

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

M. CHRENKOVÁ*, Z. ČEREŠŇÁKOVÁ, M. POLÁČIKOVÁ, Z. FORMELOVÁ, A. TOMÍKOVÁ, K. ZAUJEC

Animal Production Research Centre Nitra, Slovak Republic;

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·kg⁻¹DM), leucine (from 21.8 to 57.0 g·kg⁻¹DM) and methionine (from 5.1 to 9.2 g·kg⁻¹DM) 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

*Correspondence: E-mail: chrenkova@cvzv.sk
Mária Chrenková, Animal Production Research Centre Nitra,
951 41 Lužianky, Slovak Republic,
Tel.: +421 37 6546 217 Fax: +421 37 6546 418

Received: March 21, 2010

Accepted: April 21, 2010

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 losses were determined also. The parameters of CP degradation and effective degradation were calculated using the Neway programme based on the equations described by Rrskov 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 DISCUSSION

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 approximately 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 _M 1	DDGS _M 2	DDGS _M 3	DDGS _M 4	WDG1	WDG2	DDGS _W 1	DDGS _W 2	DDGS _W 3	DDGS _W 4	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

Feeds	Crude protein degradability			Edg	
	c (%)	b (%)	c (%·h ⁻¹)	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_w1 to 91.5% for DDGS_M1 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 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·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 ^c	89.1 ^{fg}	88.9 ^g
Met	97.2 ^a	96.1 ^b	97.2 ^a	81.4 ^c	88.6 ^d	89.9 ^c	93.4 ^f	90.9 ^g
EAA	96.6 ^a	95.5 ^b	96.4 ^a	81.2 ^c	89.2 ^{de}	89.8 ^c	92.8 ^f	90.8 ^g
NeAA	97.1 ^a	95.5 ^b	96.8 ^c	82.9 ^d	90.4 ^c	91.9 ^{fh}	94.1 ^g	91.7 ^h

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

REFERENCES

- BERK, A. – LEBZIEN, P. – FLACHOWSKY, G. 2008. Influence of use of by-products from bio-fuel production in feeds for growing-finishing pigs, In: EAAP – 59th Annual Meeting, Vilnius, August 2008, p. 265. ISBN 978-90-8686-074-6
- BOUCHER, S. E. – CALSAMIGLIA, S. – PARSON, C. M. – STEIN, H. H. – STERN, M. D. – ERICKSON, P. S. – UTTERBACK, P. L. – CHWAB, C. G. 2009. Intestinal digestibility of amino acids in rumen-undegraded protein estimated using a precision-fed caecotomised rooster bioassay: II. Distillers dried grains with solubles and fish meal. *J. Dairy Sci.*, vol. 92, 2009, p. 6056-6067.
- ČEREŠŇÁKOVÁ, Z. – SOMMER A. 1979. Untersuchungen zu Veränderungen der N-Fractionen von physikalisch und chemisch behandelten Eiweissfuttermitteln unter in vitro-Bedingungen. I. Mitt. Einfluss der Futtermittelbehandlung mit Wärme und mit Formaldehyd auf die Änderung der Stickstofflöslichkeit und den Lysinverlust. *Arch. Tierernähr.*, vol. 29, 1979, p. 305-309.
- GROFÍK, R. – FLAK, P. 1990. Statistical Methods in Agriculture. *Príroda [Nature] : Bratislava [Slovakia]*, 1990, pp. 344. ISBN 80-07-00018-6
- KLEINSCHMIT, D. H. – SCHINGOETHE, D. J. – KALSCHUR, K. F. – HIPPEN, A. R. 2006. Evaluation of various sources of corn distillers dried grains plus solubles for lactating dairy cattle. *J. Dairy Sci.*, vol. 89, 2006, p. 4784-4794.
- KLEINSCHMIT, D. H. – ANDERSON, J. L. – SCHINGOETHE, D. J. – KALSCHUR, K. F. – HIPPEN, A. R. 2007. Ruminant and intestinal degradability of distillers grains plus soluble varies by sources. *J. Dairy Sci.*, vol. 90, 2007, p. 2909-2918.
- KNOTT, J. – SHURSON, G. C. – GOIHL, J. 2004. Effects of the nutrient variability of distiller's solubles and grains within ethanol plants and the amount of distiller's solubles blended with distiller's grains on fat, protein and phosphorus content of DDGS. Available: <http://www.ddgs.umn.edu/articles-proc-storage-quality/2004-Knott-%20Nutrient%20variability.pdf>. Accessed: June, 2006.
- LICITRA, G. – HERNANDEZ, T. M. – VAN SOEST, P. J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.*, vol. 57, 1996, p. 347-358.
- LIU, C. – SCHINGOETHE, D. J. – STEGEMAN, G. A. 2000. Corn distillers grains versus a blend of protein supplements with or without ruminally protected amino acids for lactating cows. *J. Dairy Sci.*, vol. 83, 2000, p. 2075-2084.
- MAURON, J. 1990. Influence of processing on protein quality. *J. Nutr. Sci. Vitaminol. (Tokyo)*, vol. 36, p. 57-69.
- ØRSKOV, E. R. – MCDONALD, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J.*

- Agr. Sci.*, vol. 92, 1979, p. 499-503.
- SCHWAB, C. G. 1995. Protected proteins and amino acids for ruminants. In *Biotechnology in Animal Feeds and Animal Feeding*. R. J. Wallace and A. Chesson, ed., Weinheim, Germany : VCH Press, 1995, p. 115-141. ISBN 978-35-2730-065-5
- SPIEHS, M. J. – WHITNEY, M. H. – SHURSON, G. C. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *J. Anim. Sci.*, vol. 80, 2002, p. 2639-2645.
- SPSS for Windows Advanced Statistics Release 6.0 (Paperback), 1993. Publ. Prentice-Hall July 1. 1993, pp. 578, ISBN-10: 013178823X
- VAN STRAALLEN, W. M. – HUISMAN, G. 1991. The digestibility of bypass crude protein from grass silage in the intestine of dairy cows. In: *Intern rapport IVVO no. 317*, 1991. Lelystad, Netherland : Research Institute for Livestock Feeding and Nutrition.
- WALDO, G. N. O. – YU, P. 2009. Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. *J. Sci. Food Agric.*, vol. 89, 2009, p.1754-1761.