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In Vitro Yield of Microbial-N from Fermentation of Glucogenic and Lipogenic Diets Provided by Different Sources of Rumen Degradable Amino Acids

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Abstract

This experiment was conducted to evaluate the effects of different sources of rumen degradable amino acids, soybean meal versus a commercial source of hydrolyzed cottonseed cake with low molecular weight (Fortid®), on the *in vitro* ruminal fermentation responses and the microbial nitrogen yield of glucogenic (n=8) and lipogenic (n=8) diets. An *in vitro* gas production technique was used to determine the differences in microbial nitrogen yield of the diets. Sources of grain including barely and corn were used in the glucogenic diets as grinded or steam flaked, while in the lipogenic diets, corn silage, sugar beet pulp, linseed and prill fat were used to provide the energy needed for rumen fermentation. The experiment was a randomized design including two types of diets × two sources of rumen degradable amino acids factorial arrangement. The gas produced from the fermentable fraction (b) was higher for the lipogenic diets containing corn silage and F than those for the others. The fraction (b) and microbial nitrogen to diet nitrogen ratio were greater ($p<0.05$) for Fortid® versus soybean meal. The true substrate digestibility of glucogenic diets were significantly higher ($p<0.05$) compared to those of lipogenic diets. Both the grain sources used as steam flaked caused an increase in the rate of gas produced (c) and microbial nitrogen to diet nitrogen ratio. There were significant interactions ($p<0.005$) of the type of diets with either soybean meal or Fortid® on microbial nitrogen to diet nitrogen. The results indicated that pre-hydrolyzed protein sources which are rich in low molecular weight peptides may be considered as candidates to improve rumen feed fermentation and microbial nitrogen production with both glucogenic and lipogenic diets. This conclusion was arrived at from the different patterns of action of soybean meal and Fortid® in the present *in vitro* rumen fermentation responses.

Keywords: Microbial N; Glucogenic; Lipogenic; Amino acid

Abbreviations: G: Glucogenic; L: Lipogenic; SB: Soybean meal; F: Fortid® (a commercial protein source rich in low molecular weight peptide provided by hydrolyzed cottonseed cake, Mytech©); B: Barely; C: Corn; Gr: Grinded; SF: Steam flaked; CS: Corn silage; FP: Fat prill fat; LS: Linseed; SBP: Sugar beet pulp; BF: Barely grounded+Fortid®; BSB: Barely grounded+soybean meal; SFBF: Barely steam flaked+Fortid®; SFBSB: Barely steam flaked+soybean meal; CF: Grounded corn+Fortid®; CSB: Grounded corn+soybean meal; SFCF: Steam flaked corn+Fortid®; SFCSB: Steam flaked corn+soybean meal; CSF: Corn silage+Fortid®; CSSB: Corn silage+soybean meal; LF: Linseed+Fortid®; LSB: Linseed+soybean meal; SBPF: Sugar beet pulp+Fortid®; SBPSB: Sugar beet pulp+soybean meal; FPF: Prilled fat+Fortid®; FPSB: Prilled fat+soybean meal.

Introduction

All feedstuffs eaten by ruminants are first exposed to digestive activity in the rumen, the site of microbial fermentation of dietary components [1]. Dietary protein is divided into rumen-degradable (RDP) and non-degradable protein (RUP) with RDP compounds of non-protein and true protein-N in which true protein is degraded to peptides and rumen degradable amino acids (RDAA), and finally deaminated into ammonia-N or incorporated into microbial nitrogen [2]. Supplying more microbial nitrogen to the small intestine may reduce the requirement to supplement a diet with additional RUP sources; up to half of the amino acids absorbed by ruminants, and often two-thirds to three-quarters coming from microbial nitrogen [3]. Therefore, in ruminants, microbial nitrogen supply by the rumen to the small intestine must be considered as an important AA source. Ruminal microbial nitrogen synthesis depends on the supply of sufficient amounts and the type of carbohydrate as an energy source for the synthesis of peptide bonds [2]. It has been concluded that the amount of microbial nitrogen production may be influenced by the type of RDP and non-fiber carbohydrate (NFC). This may provide 30% to 45% of the diet on a dry matter basis. Different types of NFC have been shown to differ in the yields of microbial nitrogen from their *in vitro* fermentation [4].

Processing is necessary to increase the total-tract utilization of starch from grains; grains over-processing could cause an ample amount of digested starch in the rumen and maximize microbial nitrogen production flow to the small intestine. Bacteria of the rumen may be incorporated straight into amino acids and peptides from the diet [5]. Consequently, there is need to consider the proportion of peptides and amino acids in dairy cow rations. Peptides are intermediates in the transformation of protein to ammonia in the rumen, and their accumulation depends on the nature of diets [6]. The effects of starch source, dietary protein degradability, and their interactions on ruminal variables and cow behavior have been declared. The results of previous studies have shown that the addition of amino acids or peptides in the rumen significantly enhances microbial growth [7]. Therefore, it is hypothesized that the difference in ruminal fermentation and microbial synthesis between glucogenic and lipogenic diets may also be affected

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by the type and the level of RDAA. To the best of our knowledge, no previous study has implemented the effect of RDAA in glucogenic and lipogenic diets on *in vitro* microbial nitrogen production. According to this framework, the first aim of our research was to evaluate whether pre-digestion and production of peptide have an effect on microbial nitrogen production. The second objective of this experiment was to determine the kinetic parameters of gas production and microbial nitrogen production of glucogenic and lipogenic diets with different providers of amino acid sources; soybean meal versus Fortid® (hydrolyzed protein from cottonseed cake).

Materials and Methods

Experimental diets

The experimental diets are shown in Table 1. The study was carried out by using two types of diets known as glucogenic (G) and lipogenic (L), sources of rumen and highly degradable amino acids including soybean meal (SB) and Fortid® (a commercial protein source rich in low molecular weight peptide provided by hydrolyzed cottonseed cake, Mytech©) in a 2 × 2 factorial arrangement. The (G) diets were provided by the inclusion of two sources of grains including barely (B) and corn (C) used as grinded (Gr) or steam flaked (SF). To prepare the (L) diets, feed sources with high concentration of NDF including corn silage (CS) or fat (prill fat (FP) and linseed (LS)) or pectin (sugar beet pulp (SBP)) were used. Therefore, the glucogenic diets (n=8) were barely grounded+Fortid® (BF), barely grounded+soybean meal (BSB), barely steam flaked+Fortid® (SFBF), barely steam flaked+soybean meal (SFBSB), grounded corn+Fortid® (CF), grounded corn+soybean meal (CSB), steam flaked corn+Fortid® (SFCF), steam flaked corn+soybean meal (SFCSB); and the lipogenic diets (n=8) were corn silage+Fortid®(CSF), corn silage+soybean meal (CSSB), linseed+Fortid® (LF), linseed+soybean meal (LSB), sugar beet pulp+Fortid® (SBPF), sugar beet pulp+soybean meal (SBPSB), prilled fat+Fortid® (FPF), prilled fat+soybean meal (FPSB).

In vitro rumen fermentation and microbial nitrogen production

In order to evaluate the rumen fermentation responses and microbial nitrogen production, an *in vitro* experiment was conducted using the gas technique. Rumen inoculum was collected from three rumen-fistulated Holstein lactating dairy cows (620 ± 5 kg BW, 300 ± 5 DIM, mean ± SD) Prior to the morning feeding, fed with 3.2 kg of dry matter (DM) corn silage, 5.1 kg DM alfalfa hay and 12.8 kg of DM concentrate (containing: 24% corn grains, 20.5% barley grains, 27.1% soybean meal, 13.8% canola meal, 13.8% wheat bran, 0.3% calcium carbonate, 0.5% mineral and vitamin premix). The ruminal content was quickly filtered through four layers of cheese cloth to remove larger feed bits, and then moved to the laboratory. A sample of each experimental diet was weighed (250 mg), and then placed in a 125 ml serum bottle, replicated for four times and ran thrice. After that, the filtrate was used

	Glucogenica								Lipogenic							
	BF	BSB	SFBF	SFBSB	CF	CSB	SFCF	SFCSB	LF	LSB	SBPF	SBPSB	CSF	CSSB	FPF	FPSB
Corn silage	12.77	12.77	12.77	12.77	12.84	12.84	12.84	12.84	20.03	20.03	19.87	19.87	22.88	22.88	18.18	18.18
Alfalfa hay	26.45	26.45	26.45	26.45	26.59	26.59	26.59	26.59	36.01	36.01	34.91	34.91	37.16	37.16	35.95	35.95
Corn grain	14.43	14.43	14.43	14.43	23.51	23.51	0	0	10.89	10.89	9.35	9.35	11.03	11.03	10.87	10.87
Barely grain	24.54	24.54	0	0	14.71	14.71	14.71	14.71	11.05	11.05	9.49	9.49	11.21	11.21	11.03	11.03
Steam flaked corn	0	0	0	0	0	0	23.51	23.51	0	0	0	0	0	0	0	0
Steam flaked barely	0	0	24.54	24.54	0	0	0	0	0	0	0	0	0	0	0	0
Weat bran	7.63	7.63	7.63	7.63	7.67	7.67	7.67	7.67	7.62	7.62	4.38	4.38	4.39	4.39	11.24	11.24
Fortid®	5.89	0	5.89	0	5.92	0	5.92	0	6.62	0	6.01	0	5.61	0	7.31	0
XPS(Yasminomax®)b	3.48	3.48	3.48	3.5	3.5	3.5	3.5	3.5	3.48	3.48	3.79	3.79	3.8	3.8	3.84	3.84
Soybean meal	0	5.89	0	5.92	0	5.92	0	5.92	0	6.62	0	6.01	0	5.61	0	7.31
Canola meal	4.81	4.81	4.81	0	5.25	0	5.25	0	0	4.3	3.48	0	3.93	0	0	0
Linseed	0	0	0	0	0	0	0	0	4.3	0	0	8.72	0	0	0	0
Sugar beet pulp	0	0	0	0	0	0	0	0	0	0	8.72	0	0	0	0	1.57
Fat prill	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.57	0
CP (%)	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.5	17.6	17.6	17.5	17.5	17.5	17.5	17.5	17.5
NDF (%)	33.8	33.8	33.8	33.8	32.6	32.6	32.6	32.6	38.7	38.7	40.1	40.1	39	39	38.3	38.3
NFC (%)	42	42	42	42	43.7	43.7	43.7	43.7	35.4	35.4	36.5	36.5	35.8	35.8	35.1	35.1
ME (mg/ kg)	2.51	2.51	2.51	2.51	2.5	2.5	2.5	2.5	2.53	2.53	2.47	2.47	2.48	2.48	2.55	2.55

a) Glucogenic diets (n=8) containing: barely+Fortid® (BF), barely+soybean meal (BSB), steam flake barely+soybean meal (SFBSB), corn+Fortid® (CF), corn+soybean meal

(CSB), steam flake corn+Fortid (SFCF), steam flake corn+soybean meal (SFCSB), and lipogenic diets containing: corn silage+Fortid® (CSF) corn silage+soybean meal (CSSB), linseed+Fortid® (LF), linseed+soybean meal (LSB), sugar beet pulp+Fortid® (SBPF), sugar beet pulp soybean meal (SBPSB), prilled fat+Fortid® (FPF), and prilled fat soybean meal (FPSB); b) XPS: xylose protected soybean (Yasminomax®) meal containing (DM: 93%, NDF: 12.7%, ADF: 13.4%, CP: 53.4%, Ash: 8.36%, EE: 8.16%) was provided from Iranian local company named Yasnamehr

Table 1: Ingredients (% DM), chemical composition and energy content of the experimental diets.

for *in vitro* gas test described in detail by Grings et al. [8]. Cumulative gas production was measured at 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 hours of the incubation time. After the deduction of gas production from blank bottles, the data were fitted to an exponential model: $y=A \times (1-e^{-ct})$ [9], where (y) is the cumulative volume of the gas produced at time t (h), (A) is the asymptotic gas volume (ml/250 mg DM), and (c) is the fractional constant rate (ml/h). The halftime of gas production ($t_{1/2}$) was calculated as $t_{1/2}=\ln 2/c$ after the first 96-hour gas run, and then a second incubation with the diets as substrates was performed to obtain the degradability measures at substrate-specific times (i.e., $t_{1/2}$ for each substrate) [10]. The incubations were stopped at the diet-specific $t_{1/2}$ and the microbial N production at $t_{1/2}$ was determined in accordance with Grings [8] by using the "N balance" equation, which is given as follows:

Microbial N Production at $t_{1/2}$=Diet N+ΔNH_3 –N - NDFN at $t_{1/2}$

True substrate degradability was determined and calculated at $t_{1/2}$ [11]. The conversion of dietary N to microbial nitrogen (MN/DN) was determined by microbial nitrogen divided by dietary nitrogen. The concentrations of Ammonia-N per dietary nitrogen (Ammonia/DN) and ammonia-N+MN per dietary nitrogen (Ammonia+MN/DN) were measured as the rate of conversion of N to ammonia N and microbial-N in the rumen.

Chemical composition

A chemical analysis was conducted according to AOAC (2000). All the feed samples were grounded to pass through a 2 mm screen, and then analyzed for dry matter (135°C for 24 hours as per method 930.15), ash (535°C; method 942.05), CP (method 990.03), and ether extract (method 920.39) [12]. The method used to evaluate acid detergent and neutral detergent fibers (NDF and ADF, respectively) were based on Van Soest [13]. Sodium sulfite and heat stable alpha amylase were not used in the NDF and ADF assays, and were expressed without residual ash. Nitrogen fractionation was carried out as described by Higgs et al. [14]. Briefly, the proteins of SBM and Fortid® were divided into five fractions, namely A_1, A_2 B_1, B_2 and C. The A_1 fraction is made up of ammonia, and calculated by: Ammonia × (SP/100) × (CP/100) (% of CP); where SP is the soluble protein and CP is the crude protein. The fraction A_2 refers to soluble true protein and is rapidly degraded in the rumen; it is derived by: (SP × CP/100–A_1), with all of them based on the percentage of CP. The fraction B_1 refers to insoluble true protein, and it is obtained by the difference between CP and (A_1–A_2–B_2–C). The fiber-bound protein or fraction B_2 is obtained by: (NDICP–ADICP) × CP/100. Finally, the fraction C or indigestible protein is calculated as (ADICP × CP / 100).

Calculations and statistical analyses

The treatments were arranged as a randomized design including two types of diets (G and L) × two sources of RDAA (SB and F) in a factorial arrangement, as per the following statistical model:

$Yijk=\mu+\alpha i+\beta j+(\alpha\beta)ij+eijkYijk$,

when α is the main effect of diet, β is the main effect of CP source, and αβ is the interaction between them.

The data from gas production were statistically analyzed by using the general linear model's procedures of SAS 9.1 [15]. The differences between the means were assessed by the Duncan test at $p \leq 0.05$. Predesigned contrasts were used to compare the groups of treatments. The results when the contrast between the factors was significant are shown in Tables 3 and 4. The comparisons between corn silage and sugar beet pulp containing diets, prill fat versus linseed containing diets, and steam flaked corn and steam flaked barely were not significant, and therefore not shown in the tables.

Results

In this study, nitrogen fractionation was used to present the rumen degradation parameters of the amino acids used in the experimental diets. The data for the nitrogen fractionation of SB and F, according to CNCPS (version 6.5) protocols, are presented in Table 2. Fraction A_2 was significantly higher in F compared to that in SB ($p<0.05$); however, fractions B_1, B_2 and C were higher in SB than in F (A_2 in F was 4.56 folds higher than in SB, while B_2 and C were 7.8 and 2.62 folds higher in SB than in F, respectively).

The means of the gas produced (ml per 250 mg DM) from the experimental diets are shown in Figure 1. As observed, the volume of produced gas was maximum for BF and minimum for FPSB. The *in vitro* gas production parameters including the gas produced from the fermentable part (b), the constant rate of gas production (c), and halftime ($t_{1/2}$) are presented in Table 3. The main effects of RDAA and the types of diets on the (b) fraction were significant ($p<0.01$), and the main effects of RDAA and the types of diets were significant regarding the parameters (c) and ($t_{1/2}$). There was no interaction between RDAA and the diets regarding (b), (c) and ($t_{1/2}$). The contrasts between G and L, and SB and F showed significant differences between the types of diets and source of RDAA used in the present study regarding the parameter (b) ($p<0.05$). The contrasts between the types of diets, source of RDAA and forms of the grain used in the experimental diets were significant ($p<0.05$) for the parameter (c) and the halftime of gas production ($t_{1/2}$). In total, parameter (b) in the G diets was considerably higher than that in the L diets ($P<0.05$). The parameter (b) tended to be different among the treatments, with the highest values observed in CSF and the lowest values in FPSB ($p<0.05$). The (c) parameter of SFBSB was the greatest among all the diets ($p<0.05$), resulting in a significantly lower halftime of maximal gas production $t_{1/2}$ ($p<0.05$) for these diets.

The means of TSD, (MN/DN), (Ammonia/DN) and (Ammonia+MN/DN) in the types of the experimental diets, which are calculated from the incubation of the sample for $t_{1/2}$ using the gas test technique, are given in Table 4. In the case of TSD, the main effect of RDAA and diet was significant ($p<0.01$). All the main effects and interactions between RDAA and diets were significant for A+MN/DN and MN/ND. The main effect of diet was significant ($p<0.01$) for ammonia-N/DN. All the contrasts between G and L, and SB and F regarding TSD were significant ($p<0.05$). The glucogenic diets also had greater significant *in vitro* DM digestibility ($p<0.05$) compared to the others. The (MN/DN) of the diets were significantly influenced by the type of diets, RDAA and physical form of the grain used in the study ($p<0.05$). The contrasts between G and L, and SB and F and the physical type of grain showed significant differences between the types of diets, sources of RDAA, and the physical type of grain in the present study regarding (Ammonia+MN/DN) ($p<0.01$). The (MN/DN) was the minimum in the CSSB and the FPSB diets, and it was the maximum

	Soybean meal	Fortid®	SEM	p value
A1	0	0	0	-
A2	14.42	65.78	0.82	<0.001
B1	33.94	25.34	1.18	0.002
B2	42.75	5.48	2.88	0.001
C	8.87	3.38	1.88	0.02

Table 2: Nitrogen fractionation (CNCPS, version 6.5) of soybean meal and Fortid®.

	glucogenic[a)]								Lipogenic								SEM	P-value					
	Soybean meal				Fortid®				Soybean meal				Fortid®								Contrast		
	BSB	SFBSB	CSB	SFCSB	BF	SFBF	CF	SFCF	LSB	SBPSB	CSSB	FPSB	LF	SBPF	CSF	FPF		D[c)]	RDAA	D × RDAA	1[b)]	2	3
b[d)]	83.07	77.61	81.31	78.02	104.30	101.0	103.64	101.89	72.88	72.79	79.04	52.6	90.97	99.86	105.49	74.34	1.8	**	**	ns	**	**	ns
c	0.06	0.1	0.05	0.09	0.05	0.05	0.04	0.05	0.07	0.06	0.07	0.09	0.05	0.05	0.05	0.04	0.002	ns	**	ns	**	**	**
$t_{1/2}$	11.5	6.7	13.7	7.2	14.3	14.8	15.5	15.0	9.9	10.8	9.9	7.5	14.4	15.1	14.8	15.3	0.29	ns	**	ns	**	**	**

a) Glucogenic diets (n=8) containing: barely+Fortid (BF), barely+soybean meal (BSB), steam flake barely+soybean meal (SFBSB), corn+Fortid® (CF), corn+soybean meal (CSB), steam flake corn+Fortid® (SFCF), steam flake corn+soybean meal (SFCSB), and lipogenic diets containing: corn silage+Fortid® (CSF) corn silage+soybean meal (CSSB), linseed+Fortid® (LF), linseed+soybean meal (LSB), sugar beet pulp+Fortid® (SBPF), sugar beet pulp soybean meal (SBPSB), prilled fat+Fortid® (FPF), and prilled fat soybean meal (FPSB); b) Contrast 1: glucogenic versus lipogenic, 2: Fortid® versus soybean meal, and 3: steam flake versus grind; c) D; Diet, RDAA: rumen degradable amino acid, D × RDAA: interaction between RDAA and D; (*: P<0.05), (**: P< 0.01), (ns: Not significant); d) Fraction b: Gas production from fermentable part (ml per 250 mg sample), c: gas production constant (ml/h), TSD: true substrate digestibility (mg), t 1/2: halftime of gas production (h)

Table 3: *In vitro* gas production parameters of experimental diet.

	glucogenic[a)]								Lipogenic								SEM	P-value					
	Soybean meal				Fortid®				Soybean meal				Fortid®								Constract		
	BSB	SFBSB	CSB	SFCSB	BF	SFBF	CF	SFCF	LSB	SBPSB	CSSB	FPSB	LF	SBPF	CSF	FPF		D[c)]	RDAA	D × RDAA	1[b)]	2	3
TSD	613.33	570.67	608	597.33	777.33	780	778.67	758.67	474.67	673.33	541.33	342.67	722.67	738.67	710.67	729.33	21.50	**	**	ns	**	**	ns
MN/DN	0.46	0.63	0.36	0.62	0.46	0.44	0.45	0.53	0.51	0.23	0.07	0.09	0.52	0.48	0.41	0.39	0.02	**	**	**	**	**	**
Amonia/DN	0.45	0.28	0.50	0.27	0.47	0.49	0.48	0.43	0.44	0.36	0.49	0.37	0.47	0.46	0.47	0.52	0.02	**	ns	ns	**	**	**
A+MN/DN	0.89	0.89	0.87	0.9	0.93	0.95	0.93	0.96	0.95	0.59	0.56	0.46	0.99	0.95	0.87	0.91	0.001	**	**	**	**	**	**

Glucogenic diets (n=8) containing: barely+Fortid® (BF), barely+soybean meal (BSB), steam flake barely+soybean meal (SFBSB), corn+Fortid® (CF), corn+soybean meal (CSB), steam flake corn+Fortid® (SFCF), steam flake corn+soybean meal (SFCSB), and lipogenic diets containing: corn silage+Fortid® (CSF) corn silage+soybean meal (CSSB), linseed+Fortid® (LF), linseed+soybean meal (LSB), sugar beet pulp+Fortid® (SBPF), sugar beet pulp soybean meal(SBPSB), prilled fat+Fortid®(FPF), and prilled fat soybean meal (FPSB); a) Glucogenic versus lipogenic: 1, Fortid® versus soybean meal: 2, and steam flake versus grind: 3; D; Diet, RDAA: rumen degradable amino acid, D × RDAA: interaction between RDAA and D; (*: P<0.05), (**: P< 0.01), (ns: Not significant)

Table 4: True substrate digestibility (TSD, mg/g), microbial nitrogen to dietary nitrogen ratio (MN/ND), ammonia-N to dietary nitrogen ratio (Ammonia/ND), Ammonia-N+microbial nitrogen to dietary N ratio (A+MN/ND) of experimental diet.

in SFBSB and SFCSB with more starch included, resulting in a positive effect on lower ammonia N concentration. The FPF diet resulted in the highest (Ammonia/DN) (P<0.05). The ratio of ammonia N+MN to Diet N (A+MN/DN) had a tendency to increase in the LF diet (P<0.05). The response surface of NDF and NFC with microbial nitrogen is presented in Figure 2. When NDF was equal to 33 and NFC was equal to 36, microbial nitrogen was found to be the greatest.

Discussion

In the current study, we used two different sources of RDAA to evaluate the rumen fermentation potential of a wide range of glucogenic and lipogenic diets. In addition, microbial-N production was also determined. In order to evaluate the degradation potential of the amino acid sources, nitrogen fractionation [14] was used. The results indicated that F was more rumen degradable than SB as fraction A_2 in this amino acid provider was higher than that in SB. Fractions B_1, B_2 and C were higher in SB. Fraction B_1 was rapidly degraded in the rumen, and some of fraction B_2 was fermented in the rumen and some escaped to the lower gut [16]. Fraction C could not be degraded in the rumen and did not supply amino acids post-ruminally; thereby, this result showed that F and SB were different regarding the nitrogen fractions, and F was more degradable than SB.

The main aim of this study was to investigate the effects of energy and type of RDAA in glucogenic and lipogenic diets on ruminal fermentability and MCP yield by using the gas production technique. As observed in Figure 1, the highest gas volume in BF showed that F was more degradable in the rumen to provide more fermentable substrate, given the fact that the gas produced is directly proportional to the rate at which the substrate is degraded [17]. The minimum gas production in FPSB might be due to the prevention of substrate availability for bacteria by prill fat or the possible toxicity of microorganisms. As expected, the amount of gas produced by G diets was especially higher compared to that of the L diets. This was not unexpected as a lot of *in vitro* studies have reported significant differences in the fermentation characteristics of various carbohydrate sources [18]. The gas produced from the fermentable part (b) and the rate of gas production (c) were affected by the sources of RDAA; (b) was 30% higher in F and (c) was 63% higher in SB containing diets. It can be seen that SB had high values for B_2 and C fractions, leading to difficulties in attachment by microorganisms and causing lower gas production than F. Higher fraction (b) was observed in G diets as compared to that of L diets. Glucogenic dietary components were fermented in the rumen to supply energy for rumen microorganisms and produced more *in vitro* gas [19]. However, when grounded grain was used, parameter (b) increased while parameter (c) decreased in the SF containing diets. High rate of gas production was observed in SF, possibly influenced by the availability of rumen carbohydrate for the microbial population. The maximum rate of gas production (c) was observed in the SFBSB diet. Our results were consistent with the findings of other studies, which confirmed that steam flaking of grains led to greater production of *in vitro* gas compared to unprocessed grain [18]. Soluble starch of the grain in the rumen is readily susceptible to enzymatic hydrolysis, and therefore, whether amino acids and peptides can stimulate the growth of rumen bacteria, either *in vitro* or *in vivo*, will depend on the energy source [20]. The slowest gas production was observed in FPSB, indicating that prill fat was less readily available to the microbes in the rumen [21].

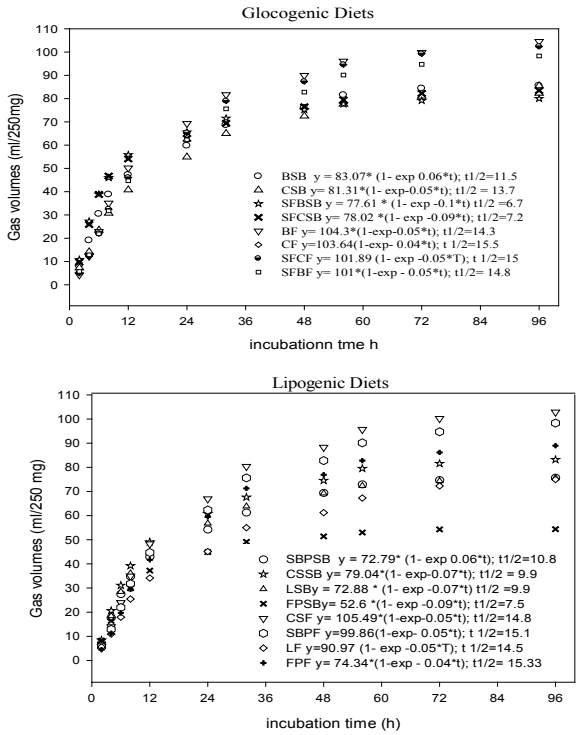

Figure 1: Gas produced (ml per 250 mg DM) from the experiment diets, including 1. Glucogenic diets (n=8) containing barely+Fortid® (BF), barely+soybean meal (BSB), steam flake barely+soybean meal (SFBSB), corn+Fortid® (CF), corn+soybean meal (CSB), steam flake corn+Fortid®(SFCF), steam flake corn+soybean meal (SFCSB), and lipogenic diets containing: corn silage+Fortid (CSF) corn silage+soybean meal (CSSB), linseed +Fortid® (LF), linseed+soybean meal (LSB), sugar beet pulp+Fortid®(SBPF), sugar beet pulp soybean meal (SBPSB), prilled fat+Fortid®(FPF), prilled fat soybean meal (FPSB).

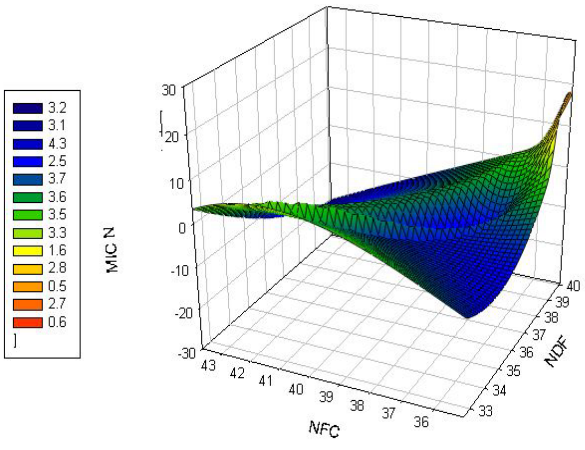

Figure 2: Response surfaces of NDF and NFC with microbial nitrogen production.

Higher TSD in the Fortid® containing diets were observed. It was probably due to the high degradability of the soluble AA from F compared to SB [2]. Additionally, *in vitro* TSD were observed to have a high correlation with gas production [22], and F containing diets had higher parameter (b) than SB. There was no significant difference between SF and Gr regarding TSD. This finding is consistent with May et al. [23], whose control had higher digestibility than the SF containing treatments. Glucogenic diets had higher (11% higher) TSD than L diets ($p<0.01$). This discrepancy was likely because of more degradability of glucogenic ingredients. It's possible that the high amount of NFC in BF diets was the reason behind the higher amount of TSD observed in this diet, because microbes that ferment NFC not only use NH_3, but peptides as well.

Higher MN/DN, Ammonia/DN and A+MN/DN were observed in F than in SB diets (0.46, 0.47, and 0.94 vs. 0.37, 0.39, and 0.77, respectively), which could likely be attributed to the high degradability of F. It can be supposed that dietary non-protein N will be of small advantage to the ruminant unless it is first converted into ammonia, and then utilized in the rumen for microbial protein synthesis, and the amount of non-protein N that can be utilized will depend upon the amount of fermentable energy available for microbial synthesis. But, F is a RDAA and causes increased production of both MN and ammonia. This effect might be related to the peptide amino acids which are more efficiently converted to cell protein than free amino acids. The production of ammonia was also faster by the peptides than the amino acids [24]. It was proposed that higher MN/DN, A+MN/DN (0.49, 0.92, and 0.34, 0.78, respectively, for glucogenic and lipogenic diets) and lower Ammonia/DN (0.42, for glucogenic, and 0.44 for lipogenic diets) was the result of more accessibility of carbon skeletons and energy from fermentable carbohydrate for the conversion of non-protein nitrogen like RDAA to microbial protein, and thus the supply of energy from glucogenic diets stimulated MN synthesis [25]. Greater MN/DN and A+MN/DN (0.56 and 0.93, respectively) in SF, and lower Ammonia/DN (0.37) versus lower MN/DN and A+MN/DN (0.43 and 0.91, respectively) in Gr diets, and high Ammonia/DN (0.47) might be influenced by the unprocessed grains which are less degraded in the rumen, thereby resulting in the reduction of energy supply and MN synthesis [26]. This processing effect was similar to that recorded in cattle fed on barley grain diets subjected to different degrees of processing [27].

The maximum MN/DN in SFBSB and SFCSB could be due to structural and solubility characteristics of the protein found in soybean meal that make it easily attachable for ruminal microorganisms. This greater microbial protein synthesis was attributed to higher starch and OM digestion in the rumen for barley diets, Increasing starch digestibility in the rumen stimulated more microbial protein synthesis. There is an appropriate balance between starch digestion in the rumen and the intestine to support microbial protein synthesis as well as a moderate escape of starch to the duodenum [28]. Steam flaking may increase the feeding value of grains by enhancing starch digestibility in the rumen, produce more microbial protein and low amount of ammonia concentration in the rumen [20]. This may be related to the synchrony between ruminal protein and carbohydrate digestion. Microbial protein synthesis depends largely on the available amount and fermentation rate of carbohydrates and N in the rumen [29]. The results of the present study were consistent with those of a previous study pertaining to the reduction of *in vitro* ammonia concentrations with steam flaking. The earlier study cited also suggested that steam flake processing could improve ruminal fermentability and energy utilization by ruminants [30]. Our results from response surfaces showed that with increasing NFC, microbial protein synthesis increased as well (Figure 2). Also, with decreasing NDF, microbial production increased. Hristove et al. found that water soluble sugars caused a decrease in ammonia-N concentration in the rumen through the decreased production of ammonia; while starch increased the uptake

of ammonia for microbial protein synthesis [29]. The rate of substrate fermentation was roughly proportional to the rate of microbial growth, with more rapidly fermented substrates yielding more microbial mass. Stock et al. reported that increasing the proportion of nonstructural carbohydrate (NSC) and reducing the NDF of diets could result in higher yields of microbial protein [25].

Conclusion

This is the first study which highlighting the role of sources of rumen degradable amino acids in both glucogenic and lipogenic diets in rumen fermentation responses and microbial protein yield. In conclusion, the inclusion of Fortid® as a rich source of the low molecular peptide with high rumen degradable amino acids used in both glucogenic and lipogenic diets was associated with *in vitro* high yield of rumen microbiota. This indicates that rumen degradable amino acids are a possible factor affecting microbial growth rate in the rumen. Although, glucogenic diets likely account for the high rate of rumen microbial growth, its possible that besides rumen degradable amino acids factors such as starch may also impact the microbial yield. The use of both steam flaked barley and corn, in overall, could provide higher rumen available energy compared with the milled grain, fermentation parameters obtained from gas production technique were improved. The results also underline the importance of glucogenic diets in contrast with regarding rumen fermentation responses.

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