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The Effects of Feeding Soybean Meal, Soybean Hulls and Raw Soybean Seed on Rumen Microbial Populations and Ruminal Fermentation in Grazing Dairy Cows

Lucia Cristina ARAKAKI[1], Mónica GAGGIOTTI[2], Miriam GALLARDO[2], Silvia VALTORTA[2], Gerardo CONTI[2], Oscar QUAINO[2], Jorge CARRILLO[1], Ruben GREGORET[2], Hiroshi KUDO[3,5]* and Burk DEHORITY[4]

[1] Institute of Pathobiology, Centro de Investigacion en Ciencias Veterinarias y Agronomia, Institute Nacional de Technologia Agropecuaria (Morón, Buenos Aires, Argentina)
[2] Estacion Experimental Agropecuaria Rafaela, Institute Nacional de Technologia Agropecuaria (Rafaela, Santa Fe, Argentina)
[3] Animal Production and Grassland Division, Japan International Research Center for Agricultural Sciences (Tsukuba, Ibaraki 305–8686, Japan)
[4] Ohio State University (Wooster, OH 44691, USA)

Abstract

The effects of soybean by-products — soybean hulls (SH), soybean meal (SM) and raw soybean seeds (RSS) — on the rumen microbial population and fermentation were studied. The concentration of total rumen bacteria was greater ($P = 0.06$) for the SH and SM diets as compared to RSS. The concentration of cellulolytic bacteria tended to be higher with the SH and SM diets. Protozoal concentrations were significantly lower for the SH diet at feeding (T0, $P < 0.05$). Generic composition of protozoa was distinctly different among the soybean by-products. The composition of protozoa at T0 and 3 h (T3) for the SH diet group primarily consisted of vestibuliferids and *Entodinium* sp. with only 1% of protozoa from different genera present in the 6 h (T6) samples. The protozoa composition was more diverse with the SM and RSS diets, in that the "Others" types of entodiniomorphs included the genera *Epidinium*, *Ophryoscolex*, *Metadinium*, and *Diplodinium*. With the SH treatment, total VFA was greater and ruminal pH and acetate:propionate ratio were lower compared with the SM and RSS diets. Supplementation with all the soybean by-products modified both the microbial population and fermentation products and had an increased total dry matter intake in grazing dairy cattle.

Discipline: Animal industry
Additional key words: bacteria, protozoa, rumen pH, VFAs

Introduction

There are several soybean by-products available for feeding to dairy cows. The list includes raw soybean seeds (RSS), soybean meal (SM) and soybean hulls (SH)[16]. SM (soybean by-product after oil extraction) is the conventional but expensive plant protein source commonly used in animal feeding[23]. SH represent the outer covering of soybean seeds and are characterized by a high neutral-detergent fiber (NDF) content, > 60%, and high ruminal digestibility, > 80%[18,25]. Both animal species and type of diet are known to influence the ruminal microbiota, i.e., bacteria, protozoa and fungi[6,8,10,15]. Since animal performance is dependent on the balance of rumen fermentation products, characterization of the rumen microorganisms present might help to understand the post ruminal effects of the different types of diets and feed

This paper reports the results obtained in the joint project between JIRCAS and INTA on use of agricultural by-products for feed in South America.

Present address:
[5] Research Planning and Coordination Division, Japan International Research Center for Agricultural Sciences (Tsukuba, Ibaraki 305–8686, Japan)
*Corresponding author: e-mail hkudo@jircas.affrc.go.jp

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supplements like agricultural by-products. A variety of by-products are used in ration formulations for dairy cows[24]. A review[22] compiled of 108 studies published throughout the world concluded that in comparison with a number of different protein supplements, soybean meal can increase the flow of microbial protein to the duodenum. Despite the large number of studies, specific groups of bacteria and protozoa involved have not been reported. In order to better understand the interactions and microorganisms involved, we investigated the effects of feeding soybean by-products on rumen bacteria, protozoa and fermentation products in dairy cows.

Materials and methods

Animals and Diets

Nine ruminally fistulated multiparous Holstein cows [body weight (BW) = 505 ± 60 kg; days in milking (DIM) = 121 ± 13] in mid lactation were used in a series of three, 3 × 3 Latin square design experiments, with rows always represented as periods and columns represented as different animals from square to square. The experiment was divided into 3 periods of 28 days each. The animals received a premix ration (PMR) formulated with corn silage, corn grain, minerals, and vitamins plus SH, SM or RSS. The PMR containing SH, SM or RSS were designed to be equal in energy, protein and fiber. SM had to be added to the SH diet to balance protein content. The cows were both fed and milked twice a day. After the morning milking (08:00 h) the cows individually received their daily PMR containing SH, SM or RSS. At noon, remaining PMR was removed and weighed. Only water was available until after the afternoon milking (15:30 h). The cows were then moved to pasture (alfalfa, white trefoil and barley) and allowed to graze daily strips. Table 1 lists the quantity of feed offered to each cow daily and Table 2 lists nutritional composition of the diets.

Microbiological Analysis

For bacterial enumeration, samples of rumen contents were collected through the ruminal cannula on day 23 and 25, just before the morning feeding (09:00 h). Rumen solids and liquid (100 g + 100 mL) were homogenised under a CO_2 atmosphere and filtered through two layers of gauze. Samples were diluted in decimal series (10^{-1} to 10^{-10}). For total bacterial concentration, 10^{-6} was diluted in decimal series (10^{-1} to 10^{-10}). For total bacterial concentration, 10^{-6}, 10^{-7} and 10^{-8} dilutions were inoculated into 10 mL of RGCSA medium according to a procedure[14] which follows the roll tube procedure[15]. Inoculated roll tubes were incubated for 5 days at 39ºC and counted under a dissecting microscope. Cellulolytic and amylolytic bacterial concentrations were estimated with a most probable number (MPN) procedure, using a basal medium with either cellulose (filter paper) or starch as the only added carbohydrate source[3,4]. All tubes were

Table 1. Daily offer of the different diet components to grazing dairy cows fed soybean by-products

Ingredients	Treatments (kg DM/d)		
	SH	SM	RSS
Pasture	15.0	15.0	15.0
Corn silage	2.4	7.0	7.0
Corn grain, ground	4.7	3.0	2.3
Soybean hulls	4.7	–	–
Soybean meal, Solv-ext, 44% CP	1.3*	2.8	–
Raw soybean seeds	–	–	2.8
Vitamins and minerals premix	0.2	0.2	0.2

SM was included in small amounts to balance the protein content of the SH diet.
SH: Soybean hulls, SM: Soybean meal (Solvent-extract, 44% CP), RSS: Raw soybean seeds, DM: Dry matter.

Table 2. Composition of diets supplemented with soybean hulls (SH), soybean meal (SM) or raw soybean seeds (RSS) for feeding grazing dairy cow

Diet	DM %	CP %	NDF %	ADF %	Lignin%	EE %	Ash%
SH	89.08	19.29	42.63	27.83	2.95	5.78	6.04
SM	89.25	23.07	18.83	9.72	1.69	5.72	7.43
RSS	89.88	23.3	19.87	11.56	2.99	12.71	8.41

DM: Dry matter, CP: Crude protein, NDF: Non detergent fiber, ADF: Acid detergent fiber, EE: Ether extract

incubated at 39ºC. Amylolytic bacteria were measured after 7 days, using Lugol's iodine reaction to determine starch digestion[20]. After 15 days incubation, cellulolytic bacterial concentrations were determined by observing the disappearance of filter paper.

For enumeration of protozoa, rumen samples were collected at three different times, 0, 3 and 6 h post feeding of the soybean by-product diet. Equal parts of rumen fluid and a saline-formalin solution (20% formalin in 0.85% NaCl solution) were mixed and stored. Prior to the analyses, a 2 mL aliquot of the fixed rumen sample was stained for at least 4 h with 2 mL of methyl green-formalin solution[19]. Protozoa quantification and generic composition were determined using a 1 mL counting chamber (Hausser Scientific Partnership, cat. No. 3800) following the procedures described by Dehority[9].

Volatile Fatty Acids Analysis

Rumen samples for VFA analyses were collected at 0, 3, 6, 9, 12, 15, 18, 21, and 24 h post feeding the soybean by-product diet. The samples were filtered through two layers of gauze, acidified with m-phosphoric acid (24%) in 3 N H_2SO_4 and kept at –20ºC before analysis. Volatile fatty acids were determined with a Shimadzu gas chromatograph GC-14B (Shimadzu Corporation, Kyoto, Japan) using a 2 m glass column packed with 10% polyethylene glycol adipate and 3% H_3PO_4 in chromosorb AW, and fitted with a flame ionization detector[12]. The working temperatures were 155ºC, 185ºC and 190ºC for the column, injector and detector, respectively. A Shimadzu CR6A integrator was used for peak quantification and identification. The internal standard was 2-methyl valeric acid.

Ruminal pH

Ruminal pH was determined in the liquid phase of rumen contents at 0, 3, 6, 9, 15, 18, 21, and 24 h after feeding the soybean by-product diet, using a digital pH meter (Orion).

Statistical analysis

For bacteria and protozoa analysis, analysis of variance (ANOVA) was performed according to the models described below[5,17]. Orthogonal contrasts were used as post ANOVA when significant effects were found.

The model for bacterial concentrations was:

$Y = u + Sqr + Per + Ani (sqr) + Treat + Sqr^* treat + e$,

where Y = response (means of two consecutive days), u = general mean, Sqr = square effect, Per = period effect, Ani (sqr) = animal effect within square, Treat = treatment effect, Sqr* treat = square by treatment interaction, and e = experimental error.

The model for protozoal concentrations was:

$Y = u + Sqr + Per + Treat + E (a) + Ti + Treat^* Ti + E (b)$,

where Y = response (means value of two consecutive days), u = general means, Sqr = square effect, Per = period effect, Treat = treatment effect, E (a) = experimental error to test treatments effects, Ti = time effect (hours), Treat* Ti = treatments by times (h) interaction, and E (b) = experimental error to test time effect and interaction. The response (in duplicate) was repeated over time (0, 3 and 6 h).

Results and discussion

Nutrient intake

Consumption differed among the animals and these data are shown in Table 3. In general, total crude protein

Table 3. Dry matter intake and nutrients intake by grazing dairy cows fed soybean by-products

	Treatments (kg/d)			SEM	Effects (P-value)
	SH	SM	RSS		
DM intake					
Pasture	7.0	7.2	6.4	0.45	0.4936
PMR	13.2	13.0	12.3	0.48	0.2684
Total	20.2	20.2	18.7	0.75	0.2731
CP intake					
Pasture	1.6	1.6	1.5	0.12	0.7933
PMR	2.3[a]	1.9[b]	1.8[b]	0.06	0.0003
Total	3.9[a]	3.5[ab]	3.4[b]	0.13	0.0360
NDF intake					
Pasture	2.3	2.2	2.1	0.19	0.8032
PMR	6.3[a]	4.6[b]	4.5[b]	0.21	<.0001
Total	8.6[a]	6.8[b]	6.6[b]	0.28	0.0007

SEM: Standard error of the means. a,b: Least square means with different superscripts differ ($P < 0.05$).

(CP) intake was higher in SH fed cows than RSS cows and total NDF intake in SH cows was greater than in the other two groups ($P < 0.05$).

Rumen pH and volatile fatty acids (VFA)

Mean values for fermentation products and pH are shown in Table 4. There was a significant treatment effect on pH ($P < 0.01$), total VFA ($P = 0.012$) and acetate:propionate ratio ($P < 0.01$). With SH in the diet, pH and the acetate:propionate ratio were lower than with either the SM or RSS supplements. Figure 1 presents the pH values at each sampling time, and shows that the SH diet was consistently lower over the entire 24 h period. Total VFA concentration was greater ($P < 0.012$) for SH (150.47 mM) compared with RSS (135.73 mM), but was not different ($P > 0.012$) from SM (139.04 mM). This is consistent with the ruminal pH data, i.e., the more acid produced, the lower the pH.

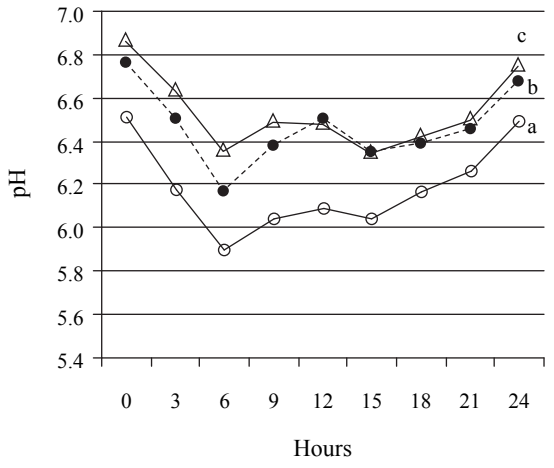

Fig. 1. Effect of supplementing different soybean by-products on rumen pH at various time intervals after feeding

—○— : SH, -●- : SM, —△— : RSS.

a,b,c: Treatments with different letters differ significantly ($P < 0.001$).

Effects on rumen bacteria

The concentrations of total, cellulolytic and amylolytic anaerobic rumen bacteria are shown in Table 5. Total bacterial concentrations were higher ($P = 0.06$) when SH and SM diets were fed. Although concentrations of cellulolytic bacteria tended to be higher with the SH and SM diets, differences were not significant. No significant effect was detected on the concentration of amylolytic bacteria. A significant period effect ($P = 0.01$) was detected in the concentration of cellulolytic bacteria, with the mean in the second period (17.93×10^7) greater than those in the first and third periods (1.97 and 9.01×10^7, respectively).

In an extensive review[22], it was reported that SM increased the flow of microbial protein to the duodenum as compared to other types of supplemental protein. In this study we observed that among the three kinds of soybean by-products used, SH and SM supported a higher concentration of total bacteria than RSS, suggesting that more microbial protein was probably available in the duodenum. Cellulolytic bacteria were also higher in the cows fed SH and SM. Although cellulolytic bacterial concentrations increased when cows were fed SH or SM, there was no marked change in the number of amylolytic bacteria. These responses can be explained on the basis that SM and SH are both essentially a protein and fiber source. Therefore SM and SH supplementation may have a positive effect on cellulolytic bacteria simply by supplying substrate. Cows supplemented with SH had the lowest acetate:propionate ratio. This fact can be partially explained because the main fiber digesting bacteria produce large amounts of succinate which can be transformed into propionate. On the other hand, the *in vitro* studies[21] demonstrated the importance of pH in the regulation of ruminal acetate to propionate ratio and methane produc-

Table 4. Ruminal pH and VFA concentrations of Holstein cows supplemented with soybean hulls (SH), soybean meal (SM) or raw soybean seeds (RSS)

	Treatment means			SEM	Effects (P-values)		
	SH	SM	RSS		T	H	T*H
pH	6.19[b]	6.46[a]	6.53[a]	0.04	<.001	<.001	0.597
Total VFA (mM)	150.47[a]	139.04[ab]	135.73[b]	3.29	0.012	<.001	0.143
Acetate (mM)	85.37	82.46	81.32	1.92	0.326	<.001	0.047
Propionate (mM)	41.90[a]	32.13[b]	30.00[b]	1.15	<.001	<.001	0.024
Butyrate (mM)	15.45	15.21	15.29	0.40	0.912	<.001	0.448
Acetone:propionate	2.09[a]	2.66[b]	2.79[b]	0.07	<.001	<.001	0.006

T: Treatment, H: Sampling time, T*H: Interaction.

a,b: Treatment means within a row with different superscripts differ at the indicated level of significance.

tion. The authors concluded that as much as 25% of the decrease in acetate to propionate ratio could be explained by the effect of low pH. In the present study, SH had a lower acetate:propionate ratio ($P < 0.001$), presumably as a result of its significantly lower pH (6.19) as compared to SM (6.46) and RSS (6.53). In a simultaneous experiment conducted in this lab, using similar experimental diets, lower milk fat % was observed with the SH treatment (3.09%) compared with SM (3.37%) and RSS (3.51%) treatments[13]. The lower milk fat % content in SH fed cows agrees with the lower ruminal acetate:propionate ratio detected in SH cows.

Effects on rumen protozoa

Table 5 shows the concentration of protozoa with the SH, SM and RSS diets. Protozoal concentrations tended to be lower for the SH treatment, 10.86×10^5 /mL, compared to SM, 15.51×10^5 /mL and RSS, 15.91×10^5 /mL. The protozoa concentrations at T0, T3 and T6 after feeding are shown in Fig. 2. It can be seen that protozoa concentrations with the SH diet were consistently lower. A significant treatment by time interaction was detected in that the concentration of protozoa was significantly lower for SH treatment only at T0. All three treatments showed similar quadratic curves, i.e., a decrease in concentration at 3 h post feeding followed by a slight increase at 6 h. This behaviour agrees with the diurnal changes reported[26,27,28] and might indicate the dilution effect from eating. However, methanogenic bacteria were not determined in this study because of the symbiotic relationship between protozoa and methanogenic bacteria; it was described[15] that the decrease in the number of protozoa when the animals were fed the SH diet could suggest a decrease in the number of methanogenic bacteria which in turn would lead to an increased production of propionic acid[15]. This fact agrees with the lower acetate:propionate ratio found in SH animals in this study.

Table 6 shows the generic composition of rumen protozoa at different hours after feeding the soybean by-prod-

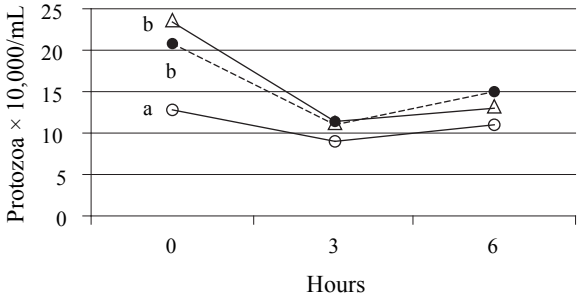

Fig. 2. Effect of different soybean by-products on rumen protozoal concentrations

—○— : SH, --●-- : SM, —△— : RSS.

Although protozoa numbers were systematically lower in SH cows with respect to SM and RSS cows, the differences were significant ($P < 0.05$) only at 0 h (prefeeding time).

a,b: Treatments with different letters differ significantly.

Table 5. Concentrations of total anaerobic bacteria, amylolytic bacteria, cellulolytic bacteria and protozoa in rumen contents of grazing dairy cow supplemented with soybean hulls (SH), soybean meal (SM) or raw soybean seeds (RSS)

	Treatment means			SEM	Effects (P-value)		
	SH	SM	RSS		T	H	T*H
Total anaerobic bacteria × 10^9/mL	6.05[a]	6.23[a]	4.41[b]	0.62	0.06	–	–
Amylolytic bacteria × 10^9/mL	5.02	4.32	3.79	0.84	0.59	–	–
Cellulolytic bacteria × 10^7/mL	12.92	12.28	7.70	2.11	0.21	–	–
Protozoa × 10^5/mL	10.86	15.51	15.91	2.64	0.27	0.001	0.013

a,b: Treatment means within a row with different superscripts differ at indicated level of significance.

Table 6. Generic composition of protozoa before feeding and at 3 and 6 hours post feeding in dairy cows fed diets containing soybean hulls (SH), soybean meal (SM) or raw soybean seeds (RSS)

Protozoa	T0 Diet[1)]			T3 Diet[1)]			T6 Diet[1)]		
	SH	SM	RSS	SH	SM	RSS	SH	SM	RSS
Vestibuliferids	32.5%	12.1%	18.9%	8.0%	5.2%	16.0%	13.0%	5.4%	6.8%
Entodinium sp.	67.5%	86.4%	75.4%	92.0%	92.0%	79.4%	86.0%	88.0%	83.7%
"Others"[2)]	–	1.5%	5.7%	–	2.8%	4.6%	1.0%	6.6%	9.5%

1): For each value, n = 3.

2): Includes the genera *Diplodinium, Ostracodinium, Epidinium,Ophryoscolex, Metadinium,* and *Eudiplodinium.*

uct diets. For easier interpretation the genus composition was combined into three groups as follows; a: vestibuliferids, (which included the genera *Isotricha* and *Dasytricha*), b: *Entodinium* sp. and c: other types of entodiniomorphs. The "Others" group refers to protozoa belonging to the genera *Diplodinium*, *Ostracodinium*, *Epidinium*, *Ophryoscolex*, *Metadinium*, and *Eudiplodinium*, all detected in the cows under the different treatments. Comparative studies (Table 6) show that the animals fed SH had a simpler or less diverse protozoal composition as compared to the animals fed SM and RSS. Except for a low percentage (1%) at 6 h, the SH diet did not support growth of the "Others" genera. The diurnal variation of vestibuliferids and entodiniomorphs observed in this study was similar to the diurnal cycles described by a number of authors[7,11,27], demonstrating that the prefeeding rise in vestibuliferid concentration and the following decrease several hours post feeding were the result of the sequestration of the vestibuliferids either on the walls of the rumen and reticulum or in the ventral portion of the rumen. Migration of the vestibuliferids back into the rumen has been explained either as a response to the incoming soluble sugars or depletion of storage carbohydrates[1,2,11]. Although supplementation with SH, SM and RSS modified the protozoal concentrations in different ways, the cyclic behavior of the vestibuliferids and entodiniomorphs was similar in all three treatments (Table 4).

Higher cellulolytic bacterial concentrations and VFA production in SH fed cows may be related to higher cellulose (60–65%) and smaller amounts of lignin contained in the SH.

Implications

Supplementation of grazing dairy cows with different soybean by-products can affect the rumen microflora and in turn their fermentative activity. Feeding SH and SM increased total bacterial concentrations. Total VFA concentration was increased and rumen pH decreased with the SH diet. The SH and SM diets tended to increase the concentration of cellulolytic but not amylolytic bacteria. Animals fed SH appeared to have lower concentrations of protozoa and less variation in the generic composition with respect to the other two diets. The lower acetate:propionate ratio detected in SH fed cows could be partially explained by its lower rumen pH, higher cellulolytic bacterial concentrations and lower concentrations of protozoa.

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