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Nutritional Value and Amino Acid Content of Four Grasses in Eastern Inner Mongolia

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Abstract: This study researched four grasses: *Hordeum brevisubulatum*, *Roegneria turczaninovii*, *Bromus inermis* and *Elymus nutans*. The study was conducted in 2006-2007 in Inner Mongolia at the Linxi seed field; it assessed the development the different growth stages of plant nutrients and analyzed plant stems and leaves of nutrients as well as their amino acid values (amino acid ratio coefficient). The results were as follows: over the entire growth period, the crude fiber content in all four types of grass changed the magnitude of coarse protein as the grass grew and its content was gradually increased. Crude ash and crude fat content barely changed during the entire growth period. The study analyzed the nutrition of the four grasses. The results showed that *Hordeum brevisubulatum* contained moderate levels of calcium and high phosphorus. *Roegneria turczaninovii*, *Elymus nutans* and *Bromus inermis* all contained low levels of calcium and moderate phosphorus. The ratios of calcium and phosphorus of *Hordeum brievisubulatum* and *Roegneria turczaninovii* were in the perfect range but those of *Elymus nutans* and *Bromus inermis* were not. EAAI values of the four grasses were higher than 0.95 indicating that the grasses were well-fed.

Key words: *Roegneria turczaninovii*, *Hordeum brevisubulatum*, *Bromus inermis*, *Elymus nutans*, nutrition value, amino acids

INTRODUCTION

Forage nutritional value mainly depends on the type and quantity of the nutrients contained (Zheng *et al*., 2006). Therefore, the content of various nutrients has become the most basic indicator for measuring the nutritional value of pasture and fodder crops. The level of amino acid concentration is an important basis for determining whether the forage quality is good or bad. Proteins constitute the basic material of animal and plant cytoplasm, nucleus, plastid and play an important role in the life activities of organisms and their functioning mechanisms. In plant composition, protein has the greatest nutritional and feed values; it is the most important nutrient for humans and animals. Agricultural analysis is often one of the most important indicators for protein determination and the level of content permits evaluation of the quality and economic value of crops and fodder (Huang and You, 2003).

With the rapid development of animal husbandry, the severe shortage of protein feed is increasingly prominent. At home and abroad, scientists are actively seeking to develop protein feed resources and one feasible solution is to develop the use of plant leaf proteins. Wu *et al*. (1999) analyzed the amino acids of the South Australian herbage leaf protein species *Thinopyrum ponticum*, *Hordeum brevisublatum* and alfalfa and found a high content of essential amino acids that is ideal for high-quality protein sources. Alfalfa leaf protein contains a similar proportion of essential amino acids to imported Peruvian fish meal which is comparable to the levels recommended by the FAO/WHO (1973) as being. Thus, it can be developed to use as a human food additive and a livestock feed additive.

In recent years, several studies have reported on the seasonal dynamic changes of forage carbohydrate and amino acid content. For instance, Xiaohong *et al*. (2005) studied the alpine region carbohydrate and amino acid content of 13 forage species and found that the total free amino acid and lysine content of Artemisia smithii Mattf, *Gentzana purdomii* Marq, *Polygonum viviparum* L. and Leguminosae showed a single peak curve over the season.

At present, the research on domestic pasture carbohydrates and amino acids has focused on mainly on alfalfa rather than grass. Li and Ma (1997) studied

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Northeast Alfalfa in North China and the amino acid analysis results showed that the eight kinds of amino acid of alfalfa varieties had similar characteristics. The highest content of amino acids is aspartic acid the lowest is histidine and all varieties of alfalfa had had the highest levels of the same five amino acids in this order: aspartic acid>glutamic acid>leucine>lysine>alanine. Wang and Du (2004) conducted an amino analysis of 12 kinds of alfalfa germplasm and found that mowing alfalfa germplasm materials contain 17 amino acids in different periods of growth. Regeneration of the trial alfalfa flowering mowing grass had significantly higher levels of total amino acids than the content of flowering grass. For the trial forage, each component of amino acids had some level of regularity; aspartic acid was the highest and cystine was the lowest.

In recent years, many researchers have used the Amino Acid Index (EAAI) for the evaluation of feed protein sources. The EAAI reflects the level of feed protein sources of essential amino acids and the essential amino acid composition of feeding objects. Thus, using the feeding of animal protein as the reference, EAAI enables researchers to evaluate the nutritional value of the animals' protein sources (Feng and Zhao, 1997). For example, Liu and Quan (2002) analyzed heat quality using EAAI evaluation. This study researched four grasses: *Hordeum brevisubulatum*, *Roegneria turczaninovii*, *Bromus inermis* and *Elymus nutans* and studied the dynamic changes of their nutrients and amino acids. The aim of this study was to provide a theoretical basis for the development and utilization of forage products as human food additives and animal feed additives.

MATERIALS AND METHODS

Profile of experimental plots: The grassland experiment was conducted in a field in Chifeng city within Linxi county of Inner Mongolia. Linxi county lies in the North part of Chifeng city and is located in the Southeast of Daxinganling. Toward the east is Balinyou town and toward the West is Keshenketeng town. South of Linxi county is the Xilamulun river and Xiwuzhumuxin town is North of Linxi county. Linxi county is located at 43°14~44°15'N and 117°37'~118°34'E, at an average of 900 m above sea level. The solar radiation during the growth period is 88~96 kcal/cm^2. There is an average of 1630 h of sunshine annually. The average annual temperature is 4-5°C about 380 mm annual rainfall. July is often the hottest month of the year and the average annual temperature is 20-22°C. The highest temperature is 40.4 degrees. January is the coldest month of the year with an average annual temperature of 13-17°C below zero. The lowest temperature is 32.2°C below zero. Average annual precipitation is 320-380 mm precipitation which is concentrated in Summer and accounts for 76.7% of the annual amount. Average annual evaporation is 1360 mm and there is a frost-free period of 125-130 days per year. The local soil is chestnut soil (Table 1).

Experimental design: Using a randomized block design test that was repeated three times, the fine soil preparation took place before sowing on a plot size of 5×2 m on May 24, 2006. This entailed sowing, artificial ditching drilling, spacing of 40 cm and sowing at a depth of 2 cm at a sowing rate of 22.5 kg/hm^2. After sowing without fertilizer, irrigating in the Autumn, weeding twice after turn green in the 2nd year.

Measurement items and methods

Determination of nutrients: Nutrients were measured according to the feed quality inspection to provide the national standard methods. Determination of project: Crude Protein (CP), Crude Fiber (CF), Ether Extract (EE), Crude Ash (CASH), Calcium (Ca), Phosphorus (P), Nitrogen Free Extract (NFE) calculated value (Wang, 2003):

$$100\% \text{ of Dry Matter basis, NFE } (\%) = 100\% - (CP + EE + CASH + CF)\%$$

Where:

DM = 105 Celcius drying method
CP = Combustion Nitrogen Analysis
CF = H_2SO_4 and NaOH Solution Boiling Digestion Method
EE = Soxhlet Extractor Method
CASH = Ashing Method
Ca = EDTA complexometry
P = Vanadate-molybdate-yellow colorimetry
NFE = Calculated by the other results

Determination of amino acids: Samples were collected from April 2007 to September 2007, respectively in the tested grass heading date, flowering period and ripening stage sampling. Then, they were taken indoors, air-dried and crushed.

Instruments and reagents: The following instruments were used: an HF-D ultrasonic cleaner, SIGMA2-16K desktop refrigerated centrifuge, RE-52C rotary evaporator and a Hitachi 835-50 type amino acid automatic analyzer. The following reagents were used: citric acid, sodium

Table 1: Source of material

Variety	Source of materials
Roegneria turczaninovii (Drob.) Nevski	Grassland stations of inner Mongolia Linxi county
Hordeum brevisubulatum (Trin.) Link	
Elymus nutans Griseb.	
Bromus inermis Leyss.	

citrate, sodium chloride, ninhydrin, concentrated hydrochloric acid and sulfosalicylic acid (both analytical grade).

Test conditions: Separation column: 2.6 mm D ×150 mm ion exchange resin 2619. In addition to the ammonia column: 2.6 mm D ×50 mm; column temperature: 57°C; column pressure for P1: 90-100 kg/cm^2 and for P2: 10-15 kg/cm^2; nitrogen pressure: 0.28 kg/cm^2; flow rate of ninhydrin: 0.13 mL min^{-1}; buffer: citric acid, sodium citrate and sodium chloride dubbed the pH 3.4 solution; flow rate of the buffer : 0.3 mL min^{-1}; amino acid standard concentrations: 10-4 mol L^{-1}; detection wavelength: 440 and 570 nm; injection volume: 50 uL; analysis time: 70 min.

Experimental methods and procedures: The amino acid of the whole plant was tested then 50 mg samples were weighed and placed into a test tube, 0.5% of acetic acid and 6 mol L^{-1} hydrochloric acid solution were added, the test tube was evacuated and sealed, then it was placed in a furnace heated to 110 degrees and hydrolysis was done for 24 h. Then, it was removed and cooled to an ambient temperature before opening, filtered at a constant volume to a 50 mL volumetric flask, 1 mL was removed and added to 5 mL of the flat-bottomed flask and a vacuum pumping system was accessed at 40-50 degrees and pumped dry. Then, 0.02 mol L^{-1} hydrochloric acid was diluted and the sample was analyzed using Japan 835-50 automatic amino acid (Wang, 1998).

Evaluation methods

Amino Acid Ratio Coefficient Method (FAO/WHO, 1973): The amino acid ratio coefficient method based on the amino acid balance theory was used with a pattern of WHO/FAO Essential Amino Acid (EAA). The amino acid ratio of the sample EAA (Ratio of Amino Acid, RAA), amino acid ratio coefficient (Ratio Coefficient of Amino Acid, RC) and score of ratio coefficient (Score of RC, SRC) were calculated:

$$RAA = \frac{A}{B}$$

Where:

A = Evaluate the protein EAA content

B = WAO/FAO formula the EAA content

RAA = Often referred to as the Amino Acid Score (AAS)

which means the corresponding EAA and the number of times some EAA content in certain food proteins is equivalent to the mode (Liu *et al*., 2005):

$$RC = \frac{RAA}{RAA\text{ of the mean}}$$

Various amino acid ratio of food is often not the same, the ratio of the amino acids' average can be calculated. If the food protein amino acid composition of the content ratio of the amino acids' pattern is consistent, the RC should be equal to 1of a variety of EAA. If the value is greater than or less than 1, it shows a deviation from the amino acid pattern, RC>1 indicate that the EAA is relative excess, RC<1 indicate that the EAA is the relative lack of the RC least for the first limiting amino acid (First Limiting Amino Acid, FLAA):

$$SRC = 100 - CV \times 100$$

where, CV is the coefficient of variation of the RC; CV = Standard deviation/average (FAO/WHO, 1973)

Essential Amino Acid Index Evaluation Method (Feng and Zhao, 1997): Researchers reference Penaflorida using the equation to calculate the EAAI value:

$$EAAI = \sqrt[n]{\prod_{i=1}^{n} \frac{aai}{AAi}}$$

The equation aai as a percentage of a required amino acids accounting for the total amount of essential amino acids in the raw material of word material; AAi the essential amino acids in protein accounting for a percentage of total essential amino acids as the reference. EAAI reflects the goodness of fit of the essential amino acid composition of the essential amino acids of the protein sources of feed composition and feeding objects. Therefore, you can use the feeding of animal protein for reference to evaluate the nutritional value of the protein source of the animals using EAAI. When n = 6-12, researchers propose a practical evaluation criteria for: EAAI>0.95 for high-quality protein sources, 0.86<EAAI<0.95 for good protein sources, 0.7<EAAI<0.86 for available protein sources, EAAI<0.75 for low-quality protein sources (Feng and Zhao, 1997).

Statistical analysis: This test adopted EXCLE, SPSS11.5 Software and SAS data processing.

RESULTS AND DISCUSSION

Analysis of forage nutrients

Different growth stages of nutrients: Researchers can see from Fig. 1-4 that in *Roegneria turczaninovii*, the crude

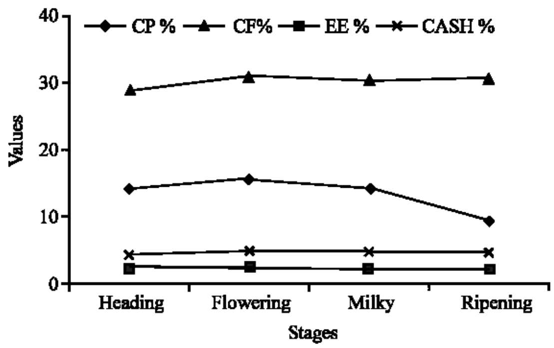

Fig. 1: The dynamic change of nutrition component of *Roegneria turczaninovii*

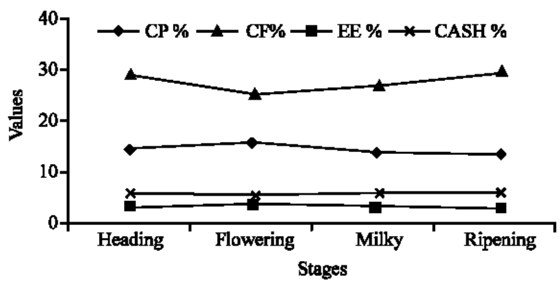

Fig. 2: The dynamic change of nutrition component of *Hordeum brevisubulatum*

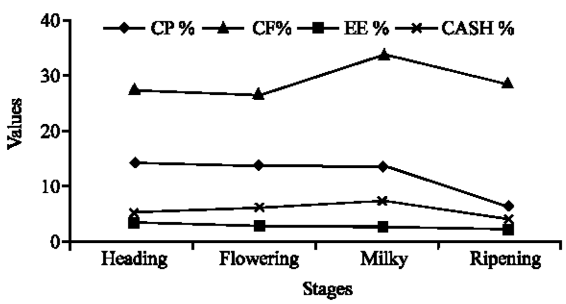

Fig. 3: The dynamic change of nutrition component of *Elymus nutans*

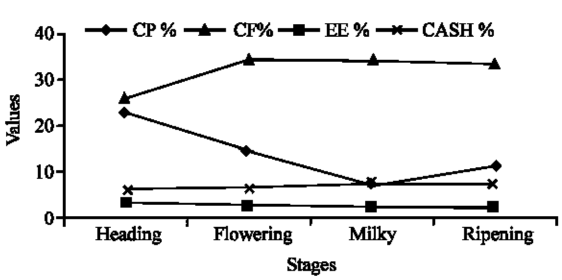

Fig. 4: The dynamic change of nutrition component of *Bromus inermisprotein*

content first increased then decreased with the growth of change having the highest content of 15.90% in the flowering stage. *Elymus nutans* the crude protein content with the change of the growth period of the downward trend having the highest content of 14.53% in heading stage. The crude protein content of *Hordeum brevisubulatum* first increased then decreased with the growth of change having the highest content of 15.27% in the flowering stage. *Bromus inermis* the crude protein content first decreased and then increased during the whole growth period; the heading stage had the highest content of 18.11%. Thus, crude fiber played an important role in the nutritional value of forage evaluation and in the four grass species, the crude fiber content changes were smaller than the crude protein in the whole growth period as grass growth and development of its content was gradually increased. Crude fat content barely changed at all during the entire growth period.

Crude ash represents the mineral content of plants. In Table 2, the crude ash content of *Roegneria turczaninovii* and *Hordeum brevisubulatum* was insignificant in the whole growth period while that of the *Elymus nutans* decreased gradually over time and that of the *Bromus inermis* increased. The average crude ash contents of the different growth stages are as follows: *Bromus inermis* (6.89%) >*Elymus nutans* (5.86%) >*Hordeum brevisubulatum* (5.60%) >*Roegneria turczaninovii* (4.96%). Mineral elements in the pasture are not only in the plant itself and they play an extremely important role in herbivore growth, reproduction and other activities. Table 2 shows that in the four grass species, calcium and phosphorus contents are both relatively stable. For the test period, the average calcium content levels were as follows: *Hordeum brevisubulatum* (0.5%) >*Elymus nutans* (0.43%) >*Bromus inermis* (0.4%) >*Roegneria turczaninovii* (0.22%). The average phosphorus content levels were: *Hordeum brevisubulatum* (0.25%) >*Elymus nutans* (0.18%) >*Bromus inermis* (0.17%) >*Roegneria turczaninovii* (0.14%). The results showed that *Hordeum brevisubulatum* contained moderate levels of calcium and high phosphorus (Enrichment standards for 0.5%<Ca<2.0%, P>0.2%). *Roegneria turczaninovii*, *Elymus nutans* and *Bromus inermis* all contained low levels of calcium and moderate phosphorus (Enrichment standards for Ca<0.5%, 0.1%<P<0.2%) (Chen, 1993) Calcium and phosphorus ratios were: *Hordeum brevisubulatum*, 2:1; *Roegneria turczaninovii*, 2.4:1; *Bromus inermis*, 2.4:1 and *Elymus nutans*, 1.6:1. The tolerable range is 1:1~7:1 while the ideal ratio of calcium ranges from 1:5~2:1 (Hao, 1999).

Grass stems and leaf nutrients: As can be seen from Table 3, there are significant differences in the flowering nutrient content of the grasses: the flowering of crude

Table 2: Change of nutritional components of the four grasses in different growth periods (%)

		Nutritional content						
Variety	Phenophase	CP	EE	CF	CASH	NFE	Ca	P
Roegneria turczaninovii	Heading stage	14.53	2.47	29.41	4.39	49.20	0.07	0.16
	Flowering stage	15.90	2.41	31.26	5.31	45.30	0.29	0.14
	Milky stage	14.25	2.24	30.49	4.84	48.19	0.29	0.17
	Ripening stage	9.46	1.97	30.54	5.48	52.56	0.65	0.11
Hordeum brevisubulatum	Heading stage	13.96	2.52	29.02	6.06	48.44	0.65	0.31
	Flowering stage	15.27	3.04	24.79	5.28	51.62	0.54	0.27
	Milky stage	13.36	2.94	26.50	5.29	51.91	0.47	0.23
	Ripening stage	12.78	2.55	29.23	5.77	49.68	0.34	0.20
Elymus nutans Griseb	Heading stage	14.53	3.48	27.15	5.27	49.57	0.51	0.21
	Flowering stage	13.63	2.71	26.44	6.08	51.14	0.38	0.16
	Milky stage	13.31	2.20	33.40	7.30	43.79	0.29	0.43
	Ripening stage	6.60	1.92	27.98	4.78	58.72	0.40	0.09
Bromus inermis Leyss	Heading stage	18.11	3.39	25.70	5.84	46.96	0.45	0.28
	Flowering stage	14.30	2.52	33.94	6.78	42.46	0.41	0.18
	Milky stage	7.00	1.97	33.34	7.39	50.30	0.36	0.08
	Ripening stage	11.06	2.01	32.88	7.55	46.50	0.36	0.15

Table 3: Nutritional components of the stems and leaves of the four grasses in florescence (%)

		Nutritional content				
Variety	Position	CP	EE	CF	CASH	NFE
Roegneria turczaninovii	Whole plant	12.64	2.41	31.26	5.31	48.56
	Stem	10.87	1.39	36.23	4.48	47.03
	Leaf	21.15	4.68	23.81	6.58	43.78
Hordeum brevisubulatum	Whole plant	15.27	3.04	24.79	5.27	51.62
	Stem	10.65	1.37	34.54	3.86	49.57
	Leaf	20.21	3.67	22.35	6.18	47.56
Elymus nutans Griseb	Whole plant	13.63	2.71	26.44	6.08	51.14
	Stem	8.62	0.91	37.76	4.13	48.59
	Leaf	20.63	4.30	23.51	5.59	45.97
Bromus inermis Leyss	Whole plant	14.30	2.52	33.94	6.78	42.46
	Stem	10.61	0.92	41.98	3.78	33.41
	Leaf	19.91	4.04	26.10	7.55	51.71

protein and crude fat content were the highest in the *Hordeum brevisubulatum*, 15.27 and 2.41%, respectively and the crude fiber content minimum was 24.79%. The crude protein content of grasses leaves was 1-1.5 times higher than the stem protein content; leaf crude fat content was significantly higher than the stem diameter and fat content (3-4 times higher). The crude fiber content of stems was significantly higher than leaf content and the average was about 1.5 times higher. Leaf crude protein content decreased in the following order by grass type: *Elymus nutans* (21.15%) >*Roegneria turczaninovii* (20.63%) >*Hordeum brevisubulatum* (20.21%) >*Bromus inermis* (19.91%). Leaf crude fat content decreased in the following order: *Elymus nutans* (4.68%) >*Roegneria turczaninovii* (4.30%) >*Bromus inermis* (4.04%) >*Hordeum brevisubulatum* (3.67%); *Elymus nutans*, *Hordeum brevisubulatum* and *Roegneria turczaninovii* stem and leaf crude fiber contents were similar while the *Bromus inermis* stems and leaves' crude fiber content was higher. The leaf crude ash content was about 0.25-1 times higher than the stem diameter ash content.

Amino acid content and evaluation

Total amino acid content: Table 4 indicates that amino acids of the four kinds of grasses are relatively complete (in addition to tryptophan that was almost completely destroyed by hydrolysis). Seventeen kinds of amino acid contents were measured and the total amino acid content of different grasses' growth periods had the following ranges: *Elymus nutans* 6.96~10.01%, *Hordeum brevisubulatum* 11.54~14.94%, *Roegneria turczaninovii* 4.64~10.54%, *Bromus inermis* 11.28~15.85%. The *Elymus nutans* had the highest amino acid content in the flowering stage. The three other grasses had the highest amino acid content in the heading stage. All four grass species had the lowest amino acid content during the ripening stage.

Essential amino acid content: The essential amino acids (threonine, valine, leucine, methionine, isoleucine, phenylalanine, lysine, tryptophan) of all four grasses changed throughout the growth period. In the *Roegneria turczaninovii*, they decreased and in the other three, they were at a maximum during the flowering stage and a minimum in the heading stage. Due to the low total amino acid content of the grass forages resulting in a higher percentage of the total amino acid content of essential amino acids. *Elymus nutans* 44.81~49.79%, *Hordeum brevisubulatum* 32.60~54.31%, *Roegneria turczaninovii* 48.34~58.27%, *Bromus inermis* 37.03~64.66% (Table 4). *Roegneria turczaninovii* and *Elymus nutans* at the same time >40% of the WHO/FAO standard requirements, *Hordeum brevisubulatum* and *Bromus inermis* reached >40% of the WHO/FAO standard requirements only during the flowering and ripening stages (Guo, 2002; Shen, 1980).

Table 4: Mass fraction of amino acids in the different growth periods (%)

Amino acid namename	*Roegneria turczaninovii* (stage) Heading	Flowering	Ripening	*Hordeum brevisubulatum* (stage) Heading	Flowering	Ripening	*Elymus nutans* (stage) Heading	Flowering	Ripening	*Bromus inermis* (stage) Heading	Flowering	Ripening
LYS*	0.77	0.93	0.60	0.69	0.74	0.43	0.48	0.45	0.30	0.79	0.53	0.53
VAL*	0.76	0.98	0.54	0.96	0.93	0.72	0.68	0.70	0.36	1.16	0.82	0.58
LEU*	0.97	1.24	0.82	1.02	1.23	0.89	0.92	0.96	0.45	1.24	0.97	0.77
MET*	0.27	0.35	0.20	0.19	0.26	0.22	0.22	0.21	0.11	0.30	0.26	0.23
ILE*	0.65	0.76	0.58	0.61	0.75	0.60	0.60	0.60	0.28	0.83	0.67	0.53
PHE*	0.68	0.81	0.46	0.77	2.13	3.02	2.88	1.89	0.56	0.90	3.92	4.34
THR*	0.41	0.54	0.23	0.63	0.63	0.40	0.36	0.38	0.19	0.65	0.45	0.31
HIS	0.21	0.24	0.15	0.26	0.29	0.19	0.18	0.23	0.10	0.54	0.23	0.14
ARG	0.70	0.67	0.33	0.81	0.65	0.36	0.43	0.52	0.18	0.69	0.50	0.25
PRO	0.58	0.75	0.59	1.18	1.03	0.59	0.71	0.97	0.47	1.47	0.74	0.55
TYR	0.18	0.25	0.03	0.40	0.23	0.13	0.11	0.13	0.00	0.25	0.15	0.04
ASP	1.04	1.36	0.49	2.53	1.27	1.16	0.87	1.10	0.40	2.87	1.40	0.98
SER	0.41	0.53	0.26	0.73	0.59	0.45	0.33	0.37	0.19	0.64	0.43	0.30
GLU	1.11	1.47	0.92	2.39	1.57	1.32	0.71	0.88	0.50	1.78	1.51	0.84
GLY	0.47	0.63	0.22	0.69	0.69	0.42	0.37	0.35	0.19	0.70	0.48	0.32
ALA	0.63	0.79	0.36	0.89	0.88	0.52	0.58	0.58	0.27	0.90	0.58	0.44
CYS	0.19	0.21	0.13	0.19	0.13	0.12	0.13	0.12	0.08	0.16	0.14	0.14
T	10.01	12.78	6.96	14.94	14.00	11.54	10.54	10.44	4.61	15.85	13.77	11.28
E	4.50	5.61	3.45	4.87	6.67	6.27	6.14	5.20	2.24	5.87	7.62	7.29
N	5.52	7.16	3.51	10.07	7.33	5.27	4.40	5.24	2.37	9.98	6.16	3.99
F	2.16	2.83	1.41	4.92	2.84	2.48	1.57	1.98	0.89	4.65	2.90	1.82
E/T	44.92	44.81	49.79	32.60	47.17	54.31	58.27	49.66	48.34	37.03	55.31	64.66
F/T	14.94	18.93	19.30	32.93	20.27	21.45	14.94	18.93	19.30	29.34	21.08	16.10

*Essential Amino Acid; T: The content of Amino Acid; N: Nonessential Amino Acid; F: Flavor Amino Acid = ASP+GLU

Table 5: Essential amino acids of the grasses (%)

Variety	Position	Essential amino acids Lys	Val	Leu	Met+Cys	Ile	Phe+Tyr	Thr
Roegneria turczaninovii	Heading stage	7.66	7.56	9.65	4.64	6.51	8.56	4.05
	Flowering stage	7.29	7.64	9.70	4.42	5.94	8.32	4.24
	Ripening stage	8.66	7.81	11.82	4.75	8.40	7.57	3.37
Hordeum brevisubulatum	Heading stage	4.62	6.43	6.83	2.54	4.08	7.83	4.22
	Flowering stage	5.29	6.67	8.77	2.79	5.38	16.86	4.47
	Ripening stage	3.70	6.20	7.71	2.97	5.18	27.34	3.44
Elymus nutans Griseb	Heading stage	4.53	6.47	8.73	3.38	5.66	28.34	3.38
	Flowering stage	4.35	6.73	9.18	3.19	5.79	19.34	3.62
	Ripening stage	6.41	7.70	9.75	4.13	6.10	12.24	4.19
Bromus inermis Leyss	Heading stage	4.97	7.31	7.81	2.92	5.23	7.27	4.08
	Flowering stage	3.85	5.92	7.03	2.96	4.86	29.50	3.29
	Ripening stage	4.68	5.14	6.84	3.27	4.69	38.80	2.79
WHO/FAO mode spectrum		5.50	5.00	7.00	3.50	4.00	6.00	4.00

Non-essential amino acids: Non-essential amino acids of forage proteins in the forage component of grass closely relate to the nature of the protein. Looking at the results from the 10 kinds of non-essential amino acids in proteins (Table 4), all four grasses had the highest concentrations of aspartate and glutamate and these two amino acids had varying concentrations throughout the growth periods. *Roegneria turczaninovii* and *Elymus nutans* had the highest content during the flowering period and the lowest content during the ripening stage, ranging from 1.41-2.83% and 0.89-1.98%, respectively; *Hordeum brevisubulatum* and *Bromus inermis* had the highest content in the heading stage and the lowest content in the ripening stage, 2.48-4.92% and 1.82- 4.65%, respectively.

The above analysis shows that in *Roegneria turczaninovii*, the total amino acid content and the content of essential amino acids and non-essential amino acids are the highest in the flowering period; *Hordeum brevisubulatum* and *Bromus inermis* had the highest total amino acid and non-essential amino acid content in the heading stage and essential amino acids in the flowering stage; *Elymus nutans* had the highest total amino acid content and essential amino acids in the heading stage and non-essential amino acid contents were the highest in the flowering stage.

Use of the amino acid ratio coefficient to compare the amino acid contents: Forage grass amino acid composition differs but the pros and cons of its nutritional value depend on the type, quantity and proportion of essential amino acids contained (Essential Amino Acid, EAA). When certain essential amino acids are lacking in the protein, other amino acids cannot be take full advantage of thereby reducing the total protein digestibility. Table 5 shows that for *Roegneria turczaninovii* and *Hordeum brevisubulatum* the mass fraction of threonine is below the mode spectrum in the ripening stage, the *Bromus inermis* is only higher than the mode spectrum in the heading stage and *Roegneria turczaninovii* methionine + cysteine and lysine have mass fractions higher than the mode spectrum. Alternatively, *Hordeum brevisubulatum* and *Bromus inermis* were lower than the mode spectrum and the *Elymus nutans* was only higher than the mode spectrum in the ripening stage. In addition, the essential amino acid quality scores of the four grass species were higher at different growth stages than the mode spectrum of the standard. It is suggested that *Roegneria turczaninovii* with an essential amino acid balance at the heading and flowering stages has has a high nutritional value. The essential amino acid balance of *Hordeum brevisubulatum* during the flowering stage, *Elymus nutans* during the ripening stage and *Bromus inermis* at the heading stage indicate that they are high in nutritional value.

Accordingly, WHO/FAO developed the essential amino acids ratio coefficient method to evaluate the nutritional value of protein in 1973. By The WHO/FAO essential amino acid pattern, calculated the sample of the EAA amino acid ratio (Ratio of Amino Acid, RAA), amino acid ratio coefficient (Ratio Coefficient of Amino Acid, RC) and the value of coefficient points (Score of the RC, SRC) that is based on the amino acid balance of theory.

As shown in Table 6, over the different stages of growth, *Roegneria turczaninovii* had a decreasing SRC value and protein nutritional value. In the heading stage, the SRC value reached 83.83%, there was a relatively high nutritional value and essential amino acids were balanced. The first limiting amino acid varied in different growth stages: both heading and ripening stages had threonine, while the flowering period had phenylalanine + tyrosine.

Table 7 shows the growth period changes for *Elymus nutans*: the SRC value gradually became larger and the protein nutritional value gradually increased. In the ripening stage SRC was 76.31%, there was a relatively high nutritional value and essential amino acids were balanced. The first limiting amino acid varied in different growth stages: both heading and flowering stages had lysine while the ripening period had threonine.

From Table 8, *Hordeum brevisubulatum* had changes in different growth periods: the SRC value became smaller and the nutritional value of protein decreased. The SCR value in the heading stage was 79.30% had a relatively high nutritional value and essential amino acids were balanced. The first limiting amino acid in different growth

Table 6: Analysis results of RAA, RC and SRC of *Roegneria turczaninovii*

	Heading stage			Flowering stage			Ripening stage		
EAA	RAA	RC	SRC	RAA	RC	SRC	RAA	RC	SRC
Lys	1.557	1.141	-	1.513	1.140	-	1.285	0.905	-
Val	1.512	1.109	-	1.528	1.151	-	1.562	1.100	-
Leu	1.379	1.011	-	1.386	1.044	-	1.689	1.190	-
Met+Cys	1.324	0.971	83.38	1.263	0.951	51.22	1.356	0.955	8.6
Ile	1.628	1.194	-	1.486	1.119	-	2.100	1.479	-
Phe+Tyr	1.134	0.831	-	1.056	0.796	-	1.102	0.777	-
Thr	1.011	0.742	-	1.061	0.799	-	0.842	0.593	-

Table 7: Analysis results of RAA, RC and SRC of *Elymus nutans*

	Heading stage			Flowering stage			Ripening stage		
EAA	RAA	RC	SRC	RAA	RC	SRC	RAA	RC	SRC
Lys	0.823	0.508	-	0.790	0.557	-	1.165	0.824	-
Val	1.295	0.799	-	1.346	0.948	-	1.541	1.091	-
Leu	1.247	0.770	-	1.311	0.924	-	1.393	0.986	-
Met+Cys	0.967	0.597	13.05	0.912	0.642	41.15	1.179	0.835	76.31
Ile	1.416	0.874	-	1.448	1.020	-	1.525	1.079	-
Phe+Tyr	4.724	2.916	-	3.223	2.270	-	2.040	1.444	-
Thr	0.845	0.522	-	0.906	0.638	-	1.047	0.741	-

Table 8: Analysis results of RAA, RC and SRC of *Hordeum brevisubulatum*

	Heading stage			Flowering stage			Ripening stage		
EAA	RAA	RC	SRC	RAA	RC	SRC	RAA	RC	SRC
Lys	0.840	0.815	-	0.961	0.702	-	0.674	0.446	-
Val	1.285	1.248	-	1.334	0.974	-	1.241	0.822	-
Leu	0.975	0.947	-	1.253	0.915	-	1.101	0.729	-
Met+Cys	0.727	0.706	79.30	0.796	0.581	51.38	0.848	0.562	9.81
Ile	1.021	0.991	-	1.344	0.981	-	1.294	0.857	-
Phe+Tyr	1.305	1.267	-	2.815	2.055	-	4.557	3.018	-
Thr	1.054	1.024	-	1.119	0.816	-	0.861	0.570	-

Table 9: Analysis results of RAA, RC and SRC of *Bromus inermis*

	Heading stage			Flowering stage			Ripening stage		
EAA	RAA	RC	SRC	RAA	RC	SRC	RAA	RC	SRC
Lys	0.904	0.806	-	0.699	0.458	-	0.851	0.492	-
Val	1.462	1.303	-	1.185	0.776	-	1.028	0.594	-
Leu	1.116	0.995	-	1.004	0.657	-	0.977	0.564	-
Met+Cys	0.833	0.742	37	0.846	0.554	1.32	0.933	0.539	-20.79
Ile	1.308	1.166	-	1.216	0.796	-	1.173	0.677	-
Phe+Tyr	1.212	1.080	-	4.917	3.220	-	6.466	3.733	-
Thr	1.019	0.908	-	0.821	0.538	-	0.697	0.403	-

Table 10: EAAI value for proteins of the four grasses

	Stage			
Variety	Heading	Flowering	Ripening	Conclusion
Roegneria turczaninovii	1.326	1.328	1.326	Quality
Hordeum brevisubulatum	1.324	1.310	1.300	Quality
Elymus nutans	1.307	1.313	1.319	Quality
Bromus inermis	1.328	1.306	1.299	Quality

stages varied: both heading and flowering stages had methionine + cysteine and in the ripening period of the first limiting amino acid was lysine.

As can be seen in Table 9, the SRC value of *Bromus inermis* was <50% and it was negative even in the ripening stage. This may be due to the excessive phenylalanine content seriously affecting the protein's nutritional value. The first limiting amino acid varied in different growth stages: the heading stage had a first limiting amino acid of methionine + cysteine; the flowering stage had lysine and ripening stage had threonine.

The use of the Essential Amino Acids Index (EAAI) to evaluate the protein source: The study done by Cowey and Tacon with Wilson and Poe shows that kinds of amino acid need by livestock is related to the amino acid composition of itself (Wilson and Cowey, 1985). Researchers reference The Penaflorida adopted formula EAAI value.

Using bovine protein (Fang, 2007) as a reference protein in the analysis of four gramineous EAAI values, the nutritional value of cows could be judged. Four kinds of grasses' EAAI values were >0.95 (Table 10), making them all high quality forage.

In recent years, many researchers have used the amino acid content and balanced level to evaluate the nutritional value of food and feed. Currently, two methods, The Amino Acid Ratio Coefficient Method and The Amino Acid Index Evaluation Method are widely used to evaluate nutritional value. At present, the study that evaluate the forage nutrition by the amino acid content is less. Modern nutritional studies suggest that not only amino acid deficiencies affect the nutritional value of protein but also amino acid excess limits the nutritional value of protein. Therefore, the Amino Acid Balance Theory has been proposed. If the EAA composition of the proportion of food protein is consistent with the EAA mode, it shows CV = 0, SRC = 100 and if the CV larger, SRC change smaller so protein nutritional value of the worse. Comparatively speaking, SRC is closer to 100 its nutritional value is relatively high.

This study uses the amino acid ratio coefficient method to compare the amino acid content of four grass species at different growth stages. Because this method is the use of FAO and WHO jointly recommended by the EAA tentative scoring mode to do the reference so suitable for amino acid of food evaluation because the use of the grass direcyly is difficult for human, therefore to further understanding the proteins use value of four grass forage in animal husbangdry using the EAAI evaluation method study. Currently, a few reach with the amino acid content evaluate nutritional value of the grass, there not have uniform and standard evaluation method, it had to reference of food and feed evaluation method. Because of certain constraints, this test does not take into can make use of the amino acid content. Therefore, it is subject to further research and discussion.

CONCLUSION

Nutrition levels of *Hordeum brevisubulatum* and *Roegneria turczaninovii* were higher in the heading stage than in other periods. The essential amino acids of *Hordeum brevisubulatum* and *Roegneria turczaninovii* were at equilibrium in the heading stage. The nutrition level of *Elymus nutans* was higher in the ripening stage than in other periods. The essential amino acids of *Elymus nutans* were at equilibrium.

NOMENCLATURE

THR = Threonine
VAL = Valine (Val)
LEU = Leucine
MET = Methionine
ISO = Isoleucine
PHE = Phenylalanine
LYS = Lysine
HIS = Histidine

ARG = Arginine
PRA = Praline
TYR = Tyrosine
ASP = Asparaginic acid
SER = Serine
GLU = Glutamic acid
GLY = Glycine
LAC = Lactamine
CYS = Cysteine
EAA = Essential Amino Acid

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