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# EFFECT OF BIOETHANOL CO-PRODUCT TYPE ON RUMINAL DEGRADATION AND AMINO ACID INTESTINAL DIGESTIBILITY

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## ABSTRACT

The objectives of this experiment were to measure the nutrient content of distiller grains (DG) with solubles (DDGS) produced from maize (4 samples of $DDGS_M$), wheat (4 samples of $DDGS_W$), triticale ($DDGS_T$) and wet distillers grains (2 samples of WDG) from maize. The ruminal CP degradability and bypass CP and amino acid (AA) intestinal digestibility were determined using *in sacco* and mobile bag technique on three dry cows with ruminal cannulae and permanent T –canulae in the duodenum. Samples of DG were incubated in the rumen for 3, 6, 9, 16, 24, 48 and 96 h. Intestinal CP and AA digestibility were measured on residue from 16 h ruminal incubation of DG. There were significant differences among DG in crude protein (CP), neutral detergent fibre (NDF), nitrogen insoluble in neutral detergent solution (N-NDF) and nitrogen insoluble in acidic detergent solution (N-ADF). The highest proportion of N bound to NDF and ADF was recorded in $DDGS_W$ (26.3% and 28.3%) and $DDGS_T$ (21.2% and 26.1%). The ruminally degradable CP fraction was variable in the range from 48.2 to 71.0% for $DDGS_W$, 52.6 to 70.0% for $DDGS_M$ and 69.4% for WDG, respectively. The content of amino acids was higher in DG from maize than in DG from wheat or triticale. Large differences were observed among the samples in the content of leucine, lysine, histidine and methionine. The lowest content of lysine and essential AA (EAA) was recorded in DDGS from wheat. The large variation in the content of lysine (from 4.6 to 9.5 $g\cdot kg^{-1}$DM), leucine (from 21.8 to 57.0 $g\cdot kg^{-1}$DM) and methionine (from 5.1 to 9.2 $g\cdot kg^{-1}$DM) in bypass CP was observed among samples of DG. Intestinal digestibility of bypass protein and EAA was greater in DDGS from maize ( 95% and 96%, resp.) than in DDGS from wheat (79-91% and 81-93%, resp.) and triticale (89% and 91%, resp.). The lowest and variable intestinal digestibility was determined for lysine (from 77.6 to 91.5% and histidine (from 77.6 to 95.6%) in all tested samples of DG. Protein degradability and digestibility differed greatly among DG sources. This phenomenon needs additional study.

**Key words:** DDGS, nutrients, ruminal CP degradation, amino acid, intestinal digestibility

## INTRODUCTION

Dramatic increase in bioethanol production needs to evaluate systematically the nutritive value of different types of distiller grains. Distiller grains (DG) are available for feeding of dairy cattle. Factors, such as grain (wheat, corn, rye etc.) variability, extent of starch extraction, amount of solubles added, and the extent of drying and temperature may significantly affect the nutrient content and digestibility (Kleinschmit et al., 2007; Berk et al. 2008). Knott et al. (2004) found that the grain fraction was less variable than solubles and the amount of solubles added to the grain fraction had a significant effect on the final DDGS nutrients composition. For cattle feeding it is very important to evaluate the ruminal CP degradability, intestinal digestibility of non-degraded protein and energy content in DDGS.

The objective of this study was to determine some

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nutritional characteristics of maize and wheat DDGS, such as nutrient content, ruminal CP degradation and intestinal amino acid digestibility.

## MATERIAL AND METHODS

Ten samples of bioethanol co-product (4 $DDGS_M$ and 1 WDG from maize, 4 $DDGS_W$ from wheat, 1 $DDGS_T$ from triticale) were milled on 3 mm screen for *in situ* and 1 mm screen for chemical analysis. Samples were analysed for dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) according to the Decree of the Ministry of Agriculture of the Slovak Republic No. 1497/4/1997-100. The neutral (N-NDF) and acid insoluble nitrogen (N-ADF) were determined according to Licitra et al. (1996).

Effective CP degradation and degradation parameters (**a**- soluble CP fraction, **b**- ruminally, degradable CP fraction, **c**- rate of degradation) were determined by the *in sacco* method. Three rumen fistulated cows (fed twice a day with a diet consisting of 70% forage and 30% concentrate on dry matter basis) were used for 3, 6, 9, 16, 24, 48 h rumen incubation of feed samples (with a minimum of three bags per animal, incubation and feed). The washing looses were determined also. The parameters of CP degradation and effective degradation were calculated using the Neway progamme based on the equations described by Řrskov and McDonald (1979). For the calculation of effective CP degradation an outflow rate of 0.06 per h was used.

Three cows with permanent T–canulae in the duodenum behind the pancreas were used for mobile bag method. The undegraded freeze dried residues after 16 h ruminal incubation were weighted into small bags of 2.5 x 3.0 cm (made from Uhelon with the pore size 47µ), incubated in pepsin-HCl solution (30 min at 39 °C), and inserted into the duodenum (Straalen and Huisman,1991).

The results were evaluated by a one-way analysis of variance and significant differences were declared at P<0.01 and P<0.05 using Tuckey T - test. The statistical analyses were performed using SPSS for Windows, Release 6, Copyright c SPSS, Inc. 1989-1993 (licensed for RIPP, Piešťany, Slovakia).

## RESULTS AND DISCUSION

The nutrient content of DDGS varies among DDGS sources (Spiehs et al., 2002). The content of selected nutrients differs significantly among individual types of DG (Table 1). The differences (P<0.05) in CP content were noted between distiller grains from maize and from wheat and triticale. Higher difference was observed between $WDG_M$ and the other values in NDF content. The highest ADF was measured in DDGS from triticale. The results of neutral and acid insoluble nitrogen showed the effect of drying process, because only the proportion of N bound to NDF and ADF was much lower in $WDG_M$ (12.2% and 8.7%, resp.). The highest N bound to NDF and ADF was in DDGS from wheat and triticale. Boucher et al. (2009) demonstrated that the heating increased the concentration of N bound to NDF and ADF aproximately twice. Kleinschmit et al. (2007) observed the same tendency. A very high variability was found in N-ADF content among samples of $DDGS_W$ (42.3%).

Amino acid (AA) composition of distiller grains protein is shown in Table 2. Concentration (g AA·kg$^{-1}$ of CP) of essential amino acids (EAA) is higher in DDGS from maize and WDG than in DDGS from wheat and triticale. The content of lysine, which is usually the first limiting AA for milk protein synthesis (Liu et al., 2000; Kleinschmit et al., 2006) ranged from 16.0 to 23.5 g·kg$^{-1}$ of CP in $DDGS_M$ and was more variable in $DDGS_W$ ( from 16.8 to 22.8 g·kg$^{-1}$ of CP). The highest concentration of lysine was measured in WDG. Similar results were demonstrated by Kleinschmit et al. (2007). According to Schwab (1995) the degree of heating has the greatest effect on lysine concentration. Lysine is the first one which is susceptible to heat damage via a Mailard reaction with the ε-amino group. The similar effect of heating was demonstrated in other feeds (Čerešňáková and Sommer, 1979). The second essential AA for dairy cattle is methionine. Its concentration ranged from 12.3 to 23.0 g·kg$^{-1}$ of CP) in all DG samples.

**Table 1: Chemical composition of DDGS from maize, wheat and triticale and WDG from maize**

| Feeds | Nutrient content g/kg DM | | | | | % of total N | |
|---|---|---|---|---|---|---|---|
| | CP | NDF | ADF | N-NDF | N-ADF | N-NDF | N-ADF |
| $DDGS_M$ | 289.9[a] | 383.4[a] | 179.8 | 8.59[a] | 6.33[a] | 18.4[a] | 13.7[a] |
| $DDGS_W$ | 356.9[b] | 367.7[a] | 223.7 | 14.99[b] | 12.14[b] | 26.3[b] | 21.2[b] |
| $DDGS_T$ | 329.0[bc] | 378.6[a] | 261.3 | 14.91[b] | 13.73[b] | 28.3[b] | 26.1[b] |
| WDG | 302.0[d] | 542.4[b] | 209.7 | 5.89[a] | 4.19[b] | 12.2[a] | 8.7[a] |

[a,b,c,d] means in columns with different superscripts differ significantly (P<0.01 and P<0.05)

**Table 2: Amino acid composition of proteins from the samples of individual types of distiller grains**

| AA | $DDGS_M1$ | $DDGS_M2$ | $DDGS_M3$ | $DDGS_M$ 4 | WDG1 | WDG2 | $DDGS_W1$ | $DDGS_W2$ | $DDGS_W3$ | $DDGS_W4$ | $DDGS_T$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | g AA·kg$^{-1}$ CP | | | | | | | | | | |
| EAA | | | | | | | | | | | |
| Thr | 37.2 | 38.2 | 36.8 | 34.0 | 34.8 | 36.4 | 27.8 | 27.3 | 28.9 | 31.8 | 29.8 |
| Val | 49.2 | 46.8 | 42.6 | 47.0 | 49.1 | 49.7 | 33.7 | 39.5 | 42.5 | 42.7 | 45.1 |
| Ileu | 34.6 | 32.8 | 32.2 | 34.5 | 36.5 | 36.3 | 26.2 | 34.3 | 34.4 | 33.4 | 35.4 |
| Leu | 123.5 | 114.8 | 120.5 | 110.6 | 119.8 | 128.9 | 55.5 | 58.0 | 61.8 | 67.7 | 62.0 |
| Tyr | 29.3 | 29.6 | 32.4 | 21.7 | 25.5 | 30.4 | 19.6 | 13.7 | 20.6 | 21.5 | 15.2 |
| Phe | 49.3 | 44.4 | 48.9 | 44.0 | 47.4 | 51.2 | 38.7 | 39.4 | 44.2 | 43.5 | 43.3 |
| His | 30.3 | 27.0 | 27.3 | 24.8 | 26.1 | 26.7 | 19.0 | 17.2 | 19.6 | 18.9 | 19.7 |
| Lys | 23.9 | 19.0 | 21.2 | 23.5 | 27.8 | 26.6 | 16.8 | 17.0 | 19.4 | 22.8 | 12.9 |
| Arg | 45.3 | 44.8 | 42.9 | 41.8 | 44.6 | 43.2 | 33.6 | 29.6 | 41.1 | 40.2 | 36.6 |
| Met | 19.1 | 23.0 | 17.7 | 18.8 | 21.0 | 19.6 | 13.7 | 14.2 | 20.9 | 12.3 | 14.6 |
| Total | 441.8 | 419.5 | 422.5 | 400.7 | 432.6 | 449.0 | 283.2 | 297.9 | 333.2 | 334.7 | 314.6 |
| NeAA | | | | | | | | | | | |
| Cys | 18.6 | 16.9 | 19,3 | 15.7 | 18.2 | 17.8 | 16.3 | 16.4 | 15.4 | 14.1 | 16.7 |
| Asp | 63.0 | 62.3 | 74,7 | 59.2 | 58.8 | 60.4 | 50.9 | 45.2 | 46.4 | 53.0 | 47.2 |
| Ser | 46.5 | 46.3 | 48,8 | 40.3 | 42.6 | 46.2 | 39.3 | 36.1 | 40.1 | 40.6 | 37.8 |
| Glu | 169.0 | 157.6 | 160,3 | 146.6 | 156.2 | 164.0 | 211.4 | 230.6 | 234.5 | 201.2 | 211.0 |
| Pro | 102.0 | 93.9 | 97,9 | 71.5 | 80.0 | 85.9 | 79.5 | 82.8 | 92.7 | 83.1 | 89.3 |
| Gly | 35.5 | 37.2 | 37,9 | 34.6 | 34.6 | 34.0 | 36.1 | 35.2 | 37.3 | 37.1 | 35.6 |
| Ala | 73.0 | 72.4 | 76.3 | 43.0 | 45.2 | 69.1 | 24.8 | 22.4 | 33.4 | 40.0 | 31.2 |
| Total | 507.7 | 491.5 | 515.4 | 410.8 | 435.6 | 477.4 | 458.3 | 468.6 | 499.85 | 469.0 | 469.1 |

AA – amino acid, EAA – essential amino acid, NeAA – non-essential amino acid

**Table 3 Effective CP degradability and degradation parameters of individual types of distiller grains**

| | Crude protein degradability | | | | |
|---|---|---|---|---|---|
| Feeds | c (%) | b (%) | c (%·h$^{-1}$) | Edg $\bar{x}$ | ±se |
| $DDGS_M$ | 19.5-26.4 | 52.6-70.0 | 0.0310-0.054 | 50.6$^a$ | 1.491 |
| $DDGS_W$ | 22.8-51.9 | 48.2-71.0 | 0.028-0.065 | 58.38$^a$ | 5.184 |
| $DDGS_T$ | 38.7 | 61.3 | 0.0497 | 76.8$^b$ | |
| WDG | 18.8 | 69.4 | 0.037 | 46.8$^c$ | |

Edg- effective CP degradability
a- soluble CP fraction,
b - ruminally degradable CP fraction,
c- rate of degradation
$^{a,b,c}$ means in column with the different superscripts differ significantly (P<0.05)

Very high concentration of glutamic acid (201.2 – 234.5 g·kg$^{-1}$ of CP ) in DG samples manifests a high proportion of prolamine and gluteline in cereal proteins.

Waldo and Yu (2009) determined in the experiment *in sacco* and *in vitro* on wheat DDGS lower intermediately degradable CP, higher a rapidly degradable non-protein nitrogen (NPN) fraction and slowly degradable CP fraction than in maize DDGS. This reflected the CP ruminal degradability. Wheat DDGS had a higher *in situ* CP degradability than maize DDGS. The similar results were obtained in our experiment, which are listed in Table 3. Effective CP degradability of $DDGS_M$ was in the range from 47.7 to 53.4% and the highest was for $DDGS_T$ (76.8%). The differences among them were significant (P<0.05 and P<0.01, resp.). Distiller grain made from maize had much lower fast degradable CP fraction than distiller grains made from wheat and triticale.

The AA profile of bypass CP (Table 4) is very important, because it is assumed, that those AA may be absorbed in the small intestine after the digestion. The profile of EAA of the protein in undegraded residues of incubated samples of distiller grains in the rumen for 16 h was changed comparing to the original feeds before incubation. Concentration of total EAA and mainly concentration of lysine decreased considerable in maize DDGS and WDG and wheat DDGS 1 and 2. Decrease in EAA concentration (mainly lysine) and slight increase in non-essential AA (NeAA) was also observed by others (Kleinschmit et al., 2007; Boucher et al., 2009).

Intestinal digestibility of by-pass EAA and NeAA was variable for the DG sample. The digestibility of EAA was lower than of NeAA in DG (Tables 4 and 5). Among all, the digestibility

**Table 4: AA contents in by-pass CP and intestinal digestibility of AA in samples of individual types of distillers grains**

| | $DDGS_M$ | | WDG | $DDGS_W$ | | | | $DDGS_T$ | $DDGS_M$ | | WDG | $DDGS_W$ | | | | $DDGS_T$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 1 | 2 | 3 | 4 | 1 | 1 | 2 | 1 | 1 | 2 | 3 | 4 | 1 |
| | $g.kg^{-1}DM$ | | | | | | | | Intestinal digestibility (%) | | | | | | | |
| CP | 43.8 | 43.2 | 45.8 | 51.6 | 37.0 | 32.5 | 33.1 | 44.9 | 94.7 | 95.4 | 95.3 | 88.1 | 79.5 | 88.6 | 91.4 | 88.9 |
| EAA | | | | | | | | | | | | | | | | |
| Thr | 15.3 | 14.7 | 15.8 | 14.8 | 10.6 | 8.8 | 10.1 | 14.8 | 93.8 | 91.9 | 92.9 | 80.2 | 87.7 | 87.3 | 90.0 | 89.3 |
| Val | 21.4 | 21.4 | 22.1 | 25.1 | 18.0 | 15.8 | 17.1 | 22.5 | 96.1 | 94.8 | 96.1 | 80.1 | 88.8 | 90.9 | 92.9 | 90.9 |
| Ileu | 16.3 | 16.6 | 17.5 | 20.5 | 15.0 | 12.8 | 13.4 | 17.8 | 96.7 | 95.6 | 96.9 | 82.2 | 89.5 | 91.3 | 93.2 | 90.8 |
| Leu | 57.0 | 56.8 | 61.4 | 35.6 | 24.4 | 21.8 | 29.4 | 32.7 | 98.1 | 97.1 | 98.1 | 82.3 | 89.5 | 91.0 | 94.2 | 90.9 |
| Tyr | 14.6 | 15.9 | 15.2 | 11.3 | 8.2 | 6.6 | 7.7 | 10.8 | 96.7 | 96.2 | 96.5 | 91.8 | 88.4 | 88.6 | 92.9 | 91.6 |
| Phe | 22.1 | 22.3 | 23.6 | 22.1 | 15.4 | 13.2 | 14.8 | 21.2 | 97.7 | 96.8 | 97.5 | 98.9 | 90.9 | 91.5 | 99.9 | 91.8 |
| His | 10.6 | 10.3 | 11.0 | 9.6 | 7.1 | 6.5 | 7.0 | 11.0 | 95.6 | 94.3 | 94.8 | 77.7 | 88.1 | 89.2 | 91.9 | 87.5 |
| Lys | 8.3 | 7.4 | 9.5 | 4.6 | 5.1 | 6.4 | 7.1 | 5.7 | 91.5 | 90.3 | 91.5 | 77.6 | 86.7 | 85.4 | 89.1 | 88.9 |
| Arg | 16.3 | 15.9 | 16.9 | 16.3 | 14.6 | 12.5 | 12.6 | 15.6 | 96.0 | 94.6 | 95.3 | 80.1 | 90.2 | 88.2 | 91.6 | 92.3 |
| Met | 8.5 | 9.0 | 9.2 | 8.6 | 6.1 | 5.1 | 5.9 | 7.4 | 97.2 | 96.1 | 97.2 | 81.4 | 88.6 | 89.9 | 93.4 | 90.9 |
| Total | 190.3 | 190.4 | 201.2 | 168.4 | 124.4 | 109.5 | 125.0 | 159.5 | 96.6 | 95.5 | 96.4 | 81.2 | 89.2 | 89.8 | 92.8 | 90.8 |
| NeAA | | | | | | | | | | | | | | | | |
| Cys | 7.0 | 7.1 | 7.6 | 9.6 | 7.0 | 6.1 | 6.0 | 8.8 | 95.5 | 94.1 | 94.7 | 79.6 | 87.0 | 86.6 | 90.7 | 87.4 |
| Asp | 26.1 | 24.8 | 26.1 | 23.8 | 19.3 | 15.2 | 17.5 | 22.4 | 95.9 | 94.3 | 95.7 | 76.9 | 86.2 | 86.6 | 90.2 | 87.5 |
| Ser | 19.9 | 18.8 | 19.9 | 19.7 | 12.6 | 11.1 | 11.8 | 19.1 | 97.3 | 95.7 | 96.2 | 83.9 | 89.4 | 88.6 | 92.2 | 90.7 |
| Glu | 74.9 | 74.6 | 80.8 | 123.3 | 77.5 | 72.9 | 66.1 | 91.1 | 98.1 | 97.0 | 98.0 | 85.5 | 92.0 | 94.8 | 96.1 | 93.3 |
| Pro | 42.2 | 41.3 | 14.3 | 50.5 | 31.1 | 31.2 | 32.8 | 39.3 | 97.3 | 96.5 | 96.8 | 86.5 | 93.0 | 96.1 | 96.8 | 94.9 |
| Gly | 14.0 | 13.9 | 32.9 | 17.7 | 13.8 | 11.3 | 11.0 | 16.1 | 92.4 | 91.2 | 91.5 | 72.9 | 84.2 | 82.1 | 87.0 | 86.7 |
| Ala | 31.1 | 31.1 | 22.1 | 17.3 | 13.7 | 10.8 | 15.3 | 17.5 | 97.6 | 96.4 | 97.5 | 76.9 | 86.1 | 87.2 | 92.5 | 89.0 |
| Total | 215.2 | 192.8 | 228.2 | 261.9 | 175.1 | 158.5 | 160.7 | 214.4 | 97.1 | 95.5 | 96.8 | 82.9 | 90.4 | 91.9 | 94.1 | 91.7 |

of lysine is the lowest and variable for distiller grain samples. Lysine digestibility is lower than total AA digestibility and generally is the least digestible AA within the DDGS (Boucher et al., 2009). Decrease in lysine digestibility is expectedly a result of the Maillard reaction (Mauron, 1990). Estimates of by-pass lysine digestibility were much lower than digestibility of the other AA. The range was from 77.6% for $DDGS_W$1 to 91.5% for $DDGS_M$1 and WDG. The digestibility of lysine and all AA was generally lower ($P<0.05$) from wheat DDGS than maize DDGS. Histidine and methionine, as a part of EAA, had also lower digestibility. The results show the need to estimate lysine digestibility in distiller grain samples, because digestibility of lysine is lower than digestibility of total bypass CP and varies widely among samples.

## CONCLUSION

In this study, lower CP degradability and a very good bypass CP and amino acids intestinal digestibility were demonstrated in distiller grains, which are an excellent source of CP for dairy cattle. Since distiller grains are a by-product of various technologies of bioethanol production, our results present large variation in degradable part of CP and intestinal digestibility of amino acids. Lysine, as the essential amino acid for milk protein synthesis, showed its sensibility to drying process, which can decrease lysine intestinal digestibility. The great variability among distiller grain sources indicates the need for estimating not only the content of CP and CP degradability but also bypass amino acid intestinal digestibility.

**Table 5: Comparison of CP, lysine, methionine, EAA and NeAA among samples of individual types of distiller grains**

| AA | $DDGS_M$ | | WDG | $DDGS_W$ | | | | $DDGS_T$ |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | | 1 | 2 | 3 | 4 | |
| | mean [1] | | mean[2] | mean[3] | | | | mean[4] |
| In intact feed | | | | g AA·kg CP | | | | |
| Lys | 21.9$^{a}$ | | 27.2$^{b}$ | 19.0$^{a}$ | | | | 12.9$^{c}$ |
| Met | 19.7$^{a}$ | | 20.3$^{b}$ | 15.3$^{ac}$ | | | | 14.6$^{c}$ |
| EAA | 421.1$^{a}$ | | 440.8$^{a}$ | 312.2$^{bc}$ | | | | 314.6$^{c}$ |
| NeAA | 481.3$^{a}$ | | 456.5$^{a}$ | 473.9$^{a}$ | | | | 469.1$^{a}$ |
| In bypass CP | $g \cdot kg^{-1}$DM | | | | | | | |
| CP | 43.8$^{a}$ | 43.2$^{a}$ | 45.8$^{b}$ | 51.6$^{c}$ | 37.0$^{d}$ | 32.5$^{ef}$ | 33.1$^{f}$ | 44.9$^{ab}$ |
| Lys | 8.3$^{a}$ | 7.4$^{b}$ | 9.5 $^{bc}$ | 4.6$^{de}$ | 5.1$^{e}$ | 6.4 $^{df}$ | 7.1$^{abg}$ | 5.7$^{bdeg}$ |
| Met | 8.5$^{a}$ | 9.0$^{a}$ | 9.2$^{a}$ | 8.6$^{a}$ | 6.1$^{b}$ | 5.1$^{c}$ | 5.9$^{cd}$ | 7.4$^{abc}$ |
| EAA | 190.3$^{a}$ | 190.4$^{b}$ | 201.2$^{c}$ | 168.4$^{d}$ | 124.4$^{eg}$ | 109.5$^{f}$ | 125.0$^{g}$ | 159.5$^{dh}$ |
| NeAA | 215.2$^{a}$ | 192.8$^{b}$ | 228.2$^{c}$ | 261.9$^{d}$ | 175.1$^{e}$ | 160.7$^{fg}$ | 160.7$^{g}$ | 214.4$^{a}$ |
| Digestibility | % | | | | | | | |
| CP | 94.7$^{a}$ | 95.4$^{a}$ | 95.3$^{a}$ | 88.1$^{bdf}$ | 79.5$^{c}$ | 88.6$^{d}$ | 91.4$^{e}$ | 88.9$^{f}$ |
| Lys | 91.5$^{a}$ | 90.3$^{b}$ | 91.5$^{a}$ | 77.6$^{c}$ | 86.7$^{de}$ | 85.4$^{e}$ | 89.1$^{fg}$ | 88.9$^{g}$ |
| Met | 97.2$^{a}$ | 96.1$^{b}$ | 97.2$^{a}$ | 81.4$^{c}$ | 88.6$^{d}$ | 89.9$^{e}$ | 93.4$^{f}$ | 90.9$^{g}$ |
| EAA | 96.6$^{a}$ | 95.5$^{b}$ | 96.4$^{a}$ | 81.2$^{c}$ | 89.2$^{de}$ | 89.8$^{e}$ | 92.8$^{f}$ | 90.8$^{g}$ |
| NeAA | 97.1$^{a}$ | 95.5$^{b}$ | 96.8$^{c}$ | 82.9$^{d}$ | 90.4$^{e}$ | 91.9$^{fh}$ | 94.1$^{g}$ | 91.7$^{h}$ |

[1]mean of 4 samples, [2]mean of 2 samples, [3]mean of 4 samples, [4]mean of 2 samples,
$^{a,b,c,d,e,f,g,h}$ means in rows with different superscripts differ significantly ($P<0.05$)

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