# Effects of Popped Soybean on Concentration of Ruminal Peptide and Blood Amino Acids in Holstein Calves*

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**ABSTRACT** : This study conducted to evaluate effects of popped soybean on levels of ruminal peptides and blood amino acids in Holstein calves fed sudan grass hay as a forage source and popped (PSB) soybean as a concentrate supplement. At 0, 2, 4 and 6 h after feeding, rumen fluid and blood samples were collected from the rumen and jugular vein, respectively, and amino acids, petides and other nitrogen-containing compounds in the rumen were analyzed. Ruminal pH tended to be higher in the RSB than in the PSB treatments, and declined upto 4 h after feeding, since then increased in both treatments. The concentrations of ammonia-N in all treatments increased upto 2 h after feeding, and then decreased gradually with time after feeding.

The concentrations of ammonia N in the rumen were not significantly different between the treatments, however, those in RSB treatment appeared to be higher. Also, protein concentrations in the rumen were not significantly different between the treatments. Peptide productions were the highest at 2 h after feeding in the group fed RSB which is rapidly degradable in rumen, whereas those in the group fed PSB which is slowly degradable in rumen were maximized at 4 h after feeding. The concentration of total free essential amino acids in plasma was higher in the RSB treatment than in the PSB, but disappearance rates of these amino acids out of plasma was higher in the PSB treatment than in the RSB treatment. Disappearance rates of free non-essential amino acids in plasma were not significantly different between the treatments. Consequently, this study implies that the production of peptide and utilization of blood amino acid may be controlled by the modification of protein degradability.

(**Key Words**: Popped Soybean, Ruminal Peptides, Blood Amino Acids)

## INTRODUCTION

Studies on the peptide production, metabolism and absorption in ruminants have been recently highlighted (Depardon et al., 1995; Wallace and McKain, 1989; Webb et al., 1992). It had been thought that peptides were produced to negligible extent and immediately degraded to amino acids in the rumen until several decades ago. This concept had been changed after the researchers revealed that significant amounts of peptides accumulated in the rumen of cows at 1 h after feeding (Wallace, 1988), and peptide production in the rumen of lamb fed feeds supplementing casein remarkably increased (Chen et al., 1987a, c).

Even though the exact roles of accumulated peptides in the rumen have not been well elucidated, it has been well documented that peptides in the rumen stimulate the growth of rumen microbes (Argyle and Baldwin, 1989; Pittman et al., 1967). It is generally accepted that most of bacteria utilize peptides more efficiently than amino acids for their growth (Wright, 1967; Rusell, 1983; Chen and Russell, 1988), and hydrophilic peptides have stronger stimulatory effects on bacterial growth than hydrophobic ones (Chen et al., 1987b). Furthermore, recent studies observed that some peptides produced in the rumen may have physiological effects on animal performance (Choung and Chamberlain, 1992a,b,c).

It has been well known that soybean is a major protein source in animal feeds (Waltz and Dtern, 1989, Titgemeyer et al., 1989). In ruminant animals, altering the physical form of the proteins in soybean, such as the popping, has been adopted to increase protein utilization by changing the protein degradability (Koeln and Paterson, 1986). However, effects of popped soybean on ruminal peptide production and amino acid utilization in ruminant

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animals have not been studied extensively. Thus, we evaluated effects of popped soybean on the concentration of nitrogen-containing compounds in the rumen and levels of blood amino acids in Holstein calves.

## MATERIALS AND METHODS

### Animals and sample collections

Two Holstein calves with an average weight of 169 kg were used in switch back design. Popped soybean (PSB) processed at 200~230℃ for 2 seconds and raw soybean (RSB) were used as an experimental diet. The feeding trial was composed of 3 periods (each period; 21 days), and the diets were switched after each period. At final day of each period, the rumen fluid was collected at 0, 2, 4 and 6 h after feeding using stomach tube, and blood were collected from jugular vein using vacuum tube. Daily feed consumption and its nutrient contents of the diet are presented in table 1. Calves were housed with continuous access to water and mineral-vitamin block.

**Table 1.** Ingredient and nutrient intake of calves[1]

| Items | Treatment[2] | |
|---|---|---|
| | RSB | PSB |
| | ..... kg/d ..... | |
| Ingredients | | |
| Concentrate[3] | 2.0 | 2.0 |
| Sudangrass hay | 2.4 | 2.4 |
| Raw soybean | 0.26 | – |
| Popped soybean | – | 0.26 |
| Nutrients | | |
| Dry matter | 4.20 | 4.20 |
| TDN | 3.09 | 3.09 |
| Crude protein | 0.68 | 0.68 |

[1] Calves were fed 95% of *ad libitum* to make sure that they have equal amount of diet.
[2] RSB: Raw soybean, PSB: Popped soybean.
[3] Nutrients composition: crude protein, 16%; crude fat, 2%; crude fiber, 10%; crude ash, 9%; calcium, 0.5%; phosphorus, 0.4%; digestible protein, 13.5%; TDN, 71%.

### Sample treatments and analysis

Nitrogen-containing compounds were determined by using the method of Chen et al. (1987c) with some modifications. In brief, rumen fluid was strained with 2 layers of cheese cloth, and pH was measured. And then, it was held on ice to prevent more fermentation. For removing large feed particles and protozoa, rumen fluid was centrifuged at 120xg for 15 minutes. At this time, the low speed centrifugation was assured not to disrupt protozoa faraction which may increase nitrogen-containing compounds in the rumen fluids. Then, rumen fluid were centrifuged at 14,000xg for 20 minutes.

Ammonia concentration of samples was analyzed according to Chaney and Marbach (1962). Ten ml of rumen fluid sample with 0.9 ml of 60% perchloric acid were then centrifuged at 8,500xg for 15 minutes to precipitate proteins. After the precipitated protein were solubilized with 0.2 N NaOH at 70℃ for 20 min., protein concentration was determined by using bovine serum albumin as a standard protein as described by Lowry et al. (1951). Supernatant was passed through ion exchange column (Dowex 50W × 2 resin, $H^+$ form from Sigma, St. Louis, USA) with the deionized water as washing solution to remove salts. Amino acids and peptides remained on the top of the column were eluted with 3 N $NH_4OH$, and concentrated using vacuum evaporator below 50℃, which step completely eliminated ammonia. Amino acids and peptides in the concentrated solution were separated using Cu-Sephadex (Pharmacy Biotech AB, Uppsala, Sweden) gel filtration chromato-graphy according to Fazakerley and Best (1965). Because amino acids are strongly bound to Cu-Sephadex gel, only peptides are eluted with the first eluting solution, borate buffer. Amino acids remained were eluted with 0.2 N HCl. These amino acids and peptides solution were separately passed through ion exchange column (Dowex 50W × 12 resin, $H^+$ form from Sigma, St. Louis, USA) to remove salts. Overall steps are presented in figure 1.

Amino acid concentrations were measured using ninhydrin reaction after hydrolyzation with 6 N HCl (Chen et al., 1987c). The changes of peptide peak with time after feeding were measured using HPLC (Waters, Millipore), after that peptides samples were mixed with the same volume of trifluoroacetic acid solution (0.2% TFA/10% acetonitrile) and filtrated with micro filter (pore size, 0.45 $\mu$m) to remove fine particles in solution.

Column of $C_{18}$ Puresil (Waters, Millipore) was used with 0.1% TFA/water and 0.1% TFA/90% acetonitrile as a mobile phase. Retention time was 50 min., and flow rate was 0.8ml/min. Twenty $\mu$l samples were injected, and absorbance was measured at 215 nm using ultra-violet detector (Waters, Millipore). Free amino acids of blood were analyzed by using amino acid analyzer (Beckman, USA) after precipitating the blood proteins.

### Statistical analysis

Data obtained were analyzed statistically using ANOVA with the general linear model procedures of SAS (1985) and significant difference of averages of two

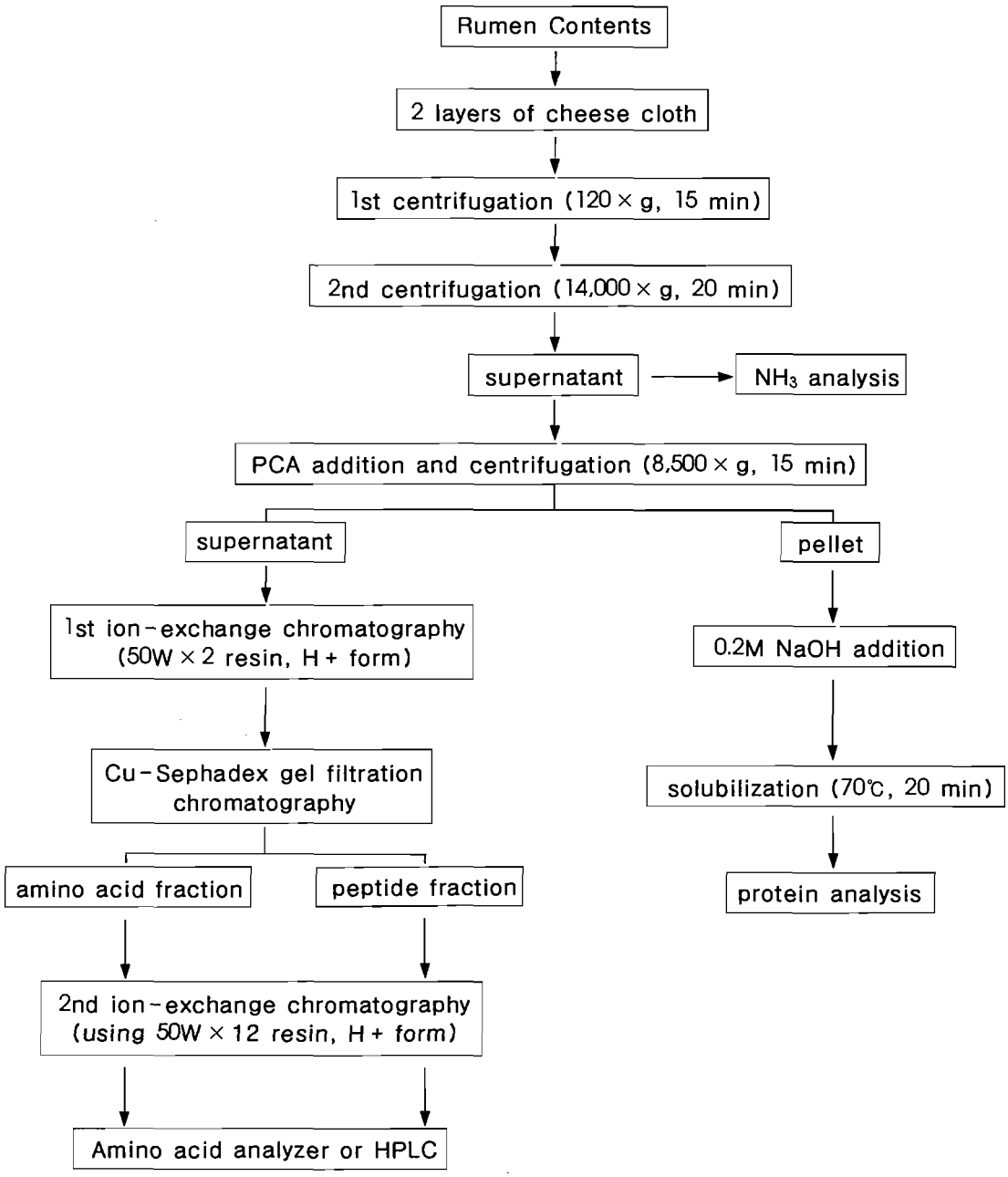


**Figure 1.** The analytical scheme for nitrogen-containing compounds in the rumen fluid.

treatments was analyzed by using Duncan's multiple range test (1955).

## RESULTS AND DISCUSSION

Changes of pH and concentrations of nitrogen-containing compounds in the rumen of Holstein calves fed RSB or PSB are presented in table 2.

At each incubation time, pH was not significantly influenced by dietary treatment, except that at 4 h after feeding, animals fed RSB showed significantly higher ruminal pH. Although there was no significant difference of ammonia concentrations between the treatments, there was a tendency of higher ammonia concentration in RSB treatment, which may partly explain higher pH value in RSB treatment.

At 0, 2 and 8 hour after feeding, the concentrations of soluble protein-N and amino acid-N were a little higher in the group of RSB than those in the group of PSB and at 4 hour after feeding, the results were reversed, but

**Table 2.** Changes of pH and concentrations of nitrogen-containing compounds in the rumen of Holstein calves fed raw or popped soybean

| Parameter | Time after feeding (h) | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6 |
| Raw Soybean | | | | |
| pH | 7.56 | 7.24 | 7.17[a] | 7.26 |
| $NH_3$-N (mg/L) | 89.93 | 113.89 | 77.08 | 72.80 |
| Protein-N (mg/L) | 88.71 | 66.84 | 58.89 | 74.81 |
| Amino acid-N (mg/L) | 51.29 | 50.42 | 41.20 | 45.01 |
| Peptide-N (mg/L) | 116.89 | 176.10[a] | 104.13 | 103.76 |
| Popped Soybean | | | | |
| pH | 7.18 | 6.89 | 6.81[b] | 6.99 |
| $NH_3$-N (mg/L) | 88.36 | 105.08 | 74.28 | 68.85 |
| Protein-N (mg/L) | 71.65 | 57.22 | 59.17 | 72.19 |
| Amino acid-N (mg/L) | 41.04 | 48.94 | 49.67 | 43.54 |
| Peptide-N (mg/L) | 89.98 | 99.31[b] | 120.56 | 104.85 |

[a,b] Means within same parameter having different superscripts are significantly different ($p < 0.05$).

there was no significant difference between these treatments. Only peptide-N production showed the significant difference between RBS and PBS treatment at 2 h after feeding. Peptide-N production was the highest at 2 h after feeding in RSB and at 4 h in PSB (table 2 and fingure 2). It has been traditionally accepted that concentration of amino acids are very low in the rumen (Wright and Hungate, 1967). Thus, researchers (Chen et al., 1987a; Annison, 1956) generally measured the ruminal peptides without separating amino acids. Interestingly enough, the result of this study showed that significant amounts of amino acids were produced in the rumen. Therefore, this study may indicate that amino acid concentration in the rumen fluids have to be determined to have more significant partition of N-compunds in the rumen content.

Chen et al. (1987a) showed that peptide-N production peaked (280 mg/L) at 1 h after feeding, and then rapidly decreased upto 3 h followed by gradual decrease afterward upto 8 h. The similar result was also observed by Broderick and Wallace (1988) who showed that peptide production was the highest (192 mg/L) at 1 h after feeding, and then decreased to the level of pre-feeding at 3 h after feeding. Primarily our result showed that peak concentration of peptide-N in the RSB treatment reached (176mg/L) at 2 h after feeding, whereas PSB treatment resulted in the highest peptide level (120 mg/L) at 4 h after feeding.

Our results imply that the rate of peptide production can be controlled by protein solubility or degradability, which is consistent with the results of previous reports of other researchers (Chen at al., 1987a; Broderick and Wallace, 1988). In fact, *in vitro* protein degradability of RSB was significantly higher than PSB (data are not shown). When the RSB or PSB was incubated with mixed rumen microbes, the concentrations of soluble protein were about three times higher in the RSB treatment than in the PSB treatment.

Not only the type of peptides but also amino acid sequences of peptides may influence peptide concentration in the rumen, thus it can not be ruled out the importance of amino acid sequences of peptides. Thus, one has to analyze amino acid sequences of peptides to challenge that possibility.

Meanwhile, when concentrations of essential or nonessential amino acids in plasma were determined, in the RSB treatment, concentrations of most essential amino acids in plasma were decreased after feeding, but those in PSB treatment decreased more rapidly after feeding (figure 3). And essential amino acid concentrations were highter in calves fed RSB than in those fed PSB (figure 4). Concentrations of non-essential amino acids in plasma also decreased with times in both treatments, although its slope was not so large as that of essential amino acid (figure 4). Thus these results implied that more free amino acids in the RSB treatment might reach to small intestine and be absorbed through epithelial cell. However, we can not rule out the possibility that amino acids absorbed might be more efficiently utilized by organs in calves fed PSB. According to Tagari and Bergman (1978), it is very difficult to elucidate the relationship of the level of plasma amino acids and other

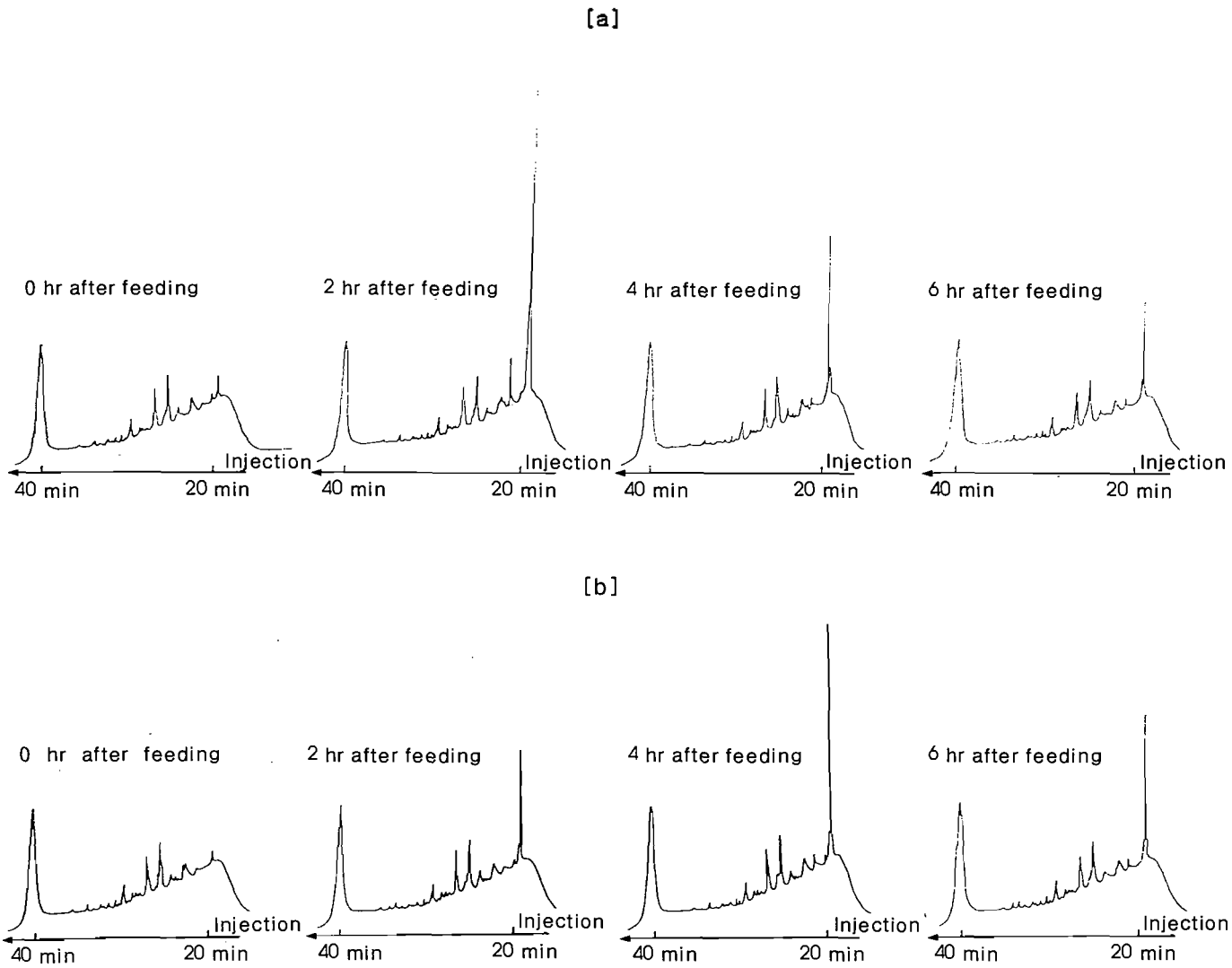


**Figure 2.** The time-course changes of HPLC chromatogram showing peptide concentration. [a] raw soybean, [b] popped soybean. Each peak indicate the peptide in the rumen fluid.

nutritional or physiological status of animal because many factors are related. These factors may include (a) the rate of absorption and utilization of individual amino acids, (b) the degradation amount of amino acids in the intestinal cell, (c) the diurnal variation of amino acids level, (d) blood flow and (e) the metabolism efficiency of the liver.

Many researchers (Crooker et al., 1986; Huntington and Prior, 1985; Lu et al., 1983; Prior et al., 1981; Santos et al., 1984; Teller et al., 1992; ) reported that the amount and degradability of protein in feed can affect concentrations of free amino acids in plasma. Our results showed that less degradable PSB in the rumen produced less amino acids and peptides than more degradable RSB did in rumen. According to Webb et al. (1992), peptides and amino acids are absorbed through the gastrointestinal tract of ruminants, and supply amino acids needed for their growth and the maintenance. Furthermore, they asserted that, quantitatively, peptide form is more important than amino acid form in supplying the amino acid to animals. Previous studies showed that 79% of amino acids in the portal vein were peptieds form (Koeln and Webb, 1982), and the size of most these peptides was below 300 Da (Schagheck and Webb, 1984). In addition, Webb et al. (1992) suggested that the concentration of peptides in plasma could be calculated as the differences between total and free amino acids. Unfortunately we did not estimate concentrations of amino acid and peptide in small intestine and those of peptide in plasma.

While, Webb et al. (1992) analyzed amino acids and peptides absorption by mesenteric flux associated with jejunum, ileum, cecum, colon, and pancreas and by nonmesenteric flux associated with rumen, reticulum,

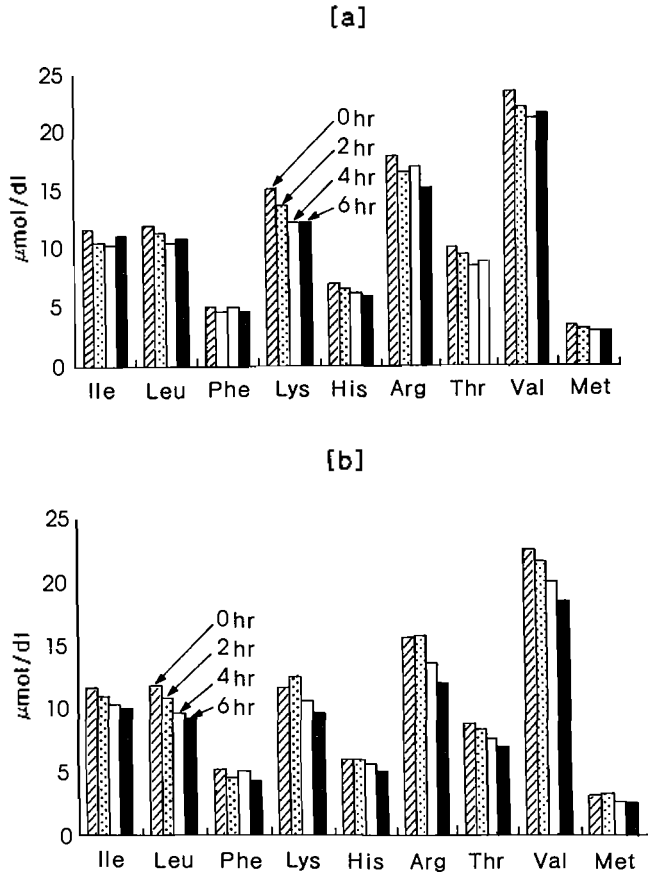


**Figure 3.** Changes of concentrations of essential free amino acids in plasma of Holstein calves fed RSB [a] or the PSB [b] at 0, 2, 4 and 6 h after feeding.

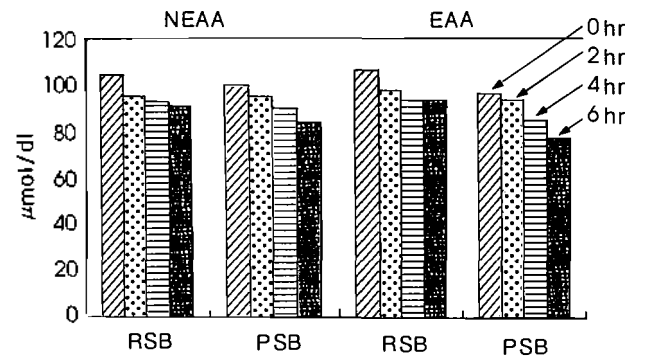


**Figure 4.** The change of concentrations of free amino acid in plasma of Holstein calves fed RSB or PSB at 0, 2, 4 and 6 h after feeding. NEAA : non-essential amino acid, EAA : essential amino acid. RSB : raw soybean, PSB : popped soybean.

omasum, abomasum, and duodenum. Regardless of essential or nonessential amino acids, free amino acids absorbed through mesenteric flux were around 50% more than peptide amino acids in lamb. However, in calves, most of amino acids absorbed through the mesenteric flux were free amino acid in essential amino acids, but were peptides in nonssential amino acids. And interestingly, amino acids absorbed across the nonmesenteric-drained viscera were mostly in the form of peptide and were 5~10 times more than amino acids absorbed through mesenteric flux, and free amino acids were negligible in both groups of animals.

From the results of this study, we may conclude that RSB produced more amino acids and peptides in the rumen, and increased the concentration of free amino acids in plasma. However, effect of processing of soybean on actual amounts of amino acids moiety absorbed into the body is not clear from the results of this study.

## REFERENCES

Broderick, G. A. and R. J. Wallace. 1988. Effects of dietary nitrogen source on concentrations of ammonia, free amino acids and fluorescamine-reactive peptides in the sheep rumen. J. Anim. Sci. 66:2233.

Chaney, A. L. and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. chem. 8:130.

Chen, G., C. J. Sniffen and J. B. Russell. 1987a. Concentration and estimated flow of peptides from the rumen of dairy cattle: effects of protein quantity, protein solubility, and feeding frequency. J. Dairy Sci. 70:983.

Chen, G. H. J. Strobel, J. B. Russell and C. J. Sniffen. 1987b. Effect of hydrophobicity on utilization of peptides by ruminal bacteria *in vitro*. Appl. Environ. Microbiol. 53:2021.

Chen, G., J. B. Russell and C. J. Sniffen. 1987c. A procedure for measuring peptides in rumen fluid and evidence that peptide uptake can be a rate-limiting step in ruminal protein degradation. J. Dairy Sci. 70:1211.

Chen, G. and J. B. Russell. 1988. Fermentation of peptides and amino acids by a monensin sensitive ruminal peptostreptococcus. Appl. Environ. Microbiol. 54:2742.

Choung, J. -J. and D. G. Chamberlain. 1992a. Protein nutrition of dairy cows receiving grass silage diets. The effects of post-ruminal supplements of proteins and amino acids. J. Sci. Food Agri. 60:25.

Choung, J. -J. and D. G. Chamberlain. 1992b. Effects of abomasal infusions of sodium caseinate, soya-protein isolate and a water extract fish meal on the utilization of nitrogen by dairy cows. J. Sci. Food Agri. 60:131.

Choung, J. -J. and D. G. Chamberlain. 1992c. Protein nutrition of dairy cows receiving grass silage diets. Effects on silage intake and milk production of postruminal supplements of casein of soya-protein isolate and the effects of intravenous infusions of a mixture of methionine, phenylalanine and tryptophan. J. Sci. Food Agri. 58:307.

Crooker, B. A., J. H. Clark, R. D. Shanks and E. E. Hatfield. 1986. Effects of ruminal exposure on the amino acid profile of heated and formaldehyde-treated soybean meal. J. Dairy Sci. 69:2648.

Depardon, N., D. Debroas and G. Blanchart. 1995. Breakdown of peptides from a soya protein hydrolysate by rumen

bacteria. Simultaneous study of enzyme activities and of two physico-chemical parameters: Molecular weight and hydrophobicity. J. Sci. Food Sci. 68:25.

Duncan, D. S. 1995. Multiple range and multiple t-test. Biometrics. 11:1.

Fazakerley, S. and D. R. Best. 1965. Separation of amino acids, as copper chelates, from amino acid, protein, and peptide mixture. Anal. Chem. 12:290.

Huntington, G. B. and R. L. Prior. 1985. Net absorption of amino acids by portal drained viscera and hind half of beef cattle fed a high concentrate diet. J. Animal Sci. 60(6):1491.

Koeln, L. L. and J. A. Paterson. 1986. Nitrogen balance and amino acid disappearance from the small inrestine in calves fed soybean meal−, toasted soybean meal− or corn gluten meal− supplemented diets. J. Anim. Sci. 63:1258.

Koeln, L. L. and Webb, Jr. 1982. Peptide, erythrocyte and plasma amino acid transport across the gastrointestinal tract and liver of calves. Fed. Proc. 41:948.

Lowry, O. H., M. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193:265.

Lu, C. D., N. A. Jorgensen and G. P. Barrington. 1983. Quantitative studies of amino acid flow in the digestive tract of sheep fed alfalfa protein concentrates. J. Nutr. 113:2390.

Pittman, K. A., S. Lakshmanan and M. P. Bryant. 1967. Oligopeptide uptake by *Bacteroides ruminicola*. J. Bacteriol. 93(5):1499.

Russell, J. B. 1983. Fermentation of peptides by *Bacteroides ruminicola* $B_4$. Appl. Environ. Microbiol. 45:1566.

SAS Institute, Inc. 1985. SAS user's suide: Statistics. 5th ed. SAS Inst., Inc., Cary, NC.

Schlagheck, T. G. and K. E. Webb, Jr. 1984. Characterization of peptides from the gastrointestinal tract of calves. Fed. Proc. 43:671.

Tagari, H. and E. N. Bergman. 1978. Intestinal disappearance and portal blood appearance of amino acids in sheep. J. Nutr. 108:790.

Teller, E., M. Vanbelle, M. Foulon, G. Collignon and B. Matatu. 1992. Nitrogen metabolism in rumen and digestive tract of lactating dairy cows fed grass silage. J. Dairy Sci. 75:1296.

Titgemeyer, E. C., N. R. Merchen and L. L. Berger. 1989. Evaluation of soyabean meal, corn gluten meal, blood meal and fish meal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim. Sci. 67:262.

Wallace, R. J. and N. McKain. 1989. Some observations on the susceptibility of peptides to degradation by rumen microorganisms. AJAS. 2(3):333.

Webb, Jr. K. E., J. C. Matthews and D. B. DiRienzo. 1992. Peptide absorption: A review of current concepts and future perspectives. J Anim. Sci. 70:3248.

Wright, D. E. and R. E. Hungate. 1967. Amino acid concentrations in rumen fluid. Appl. Microbiol. 15:148.

Wright, D. E. 1967. Metabolism of peptides by rumen microorganisms. Appl. Microbiol. 15:148.