COMPARATIVE STUDY OF THE AMINOACID'S TRUE DIGESTIBILITY OF DIFFERENT CLOVER (TRIFOLIUM) VARIETIES IN EXPERIMENTS WITH GANDERS

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ABSTRACT

The true digestibility of amino acids of 5 groups of different Trifolium varieties and hybrids was established by using the balance method adapted for geese.
Digestibility of the most important amino acids Lysine varies from 70.40 (for tetraplod red clover) to 81.78 (for medium leafy white clover varieties); for Methionine from 60.54 (for *Trifolium repens f. giganteum*) to 89.42 (diploid red clover); Cystine from 71.03 (for medium leafy white clover) to 87.72 (for *Trifolium repens f. giganteum*). Amino acids digestibility well correlates with the crude protein content, crude fiber and the total amount of amino acids in the dry matter of the forages and can be predicted with linear regression equations.

Key words: Trifolium, amino acids, digestibility, and regression equations

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DETAILED ABSTRACT:

Red and white clovers are important forages for cultivation in mountain areas as a protein source in ruminant and nonruminant nutrition.
The content and the true digestibility of amino acids of 5 groups of different Trifolium varieties and hybrids were established by using the balance method adapted for geese.
Crude protein and amino acids vary in the biomass of clovers depending on the plant genotype and morphological structure.
For the group of white clover varieties the small leafy varieties (*Trifolium repens f. silvestre*) have higher crude protein and total aminoacids concentrations in the biomass. *Trifolium repens f. hollandicum* had the lowest total aminoacids content and *Trifolium repens f. giganteum* had the lowest protein content and value.
Red clover (*Trifolium pratense* L) had significantly lower concentration of crude protein and amino acids compared to those of white clover varieties. Tetraploid varieties of red clover have higher protein and amino acid content. The advantage of tetraploid varieties to diploid one is concerning to the aminoacids into the biomass. Though tetraploid red clover varieties have higher protein and amino acid concentration, diploid red clover varieties have higher biological value due to the highest concentration of essential amino acids into the biomass of diploid varieties.
Digestibility of amino acids of the evaluated clover varieties is too high as a whole for the forage comprising high fiber content (91.41 % for Proline to 47.12 % for Glycine). The most important amino acids also demonstrate high true digestibility with ganders.
The differences in the amino acid content and true digestibility allow assuming that the average approximate coefficients for protein value for clover and the other rough forages could not be used. An exact estimation must be performed due to the specific features of the varieties.
The comparison among the true digestibility of amino acids of clover and Lucerne grass powder shows clearly that clover is almost equal to Lucerne concerning the available amino acid supply.

INTRODUCTION

Clover (Trifolium) is good forage not only for ruminants. It is well consumed also by geese as fresh forage or as flour. The interest of incorporation of clovers in the nutrition chain of animals is based on their high protein value (for white clover exceeding all leguminous plants of the group), the good feeding value and production efficiency (Pavlov, 1989). The feeding value of the clover biomass for poultry is established and mentioned in Bulgarian (Alexsiev et al., 1984) and other well-known foreign manuals (NRC 1994).

Establishing the productive potential of the nutritive substances of different clover types and varieties is of theoretical and practical importance as well.

The effect of utilization of clover in poultry feeding depends not only on the energy value but also on the availability and the true digestibility of amino acids in the biomass.

The aim of the study was to establish the content and true digestibility of amino acids of different clover types and varieties in experiments with geese.

MATERIAL AND METHODS

Amino acid content was established in the biomass of 5 groups varieties of clover from the new selection of Fodder Research Institute, Pleven and Institute of Upland Animal Husbandry and Agriculture, Trojan.

Red clover (*Trifolium pratese* L. diploid (2n) varieties – K9, KS1, Br1 and tetraploid (4n) varieties - Barfiola, Tedi, Temara; white clover *Trifolium repens* L. f. *silvestre* (small leafy)- S 184, AberCrest, AberDale, Gwenda, *Trifolium repens* L. f. *hollandicum* (medium leafy)- AberHerald, AberDai, AberVantage, AberCrown, Menna, Donna,; *Trifolium repens* L. f. *giganteum* - (large leafy) - Alice, Olwen, AberBonus, Debiut, KS 33, K 33 were cultivated in exact experiments in three replication during three year period.

The soils are Planosols, $_{P}H_{(KCL)}$ – 4.4, altitude 384m asl. Mean precipitation for 1998-2000 was 652,2 mm, average annual temperature 10.2 ^{0}C.

The biomass from the different clover varieties was collected at a "grazing stage" and dried at 65^{o} C to achieve the humidity of 12 – 15 %. The dried biomass was ground to small (< 2 mm) pieces and used for the balance experiments.

The crude protein content was determined by Weende method (AOAC 1980). Amino acid content was established by automatic aminoalyzer AAA-881 after HCl hydrolysis.

Balance experiments with ganders were performed by the methods of Sibbald (1986) adapted for waterfowl (Adeola et al., 1997) and for geese (Penkov, 1997). Six tube fed animals for each variant and 6 feed deprived analogues were used according to the scheme:

- first week – 2 tube fed ganders from each forage + 2 deprived
- second week - 2 tube fed ganders from each forage + 2 deprived
- third week - 2 tube fed ganders from each forage + 2 deprived

The true digestibility of amino acids was established as follows:

[(AA in the forage- (AA excreted from fed ganders - AA excreted from deprived ganders)]AA in the forage.

RESULTS

Concentration of crude protein and amino acids varied significantly in the biomass of clovers depending on the plant genotype. The content of crude protein in the biomass of white clover *Trifoilum repens* L. varieties varied (from 229.2 to 248.6 g kg^{-1} DM) and total amino acid (from 203.1 to 226.7 g kg^{-1} DM), (Table 1, at the end of artivcle). *Trifolium repens f. silvestre* 'small leafy' varieties exceeded medium and broad leafy varieties in crude protein and amino acid concentration. The lowest content of crude protein was measured in the biomass of medium leafy varieties white clover (229,2 g. kg^{-1}). Broad leafy white clover had the lowest content of total amino acids (203.1 g. kg^{-1}).

The crude protein content in the biomass of red clover (*Trifolium pratense* L.) varied from 143.6 to 157.1 g kg^{-1} DM and the total amino acids from 124.7 to 141.2 g kg^{-1} DM. The biomass of tetraploid red clover varieties contained higher protein concentration compared to those of diploid red clover varieties. The higher difference among tetraploid and diploid varieties was observed in the total amino acids content (141.2 and 124.7 g.kg^{-1}, respectively). The higher total amino acids content as a share of crude protein in the biomass of tetraploid red clover varieties is an advantage in comparison with diploid red clover forms.

Table 1. Amino acid content in different clover types and varieties in dry matter (DM), g kg^{-1} and in crude protein (CP), g 16 g N.

Amino acid	*Trifolium pratens,e Diploid, 2n*		*Trifolium pratense, Tetraploid, 4n*		*Trifolium repens f. hollandicum (medium leafy)*		*Trifolium repens f. giganteum (broad leafy)*		*Trifolium repens f. silvestre (small leafy)*	
	In DM	In CP	In DM	In CP	In DM	In CP	In DM	In CP	In DM	In CP
Lysine	8.5	6.82	8.5	6.02	13.0	6.35	12.1	5.96	13.9	6.13
Histidine	3.3	2.65	3.1	2.2	4.6	2.25	4.7	2.31	5.1	2.25
Arginine	6.0	4.81	6.3	4.46	10.0	4.89	10.2	5.02	12.2	5.38
Aspartic acid[3]	16.5	13.23	21.6	15.3	38.4	18.77	39.5	19.45	41.3	18.22
Threonine	6.2	4.97	6.5	4.6	9.7	4.74	9.8	4.83	10.8	4.76
Serine	6.8	5.45	7.2	5.1	11.0	5.38	11.0	5.42	12.3	5.43
Glutamic acid	13.9	11.15	14.0	9.92	21.1	10.31	20.3	10.0	23.6	10.41
Proline	17.4	13.95	25.4	17.99	22.2	10.85	24.4	12.01	21.1	9.31
Glycine	6.4	5.13	6.5	4.6	10.0	4.89	9.5	4.68	11.0	4.85
Alanine	7.3	5.85	7.9	5.59	11.7	5.72	10.9	5.37	13.4	5.91
Cystine	0.6	0.48	0.7	0.5	0.7	0.34	0.8	0.39	0.9	0.4
Valine	6.4	5.13	7.2	5.1	11.5	5.62	10.8	5.32	13.0	5.73
Methionine	0.6	0.48	0.5	0.35	0.9	0.44	0.7	0.34	1.0	0.44
Isoleucine	4.6	3.69	4.9	3.47	7.7	3.76	7.2	3.55	9.0	3.97
Leucine	9.7	7.78	9.8	6.94	14.7	7.18	14.0	6.89	17.2	7.59
Tyrosine	4.3	3.45	4.9	3.47	6.7	3.27	6.9	3.40	9.0	3.97
Phenylalanine	6.2	4.97	6.2	4.39	10.7	5.23	10.3	5.07	11.9	5.25
Total AA content, g kg-1 DM	124.7		141.2	-	204.6	-	203.1	-	226.7	-
Crude protein content, g kg-1 DM	143.6		157.1	-	229.2	-	234.9	-	248.6	-
EAAI – Essential Amino Acid Index		0.69		0.55		0.60		0.57		0.62

Table 2. True digestibility of amino acids, % (n=6):

Amino acids	*Trifolium pratense, Diploid, 2n*			*Trifolium pratense, Tetraploid, 4n*			*Trifolium repens f. hollandicum (medium leafy)*			*Trifolium repens f. giganteum (broad leafy)*			*Trifolium repens f. silvestre (small leafy)*			Average for all groups		
	x	Sx	S%	x	Sx	S%	x	Sx	S%	x	Sx	S%	x	Sx	S%	X	Sx	S%
Lysine	70.97	0.81	5.71	70.40	1.10	7.78	81.78	0.49	3.0	79.26	1.08	6.81	79.90	0.56	3.64	76.46	0.81	5.39
Histidine	69.52	0.88	6.34	70.92	1.17	8.25	84.19	0.55	3.26	83.34	0.71	4.24	75.56	0.62	4.11	76.71	0.77	5.24
Arginine	60.44	1.20	9.96	56.40	1.60	14.20	76.35	0.68	4.42	65.13	1.09	8.38	76.86	0.63	4.12	67.04	1.04	8.22
Aspartic acid	68.22	0.82	6.0	77.70	0.82	5.25	88.66	0.31	1.76	87.47	0.44	2.48	88.10	0.33	1.85	82.03	0.54	3.47
Threonine	68.02	1.49	12.22	68.83	1.57	11.42	76.24	0.82	5.36	71.61	1.12	7.81	75.44	0.81	5.36	72.03	1.16	8.43
Serine	64.53	1.25	9.71	62.99	1.56	12.37	72.54	0.74	4.91	71.96	1.05	7.26	76.18	0.73	4.79	69.64	1.07	7.81
Glutamic acid	60.59	1.11	9.16	63.72	1.42	11.16	74.17	0.69	4.63	69.96	1.03	7.34	75.18	0.68	4.53	68.72	0.99	7.36
Proline	90.58	0.76	4.17	91.74	0.62	3.40	91.25	0.57	3.14	89.66	0.64	3.55	88.81	0.64	3.58	91.41	0.65	3.57
Glycine	46.85	1.46	15.60	36.50	2.93	29.11	51.08	1.0	9.81	46.49	1.59	17.05	54.69	1.02	9.34	47.12	1.60	16.18
Alanine	57.50	1.18	10.24	61.03	1.45	11.90	65.33	0.76	5.81	62.36	1.18	9.45	71.82	0.71	4.92	63.61	1.06	8.46
Cystine	81.30	1.88	11.56	84.60	1.74	10.29	71.03	1.61	11.03	87.72	1.47	8.49	68.95	1.32	9.59	78.72	1.60	10.19
Valine	61.79	1.21	9.80	63.66	1.44	11.32	70.04	0.71	5.03	71.82	0.99	6.90	76.18	0.65	4.28	68.70	1.0	7.47
Methionine	89.42	2.24	12.52	87.02	3.02	17.38	83.37	1.43	8.55	60.54	2.36	19.47	86.58	1.30	7.48	81.39	2.07	13.18
Isoleucine	62.16	1.13	9.12	72.06	1.29	8.92	73.90	0.80	5.44	70.44	1.01	7.15	75.03	2.92	19.48	70.72	1.43	10.02
Leucine	71.44	0.93	6.47	69.90	1.22	8.71	77.27	0.72	4.63	71.84	2.95	20.50	78.70	0.58	3.67	73.83	1.28	8.70
Tyrosine	70.76	1.11	7.84	73.76	1.24	8.38	72.50	0.74	5.08	73.14	0.97	6.63	74.16	0.65	4.36	72.86	0.94	6.46
Phenylalanine	71.60	0.91	6.38	74.21	1.15	7.74	85.88	0.49	2.85	80.21	0.73	4.55	80.70	0.52	3.25	78.52	0.76	4.95
Average	68.57			69.73			76.21			73.11			76.64			72.91		

Though diploid red clover variety had the lowest content of amino acids and protein, it exceeded the other red and white clover varieties by the highest biological value of amino acids. It had the highest Essential Amino Acid Index (EAAI) - 0.69. This was due to the higher concentration of Lysine, Methionine and Leucine in the crude protein. Those amino acids are the most important essential constituents determining the biological value of the consumed protein. The biological value of tetraploid red clover and broad leafy white clover *Trifolium repens f. giganteum* was lower (0.55 and 0.57 respectively) due to the lower concentration of Lysine and Methionine in the protein.

The true digestibility of amino acids of the evaluated clover varieties was too high as a whole for the forage comprising high fiber content. The true digestibility varied from 91.41 % for Proline to 47.12 % for Glycine (Table 2, at the end of article). The most important amino acids also demonstrated high true digestibility for ganders – Lysine from 81.78 % for medium leafy varieties to 70.40 % for tetraploid red clover, Methionine from 89.42 diploid red clover to 60.54 % for broad leafy white clover varieties, Cystine from 87.72 for broad leafy varieties to 68.95 % for small leafy varieties. Digestibility of other amino acids was also high for the same type of experiments with non-ruminant animals.

DISCUSSION

When comparing the content of amino acids in the different types of clovers (Trifolium), the priority of white clover is evident. The biomass of white clover contained 2 to 4 g kg^{-1} of Lysine dry matter more than red clover. The difference was 30 – 45 %.

The higher content of amino acids in the biomass of white clover makes it a suitable and reliable source for supplying the poultry with cheap protein of high biological value.

The average true digestibility of 72.91 % was high enough for poultry. As a whole white clover varieties had higher digestibility of amino acids (from 73.11 to 76.64 %) compared to the digestibility of amino acids of the biomass of red clover (from 68.57 to 69.73). This tendency was not valid for Methionine where the higher digestibility was obtained in red clover varieties (87.02 – 89.42 %) and lower digestibility for white clover (60.54 – 86.58 %). The lowest value of Methionine digestibility was obtained for *Trifolium repens f. gigantheum*. The latest observation may be due to the specific feature of the variety.

Digestibility of amino acids correlated well with the total amount of crude protein, crude fiber and total amount of amino acids. This allowed developing regression equations for prediction of amino acids digestibility on the basis of composition as an independent variable. Coefficient of determination R^2 – 0.916 was higher when total content of amino acids was used as an independent variable (Table 3). Crude protein content was also a good independent variable for the estimation of amino acids digestibility. The accuracy of prediction of amino acids digestibility was a little lower when crude fiber was used as an independent variable but the association relation was good and it can be successfully used for estimation of amino acids digestibility.

Table 3. Regression equations for prediction of Amino acids digestibility (Y, %)

Equation	R^2	SEE*	P<
Y= 58.50669 + 0.07078 CP*	0.877	1.48	0.018
Y = 83.66004 – 0.05078 CF*	0.763	2.06	0.05
Y = 58.613 + 0.07908 AA*	0.916	1.22	0.010

*CP – crude protein, g kg^{-1} DM; CF – crude , g kg^{-1} DM; AA – amino acids, g kg^{-1} DM ; R^2 – coefficient of determination; SEE – standard error of estimation

The differences in amino acids content and true digestibility allow to assume that for clover and the other rough forages average approximate coefficients for protein value could not be used and exact estimation must be performed due to the specific features of the varieties.

Comparison among the true digestibility of amino acids of clover and Lucerne grass powder (Penkov et al., 1999) obviously demonstrated that clover was

almost equal to Lucerne concerning the available amino acids supply.

CONCLUSION

The average true digestibility of amino acids of 5 different groups (diploid and tetraploid) red clover; *Trifolium repens f. silvestre; Trifolium repens f. giganteum* and *Trifolium repens f. hollandicum* varied from 47.12 % (Glycine) to 91.41 % (Proline).
In the group of white clover small leafy (*Trifolium repens f. silvestre*) varieties had higher protein, amino acids concentration and higher biological value of the protein. The red clover tetraploid varieties exceeded diploid varieties in protein and amino acids concentration but had lower biological value of the protein.
Amino acids digestibility well correlated with total content of amino acids, crude protein and crude fiber. These parameters can be used for approximate estimation of amino acids digestibility and retention in poultry nutrition.
White clover is more suitable and could be recommended for utilization as fresh or dried forage in poultry nutrition.

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