COMPARISON OF ACTUAL AND PREDICTED AMINO ACID CONTENTS IN THE DUODENAL DIGESTA OF DAIRY COWS

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Abstract

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In this experiment on three dairy cows with the ruminal and duodenal T-cannulas, the actual and predicted amino acid (AA) profiles of the protein fraction flowed to the small intestine were compared. The prediction was calculated by two methods: with the use of mean published AA profile of microbial protein and of experimentally determined one. The actual AA profile of digesta protein was corrected for glycine (Gly) of bile origin. In comparison to the published AA profile of microbial protein the use of the actual one did not contribute to an improvement of prediction accuracy (mean prediction error: 7.36 vs. 7.54%, respectively). Inaccurate determination of AA composition of undegraded feed protein and the insufficient correction for Gly of bile origin were the probable sources of the residual variability.

amino acids, dairy cow, duodenum, microbial protein, prediction

As regards the requirement of crude protein, the diets for cattle are evaluated in the French PDI system (Jarrige, 1990) on the basis of prediction of the flow of protein truly digestible in the small intestine (PDI). This protein consists of two basic fractions: rumen undegraded feed protein (PDIA) and microbial protein (PDIM), the amount of which correlates with the fermentable part of feed. The equation to calculate the second fraction is depending upon whether the limiting factor of its formation is energy (PDIME: microbial protein digested in the intestine when rumen fermentable energy is limiting) or crude protein (PDIMN: microbial protein digested in the intestine when rumen fermentable nitrogen is limiting). The resulting predicted value of true digestible protein (PDIE, PDIN) is the sum of these two basic fractions provided by different components of the diet (Jarrige, 1989).

Even with a correct prediction of these values and subsequent balancing of PDI requirement, with some diets it is impossible to achieve maximum production of milk or meat. This is due to deficiencies of some amino acids (AA), which was confirmed e.g. by the duodenal infusion of Lys and Met in lac-

tating cows fed on maize silage based diet (Schwab *et al.*, 1992), or of His in cows fed grass silages (Vanhatalo *et al.*, 1999). For this reason, a need to define the diet occurred, not only in terms of the flow of basic protein fraction, but also in terms of representation of individual especially essential AA in PDI. For this purpose, on the basis of the existing French PDI system, a consequential AADI system was proposed (Rulquin *et al.*, 1993; Rulquin & Vérité, 1993).

The final value of AADI (% PDIE) of a given AA results from its content in the undegraded residue of feed protein and microbial mass protein formed in the rumen with contribution of the degraded feed components. Because PDI is constituted of more than 50% of microbial protein (Rulquin *et al.*, 1998) and because of large differences in AA profile of bacteria protein (Clark *et al.*, 1992; Rulquin & Vérité, 1993), the main source of variability of predicted AADI values is microbial mass.

The aim of this study was to compare the experimental and predicted AA profiles of the digesta entering the small intestine using the method described by Rulquin *et al.* (1998) and compare the accuracy of prediction when using the pub-

lished mean (Le Hénaff, 1991) and actual AA profiles of microbial protein in lactating dairy cows.

MATERIAL AND METHODS

Experiment, animals, feed

For the evaluation of actual and predicted amino acid content in the duodenal digesta, the data from previous studies (Třináctý *et al.*, 2006, Křížová *et al.*, 2007) were used. Cows, treatments and feeding regiment have been described previously (Třináctý *et al.*, 2006). Briefly, treatment consisted of administrations of either rumen-protected tablets (T) or powder (C) containing purified soya-protein HP 300 (Hamlet Protein A/S, Denmark) and amino acids Lys, Met and His into the rumen of three lactating Holstein cows (1–3 lactation, 17–35 week of lactation) of mean live weight 523 kg (SEM = 17) and milk yield 16.7 kg.day^{-1} (SEM = 0.38). Cows were fitted with ruminal and duodenal T-cannulas (Komarek, 1981). The experiment was divided into 4 periods. Each period (14 d) consisted of 10–d preliminary period and 4–d experimental period. Cows were fed individually twice daily (7.00 and 16.35 h) ad libitum a diet based on maize silage (54.7 %), lucerne hay (15.0 %) and concentrate (30.3 %). The experimental protein supplement consisted of ground soya protein HP 300 (93 %), Lys (1.6 %), Met (2.4 %) and His (3.0 %) and was prepared in two forms, either as rumen-protected tablets or powder. Tablets or powder together with chromic oxide (digesta passage marker) were wrapped into filter paper and inserted into the bottom of the rumen via ruminal cannula twice daily before feeding. Daily amount of these ingredients were as follows (g): HP 300 (306), Lys (5.7), Met (8.8), His (10.6) and chromic oxide (15.0).

In the previous study focused on the estimation of duodenal flow of soluble nitrogen fractions in the same experiment (Křížová *et al.*, 2007), it has been found that in the case of ruminally protected tablets sampling from the duodenal cannula was unrepresentative. Thus for the evaluation of actual and predicted amino acid content in the duodenal digesta only the data from the control group of animals (C) receiving the supplement in the powder form were used.

Collection, processing and analyses of samples

Feed samples were collected on the third day of each experimental period. Respective orts from each cow were collected and weighed during the four-day experimental period before each meal and stored in a freezer. After the experimental period, the orts were pooled for each cow, homogenized and the representative sample was taken.

The rumen fluid samples (1 l) were collected from each cow once a day, during the four-day experimental period with a 2h time shift between the successive days (at 9.00, 11.00, 13.00 and 15.00 h). To the 500 ml of rumen fluid sample the same volume of 0.95 % NaCl solution were added. The mixture was stirred in the blender and filtered through 4 layers of tissue, and then stored at –20 °C (Cecava *et al.*, 1990). After thawing the samples were processed according to Schwab *et al.* (1992). The samples of microbial mass were pooled for each cow and experimental period, freeze-dried and homogenized.

The samples of duodenal digesta (500 ml) were collected during the 4-day experimental period at 6 h intervals from each cow and immediately frozen to –20 °C (Schwab *et al.*, 1992).

Processing of feed, feed refusals and duodenal chymus samples for analyses has been described in details elsewhere (Křížová *et al.*, 2007). In feed and feed refusals samples the following parameters were estimated according to AOAC (1984): dry matter (DM), ash, crude protein (CP), fat (F) and crude fibre (CF). Neutral detergent fibre (NDF) with α-amylase and acid detergent fibre (ADF) were estimated according to Van Soest *et al.* (1991). Content of Cr in samples of duodenal chymus were estimated according to Williams *et al.* (1962). Ammonia nitrogen content in duodenal chymus was estimated in a water eluate (2 g.100ml^{-1} of distilled water) using a gas electrode (manufacturer Radelkis Ltd., Hungary). DM, ash and CP contents in the microbial mass and digesta were determined using the same procedure as in feeds. In samples of feeds, microbial mass and duodenal chymus content of individual AA was determined. Samples were adjusted using acidic and oxidative acidic hydrolysis. The chromatographic analysis of sample hydrolysates was performed in the analyser AAA 400 (INGOS Prague, CR) and using Na-citrate buffers and ninhydrin detection.

Calculations

PDIA, PDIN and PDIE values were calculated on the basis of chemical analysis results using regressions from the PrévAlim tool of the diet formulation software INRAtion (Baumont *et al.*, 1999) and feed tables (Jarrige, 1990). A prediction of individual AA content in duodenal digesta protein was calculated using the equations published by Rulquin *et al.* (1998) by introduction of either mean values of individual AA concentration in microbial protein reported by Le Hénaff (1991) or those determined in this experiment (Tab. II). The above mentioned mathematical model does not implicate a biliary secretion because as the only source of endogenous AA the epithelium of various forestomach compartments and abomasum is used (Ørskov *et al.*, 1986). Due to placing the duodenal T–cannula (distally to the bile duct) in this experiment the digesta samples were contaminated with bile (in particular with the conjugate of Gly and choline acids). In case of this type of endogenous fraction, there was not enough information published for us to be able to modify the mathematical prediction model. For this reason, a correction for Gly content in the bile was carried out on the side of the determined values of the duodenal AA flow before calculating the AA composition of duodenal protein. The Gly flow determined experimentally was corrected for its con-

tent in bile (Larsen *et al.*, 2000) by subtracting the following values from the total flow value:

$$GlyI_b = GlyI_E \times 0.23 \times 0.307 \times 0.892,$$

where $GlyI_b$ = the flow of Gly originating from the bile (g.day^{-1}); $GlyI_E$ = the experimental (determined) duodenal flow of Gly (g.day^{-1}); 0.23 = mean proportion of endogenous AA in the total AA duodenal flow; 0.307 = mean proportion of bile AA in the endogenous AA flow; 0.892 = mean proportion of Gly in the sum of bile AA.

Statistical analyses

For the statistical analyses the GLM procedure of the statistics software SYSTAT 11.00.01. (U.S.A.) was used. As a model for the comparison of true and predicted (by two methods) concentrations of individual AA in the duodenal digesta the following equation was used: $Y_{ijk} = \mu + M_i + C_j + R_k + \varepsilon_{ijk}$, where μ = general mean, M_i = method effect (i = 3), C_j = cow effect (j = 3), R_k = replication effect (k = 2), ε_{ijk} = residual error.

RESULTS AND DISCUSSION

Basic parameters

Basic parameters studied in this trial such as dry matter intake (DMI) (Tab. I), nitrogen intake (20.0 g.kg^{-1} of DMI, SEM = 0.39, n = 24) and non-ammonia nitrogen (NAN) duodenal flow (19.7 g.kg^{-1} of DMI, SEM = 0.81, n = 6) were similar to the values for cattle summarised from literature sources and reported by Rulquin *et al.* (1998).

I: *Dry matter and nutrients intake*

Nutrient	Unit	Values[1] (n = 24)	
		Mean	SEM
Dry matter	kg.day^{-1}	15.68	0.32
Organic matter	kg.day^{-1}	14.59	0.30
Crude protein	kg.day^{-1}	1.89	0.05
Fat	kg.day^{-1}	0.54	0.01
Crude fiber	kg.day^{-1}	2.29	0.06
NDF	kg.day^{-1}	5.23	0.12
ADF	kg.day^{-1}	3.14	0.07
Ash	kg.day^{-1}	1.09	0.02
PDIN[2]	kg.day^{-1}	1.25	0.02
PDIE[2]	kg.day^{-1}	1.31	0.02
NEL[3]	MJ.day^{-1}	98.89	2.05

[1] Values adapted from work of Třináctý *et al.* (2006)
[2] Protein truly digestible in the small intestine (Jarrige *et al.*, 1989)
[3] Net energy for lactation

Amino acid composition of microbial protein

Because of a high proportion of the microbial protein in NAN entering the small intestine ranging from 34 to 89 % (Clark *et al.* 1992), and the variability in its AA composition (Clark *et al.*, 1992; Rulquin & Vérité, 1993), this component can be regarded as the greatest source of variability in the prediction of individual AA concentrations in the duodenal digesta. The actual AA composition of microbial protein determined in our study was compared with the AA composition found by Le Hénaff (1991) and is presented in Tab. II. All the determined mean values of AA content are within the reported range.

Experimental amino acid composition of duodenal digesta protein

The experimental AA composition of duodenal digesta determined in our study ($[AAI]_E$) is presented in Tab. III. Mean values (g.100 g^{-1}of total AA) of Arg (6.18 vs. 4.92), and Gly (7.35 vs. 6.23) found in our experiment were higher and Met (1.66 vs. 1.93), Ileu (5.13 vs. 5.42) and Glu (12.6 vs. 14.6) were lower than the means reported by Rulquin *et al.* (1998), respectively. Nevertheless all mean values are within a range of values and the coefficients of variation (CV) are lower than those reported by Rulquin *et al.* (1998). A higher CV was found only for Gly (18.2 vs. 14.1 %, resp.) and Met (15.4 vs. 13.1 %, resp.). Because the diet composition was relatively constant throughout the trial it can be assumed that the main source of the increased variability of these two AA contents is their higher variability in the microbial protein found in this experiment (Tab. II).

Comparison of the prediction accuracy calculated using the published and actual AA profiles of microbial protein

The predicted values $[AAI]_{P1}$ of Arg, Ileu, Leu, Met, Phe, Thr, Val, Glu, Pro, Ser and Tyr calculated from published microbial AA profile (Le Hénaff, 1991) were significantly different (P < 0.05) from the experimentally determined ones ($[AAI]_E$). On the other hand the $[AAI]_{P1}$ and $[AAI]_E$ values of other AA (His, Leu, Lys, Thr, Val, Ala, Asp a Gly) were not significantly different (P > 0.05). This is in disagreement with Rulquin *et al.* (1998) who found that the mentioned values of only 3 AA (His, Val, Asp) did not differ significantly. Actually, mean relative difference between the values $[AAI]_{P1}$ and $[AAI]_E$ was 7.36 % and was higher than the published one (4.1 %). The main cause of a lower prediction accuracy was Arg (–25.7 %) followed by Met (22.5 %) and Gly (–17.3 %). Rulquin *et al.* (1998) reported the prediction accuracy for most AA within 5 to 6 % (13 cases). The same prediction accuracy was observed in 12 AA in this trial (Tab. III).

As to the difference between predicted AA composition calculated from experimentally determined AA composition of microbial protein $[AAI]_{P2}$ and values $[AAI]_E$, loss of significance (P > 0.05) for Ileu a Lys and its obtaining (P < 0.05) for Leu,

II: *Amino acid composition of endogenous and microbial protein (g.100g⁻¹ of total AA)*

	Endogenous[1] (n = 1)	Microbial					
		Adapted values[2] (n = 66)				Actual values (n = 6)	
		Mean	CV[3]	Minimum	Maximum	Mean	CV[3]
Arg	5.16	4.9	11.0	4.1	7.5	6.1	4.8
His	3.77	1.8	18.0	1.0	2.7	2.0	7.7
Ile	4.88	5.9	10.0	4.6	7.6	5.5	2.3
Leu	5.02	7.7	7.0	5.3	8.8	8.8	3.4
Lys	7.67	8.0	12.0	5.0	11.4	8.2	4.2
Met	1.53	2.5	24.0	1.1	3.9	2.7	14.9
Phe	4.88	5.3	12.0	4.2	8.2	5.1	1.7
Thr	6.83	5.8	9.0	4.7	7.8	5.5	3.4
Val	6.42	6.2	10.0	4.7	7.5	6.2	1.7
Ala	5.86	7.8	8.0	6.3	9.8	7.0	3.7
Asp	9.62	12.1	7.0	7.9	13.8	12.2	3.8
Glu	13.11	13.3	7.0	8.9	15.1	12.0	2.0
Gly	6.69	5.7	10.5	4.0	7.6	5.2	22.4
Pro	6.56	3.7	14.0	2.3	5.3	4.1	5.4
Ser	6.83	4.6	9.0	3.6	5.5	4.6	1.5
Tyr	5.16	4.7	12.0	2.5	5.6	4.8	2.7

[1] Adapted from work of Ørskov *et al.* (1986)
[2] Adapted from work of Le Hénaff (1991)
[3] Coefficient of variation

III: *Comparison of AA composition of the duodenal digesta (g.100g⁻¹ of total AA, n = 6) determined experimentally or predicted by two methods using published and actual microbial protein composition*

	$[AAI]_E$[1]		$[AAI]_{P1}$[2]		$[AAI]_{P2}$[3]		Deviation[4]	
	Mean	CV[5]	Mean	CV[5]	Mean	CV[5]	(P1−E)	(P2−E)
Arg	6.18[c]	2.19	4.92[a]	1.45	5.23[b]	2.35	−25.7***	−18.3***
His	2.23	4.31	2.27	2.99	2.33	4.19	1.88	4.32
Ileu	5.13[a]	2.98	5.39[b]	0.36	5.24[a]	1.15	4.76**	2.10
Leu	8.85[a]	3.05	8.72[a]	1.48	9.19[b]	2.18	−1.52	3.67*
Lys	7.25[b]	2.19	7.09[a]	1.72	7.17[ab]	1.62	−2.21*	−1.07
Met	1.66[a]	15.4	2.14[b]	4.05	2.24[b]	7.04	22.5**	25.6***
Phe	4.93[a]	2.44	5.20[b]	1.66	5.12[b]	1.87	5.13**	3.53*
Thr	5.34[b]	2.99	5.25[ab]	0.57	5.15[a]	1.60	−1.73	−3.72**
Val	6.31[b]	2.67	6.07[a]	1.86	6.05[a]	2.27	−3.99*	−4.21*
Ala	6.92	3.75	7.00	1.28	6.84	1.84	1.01	−1.34
Asp	11.2	2.41	11.1	0.82	11.1	2.20	−1.08	−0.66
Glu	12.6[a]	3.24	14.3[c]	1.29	13.9[b]	1.73	12.0***	8.97***
Gly	7.35[b]	18.2	6.27[ab]	0.37	6.04[a]	9.18	−17.3	−22.4*
Pro	4.75[a]	7.75	5.05[ab]	2.04	5.19[b]	1.71	6.01	8.58*
Ser	5.11[b]	2.57	4.82[a]	0.83	4.81[a]	1.04	−5.85***	−6.27***
Tyr	4.21[a]	2.72	4.44[b]	1.31	4.47[b]	2.06	5.09**	5.78**
Total mean[6]		4.93		1.50		2.75	7,36	7.54

[1] $[AAI]_E$ – in experiment determined AA concetration in the duodenal digesta
[2] $[AAI]_{P1}$ – predicted AA concentration using microbial AA composition according to LeHenaff (1991) in calculation
[3] $[AAI]_{P2}$ – predicted AA concentration using actual (in experiment determined) microbial AA composition in calculation
[4] Deviations (%): (P1−E) = 100 x ($[AAI]_{P1}$−$[AAI]_E$)/$[AAI]_{P1}$; (P2−E) = 100 x ($[AAI]_{P2}$−$[AAI]_E$)/$[AAI]_{P2}$; deviations statistically different from zero: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$
[5] Coefficient of variation
[6] Total average of deviations = ΣABS(Deviation)/16
[a, b, c] Means in the same row followed by the different superscripts differ significantly ($P < 0.05$)

Thr, Gly and Pro in comparison with differences of the values $[AAI]_{P1}$ was observed (Tab. III). Thus frequency of non–significant differences decreased from 7 to 5 AA. Mean relative difference between $[AAI]_{P2}$ values and $[AAI]_E$ values was 7.54%, i.e. comparable with $[AAI]_{P1}$ values (7.36%) and higher than the value of 4.1% reported by Rulquin *et al.* (1998). Using experimentally determined AA composition of microbial protein the accuracy of prediction was improved only for Arg, Ile, Lys, Phe and Asp, whereas for the rest of AA was impaired (Tab. III). In the case of Gly the accuracy of prediction declined and the difference found (–22.4%) was higher than that reported by Rulquin *et al.* (1998) being –13.3%. Because the prediction was underestimated it is probable that the correction for Gly content in the bile was insufficient. Larsen *et al.* (2000) and Olsson (1988) reported a great variability in the amount of bile formed and Gly contained in the bile both among different animals and within one animal, depending on a diet and stage of lactation. Because of low number of literature sources in this area it was impossible to consider the above--mentioned factors in the resulting mean value for Gly from bile, which might have lead to the insufficient correction.

Effect of amino acid profile of undegraded feed protein

Although the prediction accuracy for Arg concentration in $[AAI]_{P2}$ was improved the difference from the reported value (Rulquin *et al.* 1998) remained relatively great (–18.3 vs. 5.6%, resp.). In the case of Leu, the final difference between the predicted value and the one determined in this trial was relatively small (3.67%), but the prediction accuracy was two times worse by using the actual composition of microbial protein (Tab. III). Based on these results it can be assumed that beside microbial mass there is another source of variability that influences the prediction

accuracy. In this study a simplified determination of AA profile of undegraded feed protein was used assuming the same rate of degradation for each AA in whole protein. This simplification may cause either overestimation or underestimation of some AA contents in undegraded feed protein (Rulquin & Vérité, 1993). The use of the data biased in this way may worsen the resulting prediction accuracy. Rulquin *et al.* (1998) reported that for Arg, the consequence of the above–mentioned phenomenon was regular overestimation of prediction and for Leu regular underestimation, which was demonstrated by their results. However, the results of this study showed an opposite trend: prediction of Arg concentration was underestimated unlike the one (–18.3 vs. 5.6%, resp.) reported by Rulquin *et al.* (1998) and that of Leu was overestimated (3.67 vs. –5.8%, resp), which indicates a higher Arg content and lower Leu content in undegraded feed protein as compared with the expected ones. Indeed, Van Straalen *et al.* (1997) using the nylon–bag method found for maize silage (majority component of the experimental diet) a very low degree of degradation of Arg (41%) and a higher degree of degradation of Leu (80%) as compared with total feed protein degradation (74%), which is in agreement with the above mentioned trends.

CONCLUSION

In comparison to the reported mean value of microbial protein composition the use of the actual one for prediction of AA composition of protein fraction flowing into the small intestine did not contribute to an improvement of calculation accuracy. Inaccurate determination of AA composition of undegraded feed protein and the insufficient correction for Gly of bile origin were the probable sources of the residual variability.

SUMMARY

The aim of this study was to compare the experimental and predicted AA profiles of the digesta entering the small intestine and compare the accuracy of prediction when using the published mean and actual AA profiles of microbial protein in lactating dairy cows. Three lactating Holstein cows (mean live weight 523 kg, milk yield 16.7 kg.day^{-1}) fitted with ruminal and duodenal T–cannulas were fed individually twice daily *ad libitum* a diet based on maize silage (54.7%), lucerne hay (15.0%) and concentrate (30.3%). Treatment consisted of administrations of powder containing purified soya-protein HP 300 (93%), Lys (1.6%), Met (2.4%) and His (3.0%) into the rumen. Powder together with chromic oxide (digesta passage marker) were wrapped into filter paper and inserted into the bottom of the rumen via ruminal cannula twice daily before feeding. The experiment was divided into 4 periods. Each period (14 d) consisted of 10–d preliminary period and 4–d experimental period. Feed samples were collected on the third day of each experimental period. Respective orts from each cow were collected and weighed during the four-day experimental period. The rumen fluid samples (1 l) were collected from each cow once a day, during the four-day experimental period with a 2 h time shift between the successive days (at 9.00, 11.00, 13.00 and 15.00 h). To the 500 ml of rumen fluid sample the same volume of 0.95% NaCl solution were added. The mixture was stirred in the blender and filtered through 4 layers of tissue, and then stored at –20 °C. After thawing microbial mass was separated from the samples according to Schwab *et al.* (1992) and was pooled for each cow and experimental period, freeze-dried and homogenized. The samples of duodenal digesta (500 ml) were collected during the 4-day experi-

mental period at 6 h intervals from each cow and immediately frozen to –20 °C. In feed and feed refusals samples the following parameters were estimated: dry matter, ash, crude protein, fat and crude fibre, NDF (with α-amylase) and ADF. AA profiles of feeds, mikrobial mass protein and of duodenal digesta protein were determined. AA profile of duodenal protein was corrected for glycine of bile origin. Prediction of duodenal digesta AA profile according to Rulquin *et al.* (1998) was calculated using the published mean and actual AA profiles of microbial protein. Results were compared using GLM procedure according to the following model: $Y_{ijk} = \mu + M_i + C_j + R_k + \varepsilon_{ijk}$, where μ = general mean, M_i = method effect (i = 3), C_j = cow effect (j = 3), R_k = replication effect (k = 2), ε_{ijk} = residual error.

The actual AA composition of microbial protein determined in our study was compared with the AA composition found by Le Hénaff (1991). All the determined mean values of AA content were within the reported range. The predicted values of Arg, Ileu, Leu, Met, Phe, Thr, Val, Glu, Pro, Ser and Tyr calculated from published microbial AA profile ($[AAI]_{P1}$) were significantly different (P < 0.05) from the experimentally determined ones ($[AAI]_E$). Actually, mean relative difference between the values $[AAI]_{P1}$ and $[AAI]_E$ was 7.36 %. The main cause of a lower prediction accuracy was Arg (–25.7 %) followed by Met (22.5 %) and Gly (–17.3 %). As to the difference between predicted AA composition calculated from experimentally determined AA composition of microbial protein ($[AAI]_{P2}$) and values $[AAI]_E$, loss of significance (P > 0.05) for Ileu a Lys and its obtaining (P < 0.05) for Leu, Thr, Gly and Pro in comparison with differences of the values $[AAI]_{P1}$ was observed. In the case of Gly the accuracy of prediction declined from –17.3 to –22.4 %. Although the prediction accuracy for Arg concentration in $[AAI]_{P2}$ was improved the difference remained relatively great (–18.3 %). Mean relative difference between $[AAI]_{P2}$ values and $[AAI]_E$ values was 7.54 %, i.e. comparable with $[AAI]_{P1}$ values (7.36 %). Thus in comparison to the published AA profile of microbial protein the use of the actual one did not contribute to an improvement of prediction accuracy.

SOUHRN
Porovnání aktuálního a predikovaného obsahu aminokyselin v duodenální trávenině laktujících dojnic

Cílem této práce bylo porovnat stanovený a predikovaný aminokyselinový profil tráveniny vstupující do tenkého střeva a porovnání přesnosti predikce při použití průměrného publikovaného a aktuálního aminokyselinového složení mikrobiálního proteinu u laktujících dojnic. Tři dojnice holštýnského plemene (živá hmotnost 523 kg, dojivost 16,7 kg.den⁻¹) s ruminální a duodenální kanylou byly individuálně krmeny *ad libitum* krmnou dávkou složenou z kukuřičné siláže (54,7 %), vojtěškového sena (15,0 %) a směsi (30,3 %). Experimentální zásah byl realizován přídavkem práškové směsi obsahující proteinový koncentrát HP 300 (93 %), Lys (1,6 %), Met (2,4 %) a His (3,0 %) do bachoru. Směs byla spolu s oxidem chromitým (marker pasáže tráveniny) zabalena do filtračního papíru a vkládána dvakrát denně před krmením přes ruminální kanylu na dno bachoru. Experiment byl rozdělen na čtyři periody. Každá perioda (14 d) se skládala z desetidenní návykové a čtydenní experimentální periody. Vzorky krmiv byly odebírány třetí den každé experimentální periody. Odpovídající zbytky od každé dojnice byly odebírány a váženy v průběhu čtyř dnů experimentální periody. Vzorky ruminální tekutiny (1litr) byly od každé dojnice odebírány jednou denně v průběhu čtyř dnů experimentální periody s dvouhodinovým časovým posunem každý následující den (9.00, 11.00, 13.00 a 15.00 h). K 500 ml ruminální tekutiny bylo přidáno stejné množství 0,95 % roztoku NaCl. Směs byla míchána v mixeru, filtrována přes čtyři vrstvy plátna a potom zamražena na –20 °C. Po roztátí byla ze vzorků separována mikrobiální hmota dle Schwab *et al.* (1992), sloučena za každou dojnici a periodu, lyofilizována a homogenizována. Vzorky duodenální tráveniny (500 ml) byly odebírány od každé dojnice v průběhu čtyřdenní experimentální peridy v šestihodinových intervalech a ihned zamraženy na –20 °C. Ve vzorcích krmiva a zbytků byly stanoveny následující parametry: sušina, popel, N-látky, tuk, vláknina, NDF (s α-amylázou) a ADF. U vzorků krmiv, mikrobiální hmoty a duodenální tráveniny byly stanoveny obsahy jednotlivých AA. Aminokyselinový profil duodenálního proteinu byl korigován na Gly pocházející ze žluči. Predikce obsahu AA v duodenální trávenině dle Rulquin *et al.* (1998) byla vypočítána s použitím publikovaného průměrného a aktuálního aminokyselinového složení mikrobiálního proteinu. Výsledky byly porovnány GLM procedurou dle následujícího modelu: $Y_{ijk} = \mu + M_i + C_j + R_k + \varepsilon_{ijk}$, kde μ = celkový průměr, M_i = faktor metody (i = 3), C_j = faktor dojnice (j = 3), R_k = faktor opakování (k = 2), ε_{ijk} = reziduální chyba.

Aktuální AA složení mikrobiálního proteinu stanovené v naší práci bylo porovnáno s AA složením publikovaným autorem Le Hénaff (1991). Všechny průměrné hodnoty obsahu AA byly v publikovaném rozpětí. Predikované hodnoty pro Arg, Ileu, Leu, Met, Phe, Thr, Val, Glu, Pro, Ser a Tyr vypočítané z publikovaného mikrobiálního AA profilu ($[AAI]_{P1}$) byly prokazatelně odlišné (P < 0,05) od experimentálně stanovených ($[AAI]_E$). Průměrná relativní odchylka mezi hodnotami $[AAI]_{P1}$ a $[AAI]_E$ činila 7,36 %. Hlavní příčinou nižší přesnosti predikce byl Arg (–25,7 %) následovaný Met (22,5 %) a Gly (–17,3 %). Na základě porovnání predikovaného AA složení vypočítaného z experimentálně stano-

veného AA složení mikrobiálního proteinu ([AAI]$_{P2}$) s hodnotami [AAI]$_E$ byla zjištěna ztráta signifikance (P > 0,05) pro Ileu a Lys a naopak signifikantní rozdíl (P < 0,05) byl zaznamenán pro Leu, Thr, Gly a Pro. V případě Gly klesla přesnost predikce z –17.3 % na –22.4 %. Přestože se přesnost predikce u Arg v případě [AAI]$_{P2}$ zvýšila, rozdíl zůstal relativně vysoký (–18,3 %). Průměrný relativní rozdíl mezi hodnotami [AAI]$_{P2}$ a [AAI]$_E$ činil 7,54 % a byl srovnatelný s hodnotami [AAI]$_{P1}$ (7,36 %). Z výsledků vyplývá, že použití aktuálního AA profilu mikrobiálního proteinu ve srovnání publikovaným nepřispělo k zvýšení přesnosti predikce.

aminokyseliny, dojnice, duodenum, mikrobiální protein, predikce

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