Amino Acid Profiles of Tropical Legumes, Cooper (*Glycine wightii*), Tinaroo (*Neonotonia wightii*) and Siratro (*Macroptilium atropurpureum*), at Pre-blooming and Blooming Stages

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ABSTRACT : An experiment was conducted to investigate amino acid composition of three tropical legumes (Cooper (*Glycine wightii*), Tinaroo (*Neonotonia wightii*) and Siratro (*Macroptilium atropurpureum*)) at two different stages (pre-blooming and blooming stages). Chemical composition and totally 16 amino acids of these plants were analysed for comparison of their composition among species at different growing stages and characterizing the amino acid pattern of these legumes. Crude protein content of the plants ranged from 16% to 27% on a dry matter basis. The total amount of 16 amino acids analyzed in this experiment was highest at 89.7 mg/16 g N in Cooper at pre-blooming and lowest at 80.9 mg/16 g N in Glycine at blooming stage. Total amount of amino acids in each legume species tended to slightly decrease with their maturity but no statistical difference was found. The percentage of aspartic acid, glutamic acid and proline in the total amount of amino acids was dominant at 9% to 13%, and that of methionine was less than 1.6%. In this experiment it was concluded that three tropical legumes were rich in crude protein content and characterized by 16 different amino acids with lower sulfur-containing amino acid as methionine. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 5 : 651-654)***

Key Words : Amino Acid, Crude Protein, Tropical Legume, Maturity

INTRODUCTION

Nutritive value of protein in a feedstuff is determined by the amount and composition of amino acids in the absorptive small intestine (Miller, 1982). In ruminants amino acids are also supplied as microbial protein and undegraded protein through fermentation of the feed (Church and Pond, 1974; Orskov, 1982; Kellems and Church, 2003). Therefore, much research has been conducted to evaluate nutritive value of amino acids for microorganisms in the rumen as well as for the host animal. For example, amino acid intake, digestion, and absorption has been studied in sheep consuming a variety of diets under pen (Coelho et al., 1972; Harrison et al., 1973; Hogan, 1973) and free-grazing (McMeniman et al., 1986) conditions. On the other hand, knowledge of amino acid composition in feeds was limited only to cereal grains and temperate legumes (alfalfa) used in poultry and swine production. There is little information on the composition of plant amino acids except for the above feed resources. The crude protein content of tropical legumes grown in arid or semi-arid regions is high and does not decrease rapidly with plant maturity compared with tropical grasses (Minson, 1990). Tropical legumes have tolerance for drought (Kitamura and Abe, 1984), high leaf ratio, and low-appearance of dead leaves after flowering (Minson, 1990). Tropical legumes were also cultivated with grasses to supply crude protein. In the south-western part of Japan, three tropical legumes, Cooper (*Glycine wightii*), Tinaroo (*Neonotonia wightii*), and Siratro (*Macroptilium atropurpureum*) have been cultivated and their yields and nutritive values were evaluated for ruminant production (Nakanishi et al., 1987; Kitamura, 1984; Kitamura and Abe, 1984). Despite these advantages in protein content of tropical legumes, there is little information about the amino acid composition of these tropical legumes.

In this study we attempt to (1) determine the crude protein content and its amino acid composition for three tropical legumes, (2) compare their composition among species at different growing stages, and (3) characterize the amino acid pattern of these legumes.

MATERIALS AND METHODS

Preparation of plant samples and chemical analysis

Three tropical legumes, Cooper (*Glycine wightii*), Tinaroo (*Neonotonia wightii*), and Siratro (*Macroptilium atropurpureum*), were grown in an experimental field at Kyushu University, Fukuoka, Japan. Before cultivation, each seed was soaked in a diluted sulfuric acid solution for a few minutes to treat the hard shell, then washed sufficiently with tap water and dried at room temperature. Seeds were sown in each section (6 m×4 m size) in late May in the field. All plant species were cultivated in triplicate at the same time and the sections for each species

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Table 1. Chemical composition[1] and gross energy[2] of three tropical legumes at different stages of maturity

Species	Cooper		Glycine		Siratro	
Growth stage	Pre-blooming	Blooming	Pre-blooming	Blooming	Pre-blooming	Blooming
Stage (growth-days)	1-74	1-122	1-69	1-109	1-69	1-109
Crude protein	16.9[3, a]	16.6[a]	27.0[b]	24.8[b]	17.9[a]	19.1[a]
Neutral detergent fiber	57.1[a]	56.6[a]	45.9[b]	40.8[b]	51.9[a]	52.2[a]
Acid detergent fiber	36.4[ab]	39.3[a]	33.9[b]	32.5[b]	37.6[ab]	35.4[ab]
Hemicellulose	20.7[a]	17.3[a]	12.0[b]	8.3[c]	14.3[ab]	16.7[a]
Acid detergent lignin	6.1[a]	10.7[b]	5.8[a]	5.9[a]	6.9[a]	7.0[a]
Gross energy	16.8[a]	16.8[a]	17.3[a]	16.9[a]	16.2[a]	17.3[a]

[1] % on a dry matter basis. [2] kJ/g.
[3] Values with different superscript letters in a row are significantly different ($p<0.05$).

were allocated at random. Plants were cut at the pre-blooming (late July to early August) and blooming (late August to early September) stages of their first growth. The original samples were then dried in a forced-air oven at 70°C for 2 to 3 days, ground through a 1 mm screen in a Wiley Mill, and stored in sealed bottles until analyzed. Crude protein content of the samples was determined in duplicate using the standard method of the AOAC (1984). Fiber analysis was undertaken by the method of Goering and Van Soest (1970), and hemi-cellulose was calculated as a difference between neutral detergent fiber and acid detergent fiber. Gross energy content of each sample was determined by a bomb calorimeter. Each analysis was done in triplicate.

Amino acid analysis

For the amino acid analysis (Hitachi user's guide, 1984), 10 mg samples were placed in glass ampoules and 5 ml of 6 N hydrochloric acid was added. Then the tubes were placed in a dry-ice bath for 15 min. Next they were put into a freeze drier to evacuate the air in the tubes for 10 min to <5 Pa. The ampoules were sealed with a gas burner. The sealed tubes containing samples and hydrochloric acid were heated at 110±1°C for 22 h to break down the nitrogen compounds into their amino acids and cooled at room temperature. The liquid after acid hydrolysis was frozen again and evaporated *in vacuo* at <5 Pa in a freeze dryer for 12 h. Then 0.5 ml of 0.01 N sodium hydroxide solution was added to the dried residue and mixed. The mixture was allowed to stand for 10 min at room temperature. Then 1.5 ml of 0.02 N hydrochloric acid was added to make the solution acidic. After filtration (0.45 μm), 50 μl of the sample solution was injected automatically into the analyzer. The determination of amino acids was done in triplicate by using the amino acid analyzer (Hitachi model 835, Hitachi Co. Ltd, Tokyo). The method is based on the separation of the amino acids by cation exchange chromatography using five lithium acetate buffer solutions of increasing pH and ionic strength, followed by the ninhydrin color reaction. Prior to separation, sample solutions were passed through the ammonia column to eliminate ammonia. The amounts of each amino acid were calculated against a standard solution (16 amino acid mixture solution, L-type amino acid except glycine) purchased from Wako Pure Chemical Industries, Ltd.

Statistical analysis

All the data were analyzed by multiple-test as described by Snedecor and Cochran (1989). The values presented are averages of triplicate determinations.

RESULTS AND DISCUSSION

Chemical composition of the experimental plants is presented in Table 1. Crude protein content of the dry matter in the three tropical legumes in this experiment ranged from 16.6% to 27.0%. These three legumes contain slightly greater amounts of crude protein than previously reported for other locally grown forage legumes, with a protein range of 15% to 20% (Mecha and Adegbola, 1980). Gross energy content in this experiment ranged from 16.2 kJ/g to 17.3 kJ/g and there were no statistical differences among the species or the stages of maturity.

Amino acid profiles of three tropical legumes are presented in Table 2 and expressed in two ways - mg/g of crude protein content and percent of each amino acid per total amount analyzed. The total amount of amino acids expressed as mg/16g N in the three tropical legumes was 80.9 to 89.7, and the values showed little variation among different species or between stages of maturity. The total amount of amino acids was highest in Cooper at pre-blooming stage (89.7 mg/16 g N) and lowest in Glycine at blooming stage (80.9 mg/16 g N). At this time the ratio of arginine to the total amino acids was significantly lower ($p<0.05$) in blooming Glycine (3.8%) than in pre-blooming Cooper (5.4%). The total amino acid content of each legume species tended to decrease slightly with their maturity but there were no statistical differences. Since the tropical legumes used in this experiment were all grown in the same field, during the same season, and harvested at the same time, environmental effects would be minimal.

Of the total amount of amino acids, the percentage of aspartic acid, glutamic acid, and proline was higher, at 9%

Table 2. Total amino acid content[1] and its constituents[2] for three tropical legumes at different stages of maturity

Species	Cooper		Glycine		Siratro	
Growth stage	Pre-blooming	Blooming	Pre-blooming	Blooming	Pre-blooming	Blooming
Stage(growth-days)	1-74	1-122	1-69	1-109	1-69	1-109
Total amino acids	89.7[3, a]	87.5[a]	84.3[ab]	80.9[b]	86.0[a]	83.7[ab]
Glycine	5.4[a]	6.1[a]	5.6[a]	5.5[a]	5.6[a]	6.1[a]
Alanine	6.6[a]	6.0[a]	6.8[a]	6.6[a]	6.2[a]	6.1[a]
Valine	5.8[a]	5.8[a]	6.0[a]	6.0[a]	6.0[a]	6.1[a]
Leucine	8.6[a]	8.5[a]	9.0[a]	8.8[a]	8.9[a]	8.8[a]
Isoleucine	4.8[a]	5.0[a]	4.8[a]	4.9[a]	5.1[a]	5.1[a]
Serine	5.1[a]	5.1[a]	5.3[a]	5.2[a]	5.1[a]	5.0[a]
Threonine	5.1[a]	5.0[a]	5.2[a]	5.1[a]	5.4[a]	5.3[a]
Methionine	1.5[a]	1.5[a]	0.6[b]	1.1[ab]	1.5[a]	1.6[a]
Phenylalanine	5.3[a]	6.0[a]	5.1[a]	5.2[a]	6.1[a]	6.0[a]
Tyrosine	3.6[a]	3.6[a]	3.5[a]	3.5[a]	4.0[a]	3.7[a]
Proline	11.4[a]	12.6[a]	12.7[a]	12.3[a]	11.7[a]	11.6[a]
Aspartic acid	12.2[a]	12.8[a]	11.1[a]	12.2[a]	11.2[a]	12.6[a]
Glutamic acid	11.2[a]	9.2[b]	11.8[a]	12.0[a]	10.5[ab]	9.1[b]
Lysine	6.0[a]	5.5[a]	5.1[a]	5.6[a]	5.4[a]	5.4[a]
Histidine	2.2[a]	2.2[a]	2.1[a]	2.2[a]	2.0[a]	2.1[a]
Arginine	5.4[a]	4.9[ab]	5.4[a]	3.8[b]	5.4[a]	5.7[a]

[1] mg/16 g N, [2] % of total amino acids.
[3] Values with different superscript letters in a row are significantly different (p<0.05).

Table 3. Proportion[1] of classifed amino acids of three tropical legumes at different stages of maturity

Species	Cooper		Glycine		Siratro	
Growth stage	Pre-blooming	Blooming	Pre-blooming	Blooming	Pre-blooming	Blooming
Stage (growth-days)	1-74	1-122	1-69	1-109	1-69	1-109
Neutral amino acids	63.0[2, a]	65.3[a]	64.5[a]	64.2[a]	65.6[a]	65.2[a]
Acidic amino acids	23.4[a]	22.1[a]	22.9[a]	24.1[a]	21.6[a]	22.7[a]
Basic amino acids	13.6[a]	12.6[a]	12.6[a]	11.6[a]	12.8[a]	13.2[a]

[1] Percentage of total amino acids.
[2] Values with different superscript letters in a row are significantly different (p<0.05).

to 12.8%, than that of other amino acids through plant species and growth stages. Although percentages of proline and aspartic acid were not statistically different among legume species and their maturity, the percentage of glutamic acid was lower ($p<0.05$) in both Cooper and Siratro at blooming stage. The percentage of histidine and methionine was under 3%. Singleton et al. (1952) showed that the proteins of alfalfa seed contain more arginine, cystine, glutamic acid, and histidine, and less alanine, aspartic acid, and tryptophan than the proteins of the plant. Ekpenyong (1986) also reported that *Leucaena* seed contains high amount of crude protein (30.8%) and is rich in aspartic acid and glutamic acid, and low in methionine. Comparing the results in this experiment, the profiles of amino acid composition expressed as percentages were higher in aspartic acid and glutamic acid, and low in histidine and methionine.

On the other hand, according to their chemical characteristics, 16 amino acids were classified into the three groups such as neutral, acidic, and basic amino acids (Table 3). The percentage of neutral amino acids to the total amount of amino acids was highest, at 63.0 to 65.6%, in all species, medium, at 21.6% to 24.1%, in acidic amino acids and lowest, at 11.6% to 13.6%, in basic amino acids. Neutral amino acids were also classified into a further 5 groups such as aliphatic amino acids, oxy amino acids, sulfur-containing amino acids, aromatic amino acids, and imino acids (Figure 1). In this experiment the percentage of aliphatic amino acids, consisting of glycine, alanine, valine, leucine and isoleucine, was high (over 30%) in all plant species and there was a significant difference ($p<0.05$) between species and growth stages. Percentage of aromatic amino acids, consisting of phenylalanine and tyrosine, was almost 10%, similar to oxy amino acids, consisting of serine and threonine, and that of sulfur-containing amino acids as methionine was very low. Overall, it is clear that amino acids of the tropical legumes of Cooper (*Glycine wightii*), Tinaroo (*Neonotonia wightii*), and Siratro (*Macroptilium atropurpureum*) were particularly rich in neutral amino acids in terms of aliphatic amino acids, but low in their content of sulfur-containing amino acids.

Some tropical legumes have uncommon amino acids, such as mimosine, which is toxic to ruminants (NAS, 1977). In fact, Ekpenyong (1986) found an uncommon amino acid with levels as high as 36%, and Hylin (1964) reported that lysine is a precursor of the uncommon amino acid

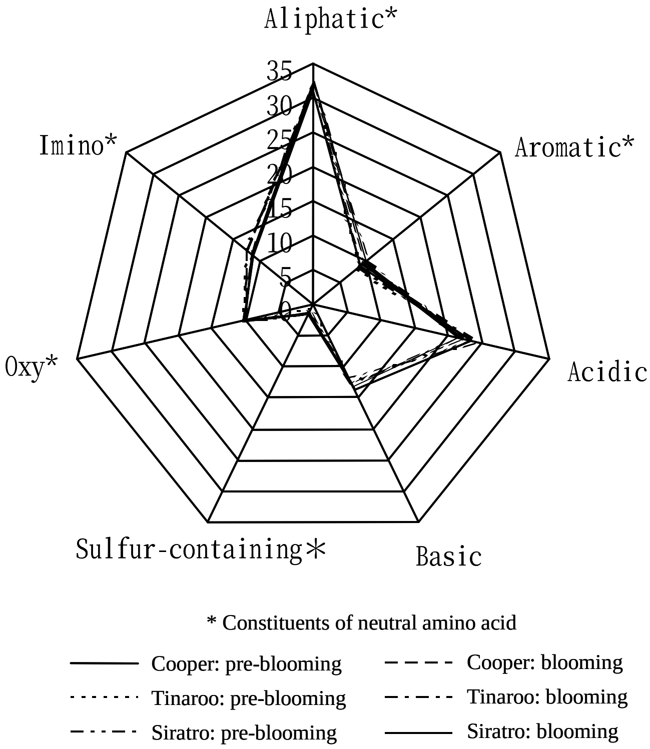

Figure 1. Distribution of major amino acid groups expressed as percentage in the tropical legumes.

mimosine. In this experiment we did not observe any unknown peak in the amino acid analysis, and lysine level in the three tropical legumes used in this study was lower than 6%. Since these data suggest that the three tropical legumes have a very low level of mimosine even at the blooming stages, the chances of adverse effects from feeding such as loss of weight, loss of appetite, or stunted growth in livestock would be rare.

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