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THE EFFECT OF SELECTED CEREALS CONTAINED IN FEED RATION ON THE AMINO ACID COMPOSITION OF COWS' MILK

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Abstract

ŠÍPALOVÁ, M., HANUŠ, O., BUŇKA, F., POZDÍŠEK, J., MRKVIČKA, V., KRÁČMAR, S.: *The effect of selected cereals contained in feed ration on the amino acid composition of cows' milk.* Acta univ. agric. et silvic. Mendel. Brun., 2010, LVIII, No. 5, pp. 369–376

The aim of this study was to evaluate the possible effects of maize replacement in feeding rations on the amino acid content in cows´ milk. Cows were fed total mix ration based on the maize, clover silage and hay. There was a difference in the concentrate of the feeding ration. The first group (fed maize) was the control group, another two groups were experimental, one fed wheat and second fed triticale. During six weeks, totally 26 milk samples were taken from dairy cows of Czech Pied breed. Feed groups were preferably balanced in terms of milk yield, stage and number of lactations. The samples of feedstuffs as well as milk were modified for the analysis using acidic and oxidative hydrolyses. The analysis of amino acids content and composition of the sample hydrolysates was performed chromatographically by an AAA 400 analyzer, using Na-citrate buffers and ninhydrin detection. Total nitrogen content was determined according to Kjehldahl and the crude protein of the samples was determined by conversion from the nitrogen content multiplied by appropriate factor. The high content of crude protein in wheat did not influenced composition of milk from dairy cows fed this type of feedstuff. With respect to resulting amino acid content and composition of milk samples, none of the tested grains can be recommended as a full-value maize replacement. Each feedstuff is an abundant source of several and typical amino acids in milk. However, triticale (cultivar Kitaro) seems to be acceptable replacement of maize owing to better crude protein efficiency, composition and health indicators of milk quality.

dairy cow, maize, wheat, triticale, amino acids

Number of studies dealing with influence of several factors on milk yield and milk composition has been published (Masoero *et al.*, 1998; Kuhn *et al.*, 2006; Strusińska *et al.*, 2006). Milk production and milk composition are affected by cow diet (e.g. Sinclair *et al.*, 2003; Leiber *et al.*, 2005; Sánchez and Ledin, 2006) and stage of the lactation (Kuhn *et al.*, 2006), as well as cow genotype. Milk composition, and milk protein composition especially, is very important for subsequent milk processing (Leiber *et al.*, 2005).

Replacing fermentable carbohydrates with fat in the diet of high-producing dairy cows may limit synthesis of microbial protein in rumen and will decrease the flow of microbial protein to the small intestine (Palmquist *et al.*, 1993). In some cases, supplementation with fat may limit the supply of amino acids to the mammary gland and reduce milk protein content (DePeters and Cant, 1992). Two independent studies (Wu and Huber, 1994; Dhiman *et al.*, 2001) confirm that an increase in dietary fat could decrease milk protein content.

As mentioned above, milk yield is affected by feeding. Effect of different amount of roughage neutral detergent fiber (NDF) in feeding ration on milk yield was evaluated by Adin *et al.*, (2008) Lower content of roughage NDF (12%) with soy hulls addition increased milk yield from 32.2 to 34.6 kg and stimulated also milk protein production. According to Sinclair *et al.*, (2003), fermented whole crop wheat can provide a comparable milk production to good quality grass silage and the harvesting at a later stage of maturity had negligible effect on milk production, although milk protein levels tended to be higher in cows fed feedstuff containing higher starch content.

Clover silage studies and studies with organically managed cows have shown that grain based supplementation increase milk protein content (Sehested *et al.*, 2003; Cohen *et al.*, 2006; Steinshamn, 2008).

The current study is related to research of Pozdíšek and Vaculová (2008), who investigated wheat (*Triticum aestivum* L.) quality utilized for feeding ruminants using *in vitro* and *in vivo* methods. It is also connected to the study conducted by Pozdíšek *et al.*, (2008), in which maize, the commonly used feedstuff, was replaced by wheat and triticale in dairy cow's feeding ration and compositional and health indicators of milk, such as somatic cell count, lactose and fat content, solid matter content, pH, density and selected macro- and micro-elements were determined. However the later study lacks an analysis of amino acids, which are considered as the building blocks of protein. Therefore, our study is aimed for determination and comparison the amino acid content in milk obtained from cows fed maize, wheat and triticale.

MATERIALS AND METHODS

Tested grain samples

Winter wheat (cultivar Sulamit) and winter triticale (cultivar Kitaro) were selected as a substitute for maize. All plants were grown in the field in the Moravia district (Agroječmínek Ltd, Chropyně, Czech Republic) in 2005.

Design of the feeding experiment

The experimental design comprised three feeding groups of cows. The experiment proceeded for six weeks and 26 cows of Czech Pied breed ($n_1 = 8$; $n_2 = 9$; $n_3 = 9$) were included into the test. Dairy cows were fed total mixed ration on the basis of maize, clover silage and hay. Otherwise, the feeding rations differed only in hard fodder as can be seen in the Table I. During the experiment, one cow from the first group was excluded because of illness. Other cows were healthy in terms of occurrence of milk secretion disorders. Feed groups were preferably balanced in terms of milk yield, stage and number of lactations. The tie stable and pipeline milking equipment were used in experiment.

Milk sampling

Animals were milked twice a day and sampled at morning milking in intervals of about seven days. On the whole, it was obtained 182 samples in the seven sampling terms using Flow milk meter (Tru-Test Ltd., New Zealand). Within groups, the individual milk samples were combined into bulk samples (three groups multiplied by seven sampling periods, n = 21).

Sample preparation

Prior to analysis, the samples of the grains were crushed with the grain mill (Bosh MUM 4 MUZ 4 GM 2, Robert Bosch GmbH, Stuttgart, Germany) to pass through the 1mm sieve.

The milk samples were transported in cold condition (< 10 °C) to the laboratory. Then they were deep-frozen at –80 °C overnight. Afterwards, the samples were lyophilisated at – 40°C and vacuum of 12 Pa for 48 hours.

Reagents

Hydrochloric acid, citric acid monohydrate, sodium citrate dihydrate, sodium chloride, thiodiglycol, boric acid, sodium azide, sodium hydroxide, ninhydrine, methylcellosolve, acetate buffer (pH 5.5) and hydrindantine were provided by Ingos, Ltd., Prague, Czech Republic. Sulphuric acid 96.0% p. a., Acetic acid, 85.0% p. a., peroxide 30%, sodium sulphate, cupric sulphate pentahydrate and mixed indicator 5 solution p.a., for ammonia titrations as well were supplied by Sigma Aldrich and Fluka, Inc., St. Louis, USA.

Hydrolysis

The sample of grain and milk were treated with acidic and oxidative hydrolysis according to Kráčmar *et al.*, (1998, 2000), Buňka *et al.*, (2009) and Official Journal (2009).

Amino acid analysis

Amino acid content and composition were determined using ion-exchange chromatography with post-column ninhydrine derivatization and spectrophotometric detection (440 nm for proline and 570 nm for other amino acids) according to Kráčmar *et al.*, (1998, 2000), and Buňka *et al.*, (2009).

Total nitrogen content and crude protein determination

Total nitrogen content was determined according to Kjehldahl using Pro-Nitro A (J. P. Selecta, s. a., Spain) apparatus. According to Pozdíšek and Vaculová (2008), the crude protein (CP) of the grain samples was determined by conversion from nitrogen content multiplied by the factor 6.25. According to Hanuš *et al.*, (1995) the CP of the milk samples was determined by conversion from nitrogen content multiplied by the factor 6.38.

Statistical methods

All obtained data were analysed with Analysis of variance (ANOVA), Duncan test and Pearson correlation coefficient (*r*). The statistical evaluation was performed using UNISTAT v. 5.5 software (Unistat Ltd, London, UK, 2003).

RESULTS AND DISCUSSION

Crude protein (CP)

The effect of maize replacing by wheat or by triticale caused the changes in milk CP content. The CP of feedstuffs (maize, wheat and triticale) was determined to be of 88.80, 145.80, and 110.50 g/1000 g, respectively. The CP content in milk samples obtained after feeding (maize, wheat, triticale) showed values of 30.8, 30.5, and 32.3 g/1000 g, respectively. The high content of CP in wheat did not influence CP in milk contrary to situation observed for triticale. Here, higher content of CP in feed resulted in higher content of CP in corresponding milk. Her-

I: *Components of feeding ration for three experimental groups (kg) and analysis of feeding ration*

Component of feeding ration	1 maize (control group)	2 wheat (tested group)	3 triticale (tested group)
clover silage	21.3	21.3	21.3
maize silage	13.1	13.1	13.1
Hay	1.0	1.0	1.0
maize silage grain	3.0	3.0	3.0
squeezing corn	3.0	3.0	3.0
feeding mixture	5.8	5.8	5.8
maize	**1.5**	-	-
wheat	-	**2.0**	-
triticale	-	-	**2.0**
Analysis of feeding ration			
NEL /kg of dry matter	6.524	6.512	6.491
NL % in dry matter	17.9	18.2	17.9
Fibre % in dry matter	15.96	15.74	15.72
PDIN/PDIE	1.189	1.189	1.191

II: *Amino acid composition and crude protein (CP) in the tested feedstuffs (maize, wheat, triticale) (g/1000 g); mean ± SE*

Amino acids	Experimental group		
	1 (maize)	2 (wheat)	3 (triticale)
Thr	2.48 ± 0.058^{a}	3.33 ± 0.032^{b}	2.70 ± 0.024^{c}
Val	4.04 ± 0.052^{a}	5.67 ± 0.094^{b}	4.44 ± 0.096^{c}
Ile	2.76 ± 0.042^{a}	4.44 ± 0.063^{b}	3.38 ± 0.037^{c}
Leu	9.19 ± 0.141^{a}	8.56 ± 0.067^{b}	6.25 ± 0.065^{c}
Phe	4.34 ± 0.073^{a}	6.14 ± 0.070^{b}	4.51 ± 0.059^{a}
His	2.96 ± 0.035^{a}	3.60 ± 0.029^{b}	2.91 ± 0.025^{a}
Lys	2.51 ± 0.038^{a}	3.42 ± 0.040^{b}	3.18 ± 0.047^{b}
Arg	4.25 ± 0.083^{a}	6.67 ± 0.075^{b}	5.34 ± 0.086^{c}
Met	3.31 ± 0.099^{a}	2.10 ± 0.019^{b}	3.02 ± 0.079^{a}
ΣEAA	$\mathbf{35.83 \pm 0.694^{a}}$	$\mathbf{43.93 \pm 0.677^{b}}$	$\mathbf{35.71 \pm 0.411^{a}}$
Asp	5.19 ± 0.079^{a}	6.01 ± 0.055^{b}	5.47 ± 0.066^{a}
Ser	3.31 ± 0.062^{a}	5.39 ± 0.058^{b}	3.96 ± 0.033^{c}
Glu	13.23 ± 0.225^{a}	35.23 ± 0.331^{b}	23.36 ± 0.172^{c}
Pro	6.91 ± 0.095^{a}	12.93 ± 0.140^{b}	8.96 ± 0.094^{c}
Gly	3.01 ± 0.040^{a}	4.94 ± 0.047^{b}	3.92 ± 0.043^{c}
Ala	5.62 ± 0.083^{a}	4.28 ± 0.039^{b}	3.63 ± 0.044^{c}
Tyr	2.94 ± 0.062^{a}	3.71 ± 0.066^{b}	2.84 ± 0.076^{a}
Cys	2.24 ± 0.007^{a}	3.18 ± 0.005^{b}	3.96 ± 0.055^{c}
ΣNEAA	$\mathbf{42.45 \pm 1.266^{a}}$	$\mathbf{75.67 \pm 3.837^{b}}$	$\mathbf{56.10 \pm 2.430^{c}}$
ΣAA	$\mathbf{78.28 \pm 0.696^{a}}$	$\mathbf{119.60 \pm 1.865^{b}}$	$\mathbf{91.82 \pm 1.185^{c}}$
CP	88.80 ± 2.21^{a}	145.80 ± 1.16^{b}	110.50 ± 2.21^{c}

ΣEAA … sum of essential and semi-essential amino acids
ΣNEAA … sum of nonessential amino acids
ΣAA … sum of all detected amino acids
SE … standard error of the mean
Means within a row (line) with the same superscript letter do not differ significantly ($P \geq 0.05$)

rera-Saldava *et al*., (1990) and Owens *et al*., (1997) interpret this discrepancy by the different digestive speed of grains. Small grains as maize, feterite, or millet are faster fermented in rumen. Wheat is considered as the one of the fastest degradable species between small grains.

Amino acids

One of the aims of this study was determination of amino acid content in milk obtained from cows fed different diet (as can be seen in Table I).

At first, content of amino acids in hard fodder (maize, wheat and triticale) of the feeding ration was analysed. The results in Table II show that in comparison to maize, wheat contained significantly higher amount ($P \leq 0.05$) of almost all detected amino acids with the exception of leucine, methionine and alanine ($P \leq 0.05$). The comparison of amino acids amount detected in maize and triticale demonstrated lower content of leucine and alanine in triticale ($P \leq 0.05$). Higher amount of methionine and cysteine ($P \leq 0.05$) was measured in triticale compared to wheat, whereas lysine showed the same content in all the tested grains.

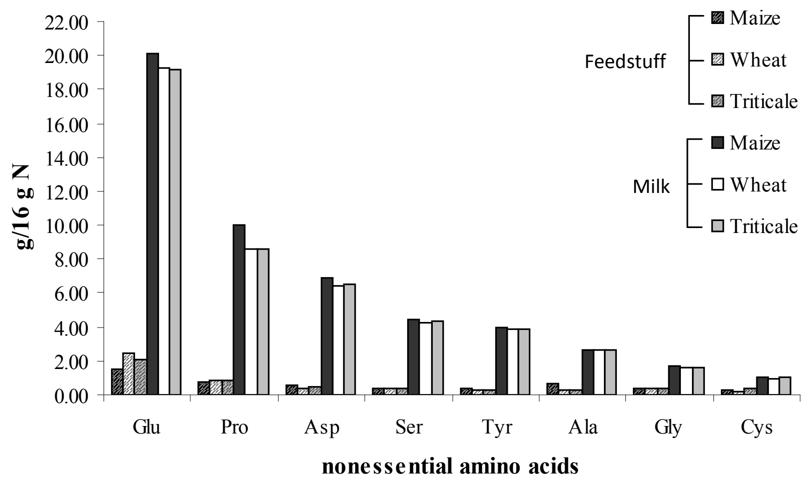
In the second step, effects of maize replacing on amino acid content in milk were studied. In Table III content of amino acids in milk obtained from tested groups is presented. Amount of all detected amino acids in milk from group fed wheat is same or lower ($P \leq 0.05$) in comparison to milk from group fed maize. On the other hand, all detected amino acids, which are contained in milk from group fed triticale are reaching, beside group fed maize, the same or higher amount ($P \leq 0.05$) with exception of proline, the content of which was significantly lower ($P \leq 0.05$). The comparison of milk from group fed wheat and milk from group fed triticale showed that triticale's milk contains higher amount of almost all detected amino acids ($P \leq 0.05$) with exception of histidine, arginine and cysteine, which are detected with no statistically significant difference in milk from all tested groups.

Amino acids detected in grain and milk samples are shown in Table II and Table III, respectively. Values in Tables II and III were used for calculation of the amino acid amount in the CP. The conversion data (g/16gN) were used to Pearson' s correlation and are summarized in Figure 1 and Figure 2 as content of nonessential and essential amino acids in feedstuffs and milk.

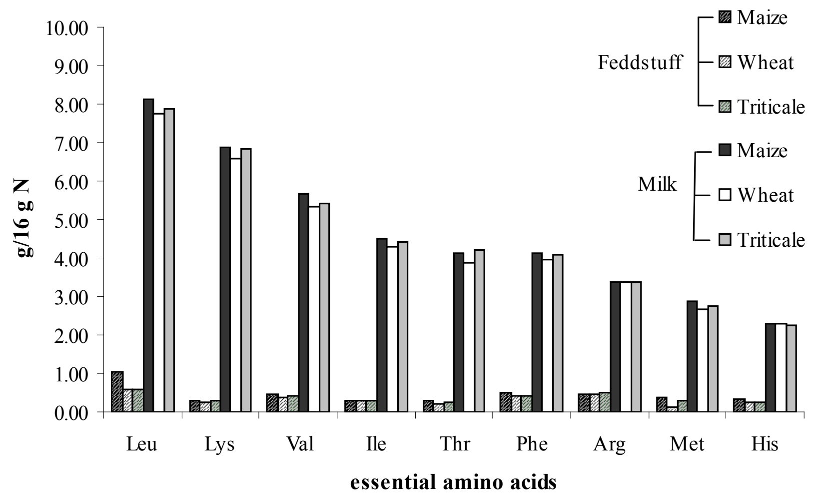
III: *Amino acids composition and crude protein (CP) of milk obtained from experimental groups (g/1000 g); mean ± SE*

Amino acid	Milk obtained from experimental group		
	1 (maize)	2 (wheat)	3 (triticale)
Thr	1.28 ± 0.014^{a}	1.19 ± 0.014^{b}	1.27 ± 0.016^{a}
Val	1.74 ± 0.017^{a}	1.63 ± 0.015^{b}	1.78 ± 0.018^{a}
Ile	1.39 ± 0.012^{a}	1.31 ± 0.010^{b}	1.45 ± 0.014^{c}
Leu	2.50 ± 0.022^{a}	2.37 ± 0.018^{b}	2.63 ± 0.025^{c}
Phe	1.27 ± 0.010^{a}	1.20 ± 0.009^{b}	1.34 ± 0.014^{c}
His	0.71 ± 0.007^{a}	0.70 ± 0.007^{a}	0.73 ± 0.008^{a}
Lys	2.12 ± 0.018^{a}	2.02 ± 0.016^{b}	2.23 ± 0.021^{c}
Arg	1.04 ± 0.010^{a}	1.03 ± 0.018^{a}	1.07 ± 0.015^{a}
Met	0.88 ± 0.011^{a}	0.82 ± 0.015^{b}	0.89 ± 0.009^{a}
ΣEAA	$\mathbf{12.93 \pm 0.195^{a}}$	$\mathbf{12.27 \pm 0.183^{b}}$	$\mathbf{13.39 \pm 0.208^{c}}$
Asp	2.11 ± 0.021^{a}	1.96 ± 0.019^{b}	2.14 ± 0.022^{a}
Ser	1.37 ± 0.015^{a}	1.31 ± 0.013^{b}	1.44 ± 0.018^{c}
Glu	6.18 ± 0.067^{a}	5.87 ± 0.071^{b}	6.28 ± 0.081^{c}
Pro	3.09 ± 0.037^{a}	2.63 ± 0.029^{b}	2.82 ± 0.034^{c}
Gly	0.51 ± 0.005^{a}	0.48 ± 0.004^{b}	0.53 ± 0.006^{a}
Ala	0.81 ± 0.008^{a}	0.82 ± 0.008^{a}	0.87 ± 0.006^{b}
Tyr	$1.22 \pm 0.010^{a, b}$	1.17 ± 0.010^{a}	1.27 ± 0.013^{b}
Cys	0.31 ± 0.005^{a}	0.30 ± 0.005^{a}	0.33 ± 0.004^{a}
ΣNEAA	$\mathbf{15.60 \pm 0.683^{a}}$	$\mathbf{14.54 \pm 0.639^{b}}$	$\mathbf{15.68 \pm 0.682^{a}}$
ΣAA	$\mathbf{28.53 \pm 0.332^{a}}$	$\mathbf{26.81 \pm 0.310^{b}}$	$\mathbf{29.07 \pm 0.333^{a}}$
CP	30.8 ± 0.67^{a}	30.5 ± 0.55^{a}	32.3 ± 0.72^{b}

Σ*EAA* … sum of essential and semi-essential amino acids
Σ*NEAA* … sum of nonessential amino acids
Σ*AA* … sum of all detected amino acids
SE … standard error of the mean
Means within a row (line) with the same superscript letter do not differ significantly ($P \geq 0.05$)



1: *Content of nonessential amino acids in feedstuffs and in milk*



2: *Content of essential amino acids in feedstuffs and in milk*

Pearson's correlation

The first Pearson's correlation analysis pointed to the question, whether the relative content (g/16gN) of amino acids in milk will rise (fall) with the rising of amino acid content in maize (shows in Table IV, column 1). For detected amino acids with the exception of isoleucine, proline and tyrosine ($P \leq 0.05$), no statistically significant correlation was found (as can be seen in Table IV, the first column). The positive correlation observed for proline and isoleucine expresses that their amount in milk will increase with the high probability ($r = 0.8202$ and $r = 0.7794$) when their amount will rise in maize. On the contrary, the negative correlation for tyrosine ($r = -0.7587$) shows the high probability of its decreasing amount in milk, while its amount in maize is growing.

The Pearson's correlation analysis presented column two of Table IV pointed to the question, whether the relative content (g/16gN) of amino acids in milk will grow (decline) with the growing relative content of amino acid in wheat. As can be seen in Table IV, serine and histidine showed the statistically significant positive correlation. Conseguently, the high possibility of increasing histidine content in milk with increasing content of this amino acid in wheat exists ($r = 0.8514$, $P \leq 0.05$). Correspondingly, the statistically significant ($P \leq 0.01$) positive correlation for serine showed its high possible increase in milk when it will increase in wheat. However, the statistically significant ($P \leq 0.05$) negative high correlation was determined for proline ($r = -0.7507$).

The last performed Pearson's correlation analysis (Table IV, column 3) pointed to the question, whether the relative content (g/16gN) of amino acids in milk will increase (decrease) with the increasing relative content of amino acid in triticale. The statistically significant positive correlation was found for tyrosine and phenylalanine. Thereby, the high possibility of tyrosine's and phenylalanine's increasing in milk with their growth in triticale exists (r = 0.8910, P ≤ 0.01; r = 0.7332, P ≤ 0.05, resp.). On the other hand, the statistically significant (P ≤ 0.05) negative high correlation was detected for arginine (r = – 0.8173).

IV: *Statistically significant Pearson's correlation coefficients (r) obtained from the correlation between amino acids content determined in feedstuff (maize, wheat, triticale) and corresponding milk samples*

Amino acid	maize vs. milk	wheat vs. milk	triticale vs. maize
His	0.3501	0.8514*	0.2538
Phe	0.6627	0.2243	0.7332*
Ile	0.7794*	0.2789	0.5123
Arg	0.2471	-0.3873	–0.8173*
Ser	–0.6888	0.9417**	0.3610
Pro	0.8202*	-0.7507*	0.4805
Tyr	–0.7587*	-0.2135	0.8910*

* … P < 0.05
** … P < 0.01

CONCLUSION

Amino acids determined in feedstuff used for feeding of tested cows were subsequently presented in milk obtained from all the three tested groups. On the other hand, the expected abundance of wheat CP was not confirmed in corresponding milk samples. With a view to amino acid content in milk samples, none of the tested grains can be recommended as a full-value maize replacement. Wheat is, according to Pearson's correlation coefficient, abundant source of histidine and serine in milk, however it cannot serve as a source of proline. According Pearson's correlation coefficient, triticale is reliable source of phenylalanine and tyrosine in milk, nevertheless it is unsuitable as a source of arginine. The supply of amino acids for absorption in the small intestine of ruminants is a mixture of undegraded dietary protein and microbial protein produced in rumen. With the view to amino acid composition in milk, it will be the best choice to make the mixture of all tested feedstuffs. Each feedstuff (maize, wheat, triticale) is an abundant and unique source of typical amino acids in milk. According to (Boisen *et al.*, 2000) it is influenced not only by dietary proteins but also by variations in the microbial population in rumen. As a summary it is possible to conclude that triticale (cultivar Kitaro) seems to be more suitable replacement of maize because of its better CP efficiency and total milk quality.

SOUHRN

Účinek vybraných obilnin obsažených v krmné dávce na obsah aminokyselin v mléce

Cílem této práce bylo zhodnocení možného účinku náhrady kukuřice v krmné dávce na obsah aminokyselin v kravském mléce. Dojnice byly krmeny směsnou krmnou dávkou složenou z kukuřice, jetelové siláže a sena. Rozdílnost v krmné dávce byla v jadrném krmivu. První skupina (krmena kukuřicí) byla skupinou kontrolní, ostatní dvě skupiny byly experimentální, jedna byla krmena pšenicí a druhá obilninou tritikale. V průběhu šesti týdnů bylo odebráno celkem 26 vzorků mléka od dojnic plemene České strakaté. Krmné skupiny byly vyváženy v dojivosti, laktačních dnech a v pořadí laktace. Vzorky krmiv a stejně tak i mléka byly upraveny pro analýzu pomocí kyselé a oxidativně-kyselé hydrolýzy. Analýza aminokyselin v hydrolysátech byla provedena pomocí kapalinové chromatografie (AAA 400 analyzátor) za použití sodno-citrátových pufrů a ninhydrinové detekce. Celkové množství dusíku bylo stanoveno dle Kjehldahla a hrubý protein daných vzorků byl stanoven pomocí vynásobení hodnoty celkového dusíku vhodným faktorem 6,38. Vysoká hodnota hrubého proteinu v pšenici neovlivnila složení mléka získaného od experimentální skupiny krmené touto obilninou. Z pohledu obsahu aminokyselin ve vzorcích mléka nemůže být žádná z testovaných obilnin doporučena jako plnohodnotná náhrada kukuřice. Každé krmivo je bohatým zdrojem mnohých a typických aminokyselin v mléce. Avšak tritikale (kultivar Kitaro) se může zdát jako vhodnou náhradou kukuřice vzhledem k lepší využitelnosti hrubého proteinu, celkovému složení mléka a zdravotním ukazatelům mléčné kvality.

dojnice, kukuřice, pšenice, tritikale, aminokyseliny

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