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Effects of Soybean Small Peptides on Nitrogen Equilibrium, Nutrient Digestibility and Levels of Glucose, Ammonia and Amino Acids in the Portal Veinous Plasma of Goats

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Summary

To study the effects of soybean small peptides and their methods of supply on nitrogen balance, digestibility of nutrients and concentrations of glucose, ammonia and amino acids in the portal veinous plasma of goats, eight Xiangdong black goats were used in a 4×4 Latin square test design. Goats were divided at random into four groups (two goats in each group). Each group received the following four treatments in a different order over four treatment periods: the SSPF treatment (soybean small peptides in the basic diet by feeding), the FAAF treatment (free amino acids in the basic diet by feeding), the SSPI treatment (soybean small peptides by infusing through the duodenum fistula), or the FAAI treatment (free amino acids by infusing through the duodenum fistula). The results showed that nitrogen retention in the goats with the SSPI and SSPF treatments were much better than in those that received the FAAF ($P<0.01$) and FAAI treatments ($P<0.05$), and the FAAI treatment was also better than the FAAF treatment ($P<0.05$). The apparent protein digestibility in the goats with the SSPI treatment was significantly higher than in those that received the FAAF ($P<0.01$) and FAAI treatments ($P<0.05$), and apparent protein digestibility in the goats treated with SSPF and FAAI was also significantly higher ($P<0.05$) than in those treated with FAAF, respectively. Moreover, the apparent biological value of protein in the goats treated with SSPI was higher by 48.71% ($p<0.01$), 32.71% ($p<0.05$) and 20.98% ($p<0.05$) than in those treated with FAAF, FAAI and SSPF, respectively, and the apparent biological value in the goats with the SSPF treatment was significantly higher ($p<0.05$) than in those that received the FAAF treatment. Regarding the digestibility of dietary nutrients, there were no significant differences in crude fat digestibility among the different treatments. However, metabolizable energy digestibility in the goats treated with SSPI was higher by 14.59% ($P<0.05$), 10.10% ($P>0.05$) and 3.40% ($P>0.05$) than in those treated with FAAF, FAAI and SSPF, respectively. But, metabolizable energy digestibility in the goats treated with FAAF was significantly lower ($P<0.05$) than in those that received the SSPF treatment. The digestibility of total amino acids in the goats treated with SSPI, FAAI, SSPF and FAAF were 78.74%, 69.55%, 73.88% and 64.69%, respectively. By contrast, dietary crude fiber digestibility in

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the goats receiving the SSPF and FAAF treatments was higher than in those that treated with SSPI or FAAI. The glucose levels in the portal veinous plasma in the goats receiving the SSPI and FAAI treatments were significantly higher ($P<0.05$) than in those receiving SSPF and FAAF treatments. Ammonia levels in the portal veinous plasma of the goats treated with SSPF and FAAF were significantly higher ($P<0.05$) than in those treated with SSPI and FAAI. The amino acid level in the portal veinous plasma of the goats treated with SSPI was significantly higher than in those treated with FAAF ($P<0.01$), FAAI ($P<0.05$) and SSPF ($P<0.05$), and both the SSPF and FAAI treatments were better ($P<0.05$) than the FAAF treatment. Our results suggest that soybean small peptides are more effective than free amino acids in promoting nitrogen balance and protein biological value, and increasing digestibility of dietary nutrients and levels of glucose and amino acids in the portal veinous plasma of goats. The method of supplying soybean small peptides or free amino acids by infusing through the duodenum fistula was more advantageous than that by feeding.

Key words: small peptide, free amino acids, nitrogen balance, digestibility, goat

Introduction

It is well known that protein is a vital nutrient to animals. Until now, many theories and evaluation systems for digestion, absorption and metabolism of protein have been established and modified [29]. Previously, scientists believed that proteins were absorbed primarily as intact molecules [25], and then considered that proteins must be hydrolyzed to amino acids prior to absorption [18, 27]. However, the general concept that amino acids in ruminants are exclusively absorbed from the small intestine and only released in the blood stream in the form of free amino acids remains questionable [19, 22, 28] because a large amount of amino acids are absorbed as small peptides, usually dipeptides and tripeptides [29]. Recent studies have shown that small peptides are important intermediates in ruminant protein metabolism. But, they are not routinely measured in nutrition experiments [13]. Moreover, their metabolism rules and their effects on protein absorption and utilization in ruminants are not very clear. Especially in goats, there is little information on the effects of small peptides as protein sources on nitrogen balance, nutrient digestion and absorption, and nutrient levels in the portal veinous plasma.

In the present study, we reported the effects of soybean small peptides and free amino acids hydrolyzed from soybean small peptides on nitrogen equilibrium, digestion and absorption of dietary nutrients, and concentrations of glucose, ammonia, amino acids in the portal veinous plasma of goats.

Materials and Methods

Materials

Soybean small peptides composed of dipeptides and tripeptides were purchased from Sanle Bio-Engineering Inc. (Haerbin, Heilongjiang, China). The amino acid content in soybean small peptide was analyzed and is shown in Table 1. Free amino acids, which were from hydrolyzed soybean small peptides, were also obtained from Sanle Bio-Engineering Inc. Other chemical reagents used in this experiment were purchased from Shanghai Chemical Reagent, Inc. (Shanghai, China).

Table 1. Amino acids in the soybean peptides

Amino acids	Concentrations (mg/g)
Aspartic acid (Asp)	81.01
Threonine (Thr)	24.73
Serine (Ser)	33.56
Glutamic acid (Glu)	160.62
Proline (Pro)	10.60
Glycin (Gly)	25.97
Alanine (Ala)	26.48
Cystine (Cys)	–
Valine (Val)	34.32
Methionine (Met)	12.17
Isoleucine (Ile)	28.99
Leucine (Leu)	39.19
Tyrosine (Tyr)	26.08
Phenylalanine (Phe)	39.14
Histidine (His)	22.50
Lysine (Lys)	47.19
Arginine (Arg)	46.10
Total amino acids (TAA)	593.38

Animals, feeding and experimental procedures

Eight castrated Xiangdong black goats (1.5 years old) with an average body weight of 22.3±2.1 kg at the start of the experiment and 25.6±3.3 kg body weight at the end of the experiment were used. Goats were fitted with a duodenum fistula made of polyamide and polyvinyl chloride (outer diameter 20mm) for infusing small peptide or free amino acids and a catheter in the portal vein for sampling blood. Surgery was performed under general anesthesia. Goats were allowed approximately 4 weeks to recover after the surgery. Each of them was housed individually in a Dracher metabolism cage under continuous lighting with controlled temperature (20℃ to 25℃).

Goats were divided into four groups (two goats in each group). Each group received the following four treatments in a different order over four treatment periods: the SSPF treatment (supplemented soybean small peptide in the basic diet by feeding), the FAAF treatment (supplemented free amino acids in the basic diet by feeding), the SSPI treatment (supplemented soybean small peptide by infusing through the duodenum fistula), FAAI treatment (supplemented free amino acids by infusing through the duodenum fistula). All goats in this experiment were fed with the same basal diet. The composition and several nutrient levels of the diets are shown in Table 2.

The experiment consisted of four periods. Each period lasted 3 weeks, comprising a 2-week adaptation stage followed by a one-week sampling stage. According to the experimental design, feed was delivered in 6 equal portions at 3-hour intervals (from 7:00 to 22:00) from an automatic feeder every day in each experimental period. Water and salt block were available *ad libitum*. Body weight was measured at 6:00 on the first day and the last day of each period to determined metabolic body size. Diet intakes were measured daily starting from the second week of the adaptation stage until the end of the experimental period by weighing feed offered and refused.

Sample collection

Samples of feces and urine were collected from goats at 7:00, 10:00, 13:00, 16:00, 19:00 and 22:00

Table 2. Composition and nutrient levels of the experimental diets

	SSPI diet	FAAI diet	SSPF diet	FAAF diet
Basic diet composition				
Corn stalk (%)	30.0	30.0	30.0	30.0
Sweet potato vine (%)	23.5	23.5	23.5	23.5
Corn starch (%)	40.0	40.0	40.0	40.0
Feed grade urea (%)	0.3	0.3	0.3	0.3
Limestone powder (%)	0.5	0.5	0.5	0.5
Dicalcium phosphate (%)	0.9	0.9	0.9	0.9
Sodium bicarbonate (%)	0.6	0.6	0.6	0.6
Rapeseed oil (%)	2.0	2.0	2.0	2.0
Salt (%)	1.2	1.2	1.2	1.2
Minerals and vitamins (%)*	1.0	1.0	1.0	1.0
Nutrients supplied by infusing				
SSP (g/d)	55	/	/	/
FAA (g/d)	/	55	/	/
Nutrients supplied by feeding				
SSP (g/d)	/	/	55	/
FAA (g/d)	/	/	/	55
Nutrient levels in diets**				
DM (%)	87.24	87.24	87.24	87.24
ME (MJ/kgDM)	8.21	8.21	8.21	8.21
CP (%)	10.13	10.13	10.13	10.13
TAA (%)	4.27	4.27	4.27	4.27
CF (%)	10.76	10.76	10.76	10.76
CE (%)	4.11	4.11	4.11	4.11
CA (%)	5.98	5.98	5.98	5.98
Ca (%)	0.95	0.95	0.95	0.95
P (%)	0.53	0.53	0.53	0.53

*Supplying (per kg diet): vitamin A, 5000 i.u; vitamin D_3, 2000 i.u; vitamin K, 1 mg; vitamin H (biotin), 1 mg; Cu, 10 mg (from $CuSo_4.5H_2O$); Zn, 30 mg (from ZnO); Fe, 50 mg (from $FeSO_4.H_2O$); Mn, 20 mg (from $MnSO_4.H_2O$); I, 0.1 mg (from KI); Se, 0.1 mg (from Na_2SeO_3) ; Co, 0.04 mg (from $CoC_2.6H_2Ol$).
**SSPI and FAAI mean soybean small peptide and free amino acids supplied by infusing through the duodenum fistula. SSPF and FAAF mean soybean small peptides and free amino acids supplied by feeding in basic diets. DM, ME, CP, TAA, CF, CE, CA, Ca and P mean dry matter, metabolizable energy, crude protein, total amino acids, crude fiber, crude ash, calcium and phosphorus, respectively.

every day in each experimental period. Each of the samples was weighed and then stored in a refrigerator at 4℃ until use. Blood samples were collected from goats at 6:00, 10:00, 14:00, 18:00 and 22:00 on the sixth day of each sampling stage in the experiment, and at 8:00, 12:00, 16:00, 20:00 and 24:00 on the next day (the last day of each sampling stage in the experiment). Five ml of blood were collected with a catheter in the portal vein of goats into cold syringes containing anticoagulant. Samples were rapidly placed on ice and plasma was separated from the blood samples by centrifugation at 2000×g for 10 min at 4℃. The plasma samples were stored in a refrigerator at -20℃ until use.

Chemical analysis

The diets and feces of goats were dried at 60℃ and grounded using a cyclone mill to pass through a 1-mm screen. Dry matter (DM) was estimated by drying a 1g sample in duplicate at

100℃ in a conventional oven for 24 hours, and crude ash was analyzed by burning a 2g sample in duplicate at 600℃ for 2 hours in a muffle furnace.

The concentrations of total amino acids and free amino acids in diet, plasma, feces and urine of goats were determined by using an amino acid analyzer. Metabolizable energy was measured using a WZR-1 oxygen-bomb thermal energy machine. Calcium was estimated with an atomic absorption spectrophotometer [4]. Phosphorus was analyzed with a colorimeter [4]. Analyses of crude fat [4], nitrogen [4], crude protein calculated as N × 6.25[4], ash [4], and crude fiber [26] were also carried out. Ammonia levels of the portal veinous plasma were analyzed with a colorimeter [31]. The concentration of glucose in the portal veinous plasma was measured at a wavelength of 620 nm with a Hitachi UV-Vis spectrophotometer [31, 32].

Calculation and statistical analysis

The apparent digestibility and apparent biological value of protein were calculated respectively as:

$$aD_N\ (\%) = (N_I - N_F)\ /\ N_I \times 100$$

$$aBV\ (\%) = (N_I - N_F - N_U) \div (N_I - N_F) \times 100 = N_R \div N_D \times 100$$

Where aD_N is apparent digestibility of protein in the diet; aBV is apparent biological value of protein in the diet; N_I is the intake of nitrogen in the diet; N_F is the amount of nitrogen excreted in feces; and N_U is the amount of the nitrogen excreted in urine ; N_R is the amount of retention nitrogen ; N_D is the amount of digestible nitrogen.

The digestibility of dietary nutrients such as matebolizable energy, crude fiber, crude fat were calculated as:

$$D_R\ (\%) = (N_I - N_F)\ /\ N_I \times 100$$

Where D_R is the digestibility of a nutrient in the diet; N_I is the intake of a nutrient in the diet; N_F is the amount of the nutrient excreted in feces.

The absorptivity of nutrients in the diets were calculated as:

$$A\ (\%) = (R_E \div I_N) \times 100 = [I_N - (E_F + E_U)] \div I_N \times 100$$

Where A is the absorptivity of a nutrient in the diet; R_E is the amount of a dietary nutrient retained inside the goat's body; I_N is the intake of a nutrient in the diet; E_F is the amount of the nutrient excreted in feces; and E_U is the amount of the nutrient excreted in urine.

The experiments were designed with a 4×4 Latin square procedure, in which animals were randomly divided into four groups and were assigned to one of each of the four treatments. Data were first subjected to an analysis of variance (ANOVA) with the General Linear Model Procedure of SAS Institute [24] for a Latin square design. Sources of variation were treatments (n = 4), periods (n = 4), and goat groups (n = 4). Where appropriate, treatment means were compared using the Student-Newman Keuls' multiple range tests.

Table 3. 4×4 Latin square experimental design

	Group 1	Group 2	Group 3	Group 4
Period 1	SSPI	FAAI	SSPF	FAAF
Period 2	FAAI	SSPI	FAAF	SSPF
Period 3	SSPF	FAAF	SSPI	FAAI
Period 4	FAAF	SSPF	FAAI	SSPI

Results

Nutrient intakes

Nutrient intakes by metabolic body size of goats in the experiments are shown in Table 4. Some differences were observed in several nutrient intakes of goats between soybean small peptide treatment and free amino acid treatment, and between methods of supply (by feeding or by infusing). In general, the intakes of DM, ME, CP, TAA, CF and CE in the goats receiving soybean small peptide treatment were higher than in those receiving free amino acid treatment. The intakes of DM, ME, CP, TAA, CF and CE in the goats receiving the SSPF and FAAF treatments in which soybean small peptides or free amino acids were supplemented in the basic diet, were also higher than in those treated with soybean small peptides or free amino acids by infusing through the duodenum fistula, respectively. However, there were no significant differences (P>0.05) between soybean small peptide treatment and free amino acid treatment, or between ways of supplying soybean small peptides and free amino acids. The data suggest that treatment was not a major factor affecting nutrient intake of the goats in this study. Our results were in close agreement with a previous report [17], in which the interrelationships between feed intake and ruminal characteristics as well as intestinal amino acids were reported in the growing beef steers.

Table 4. Nutrient intakes in the experimental diets*

	SSPI diet	FAAI diet	SSPF diet	FAAF diet
BW (kg)	22.14	22.63	23.41	22.89
W$^{0.75}$ (kg)	10.21	10.38	10.64	10.47
DM (g/kg W$^{0.75}$/d)	75.31	73.08	78.66	77.12
ME (MJ/Kg W$^{0.75}$)	0.62	0.60	0.65	0.63
CP (g/kg W$^{0.75}$/d)	7.63	7.48	7.95	7.89
TAA (g/kg W$^{0.75}$/d)	3.21	3.12	3.36	3.29
CF (g/kg W$^{0.75}$/d)	8.10	7.86	8.46	8.29
CE (g/kg W$^{0.75}$/d)	3.09	3.00	3.23	3.16

*BW, W$^{0.75}$, DM, ME, CP, CF, TAA and CE mean body weight, metabolic body size, dry matter, metabolizable energy, crude protein, crude fiber, total amino acids and crude fat, respectively.

Nitrogen retention and protein utilization

As shown in Table 5, nitrogen intakes and the amount of the nitrogen excreted in the urine of goats were quite similar among the different treatments (P>0.05). But there are some differences among the treatments regarding the amount of the nitrogen excreted in feces, the amount of digestible nitrogen, the amount of retained nitrogen, the apparent digestibility and apparent biological value of protein in the diet. The goats receiving the SSPI treatment had lower nitrogen excretion in feces than those treated with the SSPF (P<0.05), FAAF (P<0.05) and FAAI treatments (P>0.05), while there were no significant differences (P>0.05) in nitrogen excretion among the FAAI, SSPF and FAAF treatments. The nitrogen retention in both SSPI and SSPF treatments was much better than that in the FAAF (P<0.01) and FAAI treatments (P<0.05), and the FAAI treatment was also better than the FAAF treatment (P<0.05). Apparent protein digestibility in the goats treated with SSPI was significantly higher than in those receiving FAAF (P<0.01) and FAAI treatments (P<0.05), but there was no significant difference with the SSPF treatment (P>0.05). The apparent protein digestibility in the goats treated with SSPF and FAAI was also significantly higher (P<0.05) than in those treated with FAAF. Moreover, the apparent biological value of protein in the goats treated with SSPI was higher by 48.71% ($p<0.01$), 32.71% ($p<0.05$) and 20.98% ($p<0.05$) than in those treated with FAAF, FAAI and SSPF, respectively, and the apparent biological value in the goats receiving SSPF treatment was significantly higher ($p<0.05$) than in those receiving FAAF treatment. These results indicated that the method of supplying soybean small peptides or free amino acids by infusing through the duodenum fistula was more advantageous than that by feeding in diet. Moreover, small peptides had a positive effect on the nitrogen retention and protein utilization in the goats, in other words, when small peptides were used as protein sources, protein digestibility and protein biological value were much better than when other protein sources were used.

Table 5. Effects of different treatments on nitrogen retention in the goats*

	SSPI	FAAI	SSPF	FAAF
N_I (g/d)	12.46	12.42	13.53	13.32
N_F (g/d)	6.62^{a}	7.41^{ab}	7.78^{b}	8.73^{b}
N_U (g/d)	3.21	3.31	3.61	3.20
N_D (g/d)	5.84^{b}	5.01^{ab}	5.75^{b}	4.59^{a}
N_R (g/d)	2.63^{c}	1.70^{b}	2.41^{c}	1.39^{a}
aD_N (%)	46.87^{c}	40.38^{b}	42.50^{bc}	34.46^{a}
Ad_v (%)	45.03^{c}	33.93^{ab}	37.22^{b}	30.28^{a}

*N_I, N_F, N_U, N_D, N_R, aD_N and Ad_v mean intake of nitrogen in the diet, the amount of the nitrogen excreted in feces, the amount of the nitrogen excreted in urine, the amount of digestible nitrogen, the amount of retained nitrogen, apparent digestibility of protein in the diet and apparent biological value of protein in the diet, respectively.

**There are significant differences between figures in the same row with different supercripts such as ab and bc ($P<0.05$) or ac ($P<0.01$).

Dietary nutrient digestibility

Digestibility of dietary nutrients in the goats according to treatment is shown in Table 6. There was no significant difference in crude fat digestibility among the different treatments. Metabolizable energy digestibility in the goats treated with SSPI was the highest among the four treatments; it was higher by 14.59% ($P<0.05$), 10.10% ($P>0.05$) and 3.40% ($P>0.05$) than in those treated with FAAF, FAAI and SSPF, respectively. On the other hand, metabolizable energy and digestibility in the goats treated with FAAF were significantly lower ($P<0.05$) than in those receiving the SSPF treatment. The digestibility of total amino acids was 78.74%, 69.55%, 73.88% and 64.69% in the goats treated with SSPI, FAAI, SSPF and FAAF, respectively. Some significant differences were observed in digestibility of total amino acids between the SSPI and FAAF treatments ($P<0.01$), between the SSPI and FAAI treatments ($P<0.05$), and between the SSPF and FAAF treatments ($P<0.05$). By contrast, dietary crude fiber digestibility in the goats receiving the SSPF and FAAF treatments by feeding was higher than in those treated with SSPI or FAAI by infusing through the duodenum fistula, especially in the case of the SSPI treatment which had the highest digestibility among the treatments, higher by 15.28% ($P<0.05$), 12.47% ($P<0.05$) and 9.73% ($P>0.05$) than the levels recorded for the FAAI, SSPI and FAAF treatments, respectively. Thus, treatment with soybean small peptide was much better than that with free amino acid treatment, and treatment effect was not influenced significantly by methods of supply (whether by infusing or by feeding). By contrast, the digestibility of crude fiber in the goats treated with SSPF or FAAF by feeding was higher than in those treated with SSPI or FAAI by infusing through the duodenum fistula.

Table 6. Digestibility of dietary nutrients in goats receiving different treatments*

	SSPI	FAAI	SSPF	FAAF
ME (%)	83.08±8.4^{b}	74.85±8.21^{ab}	80.35±7.76^{b}	72.50±7.81^{a}
TAA (%)	78.74±7.73^{c}	69.55±7.06^{ab}	73.88±7.17^{bc}	64.69±6.54^{a}
CE (%)	89.82±9.23	85.61±8.65	88.85±10.11	85.30±8.64
CF (%)	43.40±4.15^{a}	42.34±4.56^{a}	47.81±4.74^{b}	44.75±5.10^{ab}

*ME, TAA , CE and CF mean metabolizable energy, total amino acids, crude fat and crude fiber, respectively.
**There are significant differences between figures in the same row with different supercripts such as ab and bc (P<0.05) or ac (P<0.01).

Fluctuation of glucose levels in the portal veinous plasma

Concentrations of glucose in the portal veinous plasma of the goats receiving different treatments from 6:00 to 24:00 (at 2-hour intervals) on the sampling days are shown in Table 7. The fluctuation curve is shown in Figure 1. The fluctuation trends of glucose levels in the portal veinous plasma of the goats receiving different treatments were similar to each other. The lowest point of the fluctuation curve in the case of all treatments was at 6:00 when the goats had not received their first feed of the day. After the first feed, the curves ascended step by step until 14:00 when their flux peaks appeared, except in the case of the FAAI treatment where the peak was reached at 16:00. After 14:00

Table 7. Glucose levels over time in the portal veinous plasma of the goats receiving different treatments* (mmol/L)

Time	SSPI	FAAI	SSPF	FAAF
06 : 00	2.14±0.20	2.21±0.30	2.17±0.26	2.07±0.27
08 : 00	4.32±0.51^{c}	3.75±0.41^{b}	3.54±0.27^{b}	2.87±0.33^{a}
10 : 00	4.77±0.37^{b}	4.43±0.49^{b}	3.46±0.39^{a}	3.30±0.29^{a}
12 : 00	4.83±0.43^{c}	4.62±0.44^{bc}	3.70±0.39^{ab}	3.48±0.40^{a}
14 : 00	5.11±0.48^{c}	4.69±0.51^{b}	3.88±0.52^{a}	3.62±0.46^{a}
16 : 00	4.87±0.54^{c}	4.86±0.49^{c}	3.76±0.41^{a}	3.57±0.35^{a}
18 : 00	4.66±0.60^{b}	4.63±0.62^{b}	3.59±0.38^{a}	3.44±0.36^{a}
20 : 00	4.92±0.53^{c}	4.46±0.48^{b}	3.49±0.39^{a}	3.46±0.42^{a}
22 : 00	4.61±0.49^{c}	4.31±0.54^{bc}	3.51±0.49^{a}	3.22±0.34^{a}
24 : 00	4.25±0.62^{b}	4.17±0.39^{b}	3.26±0.27^{a}	3.18±0.41^{a}
Average	4.448^{b}	4.213^{b}	3.433^{a}	3.221^{a}

*There are significant differences between figures in the same row with different supercripts such as ab and bc (P<0.05) or ac (P<0.01).

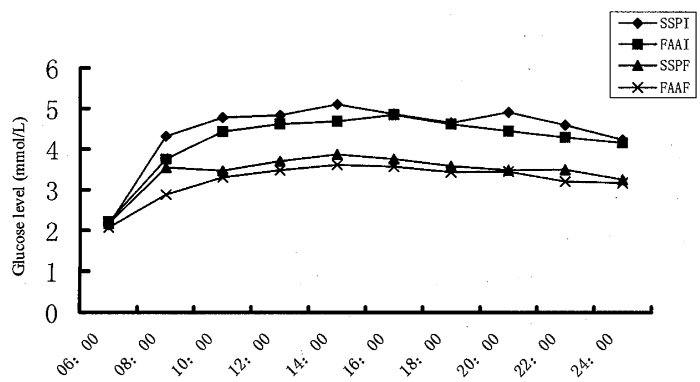

Figure 1. Fluctuation of glucose levels over time in the portal veinous plasma of the goats receiving different treatments.

Table 8. Ammonia levels over time in the portal veinous plasma of the goats receiving different treatments* (mmol/L)

Time	SSPI	FAAI	SSPF	FAAF
06：00	0.167	0.173	0.179	0.182
08：00	0.174[a]	0.176[a]	0.202[b]	0.236[c]
10：00	0.388[b]	0.340[a]	0.354[ab]	0.352[ab]
12：00	0.312[a]	0.323[ab]	0.363[ab]	0.374[b]
14：00	0.264[a]	0.277[a]	0.376[b]	0.383[b]
16：00	0.242[a]	0.235[a]	0.355[c]	0.367[c]
18：00	0.230[a]	0.226[a]	0.320[b]	0.339[c]
20：00	0.237[a]	0.227[a]	0.274[b]	0.283[b]
22：00	0.221[a]	0.234[a]	0.261[b]	0.267[b]
24：00	0.218	0.220	0.235	0.240
Average	0.245[a]	0.243[a]	0.292[b]	0.302[b]

*There are significant differences between figures in the same row with different supercripts such as ab and bc (P<0.05) or ac (P<0.01).

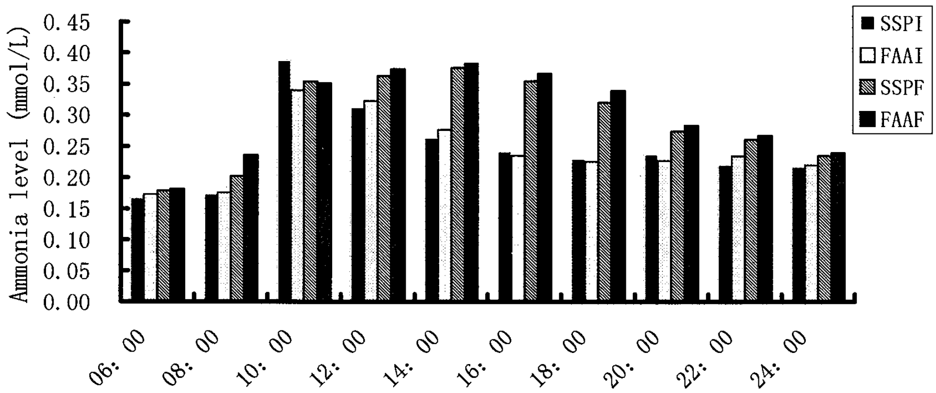

Figure 2. Fluctuation of ammonia levels over time in the portal veinous plasma of the goats receiving different treatments.

or 16:00, they all descended gently. But there were some differences in glucose levels in the portal veinous plasma depending on the treatment received. The average of glucose level in the portal veinous plasma of the goats treated with SSPI was similar to that of those treated with FAAI although it was 5.58% higher in the former. Glucose level in the goats receiving these treatments was significantly higher (P<0.05) than in those treated with SSPF or FAAF. There was no significant difference (P<0.05) in average of glucose level in the portal veinous plasma between the SSPF and FAAF treatments. These results indicate that the supplement of SSP or FAA by infusing through the duodenum fistula was more efficacious for glucose absorption than that by feeding in diets.

Fluctuation of ammonia levels in portal veinous plasma

Concentrations of ammonia in the portal veinous plasma of the goats receiving different treatments from 6:00 to 24:00 (at 2-hour intervals) on the sampling days are shown in Table 8, and the corresponding fluctuation curve is shown in Figure 2. At 6:00 each day, the ammonia in the portal veinous plasma of all the goats was also at its lowest level of the day. After the first feeding, it began rising sharply until reaching peaks of about 0.388 mmol/L (SSPI treatment) and 0.340 mmol/L (FAAI treatment) at 10:00, and 0.376 mmol/L (SSPF treatment) and 0.383 mmol/L (FAAF treatment) at 14:00. Then it declined slowly. As shown in Table 8, depending on treatment there were significant differences in ammonia levels in the portal veinous plasma of the goats at different times, except at 6:00 and 24:00. Ammonia levels in the portal veinous plasma of the goats treated with FAAF and SSPF were higher (P<0.05) than that treated with FAAI and SSPI. Thus, our data suggest that the way of supplying SSP and FAA influences ammonia levels in the portal veinous plasma of the goats. The feeding method led to higher ammonia levels in the portal veinous plasma than the infusing method.

Fluctuation of amino acid levels in the portal veinous plasma

As shown in Table 9, there were significant differences (P<0.05 or P<0.01) in amino acid levels among the different treatments except in the case of isoleucine. The concentrations of most amino acids in the portal veinous plasma of the goats receiving the SSPI treatment were significantly higher (P<0.05 or P<0.01) than in those receiving the other treatments. Both SSPF and FAAI treatments resulted in considerably higher (P<0.05) levels of most amino acids in the portal veinous plasma than the FAAF treatment. Significant distinctions in the levels of total amino acids among the different treatments were observed. The goats receiving the SSPI treatment showed the highest levels of total amino acids in their portal veinous plasma, these levels being significantly higher than in those treated with FAAF (P<0.01), FAAI (P<0.05) and SSPF (P<0.05), respectively. The levels of total amino acids in the portal veinous plasma of the goats treated with SSPF were higher by 8.88% (P>0.05) than in the goats treated with FAAI although there was no significant difference between them. The levels of total amino acids in the portal veinous plasma of the goats treated with SSPF and FAAI were also significantly higher (P<0.05) than in those treated with FAAF. The results indicated that the treatments with soybean small peptides could improve absorption of amino acids, thereby enhancing amino acid levels in the portal veinous plasma of the goats. Furthermore, SSPI treatment was more effective than SSPF treatment.

Table 9. Amino acids levels in the portal veinous plasma of the goats receiving different treatments*

	SSPI	FAAI	SSPF	FAAF
Asp (mmol/L)	0.211^{c}	0.190^{bc}	0.187^{bc}	0.147^{a}
Thr (mmol/L)	1.027^{c}	0.793^{ab}	0.920^{bc}	0.724^{a}
Ser (mmol/L)	0.252^{b}	0.213^{a}	0.232^{b}	0.185^{a}
Glu (mmol/L)	0.066^{c}	0.051^{b}	0.048^{a}	0.042^{a}
Gly (mmol/L)	0.890^{c}	0.771^{b}	0.808^{bc}	0.643^{a}
Ala (mmol/L)	1.130^{c}	0.676^{b}	0.970^{c}	0.524^{a}
Val (mmol/L)	0.414^{c}	0.328^{ab}	0.354^{bc}	0.287^{a}
Met (mmol/L)	0.115^{c}	0.103^{c}	0.090^{b}	0.062^{a}
Ile (mmol/L)	0.221	0.204	0.182	0.202
Leu (mmol/L)	0.601^{c}	0.486^{b}	0.454^{ab}	0.393^{a}
Tyr (mmol/L)	0.072^{c}	0.043^{a}	0.052^{b}	0.037^{a}
Phe (mmol/L)	0.142^{b}	0.111^{a}	0.125^{ab}	0.108^{a}
His (mmol/L)	0.074^{c}	0.060^{b}	0.047^{a}	0.044^{a}
Arg (mmol/L)	1.091^{c}	0.873^{b}	0.894^{b}	0.656^{a}
Pro (mmol/L)	0.141^{b}	0.121^{b}	0.101^{a}	0.118^{ab}
TAA (mmol/L)**	6.447^{c}	5.023^{b}	5.469^{b}	4.282^{a}

*There are significant differences between figures in the same row with different supercripts such as ab and bc (P<0.05) or ac (P<0.01).

**TAA means total amino acids.

Discussion

Effects of soybean small peptides and their methods of supply on nitrogen retention and protein utilization in goats

As summarized by Webb *et al* [29], the primary form of amino acid absorption in the ruminant is in peptides, not in free amino acids. Recent studies suggested that small peptides had a positive effect on nitrogen retention and protein utilization in ruminants. When small peptide was used as a protein source in ruminants, protein digestibility and protein biological value were much better than when other protein sources were used [5, 20]. Our results demonstrated that soybean small peptides could lead to better nitrogen retention, protein digestibility and protein biological value than free amino acids, a finding that coincides well with those of previous reports. Furthermore, nitrogen retention and protein utilization were also influenced by the way of supplying soybean small peptides or free amino acids to goats. It was found that infusion of soybean small peptides or free amino acids through the duodenum fistula was more advantageous than the feeding method. This difference may result from the effect of rumen fermentation. Rumen fermentation is a significant source of nutrients including nitrogen for the goats [8]. The goats rely on flora residing in rumen to carry this out. In the anaerobic environment of the rumen, the fermenting bacteria use nitrogen from the diet to produce amino acids and proteins, which can be used by goats. Bacteria deamminate from dietary proteins, peptides, amino acids, nonprotein nitrogen and endogenous nitrogenous materials in order to produce energy or to restructure amino acids for their own use. Dietary amino acids entering the rumen are partly used by bacteria, and partly absorbed into the intestine directly, while others are excreted from the body in urea [2, 14]. Several studies have shown that ruminant animals fed high-N-containing diets have a reduced efficiency of protein deposition [6, 23]. On the other hand, when nitrogenous materials such as small peptides and amino acids are infused into the intestine directly, their absorptivities and protein biological values should be greatly enhanced. That is why the infusing method was much better than the feeding method in this experiment. Another problem concerns the site of absorption of these peptides, a point on which there has been much disagreement. Dirizenzo [9] claimed that absorption of small peptides and free amino acids in ruminants occurs primarily in the nonmesenteric-drained viscera system including the rumen, reticulum, omasum, abomasums, duodenum, and spleen. Since small peptides (primarily dipeptides and tripeptides) and amino acids are absorbed from the small intestine [28], the mesenteric-drained viscera system consisting of the jejunum, ileum, cecum, colon, and pancreas should play an important role in absorption of small peptides and amino acids. In our study, higher levels of nitrogen retention, protein digestibility and protein biological value were achieved in the goats by infuse of soybean small peptides or free amino acids into the small intestine through the duodenum fistula than by feeding soybean small peptides or free amino acids in diets. Our results indicated that the nonmesenteric-drained viscera system in ruminant appears to be an important site of absorption of these small peptides. Further study on peptide absorption is required.

Effects of soybean small peptides and their methods of supply on nutrient digestibility in goats

Our data show that there were some significant differences in digestibility of metabolizable energy and total amino acids between the treatments with soybean small peptides and free amino acids. Soybean small peptides were much better than free amino acids. By contrast, digestibility of crude fiber in the goats treated with SSPF or FAAF by feeding was significantly higher than in those treated with SSPI or FAAI by infusing through the duodenum fistula. However, there were no significant differences in digestibility of metabolizable energy and total amino acids between the methods of supply, and there was no significant difference in crude fiber digestibility between the treatments with soybean small peptides and free amino acids.

Protein is a vital component of cells and also participates in large numbers of chemical reactions within organisms. Thus, protein plays an important role in life. The source, quantity and quality of protein in a diet impact on digestion, absorption and metabolism of other nutrients. Li *et al* [16] found that energy utilization in the goats improved remarkably ($p<0.05$) after replacing plant protein with small peptides. Recent studies have shown that there was a significant difference in amino acid absorptivity among different protein sources. The differences appear to result from the absorption form of amino acids after dietary protein degradation in the gastrointestinal tract. If the absorption form of amino acids is as peptides, not as free amino acids, The absorptivity of amino acids will be much higher [10, 20]. Our study proved these conclusions concerning digestibility of metabolizable energy and total amino acids.

The digestibility of crude fiber in the diet of the goats in this experiment was influenced primarily by the methods of supply, not by the additives, soybean small peptides or free amino acids. That may also result from the effect of rumen fermentation. As a species of herbivorous animal, goats possess one large organ (the rumen) ahead of the gastric organ (the true stomach), and consume diets that contain vast quantities of cellulose and other fibrous materials. These materials are difficult for carnivores to consume. Although energy can be derived from cellulose, goats do not produce the enzymes to break down these materials. Therefore, they rely on bacteria residing in the rumen to break down the cellulose. In this fermentation process, the bacteria need adequate energy and nitrogen to accomplish their task of breaking down the crude fiber in the diet [8]. In our experiments, the supply of soybean small peptides or free amino acids by feeding in the diets satisfied the demand of rumen flora. As a result, digestibility of crude fiber with SSPF and FAAF treatments was significantly higher than that with SSPI and FAAI treatments.

Effects of soybean small peptides and their methods of supply on glucose, ammonia and amino acid levels in the portal veinous plasma of the goats

Pathways and mechanisms of glucose transport in the small intestine have been extensively reviewed [3, 11, 12, 15, 21, 30]. In grazing ruminants, ruminal fermentation converts most cellular wall polysaccharides and all of the intracellular carbohydrates present in the forage to short-chain volatile fatty acids, which are then absorbed by the rumen epithelium. Negligible quantities of glucose polysaccharides flow from the rumen into the small intestine in ruminants fed high roughage diets [7]. Furthermore, in ruminal fermentation, the rumen bacteria consume glucose, nitrogenous materials and other nutrients for their own metabolic needs. Thus, glucose absorptivity in the small intestine and its concentration in blood decrease. In our study, we found that supplying small soybean peptides or free amino acids by infusing through the duodenum fistula was more efficacious for enhancing glucose levels in the portal veinous plasma of the goats than feeding in diets.

Moreover, the feeding method produced a higher ammonia level in the portal veinous plasma than the infusing method. The reason could be that soybean small peptides and free amino acids supplied by feeding were broken down partly to ammonia by rumen bacteria, and ammonia was then absorbed and entered into the blood. Therefore, ammonia levels in the portal veinous plasma of the goats treated with SSPF and FAAF were significantly higher than in those treated with SSPI and FAAI.

Recent studies have shown that small peptides and amino acids in blood circulation come mainly from the gastrointestinal tract where they were absorbed [1, 20]. Amino acid concentration in blood reflects their absorptivity. In our study, total amino acid concentration in the portal veinous plasma of the goats treated with SSP was significantly higher than in those treated with free amino acids. Moreover, the goats supplying small soybean peptides and FAA by infusing through the duodenum fistula had significantly higher levels of total amino acids in the portal veinous plasma than those by feeding in diets. Thus, our results suggest that soybean small peptides had relatively high rumen degradation escape values when they were used for goats.

In summary, our results indicated that supplying soybean small peptides or free amino acids for goats by infusing through the duodenum fistula was more advantageous than feeding in the diet. Soybean small peptide was more efficacious in improving nitrogen balance, digestibility of dietary nutrients and levels of glucose and amino acids in the portal veinous plasma of the goats than free amino acids. The nonmesenteric-drained viscera system in ruminants appears to be an important site of absorption of soybean small peptides.

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