Journal of the Korean Society of Grassland and Forage Science 42(3): 183~187, 2022
https://doi.org/10.5333/KGFS.2022.42.3.183
ISSN 2287-5824 (Print)
ISSN 2287-5832 (Online)

Research Article

Determination of Amino Acid Composition in Leaf, Stem, and Inflorescence of Alfalfa (*Medicago sativa* L.)

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ABSTRACT

Alfalfa is one of the most useful forage crops worldwide, containing a high level of amino acids that are essential to both human and animal health. However, amino acids and their concentrations may differ between plant parts. Hence, detecting amino acids in different plant parts would be useful in the development of diet supplements. The purpose of this study was to determine the amino acid content in alfalfa leaves, stems, and inflorescences using an amino acid analyzer. Asparagine and glutamic acid were the most abundant amino acids found in stems, leaves, and inflorescences than other amino acids. All parts of alfalfa had low concentrations of cysteine and methionine. All amino acids except asparagine were present in the highest concentration in leaves followed by inflorescences. Leaf had a rich amino acid content, namely asparagine, glutamic acid, leucine, proline, and lysine. However, the stem had a lower amino acid composition than the leaf or inflorescence. Overall, the data showed determining the amino acid content of forages provides a good approach to making animal feed with essential and specific amino acids and preventing excessive inclusion of amino acids.

(Key words: Alfalfa, Amino acid, Inflorescence, Leaf, Stem production)

Ⅰ. INTRODUCTION

In general, protein is an essential molecule and plays a key role in the metabolism of human, animal, and their growth process. The demand for a protein required worldwide has been increased due to the growth of animals and the human population. Due to the dense population, limited cultivation land leads to make a deficit of feed protein supply in many countries. It seriously affects the development of the feed industries and aquaculture (Guo et al., 2007; Zhang et al., 2017). Alfalfa contains various minerals, vitamins, and β -carotene. In addition, alfalfa concentrate consist of different amino acid content such as threonine, lysine, leucine, Isoleucine, valine, methionine, phenylalanine, histidine, aspartic amino acid, tyrosine, cysteine, alanine, glycine, proline, arginine, serine and glutamic acid (Apostol et al., 2017). Comparatively, alfalfa has high concentrations of lysine and methionine therefore it qualifies alfalfa as a rich protein source for pigs and poultry (Van Krimpen et al., 2013; Wüstholz et al., 2017). Alfalfa can be used as a good functional dietary ingredient in prevention and treatment of several metabolic diseases and disorders (Bora, 2011; Sadeghi et al., 2016). Many researchers have claimed that alfalfa has a higher protein yield than the other legumes (Arlabosse and Blanc, 2011; Chiesa and Gnansounou, 2011). A more attention is being paid to plant-based proteins as dietary supplements than to animal-based proteins. As a nutritional diet, alfalfa is now available as capsules, tablets, and powder. Therefore, the quantification of protein content and amino acids compositions in the alfalfa could provide essential information about precise uses in the improvement of animals and human health. Many researchers have reported protein and amino acids contents in aerial parts, leaves, whole powder, and the sprouted seed of alfalfa (Livingston, 1971; Blume, 2012; Zhang, 2017). The key aim of this study is to determine the precise concentration of amino acid in different parts of alfalfa which could provide concise information's about parts that are needed more for human and animal health improvement. In this study, we collected the

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leaves, flower and stem from alfalfa and extracted their proteins from each part and used for determination of amino acids content.

Ⅱ. MATERIALS AND METHODS

1. Cultivation of alfalfa

Alfalfa (Common) cultivation was performed according to the agricultural guidelines of the Rural Development Administration (RDA). In brief, Alfalfa (*Medicago sativa*) was sown at a seeding density of 20 kg/ha in the experimental field (3×5m^2 plot) of Rural Development Administration (RDA), Seonghwan-eup, Cheonan, Korea in early August, 2019. Seeding was performed by seed drilling machine (seeding width was fixed 20cm). The fertilizers such as nitrogen (100kg/ha), phosphate (300kg/ha), potassium (300 kg/ha), boron trioxide (B_2O_3, 20 kg/ha) and lime (300 kg/ha) were applied on a day of sowing. The chemical properties of the alfalfa cultivation soil used in this experiment were pH 7.26, Total nitrogen 0.25%, Soil organic matter 30.10 g/kg, Available P_2O_5 618.44 mg/kg and cation exchange capacity 14.02 cmol+/kg.

2. Determination of Amino acids content

Alfalfa was cut at full bloom and then each stem, leaf, and inflorescence in alfalfa was collected and measured fresh and dry matter content. For amino acid determination, samples were collected washed with sterilized distilled water, and then dried completely at 65°C. The samples were then ground to a fine powder using an electric mill. Powder samples were used for amino acid analysis (Choi et al., 2012). Five grams of powder were hydrolyzed with 6 N HCl at 110°C for 24 h. After hydrolysis, the acid was removed at 50°C by a rotary evaporator. Samples were then suspended in 50 mL of sodium citrate buffer (pH 2.2) and filtered through filter paper (0.45μm). Thirty microliters of each sample were used for the analysis of the amino acid contents by the amino acid analyzer (Model 835, Hitachi, Japan).

3. Statistical Analysis

The obtained data were subjected into statistical analysis using a statistical Package for the Social Science-16 (SPSS-16, Chicago; SPSS Inc). Means and standard errors were calculated for all the amino acid content using the means procedure of the SPSS. The significant between amino acids was performed by the general linear model containing multivariate analysis with Duncan's multiple range tests. Significance was defined at $p<0.01$.

Ⅲ. RESULTS AND DISCUSSION

Table 1 showed morphological, fresh and dry matter content of various parts of alfalfa. The average length of the plant was 78.9 ± 1.34 cm and stem diameter was 4.11 ± 0.07 cm. The average fresh weight for stem, leaves and inflorescence were 73.57 ± 9.2, 52.08 ± 5.4, and 15.9 ± 4.7 g/plant, respectively. The fresh total weight was 141.6 ± 8.9 g/plant. Dry matter yields for stem, leaves and inflorescence were 28.83 ± 3.7, 16.5 ± 1.7, and 3.83 ± 1.1 g/plant, respectively and total dry matter content was 49.2 ± 2.1 g/plant.

Table 1. Morphological, fresh and dry matter yields of different parts of alfalfa

Plant parts	
Height(cm)	78.9 ± 1.34
Stem (mm)	4.11 ± 0.07
Fresh matter yields (g/plant)	
Stem	73.5 ± 9.2
Leaf	52.0 ± 5.4
Inflorescence	15.9 ± 4.7
Total	141.6 ± 8.9
Dry matter yields (g/plant)	
Stem	28.8 ± 3.7
Leaf	16.5 ± 1.7
Inflorescence	3.83 ± 1.1
Total	49.2 ± 2.1

The data are represented as mean ± S.E. of three replicates.

Good protein quality, high protein content, complete and reasonable proportion of amino acid composition and proving the exact concentration of fat, sugar, and soluble starch, different minerals and vitamins, alfalfa could be employed for feed additives. The use of alfalfa as a protein source is well established for ruminants and horses (Radovic et al., 2009). The higher nitrogen supply to the livestock could generate more environmental pollution with bad odors. In order to minimize these problems, many studies have been performed to provide the precise requirement of the protein content in diet and amino acid availability for ruminants (Manjarin et al., 2014; Patton et al., 2014). Therefore, the determination of amino acid content in different parts of plants used for animal feed production is helping to develop the animal with a necessary concentration of amino acids and to avoid excessive usage which helps to prevent nitrogen source-based environmental pollution. Alfalfa protein constitutes an essential food ingredient and is commonly used in food technology due to its high nutritive value with protein content. Alfalfa is widely used in animal and human health. So, hence that it is considered a valuable approach with great interest to identify the amino acid contents in various parts of alfalfa. Table 2 represents percentage of amino acid content in stem, leaf and inflorescence of alfalfa. Asparagine (2.205± 0.853%), leucine (0.667±0.192%) and glutamic acid (0.888± 0.268%) were the most dominant amino acids (greater than 0.5%) found in the stem of alfalfa. Asparagine was highest concentration found in the stem followed by glutamic acid, leucine and proline. Methionine was the lowest concentration found in the stem of alfalfa. Asparagine (2.412±0.036%), glutamic acid (1.793±0.033%), and leucine (1.429±0.030%) were found higher concentration (>1%) in the leaf. The other amino acids such as threonine serine, glycine, valine, isoleucine, tyrosine, and arginine were the next most common amino acid found in leaf at the concentration between 0.6% and 0.9%. Cysteine and methionine were found lowest level in the leaves of alfalfa (Table 2). Asparagine (3.911±0.024), glutamic acid (1.456±0.022), and leucine (1.088±0.019) were the highest concentration (greater than 1% of amino acid noted in the inflorescence. Threonine, Serine, Glycine, Alanine, Valine, Phenylalanine, Lysine, Arginine were found at the concentration between 0.6 - 0.9 % in inflorescence (Table 2).

Alfalfa leaf protein is considered an important food ingredient and is commonly used in food industries (Zhang et al., 2017). Sahni et al., 2020 reported that the amino acids compositions in processed and non-processed protein isolate from alfalfa seeds. Aspartic acid, leucine, isoleucine and alanine were found highest in both processed and non-processed seeds (Sahni et al., 2020).

Table 2. Amino acids contents in stem, leaf and inflorescence of alfalfa

Amino acids (%)	Stem (%)	Leaf (%)	Inflorescence (%)
Cysteine	0.13 ± 0.054^{b}	0.30 ± 0.005^{i}	0.23 ± 0.002^{j}
Methionine	0.11 ± 0.049^{b}	0.30 ± 0.006^{i}	0.21 ± 0.004^{j}
Asparagine	2.20 ± 0.853^{a}	2.41 ± 0.036^{a}	3.91 ± 0.024^{a}
Threonine	0.47 ± 0.146^{b}	0.90 ± 0.015^{e}	0.78 ± 0.009^{f}
Serine	0.56 ± 0.165^{b}	0.89 ± 0.008	0.89 ± 0.005^{e}
Glutamic acid	0.88 ± 0.268^{b}	1.79 ± 0.033^{b}	1.45 ± 0.022^{b}
Glycine	0.44 ± 0.124^{b}	0.91 ± 0.016^{e}	0.71 ± 0.016^{fg}
Alanine	0.55 ± 0.174^{b}	1.13 ± 0.026^{d}	0.88 ± 0.005^{e}
Valine	0.44 ± 0.133^{b}	0.85 ± 0.020^{e}	0.74 ± 0.018^{fg}
Isoleucine	0.34 ± 0.115^{b}	0.66 ± 0.020^{f}	0.58 ± 0.007^{h}
Leucine	0.66 ± 0.192^{b}	1.42 ± 0.030^{d}	1.08 ± 0.019^{c}
Tyrosine	0.23 ± 0.081^{b}	0.55 ± 0.003^{g}	0.41 ± 0.011^{i}
Phenylalanine	0.43 ± 0.122^{b}	0.93 ± 0.011^{e}	0.72 ± 0.024^{fg}
Lysine	0.55 ± 0.157^{b}	1.12 ± 0.026^{d}	0.91 ± 0.026^{e}
Histidine	0.23 ± 0.071^{b}	0.39 ± 0.005^{h}	0.38 ± 0.009^{i}
Arginine	0.37 ± 0.146^{b}	0.88 ± 0.015^{e}	0.68 ± 0.012^{g}
Proline	0.63 ± 0.171^{b}	1.19 ± 0.025^{d}	1.00 ± 0.027^{d}
Total (%)	9.30 ± 0.110	16.69 ±0.125	15.65 ±0.195

The data are represented as mean ± S.E. of three replicates. $^{a\text{-}j}$*p<0.01* within a same row with different alphabets are significantly different.

The present study revealed that stem had the highest concentration of asparagine compared to other amino acids. But leaves had many amino acids greater than 1% such as asparagine, glutamic acid, leucine, proline, histidine, and alanine. The inflorescence had asparagine, glutamic acid, leucine, and proline at the highest level compared to others. Comparison of amino acids concentration between parts of alfalfa showed asparagine was a dominant amino acid found in all parts of plats such stem, leaves, and inflorescence but the maximum concentration of asparagine was found at inflorescence followed by leaves and stem. Leaves had the most of the tested amino acids at higher level compared to other parts of alfalfa. Leaves contain rich crude protein content that the other parts. It is the key reason for various amino acids at the higher levels was found in the leaves. This result was consistent with previous findings, suggesting leaf had higher crude protein concentration than in the other parts such stem and whole plant (Arinze et al., 2003; Hojilla-Evangelista et al., 2020). Another finding indicated that the leaf had glutamic acid (83.2 g kg^{-1}), aspartic acid (71.2 g kg^{-1}), leucine (67.6 g kg^{-1}), and arginine (53.2 g kg^{-1}) (Giner-Chavez et al., 1997). Similarly, glutamic acid, leucine, and proline were the top three concentrations found in the leaf of alfalfa. Another report has claimed that leucine, phenylalanine, arginine and lysine were the prominent amino acid in the leaf (Hsu and Allee, 1980).

Ⅳ. CONCLUSIONS

This study demonstrated that the amino acid content shows marked differences depending on the part of the alfalfa. Leaves had the highest amino acid concentrations followed by inflorescence and stem. It gives good strategy to develop a feed for the livestock with the desired concentration of amino acids from different parts of alfalfa.

Ⅴ. ACKNOWLEDGMENTS

Cooperative Research Program for Agriculture Science and Technology Development supported funds for this research work (Project No. PJ01358902). The project titled “technique development for the manufacture of high-quality legume silage “sponsored by RDA, Korea. This study was also supported by the Postdoctoral Fellowship Program of the National Institute of Animal Science funded by RDA, Korea.

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(Received : September 02, 2021 | Revised : September 26, 2022 | Accepted : September 26, 2022)