ns	ns	ns
Neutral Detergent Fiber									
Intake	g/d	334	351	367	357	10.01	ns	ns	ns
Total tract digestibility	″	197	211	215	207	12.11	ns	ns	ns
Acid Detergent Fiber									
Intake	g/d	225	229	225	215	8.09	ns	ns	ns
Total tract digestibility	″	126	129	121	115	4.05	ns	ns	ns

SSSP: Slowly degradable starch, slowly degradable protein; SSRP: Slowly degradable starch, rapidly degradable protein; RSRP: Rapidly degradable starch, rapidly degradable protein; RSSP: Rapidly degradable starch, slowly degradable protein.
SEM: Standard error mean; ns: not significant.

Experimental periods were 29 d with d 1 to 19 for adaptation diets and d 20 to 29 for sample collection. Total fecal output was collected daily with fecal collection bags, weighed, and, subsamples (10%) were taken and stored at -20°C until laboratory analysis.

Diets were evaluated *in situ* to determine ruminal availability and rate of degradation of organic matter (OM), CP, and starch, on day six and nine of the collection period (Orskov and McDonald, 1979). Dacron bags were purchased (5x15 cm, 40 μm pore size: Ankom, Fairport NY., USA). Samples were ground to pass through a 2.5 mm screen, and approximately 4-5 g of dry matter of sample was added to each bag. Samples incubated were SSSP, SSRP, RSSP, and RSRP (Table 1). The bags were placed into the rumen after morning feed and removed after intervals of 2, 4, 6, 8, 12, 24, 48, 72, and 96 h. Following incubation, the bags were washed through the cold water for 12 h. Two quadruplicate sets of each sample were soaked and rinsed without ruminal incubation to determine degradation at 0 h within the rumen. After washing bags and contents were dried in an

Table 3. The effect of starch and protein degradation on rumen fermentation parameters.

		SSSP	SSRP	RSSP	RSRP	SEM	S	CP	SxCP
pH		5.94	5.81	5.62	5.63	0.039	*	ns	ns
Ammonia-N	mg/dl	32.65	30.90	32.50	31.75	1.62	ns	ns	ns
Acetate (A)	mol/100 mol	55.84	54.93	51.66	51.14	3.38	ns	ns	ns
Propionate(P)	"	27.03	28.57	32.74	33.78	4.04	*	ns	ns
Butyrate	"	11.33	10.66	9.69	9.49	0.95	ns	ns	ns
TVFA	mmol/l	107.1	106.16	104.9	120.0	8.09	ns	ns	ns
A/P ratio		2.06	1.92	1.57	1.51	0.11	*	ns	ns

SSSP: Slowly degradable starch, slowly degradable protein; SSRP: Slowly degradable starch, rapidly degradable protein; RSRP: Rapidly degradable starch, rapidly degradable protein; RSSP: Rapidly degradable starch, slowly degradable protein.
TVFA: Total volatile fatty acids
**P< 0.05*
SEM: Standard error mean; ns: not significant

oven at 60°C for 48 h, and reweighed. Sufficient bags of each feed ingredient were incubated to provide a pooled residue of approximately 8 g of dry matter for each incubation time for each sheep. The contents of bags were combined, mixed and ground through a 1 mm screen. A composite sample was analyzed for residual CP (AOAC, 1990). Starch content was determined by the colorimetric method of Bal *et al.* (2000).

Diet samples were taken weekly and these samples were used for feed analysis. Feed, content of incubation bags, and fecal samples were oven-dried (60°C, 48 h) and ground to pass a 1 mm screen. Dry matter, ash and CP were analyzed by standard methods (AOAC, 1990). Determination of the neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin content in feed, and feces were done according to the methods of Van Soest *et al.* (1991).

The total tract digestibility (D) of nutrient (x) was calculated according to standard equation: Dx (%) = [Intake of x (gram/day) – Fecal output of x (gram/day)/Intake of x (gram/day)] x100.

Rumen fluid samples were collected on d 28 at 0 h (before feeding) and at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h post feeding. Each sample was strained immediately through four layers of cheesecloth, the pH was measured, and the samples at 0, 3, 6, 9 and 12 h were divided into subsamples for determination of volatile fatty acids (VFA) and ammonia-N. The samples for VFA were acidified with 2 ml of 25% metaphosphoric acid, and centrifuged at 5000xrpm for 10 min. Supernatant was frozen (-20°C) for subsequent analyses. Rumen NH_3-N was determined by the hypochlorite phenol procedure (Beecher and Whitton, 1970). Volatile fatty acids were determined using a gas chromatograph (Hewlett Packard Series II 5890, Gas Chromatograph). A column (6x2mm ID glass) was packed with 10% SP-1200/1% H_3PO_4 80/100 Chromosorb WAW (Supelco, Inc., Bellefonte, PA, USA).

Statistical analysis

All data were subjected to least squares ANOVA for a 4x4 Latin square design with a 2x2 factorial arrangement of treatments using the general linear models procedure of SPSS software (SPSS, 2005). The model

included time, sheep, periods, starch (Slowly, Rapidly), CP (Slowly, Rapidly) and starchxCP interaction (SPSS, 2005). When interactions of main effects were significant, treatment means were compared using Tukey's Test (Snedecor and Cochran, 1980). Significance was declared at $P< 0.05$ unless otherwise noted.

Results

The chemical analysis and ingredient composition of the diets are presented in Table 1. The CP and starch contents were similar across the four diets (mean 175 and 337 g/kg of DM, respectively). The highest levels of NDF were in the RSSP diet with value 299 g/kg of DM, whereas the lowest values in the SSSP diet at 269 g/kg of DM. The mean ADF and OM of the four diets were very similar, with a mean value of 179, and 923 g/kg of DM, respectively.

Dry matter intake, total tract and rumen digestibility of the diets are presented in Table 2. There were no significant effects of treatments on digestibility, degradability or intake of any of the dietary components measured. The quantities of OM, CP, NDF, and ADF digested in the rumen were similar for the diets, calculated using the *in situ* degradability data, and in accordance with that predicted from total tract digestibility data (Table 2). The quantity of starch total tract digestibility in SSSP was less than those in other diets although not significantly different. Sheep on the slowly degradable starch diets had numerically higher ADF digestibility than sheep on the rapidly degradable starch diets, with mean value of 127 g/d for ADF digestibility.

Ammonia-N and VFA in rumen fluid mean values are given in Table 3. Ruminal fluid total VFA concentrations were not affected by treatment but the molar proportion of propionic acid were 27.82 and 33.26 mol/100 mol of total VFA for sheep fed the rapidly degradable and slowly degradable starch diets, respectively ($P< 0.05$). The ratio of acetic acid to propionic acid was 1.99 and 1.54 for the slowly degradable and rapidly degradable starch diets, respectively ($P< 0.05$). Molar proportion of ruminal acetate and butyrate were not affected by either starch or protein degradation of diets. The rumen pH of slowly degradable starch diets was higher than for rapidly degradable starch diets ($P< 0.05$) (Table 3). The rumen pH had the highest values at 0 hours (6.97 ± 0.7) and the lowest values at 4 hours after feeding (5.06 ± 0.7) in RSSP ($P< 0.05$).

There was no significant effect of treatment on ruminal NH_3-N concentration; however, there was a numerical trend whereby NH_3-N was lower in slowly degradable protein treatments than in rapidly degradable protein treatments.

Discussion

In this study, the digestibility of DM and DM intake (DMI) was not altered by starch and/or protein degradation (Table 2). This is in agreement with the work by others (Aldrich *et al.*, 1993; Khorasani *et al.*, 1994; Arieli, *et al.*, 1996; Kolver *et al.*, 1998; Shabi *et al.*, 1998). However, Kyriazakis and Oldham (1997) reported a greater DMI in lambs fed on a rapidly, when compared with a slowly, degradable energy source and that increasing the supply of rumen degradable protein enhanced daily intake only in animals offered the slow rate of energy release. Reasons for this situation in the consumption of DM in response to changes in nutrient degradability are still unclear.

Kolver *et al.*, (1998) noticed no change in apparent total tract digestibilities of DM, OM, CP, and NDF when microbial protein synthesis was increased by the release of carbohydrate rich supplements with availability of pasture N in the rumen. These findings are in line with the findings of this study (Table 2). Increased dietary starch as rapidly degradable carbohydrate depresses rumen fiber digestibility, partly by depressing ruminal pH (Khalili and Huhtanen, 1991). No

analysis of the rumen digestibility of NDF and ADF was conducted in the present study; however, total tract digestibilities were not influenced by treatments with different diets or changes in rumen pH.

The lower ruminal pH of sheep fed the rapidly degradable starch diets reflected the higher degradation rates of starch for these diets and agree with the results of Aldrich *et al.*, (1993). However, a reduction in pH has not been observed in all studies (Stokes *et al.*, 1991; Shabi *et al.*, 1998; Casper *et al.*, 1999). There was no significant effect of dietary treatment on total VFA. This finding was similar to the findings of Witt *et al.* (1999). However, higher ruminal concentrations of propionate for sheep fed diets including rapidly degradable starch might have resulted from the difference in ruminal starch and OM degradation (Table 3). Rapidly degradable starch and OM in the rumen potentially leads to increased ruminal propionate concentrations. In addition, an increase in starch availability in the rumen using grains usually increases VFA concentration especially that of propionate concentration (McCarthy *et al.*, 1989). In this study, no response in total VFA concentration was observed when sheep fed on diets containing wheat or corn grain. However, propionate was higher in rapidly degradable starch diets than in slowly degradable starch diets ($P< 0.05$), (Witt *et al.*, 1999). Varying the starch and CP degradability resulted in similar molar concentrations of acetate and butyrate, although in comparison with sheep fed diets with slowly degradable starch, sheep fed the diets of rapidly degradable starch had a greater ratio of acetate to propionate ($P< 0.05$).

In this study, ruminal ammonia-N concentrations were not influenced by starch or protein degradation in diets (Table 3). The minimum concentration needed to support maximum rates of microbial protein synthesis is not known but the earlier study of 5 mg/dl ammonia-N (Satter and Slyter, 1974) has been challenged in later publications (Balcells *et al.*, 1993). In the present study, we observed no effect of dietary treatments on ammonia-N, which might have been due to protein degradation being similar between the diets. All of the diets had ammonia-N up well above 5 mg/dl in rumen fluid. Ammonia produced in the rumen in excess of the ability to be incorporated into ruminal microbes is absorbed across the rumen wall and converted to urea in the liver (Lobley *et al.*, 1995). In the current work, fast absorption across the rumen wall might be a reason for no differences in ammonia-N between diets.

Conclusions

Sheep given wheat-based diets with a rapid rate of starch degradation had a decreased rumen pH and an increased rumen propionate fraction compared with those given corn grain-based diets with a slow rate of starch degradation. However, there were no effects of starch source on total tract digestibility of OM. Providing CP mainly as sunflower meal (slowly degraded) or as a combination of mainly corn gluten meal and hazelnut meal (rapidly degraded) had no effect on rumen characteristics or total tract digestibility. It was concluded that the rate of starch degradability affected rumen fermentation and utilization of nutrients in the rumen more than protein degradability. Synchronizing starch and protein degradation in rumen had no effect on the intake, rumen and total tract digestibility of nutrients in sheep. Further research is required to support this conclusion.

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