



Effects of Soybean Small Peptides on Rumen Fermentation and on Intestinal and Total Tract Digestion of Luxi Yellow Cattle

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ABSTRACT: Four Luxi beef cattle (400±10 kg) fitted with ruminal, duodenal and ileal cannulas were used in a 4×4 Latin square to assess the effects of soybean small peptide (SSP) infusion on rumen fermentation, diet digestion and flow of nutrient in the gastrointestinal tract. The ruminal infusion of SSP was 0 (control), 100, 200 and 300 g/d. Ruminal SSP infusion linearly ($p < 0.01$) and quadratically ($p < 0.01$) increased microbial protein synthesis and rumen ammonia-N concentration. Concentrations of total volatile fatty acid were linearly increased ($p = 0.029$) by infusion SSP. Rumen samples were obtained for analysis of microbial ecology by real-time PCR. Populations of rumen *Butyrivibrio fibrisolvens*, *Streptococcus bovis*, *Ciliate protozoa*, *Ruminococcus flavefaciens*, and *Prevotella ruminicola* were expressed as a proportion of total Rumen bacterial 16S ribosomal deoxyribonucleic acid (rDNA). *Butyrivibrio fibrisolvens* populations which related to total bacterial 16S rDNA were increased ($p < 0.05$), while *Streptococcus bovis* populations were linearly ($p = 0.049$) and quadratically ($p = 0.020$) decreased by infusion of SSP. Apparent rumen digestibility of DM and NDF were (Q , $p < 0.05$; L , $p < 0.05$) increased with infusion SSP. Total tract digestion of DM, OM and NDF were linearly ($p < 0.01$) and quadratically ($p < 0.01$) increased by infusing SSP. The flow of total amino acids (AA), essential amino acids (EAA) and individual amino acids were linearly ($p < 0.01$) and quadratically ($p < 0.01$) increased with infusion SSP. The digestibility of Lysine was quadratically ($p = 0.033$) increased and apparent degradability of Arginine was linearly ($p = 0.032$) and quadratically ($p = 0.042$) increased with infusion SSP. The results indicated that infusion SSP could improve nutrient digestion, ruminal fermentation and AA availability. (**Key Words:** Cattle, Digestibility, Flow, Microbial, Rumen Fermentation, Soybean Small Peptide)

INTRODUCTION

It has been demonstrated that rumen bacteria use both ammonia-N and non-ammonia-N (NAN; e.g. amino acid, peptides) for bacterial protein synthesis and some species of bacteria prefer NAN to ammonia-N (Russell et al., 1992; Cruz Soto et al., 1994; Griswold et al., 1996). Lindemann et al. (2000) and Dabrowski et al. (2003) showed that supplying peptides offered an advantage over free AA that is easily deaminated in the rumen. However, discrepant results were reported from *in vitro* studies that assessed the

effects of peptides on the rumen metabolism. Whilst some studies showed that rumen bacteria growth, fibre digestion and overall rumen fermentation were improved by supplementation of peptides (Griswold et al., 1996; Carro and Miller, 1999; Russi et al., 2002), this was not observed in other studies (Cruz Soto et al., 1994; Jones et al., 1998). However, there is little information about the effects of supplementation of peptide on rumen microbial activity and on ruminal metabolism *in vivo*.

Small peptides obtained from proteolytic hydrolysis of different plant proteins have been shown to have various biological and pharmaceutical activities including enhancing growth performance and immunological status (Kotzamanis et al., 2007), offering a more cost-effective way of supplementing AA (Zimmerman et al., 1997; Lindemann et al., 2000; Dabrowski et al., 2003), improving nitrogen retention (Adibi et al., 1993), and increasing mohair fiber growth (Puchala et al., 2002). Zhang et al. (2007) reported that feeding or duodenal infusing SSP, improved nitrogen balance and protein biological value and

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increased digestibility of dietary nutrients in goats and that supplying SSP by infusing through a duodenum fistula was more efficient than feeding. Liu et al. (2009) also observed increased absorption of small peptides from small the intestine and increased milk protein synthesis by duodenal infusion of SSP. However, information of the effects of SSP on rumen fermentation, microbes and digestive tract metabolism is limited. The objective of this study was to determine the effects of SSP on ruminal fermentation and nutrition digestibility of beef cattle.

MATERIALS AND METHODS

Experimental design and diets

The experiment consisted of four treatments and was conducted in a 4×4 Latin square arrangement using four Luxi Cattle steers (approximate 30 months old) averaging 400±10 kg body weight and fitted with ruminal, proximate duodenal (simple T-piece) and terminal ileal cannula. The fistulation procedures and animal care were according to the experimental protocol that was reviewed and approved by Shandong Academy of Agriculture Sciences. Cattle were fed indoors in individual stalls. Each period lasted for 20 d, comprising a 10-d adaptation and 10-d experiment and sampling. The four treatments were basal diet supplemented with SSP which was produced from proteolytic hydrolysis (ZS Duqing Biotech CO., Ltd., Shandong, China) and dosed by ruminal infusion at the levels of 0, 100, 200 and 300 g/d (denoted as Control, SSP100, SSP200 and SSP300 respectively). The AA composition of the SSP is shown in

Table 1. The concentration (mg/g) of total amino acids and peptide amino acids in soybean small peptides

Items	Total amino acid content	Peptide amino acid content
Aspartic acid	90.91	89.06
Glutamic acid	196.10	189.48
Serine	46.60	46.06
Histidine	19.70	18.97
Glycine	32.60	32.09
Threonine	32.00	30.72
Arginine	62.00	60.85
Alanine	34.90	32.85
Tyrosine	27.60	26.19
Cystine	6.70	6.19
Valine	32.00	30.65
Methionine	12.50	11.45
Phenylalanine	38.20	35.49
Isoleucine	30.80	30.15
Leucine	61.10	55.77
Lysine	55.40	54.59
Proline	52.30	52.29
TAA ¹	831.41	802.85

¹ TAA = Total amino acids.

Table 1. The basal diet (Table 2) was a total mixed ration (TMR) formulated to meet the nutrient requirements of cattle (Feeding Standard of Cattle of the People's Republic of China, NY/T815- 2004). The TMR was offered in equal amount twice daily at 07:00 and 19:00 for 95% of the *ad libitum* intake and all cattle had free access to fresh water. The daily amount of SSP of each treatment was dissolved in 1,000 ml of 0.9% physiological saline and was infused using a peristaltic pump (BL-100, Prefluid Technology Company, Jiangsu, China) into the rumen through the ruminal cannula upon morning feeding from d 11 to d 20 at 7:00 clock everyday. Cattle without SSP were infused 1.0 L of physiological saline only (Control).

Sampling and preparations

Samples of feed offered were taken daily during the entire experimental period, composted, oven dried (55°C) and ground to pass 1.0 mm screen for chemical analysis. Duodenal and ileal flow and fecal output were measured by 15 g/d of chromic oxide (Uden et al., 1980), which was given as three boluses of 2.5 g each (7.5 g twice daily at 07:00 and 19:00 h) and administered into the rumen via fistula before feeding on d 11 through 20. On d 18 to 20, samples of duodenal (300 ml) and ileal (200 ml) chyme, faeces (300 g wet basis) and ruminal fluid (200 ml) from the rumen were collected every 6 h daily. The sampling time was adjusted 2 h ahead of the previous day so that samples obtained represented every 2 h during 24-h period

Table 2. Ingredients and chemical composition of the basal diet (% DM) fed during the experiment

Ingredients	Content
Chinese wild rye	61.61
Corn	27.00
Wheat bran	2.89
Cottonseed meal	6.75
Calcium hydrogen phosphate	0.35
Calcium carbonate	0.58
Sodium chlorid	0.39
Sodium bicarbonate	0.39
Minerals premix ¹	0.04
Vitamins premix ²	0.19
Total	100.00
Analyzed composition	
CP %	9.55
ADF %	27.68
NDF %	52.27
Ca %	0.86
Calculated composition	
NEm/(MJ/kg)	6.48

¹ Provides per kg of minerals premix: 40 g of Mn, 60 g of Zn, 20 g of Fe, 30 g of Cu, 1.5 g of I, 1 g of Se, 400 mg of Co.

² Provides per kg of minerals vitamins premix: 30,000,000 IU of vitamin A, 100,000,000 IU of vitamin D, 6,000 mg of vitamin E.

(12 total samples). The duodenal, ileal and fecal samples were immediately stored at -20°C, lyophilized and ground in a Wiley mill to pass a 1.00 mm screen for laboratory analysis. Sub-samples of rumen fluid were also strained through four layers of cheesecloth, pH measured immediately using a pH meter (Model PB-20, Sartorius, Germany), and were then stored at -20°C for analysis of ammonia-N, microbial crude protein (MCP) and volatile fatty acids (VFA).

Approximately 200 ml of rumen fluid collected at 6 h after feeding on d 20 of each period was immediately stored at -80°C for determination of the relative quantity to total bacterial 16 S rDNA of *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, *Ciliate protozoa*, *Butyrivibrio fibrisolvens* and *Streptococcus bovis*.

Chemical analysis

Samples were analyzed for total N, DM, OM, Ca and P (AOAC, 1997), and for NDF and ADF (Van Soest et al., 1991). Amino acid analyses were performed according to the methods of the AOAC (1997) using the cationic HPLC system that utilizes post derivatization with ninhydrin followed by UV.

Chromium concentrations of duodenal, ileal, and fecal samples were determined by atomic absorption spectrophotometry with an acetylene/air flame according to the methods of Williams et al. (1962). Rumen fluid samples were analyzed for ammonia N and VFA concentration using methods described by Hu et al. (2005) and for MCP by analyzing purine concentration using method described by Zinn and Owens (1986).

Quantification of microbial 16S rRNA marker genes by RT-PCR: Total DNA was extracted from rumen fluid by a SDS-based DNA extraction method as described by Zhou et al. (1996). The primers of total bacteria, *ciliate protozoa*, *Prevotella ruminicola*, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens* and *Streptococcus bovis* are listed

in Table 3, as described by Khafipour et al. (2009) and Stuart and McSweeney (2006). The species-specific real-time quantitative PCR was performed using the Roche 4500 real time PCR system with fluorescence detection of SYBR green dye. Amplification conditions were as follows: one cycle at 95°C for 30 s for initial denaturation and followed by 40 cycles of 95°C for 5 s and 60°C for 20 s. Specificity of amplified products was confirmed by melting temperature and dissociation curves after each amplification. Amplification efficiencies for each primer pair were investigated by examining dilution series of total ruminal microbial DNA template in triplicate.

Data calculation and statistical analysis

Duodenal, ileal, and fecal output for each cattle were calculated as the amount of chromic oxide consumed (g/d) divided by the chromic oxide concentration (g/kg of DM) in the duodenal and ileal chyme or feces (Wang et al., 1998). Nutrient apparently digested in the rumen was calculated as the difference between the nutrient intake (kg/d) and its duodenal flow (kg/d). Nutrient apparently digested in the small intestinal was calculated as the difference between the duodenal flow (kg/d) and ileum flow (kg/d). Nutrient apparently digested in the large intestinal was calculated as the difference between the ileum flow and fecal output (kg/d). Nutrient apparently digested in the total tract was calculated as the difference between the intake and fecal output (kg/d).

The abundance of 16S rRNA marker gene copy number for the ciliate protozoa, *P. ruminicola*, *B. fibrisolvens*, *R. flavefacien* and *S.bovis* were expressed relative to the copy number of total rumen bacterial 16S rRNA according to the following equation:

$$\text{Relative quantification of target} = 2^{-(Ct \text{ target} - Ct \text{ total bacteria})}$$

Where Ct represents threshold cycle.

Table 3. PCR primers for real-time PCR assay

Target species	Forward/reverses	Primer sequence
<i>Butyrivibrio fibrisolvens</i> ¹	F	ACCGCATAAGCGCACGGA
	R	CGGGTCCAICTTGTACCGATAAAT
<i>Prevotella ruminicola</i> ¹	F	GCGAAAGTCGGATTAATGCTCTATG
	R	CCCATCCTATAGCGGTAAACCTTTG
<i>Streptococcus bovis</i> ¹	F	TTCCTAGAGATAGGAAGTTTCTTCGG
	R	ATGATGGCAACTAACAATAGGGGT
<i>Ruminococcus flavefaciens</i> ¹	F	GAGTGAAGTAGAGGTAAGCGGAATTC
	R	GCCGTACTCCCCAGGTGG
<i>Ciliate protozoa</i> ¹	F	GCTTTCGWTTGGTAGTGTATT
	R	CTTGCCCTCYAATCGTWCT
Total bacteria ²	F	CGGCAACGAGCGCAACCC
	R	CCATTGTAGCACGTGTGTAGCC

¹ Khafipour et al. (2009). ² Denman and McSweeney (2006).

Data were analyzed as 4×4 Latin square using Mixed procedure of SAS (version 8.0, 2000). The following model was fitted to all variables that did not have repeated measurements over time.

$$Y_{ijkl} = \mu_i + C_i + P_j + T_k + Z_l + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the dependent variable, μ is the overall mean, C_i is the effect of cattle ($i = 1$ to 4), P_j is the effect of period ($j = 1$ to 4), T_k is the effect of treatment ($k = 1$ to 4), Z_l is the effect of sample time l , and ε_{ijkl} is the residual error. All terms were considered fixed except for ε_{ijkl} which was considered random. Polynomial contrasts were used to assess linear and/or quadratic dose-effects of SSP. Differences among treatments were declared significant at $p < 0.05$ using LSMEANS with PDIF and adjusted with a Dunnett's test of SAS (2000).

RESULTS

Ruminal fermentation characteristics

In general, increasing the rate of SSP infusion from 100 to 300 g/d increased concentrations of ammonia-N (L, Q; $p < 0.001$), MCP (L, Q; $p < 0.001$) and VFA (L; $p = 0.029$) and molar proportions of acetate (Q; $p < 0.05$) and propionate ($p < 0.001$), but reduced molar proportion of butyrate (L, $p < 0.05$; Q, $p < 0.001$) and acetate:propionate (A:P) ratio (L, Q; $p < 0.001$; Table 4). Compared to Control, rumen fluid from all SSP infused cattle had higher ($p < 0.001$) concentrations of ammonia-N and MCP, lower ($p < 0.001$) A:P ratio but similar pH. In contrast, effects of

SSP on ruminal VFA profile depended on the rate of SSP infusion. Rumen fluid from cattle in SSP200 and SSP300 had higher ($p < 0.05$) concentrations of VFA and higher ($p < 0.05$) molar proportions of propionate than that in Control, whereas these differences were not found between SSP100 and Control.

Infusion of SSP at the rates of 100, 200 and 300 g/d increased ($p < 0.05$) percentage of 16S rRNA gene copies of *S. bovis*, but did not affect percentages of 16S rRNA gene copies of *P. ruminicola*, *R. flavefaciens* or ciliate protozoa in the total bacterial 16S rRNA gene copies as compared to that of Control. In contrast, percentage of 16S rRNA gene copies of *B. fibrisolvans* was increased ($p < 0.05$) by SSP infused at the rate of 200 g/d but not others.

Nutrient intake, flow and digestibility in gastrointestinal tract

Nutrient intakes and apparent total tract digestibility are presented in Table 5. Because DMI was restrained, intake of DM, OM, NDF, and ADF did not differ among treatments. Nitrogen intake increased as the levels of SSP infusion increasing ($p < 0.05$). The duodenal flow (kg/d) of DM was linearly ($p < 0.001$) and quadratically ($p < 0.001$) decreased and the duodenal flow of NDF was linearly decreased ($p = 0.042$) by the infusion of SSP. On the contrary, duodenal flow of ADF increased ($p < 0.05$) in cattle receiving 300 g/d of SSP infusion as compared to the control.

Apparent rumen digestibility of DM was quadratically ($p < 0.05$) increased, and apparent rumen digestibility of NDF was linearly ($p < 0.05$) increased, whereas the ileum flow of NDF decreased (L, Q, $p < 0.05$) with the increasing

Table 4. Effects of soybean small peptide (SSP) infusion into rumen at the rates of 0 (Control), 100 (SSP100), 200 (SSP200) and 300 (SSP300) g/d on characteristics of rumen fermentation and microbial abundance as determined by marker gene copy number in total bacterial 16 S rRNA (percentage of total bacterial 16 S rRNA) ($n = 4$)

Item	Treatment				SEM ¹	Contrast ²	
	Control	SSP100	SSP200	SSP300		L	Q
pH	6.54	6.48	6.54	6.56	0.618	0.686	0.820
Ammonia N (mg/L)	66.91 ^c	84.39 ^b	96.79 ^b	116.31 ^a	9.363	<0.001	<0.001
Microbial protein (mg/L)	62.49 ^c	74.99 ^b	82.27 ^b	92.45 ^a	8.594	<0.001	<0.001
Total VFA (mmol/L)	86.03 ^b	89.92 ^{ab}	91.74 ^a	92.01 ^a	1.313	0.029	0.092
Acetate (mol/100 mol)	75.97 ^a	71.93 ^c	73.74 ^{bc}	74.35 ^{ab}	1.035	0.423	0.038
Propionate (mol/100 mol)	13.98 ^c	14.53 ^c	16.48 ^b	19.14 ^a	0.8	<0.001	<0.001
Butyrate (mol/100mol)	10.05 ^b	13.55 ^a	9.78 ^b	6.51 ^c	1.004	0.022	<0.001
Acetate:propionate	5.45 ^a	4.95 ^b	4.49 ^c	3.92 ^d	0.672	<0.001	<0.001
Ruminal microbial							
<i>P. ruminicola</i> ($\times 10^{-2}$)	1.94	2.18	2.03	2.21	0.732	0.231	0.437
<i>R. flavefaciens</i> ($\times 10^{-2}$)	5.51	5.91	5.78	5.75	0.832	0.699	0.93
<i>B. fibrisolvans</i> ($\times 10^{-4}$)	1.50 ^b	1.68 ^{ab}	1.82 ^a	1.70 ^{ab}	0.622	0.162	0.194
<i>S. bovis</i> ($\times 10^{-6}$)	5.91 ^a	4.87 ^b	4.85 ^b	4.94 ^b	0.801	0.049	0.020
Ciliate protozoa ($\times 10^{-2}$)	3.82	3.83	3.98	3.87	0.771	0.686	0.866

¹ SEM = Standard error of mean. ² p values for the linear (L) or quadratic (Q) effects of SSP infused at the rates 0, 100, 200, 300 g cattle⁻¹ d⁻¹.

^{a-d} Means within a row with different superscript letter differ ($p < 0.05$).

Table 5. Effects of soybean small peptide (SSP) infusion into rumen at the rates of 0 (Control), 100 (SSP100), 200 (SSP200) and 300 (SSP300) g/d on digestion of nutrient at different digestive sites of Luxi Yellow cattle (n = 4)

Item	Treatment				SEM ¹	Contrast ²	
	Control	SSP100	SSP200	SSP300		L	Q
DM							
Flux to duodenum (kg/d)	4.15 ^a	4.15 ^a	4.07 ^a	3.97 ^b	2.675	<0.001	<0.001
Flux to ileum (kg/d)	3.21	3.18	3.15	3.14	3.227	0.381	0.677
Fecal flux (kg/d)	2.71 ^a	2.62 ^b	2.59 ^{bc}	2.53 ^c	2.569	0.001	0.002
Apparent digestion, of intake (g/100 g)							
Rumen	43.02 ^b	43.14 ^b	44.12 ^b	45.49 ^a	0.918	0.067	0.021
Small intestine	12.92	13.48	12.63	11.4	1.092	0.184	0.275
Large intestine	6.94	7.79	7.65	8.39	1.055	0.189	0.436
Total tract	62.88 ^b	64.41 ^a	64.40 ^a	65.28 ^a	0.905	<0.001	0.002
OM							
Flux to duodenum (kg/d)	3.93 ^a	3.97 ^a	3.91 ^a	3.80 ^b	2.373	0.123	0.153
Flux to ileum (kg/d)	3.05	3	2.97	2.96	3.319	0.183	0.399
Fecal flux (kg/d)	2.61 ^a	2.47 ^b	2.47 ^b	2.42 ^b	2.774	<0.001	0.001
Apparent digestion, of intake (g/100 g)							
Rumen	47.54 ^b	47.04 ^b	47.80 ^b	49.26 ^a	0.807	0.981	0.25
Small intestine	11.77	12.83	12.64	11.23	1.15	0.713	0.484
Large intestine	5.83	7.13	6.62	7.29	1.072	<0.001	0.001
Total tract	65.13 ^b	67.00 ^a	67.05 ^a	67.79 ^a	0.943	<0.001	0.001
NDF							
Flux to duodenum (kg/d)	2.17 ^a	2.11 ^{ab}	2.15 ^{ab}	2.09 ^b	2.532	0.042	0.136
Flux to ileum (kg/d)	2.08 ^a	1.98 ^{ab}	1.99 ^{ab}	1.94 ^b	2.848	0.022	0.04
Fecal flux (kg/d)	1.73 ^a	1.71 ^a	1.66 ^b	1.61 ^c	2.224	0.001	0.004
Apparent digestion, of intake (g/100 g)							
Rumen	48.77	50.28	49.26	50.81	0.992	0.042	0.136
Small intestine	2.22	2.91	3.85	3.42	1.13	0.177	0.31
Large intestine	8.27	6.22	7.76	7.68	1.106	0.951	0.582
Total tract	59.26 ^c	59.42 ^c	60.87 ^b	61.91 ^a	0.863	0.001	0.004
ADF							
Flux to duodenum (kg/d)	1.32 ^b	1.34 ^{ab}	1.34 ^{ab}	1.38 ^a	2.328	0.068	0.183
Flux to ileum (kg/d)	1.26	1.24	1.22	1.26	2.627	0.95	0.66
Fecal flux (kg/d)	1.09	1.05	1.04	1.05	2.366	0.122	0.084
Apparent digestion, of intake (g/100 g)							
Rumen	34.80 ^a	33.70 ^{ab}	33.84 ^{ab}	31.84 ^b	1.098	0.068	0.183
Small intestine	3.07	4.84	5.55	5.61	1.246	0.068	0.137
Large intestine	8.13	9.52	9.13	10.44	1.161	0.145	0.36
Total tract	45.99	48.06	48.53	47.90	1.116	0.122	0.085
N							
Intake (g/d)	119.1 ^d	130.4 ^c	142.1 ^a	153.2 ^b	0.71	<0.001	<0.001
Flux to duodenum (g/d)	121.7	125.0	129.6	132.9	1.71	0.019	0.072
Flux to ileum (g/d)	51.84	53.31	58.29	62.3	1.611	0.008	0.032
Fecal flux (g/d)	48.07	47.18	48.91	49.39	1.152	0.406	0.649
Urinary (g/d)	52.41 ^a	57.65 ^b	55.37 ^c	63.94 ^d	0.908	<0.001	<0.001
Retain nitrogen (g/d)	18.65 ^c	25.54 ^b	37.77 ^a	39.83 ^a	1.057	<0.001	<0.001

¹ SEM = Standard error of mean. ² p values for the linear (L) or quadratic (Q) effects of SSP infused at the rates 0, 100, 200, 300 g cattle⁻¹ d⁻¹.

^{a-d} Means within a row with different superscript letter differ (p<0.05).

rate of SSP infusion. Similarly, OM digestion in the large intestine and the total tract digestion of DM, OM, and NDF were also linearly and quadratically (p<0.01) increased as the rate of SSP infusion increased.

Ruminal infusion of SSP at the rates up to 300 g/d did not affect N flow to duodenum and ileum or fecal N output,

but increased urinary N excretion (L, Q; p<0.001) and N retention (L, Q; p<0.001).

Flux and digestion of AA at different digestive sites

Ruminal infusion of SSP increased (p<0.05) duodenal flux of all 17 AA examined in this study as compared to

Table 6. Effects of soybean small peptide (SSP) infusion into rumen at the rates of 0 (Control), 100 (SSP100), 200 (SSP200) and 300 (SSP300) g/d on flux of amino acids at proximate duodenum of Luxi Yellow cattle (n = 4)

Item	Treatment				SEM ¹	Contrast ²	
	Control	SSP100	SSP200	SSP300		L	Q
Aspartic acid	43.20 ^b	45.59 ^b	54.87 ^a	57.74 ^a	1.308	<0.001	<0.001
Glutamic acid	72.26 ^c	78.00 ^b	89.87 ^a	94.24 ^a	1.330	<0.001	<0.001
Serine	29.67 ^c	31.73 ^c	40.66 ^b	44.46 ^a	1.106	<0.001	<0.001
Glycine	23.26 ^{bc}	22.10 ^c	26.78 ^b	31.57 ^a	1.240	<0.001	<0.001
Histidine	13.86 ^c	17.42 ^b	18.21 ^{ab}	19.71 ^a	0.968	<0.001	<0.001
Arginine	23.01 ^c	24.95 ^b	27.96 ^a	28.49 ^a	1.010	<0.001	0.002
Threonine	25.04 ^c	26.62 ^{bc}	29.36 ^{ab}	30.95 ^a	1.130	<0.001	<0.001
Alanine	30.10 ^b	30.14 ^b	29.76 ^b	36.73 ^a	0.922	0.004	<0.001
Proline	27.79 ^c	31.43 ^b	33.97 ^b	38.12 ^a	1.127	<0.001	<0.001
Tyrosine	17.80 ^{ab}	15.87 ^c	16.86 ^{bc}	19.21 ^a	0.979	0.161	0.003
Valine	30.74 ^c	31.96 ^{bc}	33.39 ^{ba}	34.38 ^a	1.053	<0.001	0.002
Methionine	8.66 ^b	9.68 ^{ba}	10.54 ^{ba}	10.82 ^a	1.019	0.003	0.009
Cystine	8.96 ^c	8.97 ^c	10.17 ^b	12.32 ^a	0.849	<0.001	<0.001
Isoleucine	26.37 ^b	28.36 ^{ab}	30.27 ^a	30.31 ^a	1.154	0.004	0.009
Leucine	42.02 ^b	44.74 ^b	49.06 ^a	49.93 ^a	1.232	<0.001	<0.001
Phenylalanine	20.59 ^c	23.39 ^b	26.30 ^a	25.07 ^a	0.964	0.002	<0.001
Lysine	25.38 ^d	29.88 ^c	33.50 ^b	35.78 ^a	1.067	<0.001	<0.001
TAA ³	468.43 ^b	495.15 ^b	548.73 ^a	583.85 ^a	2.181	<0.001	<0.001
NEAA ⁴	244.08 ^d	254.85 ^c	292.75 ^b	322.07 ^a	1.509	<0.001	<0.001
EAA ⁵	224.63 ^c	245.97 ^b	269.62 ^a	276.85 ^a	1.604	<0.001	<0.001

¹ SEM = Standard error of mean. ² p values for the linear (L) or quadratic (Q) effects of SSP infused at the rates 0, 100, 200, 300 g cattle⁻¹ d⁻¹.

³ TAA = Total amino acids. ⁴ NEAA = Non-essential amino acids. ⁵ EAA = Essential amino acids.

^{a-d} Means within a row with different superscript letter differ (p<0.05).

Control (Table 6). The efficacy of different amounts of SSP on the AA duodenal flux depended on the individual AA with the greatest effects observed at the higher dosage. In general, increasing the amount of infused SSP from 100 to 300 g/d linearly and quadratically increased (p<0.01) increased duodenal flux of 17 AA with the exception of Tyrosine that was only quadratically (p<0.01) increased by the infusion of SSP. Similarly, flux at the terminal ileum of AA examined were also increased (p<0.05) by the ruminal infusion of SSP compared to Control with the exception of Arginine, Threonine, Tyrosine, and Methionine (Table 7). The increased flux of these AA by SSP was also observed in a dose dependent manner and most of them were linearly and/or quadratically (p<0.05) increased as the SSP increased from 100 to 300 g/d. In contrast, the digestibilities of most of the AA examined in the small intestine were not affected by SSP (Table 8) and increased AA digestibility was only observed for Arginine at SSP100, SSP200 and SSP300 and for Lysine at SSP200 (p<0.05).

DISCUSSION

Effect of ruminal SSP infusion on rumen fermentation

In the current study, infusion SSP into the rumen had no effect on ruminal pH, which is consistent with other reports using peptides or sodium caseinate (Cruz Soto et al., 1994;

Kazemi et al., 2010). However, ammonia-N was linearly increased as the SSP infusion rate increased, indicating a portion of the infused SSP was degraded and deaminated to ammonia. Increasing crude protein concentration or degradation of dietary protein usually results in increased ammonia concentrations in the rumen (Davidson et al., 2003). Increased ammonia concentration in the rumen was also observed when casein was infused into rumen (Khalili and Huhnen, 2002; Kazemi et al., 2010). Microbial protein synthesized in the rumen is influenced by many factors such as fermentative synchrony of degradable carbohydrate and ruminally degradable protein, ammonia concentration and pH (Fu et al, 2001) and it has been reported that between 18 and 100% microbial protein was derived from ammonia-N Salter et al. (1979). Providing peptides has been shown to stimulate the growth of rumen bacteria (Cruz Soto et al., 1994; Chikunya et al., 1996), and to enhance digestion of fiber (McAllan, 1991; Griswold et al., 1996). The results of this study demonstrated that infusion of SSP in the rumen positively increased microbial crude protein synthesis, which is consistent with another report that showed that supplying peptides and free amino acid to the rumen stimulated microbial yield and fermentation (Chikunya et al., 1996). The enhanced MCP synthesis by SSP infusion may partly due to the increased availability of ammonia-N and the improved synchronization between fermentative

Table 7. Effects of soybean small peptide (SSP) infusion into rumen at the rates of 0 (Control), 100 (SSP100), 200 (SSP200) and 300 (SSP300) g/d on flux of amino acids at the terminal ileum of Luxi Yellow cattle (n = 4)

Item	Treatment				SEM ¹	Contrast ²	
	Control	SSP100	SSP200	SSP300		L	Q
Aspartic acid	13.76 ^b	14.49 ^b	17.89 ^a	18.87 ^a	1.015	<0.001	0.005
Glutamic acid	25.26 ^b	26.91 ^{ab}	31.29 ^{ab}	32.57 ^a	1.393	0.004	0.018
Serine	12.22 ^c	12.80 ^c	15.19 ^b	17.80 ^a	0.946	<0.001	0.002
Glycine	9.65 ^b	9.02 ^b	10.69 ^{ab}	12.47 ^a	1.034	0.001	<0.001
Histidine	3.10 ^b	4.06 ^a	4.57 ^a	4.53 ^a	0.790	<0.001	<0.001
Arginine	6.74	6.46	6.28	6.93	0.856	0.780	0.328
Threonine	9.325	9.325	9.88	10.77	1.005	0.049	0.123
Alanine	10.37 ^b	10.18 ^b	10.78 ^{ab}	13.16 ^a	1.099	0.020	0.018
Proline	12.13 ^b	13.33 ^b	14.09 ^{ab}	16.27 ^a	1.125	<0.001	0.001
Tyrosine	6.30 ^b	5.62 ^b	6.02 ^b	7.20 ^a	0.807	0.340	0.268
Valine	10.18	10.74	11.40	12.05	1.008	0.007	0.031
Methionine	2.4	2.38	2.58	2.75	0.786	0.107	0.242
Cystine	2.96 ^b	2.91 ^b	2.76 ^b	3.86 ^a	0.836	0.062	0.017
Isoleucine	6.83	7.45	7.74	8.49	0.990	0.018	0.067
Leucine	11.79 ^c	12.38 ^{bc}	13.13 ^{ab}	14.34 ^a	0.922	<0.001	0.004
Phenylalanine	6.68 ^c	7.18 ^{bc}	7.82 ^{ab}	8.64 ^a	0.853	<0.001	0.001
Lysine	6.43 ^b	6.83 ^{ab}	7.07 ^{ab}	8.04 ^a	0.915	0.002	0.007
TAA ³	158.04 ^c	162.25 ^{bc}	179.13 ^b	198.73 ^a	1.787	<0.001	0.001
NEAA ⁴	89.68 ^b	92.35 ^b	105.93 ^a	118.33 ^a	1.658	<0.001	<0.001
EAA ⁵	66.43 ^c	69.91 ^{bc}	73.20 ^b	80.40 ^a	1.334	<0.001	<0.001

¹ SEM = Standard error of mean. ² p values for the linear (L) or quadratic (Q) effects of SSP infused at the rates 0, 100, 200, 300 g cattle⁻¹ d⁻¹.

³ TAA = Total amino acids. ⁴ NEAA = Non-essential amino acids. ⁵ EAA = Essential amino acids.

^{a-c} Means within a row with different superscript letter differ (p<0.05).

Table 8. Effects of soybean small peptide (SSP) infusion into rumen at the rates of 0 (Control), 100 (SSP100), 200 (SSP200) and 300 (SSP300) g/d on digestion (g/g) of amino acids in the small intestine of Luxi Yellow cattle (n = 4)

Item	Treatment				SEM ¹	Contrast ²	
	Control	SSP100	SSP200	SSP300		L	Q
Aspartic acid	68.00	68.25	68.34	67.22	1.285	0.811	0.924
Glutamic acid	64.94	65.52	62.21	65.35	1.470	0.843	0.930
Serine	58.89	59.66	60.58	59.83	1.316	0.675	0.857
Glycine	59.35	59.11	61.76	60.17	1.531	0.419	0.638
Histidine	77.46	76.61	76.55	76.93	1.331	0.827	0.916
Arginine	70.71 ^b	70.01 ^a	76.74 ^a	75.54 ^a	1.117	0.032	0.042
Threonine	62.74	64.20	64.63	65.18	1.425	0.300	0.575
Alanine	65.44	66.16	65.06	64.18	1.413	0.633	0.845
Proline	56.34	27.48	58.28	57.35	1.540	0.681	0.816
Tyrosine	64.28	64.43	65.82	61.99	1.409	0.784	0.876
Valine	66.79	66.34	65.03	64.97	1.416	0.325	0.622
Methionine	72.31	75.06	76.54	74.61	1.437	0.332	0.291
Cystine	66.87	67.76	68.61	73.22	1.509	0.311	0.299
Isoleucine	74.04	73.69	74.85	71.94	1.463	0.561	0.692
Leucine	71.86	72.25	72.78	70.64	1.328	0.625	0.603
Phenylalanine	67.56 ^{ab}	69.24 ^{ab}	70.23 ^a	65.48 ^b	1.212	0.510	0.131
Lysine	74.63 ^b	77.10 ^{ab}	79.35 ^a	77.52 ^{ab}	1.232	0.060	0.033
TAA ³	66.25	67.12	67.46	65.81	1.228	0.887	0.711
NEAA ⁴	63.23	63.77	63.79	63.23	1.312	0.998	0.948
EAA ⁵	70.42	71.54	72.83	70.95	1.164	0.458	0.149

¹ SEM = Standard error of mean. ² p values for the linear (L) or quadratic (Q) effects of SSP infused at the rates 0, 100, 200, 300 g cattle⁻¹ d⁻¹.

³ TAA = Total amino acids. ⁴ NEAA = Non-essential amino acids. ⁵ EAA = Essential amino acids.

^{a-b} Means within a row with different superscript letter differ (p<0.05).

energy and ammonia-N (Cruz Soto et al., 1994; Chikunya et al., 1996).

In the present study, rumen concentration of total VFA and molar proportions of propionate were increased but molar proportion of butyrate and acetate: propionate ratio were reduced by SSP infusion. This indicated that SSP infusion increased energy supply to the animal as well as improved energy efficiency. Griswold et al. (1996) found total VFA, acetate and butyrate were all increased by adding different amounts and sources of rumen degradable protein. However, Hristov et al. (2004) reported no effect of different rumen degradable protein levels on concentrations of total VFA, acetate, butyrate or propionate in rumen fluid. The discrepancy in the effects of the various forms of rumen degradable proteins on the rumen VFA profile among these studies might be due to the differences in type of animals, diet and the sources of rumen degradable proteins.

Effect of SSP infusion on ruminal microbial communities

This study found that infusion of SSP had no effect on the population of *R. flavefaciens*, increased the population of *B. Fibrisolvens* but reduced population of *S. bovis*. It has been shown that some hemicellulolytic bacteria (e.g., *B. fibrisolvens*) utilize peptide or AA N directly for microbial protein synthesis (Cotta and Hespell, 1986). *F. succinogenes*, *R. flavefaciens* and *R. albus* are the main cellulolytic bacteria in the rumen (Forsberg et al., 1997) and all of them utilize ammonia-N. The results of this study may suggest that effect of ruminal infusion of SSP on rumen bacteria depend on bacterial capability in utilizing peptide- AA- and ammonia-N.

Effect of SSP infusion on nutrient digestibility

It is generally regarded that ruminally available N in the form of peptides and AA can improve the digestion of both structural carbohydrate and nonstructural carbohydrate by rumen microorganisms. However, the Cornell net carbohydrate and protein system assumes that bacteria fermenting structural carbohydrates do not utilize peptides or amino acid and all their N must come from NH₃ (Russell et al., 1992). The improved ruminal digestion of NDF by SSP infusion observed in this study indicated that microbial activity relating to fibre digestion was enhanced by the SSP infusion and this is consistent with the finding of increased microbial protein concentration by infusion of SSP in this study. The digestibility of crude fiber was also increased by addition of SSP in the diet of the goats (Zhang et al., 2004). The exact mechanism by which the microbial activity was increased by supply of SSP is not clear. However, the result that the linear increase of microbial protein was positively

associated with the linear increase of ruminal ammonia-N by SSP infusion suggests that increasing the rate of SSP infusion increased the production of ammonia-N thereby enhanced MCP synthesis. Bryant (1973) proposed that ammonia-N in rumen fluid could stimulate the activity of cellulolytic bacteria. On the other hand, Carro and Miller (1999) argued that N forms other than NH₃-N are needed for both maximum growth of fiber digesting ruminal microorganisms for maximum fiber digestion. Therefore the increased MCP by SSP infusion might be a combined result of increased ammonia-N and NAN.

Overall, the results of this study showed ruminal infusion of SSP at the rates of 100, 200 and 300 g/d increased the apparent digestibilities of DM, OM, NDF and CP in the total digestive tract of beef cattle. The fact that the improvement in digestibilities of the nutrient by SSP mainly occurred in the rumen indicated that the beneficial effects of supplying SSP on nutrient digestion were mainly through improving rumen fermentation efficiency.

Effect of infusion SSP on AA flow and apparent digestibility of small intestinal

Ruminal infusion of SSP increased all measured AA flux to the small intestine without affecting their digestibility in the small intestine. This suggests that amount of AA absorbed from the small intestine was increased, which is consistent with the observation of an increased amount of N retained due to SSP infusion. The increased AA flux to the small intestine by SPP infusion is likely a combined result of increased flux of ruminal undegradable SPP and MCP. The similar small intestinal digestibility of AA across all the treatments also indicate that the small intestinal digestibility of AA in SPP is similar to that of other feed components used in this study.

IMPLICATIONS

In this experiment, infusion soybean small peptide profoundly influenced ruminal fermentation, duodenal and ileal amino acid flow, and digestion of dry matter, organic matter and fiber. Soybean small peptide may improve the absorption of individual amino acids in the small intestine. But there are still many questions to be answered, including the ruminal degradability of small peptides, the net absorption of intact peptides in the gastrointestinal tract and the influence on microbial population of infusion soybean small peptides, specially the relationship between peptide nitrogen and fiber. More research is needed in this area to determine the intake at which undegraded intake soybean small peptide supplementation becomes beneficial. Although practical application of peptides to livestock nutrition is lacking, further investigations will provide more

information, which may enable the improvement of nutritional management decisions.

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