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Сборник составлен по итогам работы Межвузовского научного конгресса. Включает в себя доклады российских и зарубежных представителей высшей научной школы, в которых рассматриваются современные научные тенденции, новые научные и прикладные решения в различных областях науки, практика применения результатов научных разработок. Служит инструментом обмена опыта научных работников, апробации исследований путем их публичного обсуждения.

Предназначено для научных работников, профессорско-преподавательского состава, соискателей ученой степени и студентов вузов.

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STUDY OF THE AMINO ACIDS OF MEDICAGO SATIVA, ALHAGI PSEUDALHAGI, COUSINA PLANTS

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Annotation. *One of the main objectives of livestock development is to increase productivity while minimizing feed consumption. The aim of the study was to determine the chemical composition, namely amino acids, of Medicago sativa, Alhagi pseudalhagi and Cousina plants using high-performance liquid chromatography (HPLC). High-performance liquid chromatography (HPLC) is considered the most effective method for analyzing complex organic samples.*

Keywords: *Alhagi pseudalhagi, amino acids, Cousina, feed, high-performance liquid chromatography (HPLC), Medicago sativa, plant.*

Introduction: The nutritional value of feed plays an important role in ensuring the abundance and quality of products obtained from agricultural animals. This is because livestock consume various plant-based feeds, which are then converted into milk and meat products. Feed serves as the main source of energy for animals. Field and pasture grasses have a significant impact on improving the productivity, health, and reproductive functions of cattle [1].

In achieving efficiency in animal husbandry, strengthening the livestock feed base, increasing the production of animal products, and expanding the areas for growing forage crops are among the most urgent issues. According to estimates, approximately 70-80% of agricultural land worldwide is used for producing feed for livestock [2].

Medicago sativa is an annual and perennial herbaceous plant belonging to the legume family. Its origin is in Iran, and around 2-2.5 thousand years ago, it was introduced to Greece, Ancient Rome, and North Africa. Later, alfalfa became a cultivated crop and spread to Europe, North and South America, and Australia.

There are about 100 wild and cultivated species of plants belonging to the Medicago genus. Varieties such as alfalfa (Medicago sativa), yellow alfalfa (Medicago falcata L), hybrid alfalfa (Medicago media L), blue alfalfa (Medicago coerulea L), and hop-like alfalfa (Medicago lupulina L) are widely planted.

Medicago sativa is a perennial leguminous plant that grows to a height of 70-100 cm. Its leaves are elongated and consist of three leaflets. It blooms 60 days after sprouting in early spring, and after subsequent harvests, it blooms again 40-45 days later. Its fruits ripen in June-July [3].

Figure 1. *Appearance of the Medicago sativa plant*

It significantly enhances soil fertility. Due to its high protein content, it is primarily used as feed for livestock. Medicago sativa is also suitable for restoring degraded lands, low-fertility soils, and steppe meadows. This plant is known for its high productivity, nutritional value, and strong drought resistance [4].

Alhagi sparsifolia is a perennial plant from the legume family, reaching a height of 25-80 cm. It blooms from July to August. Its flowers are pink, hairless, about 1 cm long, and grow in clusters of 3-8 on branches. Its fruits ripen between August and October. The root of Alhagi pseudalhagi is a taproot that can grow up to 15-20 meters long. It is a perennial plant whose above- ground part dies off. Its thorns form a complex branching bush [5].

Figure 2. *Appearance of the Alhagi pseudalhagi plant*

Species of the Cousinia plant found in nature include C. allolepis, C. adenophora, C. butkovii, C. vvedenskyi, C. platystegia, C. dshisakensis, C. umbilicata, C. candicans, and C. rhodantha [6].

The Cousinia plant belongs to the Asteraceae family and is a perennial plant that reaches a height of 60-100 cm. Its leaves are leathery, gray-green on both sides,

and densely hairy. The leaves near the stem shrink as they ascend, with the lower leaves being sessile or short-stemmed, reverse-lanceolate, and pinnately lobed, with curly edges. The flower heads are round, with a diameter of 18–20 mm, and are densely covered with tangled hairs. The involucral bracts are numerous, fluffy, and ridged. The plant flowers in June-July, with the fruits ripening in August.

It grows on the small stony slopes of mid-altitude mountains and in gray loamy soils. Cousinia is distributed in the Kashkadarya basin, the western part of the Hisar Range, and the southern slopes of the Zarafshan Range, particularly in the Ayakchi River Valley [7].

Figure 3. *Appearance of the Cousinia plant*

The Cousinia plant, one of the field grasses, can serve as important feed for both ruminant and non-ruminant animals. Cousinia is a versatile plant that grows on various continents, in different soils, and under diverse ecological conditions [8].

One of the main tasks in the development of animal husbandry is to increase productivity while minimizing the consumption of feed. Using Cousinia, Medicago sativa, and Alhagi plants as mixed feed for livestock is appropriate.

EXPERIMENTAL SECTION

Equipment used: Amino acids in Cousinia, Medicago sativa and Alhagi plants were determined using HPLC (high performance liquid chromatography).

The amino acids in Cousinia, Medicago sativa, Alhagi plants, and cottonseed husks were identified. Methodology for Isolation of Free Amino Acids. Isolation of free amino acids was carried out from the aqueous extracts of samples. The protein and peptide precipitation was performed in centrifugal tubes. For this, 1 ml of the test sample was mixed with 1 ml of 20% trichloroacetic acid (TCA) (a known volume). After 10 minutes, the precipitate was separated by centrifugation at a speed of 8000 rpm for 15 minutes. A volume of 0.1 ml of the supernatant was taken and lyophilized. The hydrolysate was evaporated and dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1), then dried. To neutralize the acid, this process was repeated twice. The phenylthiocarbamyl derivatives of amino acids were obtained by reacting with phenyl isothiocyanate according to the methods of Stephen A. and Cohen David.

RESULTS OBTAINED AND THEIR ANALYSIS

The identification of amino acid derivatives was performed by HPLC. HPLC conditions: Agilent Technologies 1200 chromatograph with a DAD detector, Discovery HC C18 column (75 x 4.6 mm). Solvent A: 0.14 M sodium acetate + 0.05% triethylamine, pH 6.4; Solvent B: acetonitrile. The flow rate was 1.2 ml/min, and the detection wavelength was 269 nm. Gradient %B/min: 1-6% from 0 to 2.5 min; 6-30% from 2.51 to 40 min; 30-60% from 40.1 to 45 min; 60-60% from 45.1 to 50 min; 60-0% from 50.1 to 55 min.

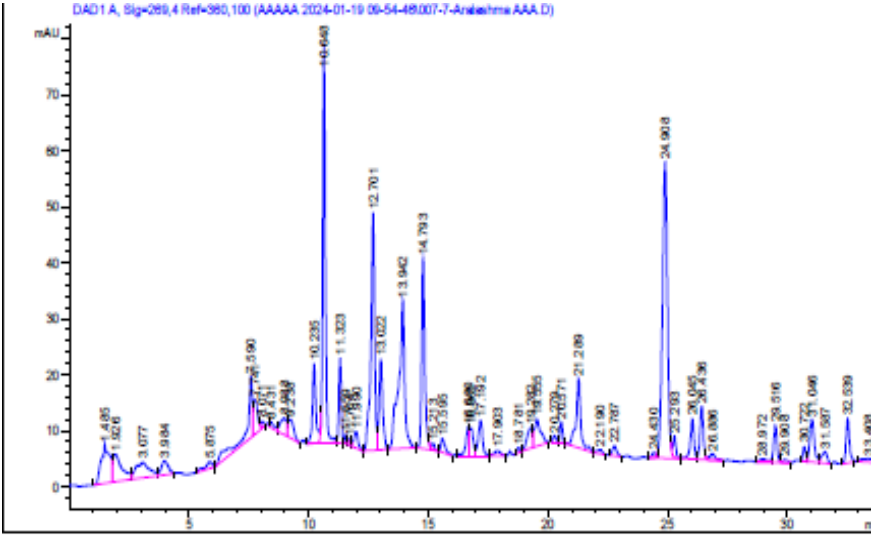

Figure 4. *Chromatographic spectrum of amino acids from Cousina, Medicago sativa, Alhagi and cotton husk plants.*

Table 1

Amino acids in Cousina, Medicago sativa, Alhagi plants and cotton seed husks

№	Amino acids	Amount of amino acids (mg/g)
Interchangeable amino acids		
Monoaminocarboxylic acid		
1	Alanine (Ala)	0,71
2	Glycine (Gly)	0.1
Monoaminodicarboxylic acid		
3	Aspartic acid (Asp)	0,55
4	Glutamic acid (Glu)	0,24
Hydroxymonoaminocarboxylic acid		
5	Serine (Ser)	0,17

Aromatic acid		
6	Tyrosine (Tyr)	0,37
Oxydiaminocarboxylic acid		
7	Asparagine (Asp)	0,45
8	Glutamine (Gln)	0,56
Heterocyclic amino acids		
9	Proline (Pro)	1,39
Sulfur-containing amino acids		
10	Cysteine (Sys)	0,37
Essential amino acids		
Monoaminomonocarboxylic acid		
1	Valine (Val)	0,51
2	Leucine (Ley)	0,30
3	Isoleucine (Ile)	0,19
Hydroxymonoaminocarboxylic acid		
4	Threonine (Thr)	1,84
Diaminomonocarboxylic acid		
5	Arginine (Arg)	0,32
6	Lysine (Lys)	0,15
Aromatic amino acid		
7	Phenylalanine (Phe)	0,20
Sulfur-containing amino acids		
8	Methionine (Met)	0,47
Heterocyclic amino acids		
9	Histidine (His)	0,19
10	Tryptophan (Trp)	0,20

Discussion: High-performance liquid chromatography (HPLC) revealed the presence of 20 amino acids in the mixed feed, 10 of which belong to the group of exchangeable amino acids.

The amino acids in the mixed feed are quantitatively arranged in the following order:

Interchangeable amino acids: Pro > Ala > Gln > Asp > Asn > Tyr = Sys > Glu > Ser > Gly

Non-exchangeable amino acids: Thr > Val > Met > Arg > Leu > Phe = Trp > Ile = His > Lys

All amino acids: Thr > Pro > Ala > Gln > Asp > Val > Met > Asn > Tyr = Sys > Arg > Leu > Glu > Phe = Trp > Ile = His > Ser > Lys > Gly.

Table 2 shows the chemical properties of some amino acids and their importance in animal development.

Table 2

The role of amino acids and their structural formula.

Amino acid	Symbol	Chemical formula	The role of amino acids in animal organism	Formula	References
Alanine	Ala	$C_3H_7NO_2$	Alanine plays a significant role during early starvation, exposure to high-fat and high-protein diets, and diabetes.	NH_2-CH(CH_3)-COOH	9
Glycine	Gly	$C_2H_5NO_2$	Glycine plays an important role in metabolic regulation, anti-oxidative reactions, and neurological function.	NH_2 —CH_2 — COOH	10
Aspartic acid	Asp	$C_4H_7NO_4$	Aspartic acid increases testosterone levels in the blood	$HO_2CCH(NH_2)$ CH_2CO_2H	11
Serine	Ser	$C_3H_7NO_3$	Serine plays an important role in immune function, growth stimulation and treatment of cancer in animals.	HO_2C-CH(NH2) CH_2OH	12
Cysteine	Sys	$C_3H_7NO_2S$	Cysteine – plays an important role in the functioning of the visual system. It participates in the metabolism of the eye lens and improves vision	$HO_2CCH(NH_2)$ CH_2SH	13
Valine	Val	$C_5H_{11}NO_2$	valine enhances growth and reproductive functions, regulates intestinal microbiota and immune functions.	$HO_2CCH(NH_2)$ $CH(CH_3)_2$	14
Leucine	Ley	$C_6H_{13}NO_2$	Leucine helps maintain strong bones, controls blood sugar levels and helps strengthen the immune system.	$HO_2CCH(NH_2)$ $CH_2CH(CH_3)_2$	15
Threonine	Thr	$C_4H_9NO_3$	Threonine effects on protein synthesis, energy metabolism and nutrient absorption.	$HO_2CCH(NH_2)$ $CH(OH)CH_3$	16

Methionine	Met	$C_5H_{11}NO_2S$	Methionine serves as a functional nutrient necessary for the production of antioxidants and taurine and is also required for protein synthesis	$HO_2CCH(NH_2)CH_2CH_2SCH_3$	17
Glutamine	Gln	$C_5H_{10}N_2O_3$	Glutamine is a nonessential amino acid that is important as a constituent of proteins and as a central metabolite for amino acid transamination.	O=C(NH2)-CH2-CH2-CH(NH2)-COOH	18
Arginine	Arg	$C_6H_{14}N_4O_2$	Arg regulates cellular signaling pathways and gene expression, improving cardiovascular function, increasing insulin sensitivity, increasing lean tissue mass and reducing obesity.	$NH\text{-}C(NH_2)NH(CH_2)_3CH(NH_2)$-COOH)	19

Conclusion: Of the 20 amino acids found in animals, eight cannot be produced or stored in the body and therefore must be obtained through the food animals eat. This group includes lysine, methionine, tryptophan, threonine, phenylalanine, leucine, isoleucine, arginine, histidine and valine.

Using the method of high-performance liquid chromatography, it was established that 20 types of amino acids were identified in the composition of the proposed feed consisting of Cousina, Medicago sativa and Alhagi pseudalhagi plants and cotton seed husks. It should be noted that the feed contains the amino acids lysine, methionine, tryptophan, threonine, phenylalanine, leucine, isoleucine, arginine, histidine and valine, which are not absorbed in the body of animals.

Analysis of the results shows that feed prepared from Cousina, Medicago sativa and Alhagi pseudalhagi plants and cotton seed husks is the most effective feed for domestic animals.

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