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EFFECT OF BETAINE SUPPLEMENTATION AND BREED ON THE FATTY ACID COMPOSITION AND ADIPOCYTE SIZE AND DISTRIBUTION IN BACKFAT AND MUSCLE LIPIDS IN PIGS

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A comparison was made between pigs from the Danube white breed (DW) and the cross breed Danube white × Landrace (DW × L) to study the effect of betaine supplementation and breed on the fatty acid composition of the triacylglycerols in the backfat and m. longissimus dorsi, as well as the adipocyte size and distribution in these depots. The fatty acid composition was mostly influenced by the animal breed, compared to the betaine supplementation. Pigs from DW × L showed significantly lower amounts of 18:0 (P<0.01) in the backfat and 14:0 in the muscle triacylglicerols (P<0.01) than DW. The proportion of 16:0 was influenced by both betaine (P<0.05) and breed (P<0.01) in muscle triacylglycerols. Breed affected significantly the content of monounsaturated fatty acids in the backfat as the pigs of DW breed had lower amounts of 16:1 (P<0.05) and 18:1 (P<0.01), but the content of the latter was dependent on the presence of betaine as well (P<0.05). After betaine supplementation the average size of the adipocytes in the backfat was lower in DW and higher in DW × L (P<0.05). Compared to the DW the crossbred pigs had significantly higher diameter of the adipose cells (P<0.001). Breed influence on the adipocytes distribution in backfat and two of the cell populations in the muscle was highly significant (P<0.001). Dietary betaine supplementation affected only one cell population for both backfat and longissimus muscle (P<0.05).

Key words: pigs; betaine; breed; fatty acids; adipocytes

ЕФЕКТ НА РАСАТА И НА ДОДАВАЊЕ БЕТАИН ВО ХРАНАТА ВРЗ СОСТАВОТ НА МАСНИТЕ КИСЕЛИНИ, ГОЛЕМИНАТА НА АДИПОЦИТИТЕ И РАСПРЕДЕЛБАТА НА МАСТИТЕ ВО ГРБОТ И МУСКУЛНИТЕ ЛИПИДИ КАЈ СВИЊИТЕ

Направена е споредба помеѓу свињи од расата бела дунавска (БД) и мелези помеѓу бела дунавска и ландрас (БД × Л) со цел да се проучи ефектот од додавање бетаин во храната врз составот на масната киселина на триацилглицеролите во грбните масти и во мускулот longissimus dorsi, како и големината на адипоцитите и нивната распределба во овие депоа. Врз составот на масните киселини најмногу влијаеше расата, во споредба со додавањето на бетаинот. Свињите од БД × Л покажаа значително пониски количини од 18:0 (P<0,01) во грбните масти и 14:0 во мускулните триацилглицероли (P<0,01) во споредба со БД. На соодносот 16:0 во мускулните триацилглицероли влијаеше бетаинот (P<0,05) и расата (P<0,01). Расата влијаеше значително врз содржината на мононезаситените масни киселини во грбните масти, затоа што свињите од БД имаа помали количини од 16:1 (P<0,05) и 18:1 (P<0,01), но содржината кај вторите беше зависна и од присутноста на бетаинот (P<0,05). По додавањето на бетаинот просечната големина на адипоцитите во грбните масти беше пониска кај БД и повисока кај БД × Л (P<0,05). Во споредба со БД, мелезите имаа значително поголем дијаметар на масните клетки (P<0,001). Влијанието на расата врз распределбата на адипоцитите во грбните масти беше од исклучително значење (P<0,001). Додавањето на бетаин во храната влијаеше само врз една популација клетки: за грбните масти и за мускулот longissimus dorsi (P<0,05).

Клучни зборови: свињи; бетаин; раса; масни киселини; адипоцити

1. INTRODUCTION

Betaine is a trimethyl derivative of the amino-acid glycine, widely spread in nature. In living organisms betaine functions as an organic osmoprotectant (Clarke et al., 1994). Furthermore the availability of three chemically active methil groups in its molecule allows it to act as a methyl donor via transmethilation, thus partially reducing the requirements for other methyl donors such as methionine and choline and participating in the protein and lipid metabolism (Kidd et al., 1997; Simon et al., 1999; Huang et al., 2006).

Betaine is commercially available as an additive in animal feed, since it is a by-product of sugarbeet processing. As one of the main objects of meat production is to decrease fat content, the use of betaine in the swine diets has increased since several studies reported decrease in the backfat thickness and increase of the lean meat percentage (Casarin et al., 1997; Matthews et al., 2001a, Lawrence et al., 2002) and hence presumed that betaine could be used as a carcass modifier. On the other hand fat content of carcass and meat depends on endogenous factors such as animal genetic. As a result of animal selection for carcass fat content mostly in pigs there are breeds, crossbreeds and hybrids with decreased backfat and high carcass quality.

Feed additives and breed characteristics of the animals could as well influence the fat composition by affecting the fatty acid synthesis in both adypose and muscle tissue (Vernon and Flint, 1988). The ways of modifying the fatty acid composition of meat have been of much interest (Wood and Enser, 1997; Nürnberg et al., 1998; Jakobsen, 1999; Lauridsen et al., 1999b; Lauridsen et al., 1999c), since it is known that meat is an important source of fat in the human diet and particularly of saturated fatty acids that are related to cardiovascular diseases and diabetes.

The aim of this study is to examine the effect of betaine supplemetation and breed on the fatty acid composition and adipocyte size and distribution of backfat and muscle lipids in pigs.

2. MATERIAL AND METHODS

The object of the study was 20 male castrated pigs – 10 from the Danube white breed (DW) and 10- crossbred Danube white × Landrace (DW × L) with a mean initial body weight of 75.11 ± 6.31 kg. The pigs from each genetic population were divided into 2 groups – control and experimental. Both groups received concentrate (CP 13.71%; ME 12.21 MJ/kg) and vitamin/mineral mixture as the diet of the experimental group was additionally supplemented with 0.1 % betaine for a period of 30 days before slaughter. At the end of the trial the animals were slaughtered at a mean body weight of 91.7 kg.

Samples for fatty acid and histological analyses were taken from the upper backfat layer and *m. longissimus dorsi* (m.LD).

Lipids were extracted from the adipose and muscle tissues according to the method of Bligh and Dyer (1959). The triacylglycerols were isolated by preparative thin layer chromatography on silicagel G in a system of solvents: hexane: diethyl ether: acetic acid, 80:20:1; v/v. Methyl esthers of triacyglycerols were prepared using 0.01 % sulphuric acid in dry methanol at 47 °C for 14 h as described by Christie (1973). The fatty acid composition was determined using gas chromatograph Carlo Erba equipped with a capillary column (DB-WAX, 30 m length, 0.32 mm internal diameter and 0.25 μm film thickness) and hydrogen as a carrier gas. The output from the flame ionization detector was quantified by a computer integrator (Spectra Physics 4100). Fatty acids methyl esthers were identified by comparing the retention times of the standards and were expressed as percentages of the total fatty acid methyl esthers.

For determination of adipocyte size samples of 1 cm^3 were taken from the upper backfat layer and m. LD. Microscope cuts from muscle (10 μm thickness) and adipose (16 μm thickness) tissues were made using microtome Cryo-cut. The cuts were dyed by Sudan III and their mean diameter was determined in 3–5 visible fields on 100 cells/sample, using an eyepiece micrometer.

The data were analyzed by least squares procedures (JMP7, 2007). The mathematical model included fixed effects due to treatment (control vs. betaine supplementation) and breed. The interaction was included where significant ($P<0.05$).

3. RESULTS AND DISCUSSION

Betaine supplementation had little influence on the fatty acid composition of the upper backfat layer of the pigs from the two genotypes (Table 1) as it increased the proportion of 18:2 ($P<0.05$) and hence the total amount of PUFA (P<0.05). Since 18:2 could not be synthesized in the organism the higher amount in the experimental groups could be explained by their increased daily consumption as reported by Vasileva et al.(2008).

Table 1

Fatty acid composition of triacylglycerols in the upper backfat layer in pigs from the Danube white and Danube white × Landrace

Fatty acids, %	Backfat				S.E	Significance		
	DW		DW × L			Treatment	Breed	Interaction
	Control	Experimental	Control	Experimental				
14:0	1.50	1.51	1.51	1.51	0.16	NS	NS	NS
16:0	24.70	23.95	23.62	23.44	1.04	NS	NS	NS
16:1	3.13	2.94	3.45	3.37	0.38	NS	*	NS
18:0	10.58	10.87	9.79	9.49	0.79	NS	**	NS
18:1	46.62	44.82	47.29	47.74	1.14	NS	**	*
18:2	13.03	15.41	13.84	14.04	1.24	*	NS	NS
18:3	0.44	0.51	0.51	0.42	0.08	NS	NS	*
PUFA	13.47	15.92	14.35	14.46	1.28	*	NS	NS
MUFA	49.75	47.76	50.74	51.10	1.16	NS	**	*
PUFA/SFA	0.27	0.33	0.28	0.28	0.02	*	NS	*
SFA	36.78	36.32	34.91	34.44	1.66	NS	*	NS
UFA	63.22	63.68	65.09	65.56	1.66	NS	*	NS
SFA/UFA	1.72	1.76	1.87	1.91	0.13	NS	*	NS
Stearoyl CoA desaturase index[1]	0.58	0.57	0.60	0.60	0.01	NS	**	NS
Thioesterase index[2]	16.46	15.86	15.64	15.52	1.40	NS	NS	NS
Elongase index[3]	0.42	0.45	0.41	0.40	0.03	NS	*	NS

[1] Calculated as (C16:1+C18:1)/(C16:1+C16:0+C18:1+C18:0)
[2] Calculated as 16:0/14:0
[3] Calculated as 18:0/16:0
* – P<0.05; ** – P<0.01; NS – no significance

The genotype of the animals had more pronounced impact on the fatty acid compostion in the backfat. The proportion of the major saturated fatty acid (16:0) in the backfat ranged from 23.44 to 24.70 % and was not influenced by betaine supplementation or breed. Changes were observed in the content of 18:0 as the pigs from the Danube white exhibited higher proportions (P<0.01) which is the reason for the significant difference (P<0.05) in the total saturated fatty acid amount between the pure and the crossbred animals. Breed influence was found in the content of monounsaturated fatty acids. Higher content of both 16:1 (P<0.05) and 18:1 (P<0.01) was observed in pigs from the Danube white × Landrace, as the content of the latter depended on the betaine supplementation as well (P<0.05). Hence the significant effect (P<0.05) of the breed on the total amount of the unsaturated fatty acids could be attributed to the difference of the content in the monounsaturated fatty acids between the pigs from the two genotypes.

Stearoyl-CoA desaturase catalyzes the conversion of the saturated 16:0 and 18:0 to 16:1 and 18:1, which are the major monounsaturated fatty acids in the pork lipids (Warnants et al., 1996). Its activity, described by the SCD index corresponds to the changes in the above mentioned fatty acids and is significantly higher in the pigs from the Danube white × Landrace (P<0.01) and indicates breed dependence.

Breed had significant impact on the elongase index, described by the ratio 18:0/16:0 as it was higher for the crossbred animals. The main site for fatty acid elongation is the endoplasmic reticulum membrane, though it occurs in the mitochondria and microsomal membranes as well (Zhang et al., 2007). Generally, fatty acyl-CoA substrates in the range of C10-C14 are used by the mitochondrial elongation system, whereas microsomal elongases act on C16 and longer fatty acids (Harwood, 1994).

Table 2

Fatty acid composition of triacylglycerols in m. longissimus dorsi in pigs from the Danube white and Danube white × Landrace

Fatty acids, %	m.LD				S.E	Significance		
	DW		DW × L			Treatment	Breed	Inter action
	Control	Experimental	Control	Experimental				
14:0	1.72	1.83	1.54	1.55	0.14	NS	**	NS
16:0	25.84	25.23	24.95	23.68	0.8	*	**	NS
16:1	5.26	5.26	5.37	5.40	0.42	NS	NS	NS
18:0	9.43	9.66	9.81	9.00	0.66	NS	NS	NS
18:1	54.45	53.88	54.35	55.52	0.83	NS	NS	*
18:2	3.20	4.02	3.86	4.68	0.93	NS	NS	NS
18:3	0.10	0.12	0.16	0.17	0.05	NS	NS	NS
PUFA	3.30	4.14	3.99	4.85	0.95	NS	*	NS
MUFA	59.71	59.14	59.71	60.93	1.02	NS	*	NS
PUFA/SFA	0.06	0.07	0.07	0.08	0.01	NS	NS	NS
SFA	36.99	36.72	36.30	34.23	1.26	NS	NS	NS
UFA	63.01	63.28	63.70	65.77	1.26	NS	NS	NS
SFA/UFA	1.70	1.73	1.76	1.93	0.09	NS	NS	NS
Stearoyl CoA desaturase index[1]	0.63	0.63	0.63	0.65	0.01	NS	***	NS
Thioesterase index[2]	15.02	13.78	16.20	15.27	1.19	*	*	NS
Elongase index[3]	0.36	0.38	0.39	0.38	0.02	NS	NS	NS

[1] Calculated as (16:1+18:1)/(16:1+16:0+18:1+18:0)
[2] Calculated as 16:0/14:0
[3] Calculated as 18:0/16:0
* – P<0.05; ** – P<0.01; *** – P<0.001, NS – no significance

The difference in fatty acid composition of muscle triacylglycerols (Table 2) was due mainly to breed as it was found in the adipose tissue.

The Danube white pigs had significantly higher proportions of 14:0 and 16:0 (P<0.01). The content of the latter was lower in betaine supplemented groups of both Danube white and Danube White × Landrace (P<0.05).

The individual proportions of mono- and polyunsaturated fatty acids were not influenced by treatment or breed whereas their total amounts were significantly higher in crossbred animals (P<0.05).

Stearoyl-CoA desaturase activity was higher (P<0.001) in the Danube white × Landrace similarly to the backfat. Breed influenced significantly the index of thioestherase activity described as the ratio between 16:0 and 14:0. Thioesterase in the fatty acid synthase complex is responsible for terminating the cycles of fatty acid synthesis and release of the newly synthesized fatty acid. The enzyme has for substrates both C14-acyl ACP and C16-acyl ACP, as 16:0 is the major product. The ratio of 16:0 to 14:0 was utilized to reflect the selective cleavage of thioesterase on C14-acyl ACP or C16-acyl ACP as the greater the thioesterase index, the less cleavage of C14-acyl ACP. Thioesterase index is influenced by betaine supplementation as it is lower for the experimental groups (P<0.05).

As it was seen most changes in the fatty acid composition in both backfat and muscle were mainly attributable to the genetic difference between the pigs compared to the betaine supplementation. In contrast Fernandez-Figarez et al. (2008) observed lower percentage of 16:0 and higher of 18:1 in backfat of pigs received 0.5% betaine. Other studies (Hur et al., 2007) reported a decrease in the quantities of 14:0 and 18:2 and increase in 18:0 in the longissimus muscle in pigs in response to betaine supplementation. The lack to observe stronger influence of betaine on the fatty acid composition in both backfat and muscle may be due to the lower concentration of the additive as well as the duration of the trial compared to other experiments.

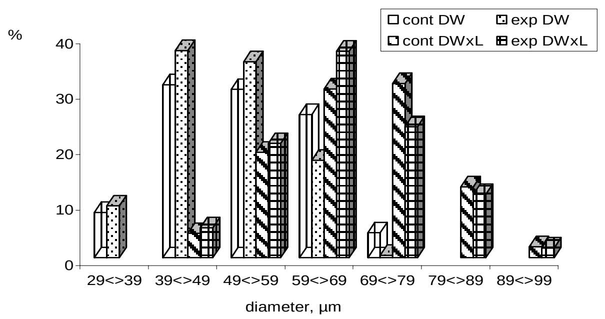
The results in Table 3 show significant influence of both factors on the average size of the adipocytes in the upper backfat layer. Betaine supplementation decreased the average diameter of the fat cells in the Danube white pigs which corresponded to the decresed thickness of the backfat in the same location as reported by Vasileva et al. (2008). The pigs from the Danube white × Landrace cross had significantly larger adipose cells when compared to the Danube white (P<0.001). This corresponded to the highy significant influence of the breed on the adipocyte distribution in the backfat layer (Fig. 1).

Table 3

Average size of the adipocytes in the backfat and m. longissimus dorsi in pigs from the Danube white and Danube white × Landrace

Average size of the adipocytes, µm	DW		DW × L		S.E	Significance		
	Control	Experiemental	Control	Experimental		Treatment	Breed	Interaction
Backfat	52.26	49.73	69.32	71.01	2.87	*	***	NS
m.LD	40.43	47.36	46.38	44.99	5.93	NS	NS	NS

* – P<0.05; *** – P<0.001; NS – no significance



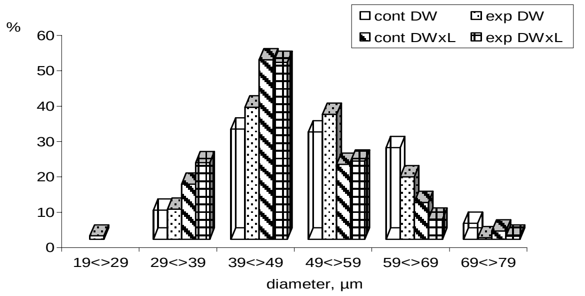
Cell population with size	Significance		
	Treatment	Breed	Interaction
39 µm <>49 µm	NS	***	NS
49 µm <>59 µm	NS	*	NS
59 µm <>69 µm	NS	***	*
69 µm <>79 µm	*	***	NS

Fig. 1. Adipocyte size distribution in the upper backfat layer

In the Danube white pigs the cell population with size up to 59 µm made a significant part. and is 70% and 80 % for the control and betaine supplemented groups respectively. The proportion of the adipocytes with a larger size (between 59–99 µm) was significantly higher in the pigs from the Danube white × Landrace compared to the Danube white. For the crossbred pigs the biggest relative part was formed by the adipocytes at the size of 49–79 µm. as it is 81% for both control and experimental pigs.

The population of the adipocytes at the size of 69–79 µm in the backfat layer was significantly influenced by the betaine supplementation as it was lower in the experimental groups for the pigs of the two genotypes (P<0.05). This could indicate changes in the lipogenesis in response to betaine since Huang et al. (2008) reported decreased activities of lipogenic enzymes in betain supplemented pigs. The larger number of adipose cells with a higher size in the backfat of the Danube white × Landrace pigs corresponds to the higher proportions of monounsaturated C16:1 and C18:1 and SCD index in the crossbred pigs. The amount of SCD is known to specifically affect the adipocyte size in the fat depots (Barber et al., 2000; Daniel et al., 2004).

Breed had a significant influence on the adipocyte distribution in m.LD as well (Fig 2). Intramuscular fat in pigs from the Danube white had a smaller relative part of the cell populations at the size of 29-49 µm and bigger of the formations at the size of 49-69 µm compared to the Danube white × Landrace. Betaine supplementation decreased significantly the group of adipocytes at the size 59–69 µm in both pure and crossebred animals.



Cell population with size	Significance		
	Treatment	Breed	Interaction
29 µm -39 µm	NS	*	NS
39 µm <>49 µm	NS	**	NS
49 µm <>59 µm	NS	*	NS
59 µm <>69 µm	*	***	NS
69 µm <>79 µm	NS	NS	NS

Fig. 2. Adipocyte size distribution in m.LD

4. CONCLUSIONS

In the present study the major influence on the fatty acid composition and adipocyte size and distribution in both backfat and muscle was due to breed differences compared to betaine supplementation. In order to fully clarify the effects of betaine on the fatty acid composition and morphological characteristics in lipids in pigs, raised in Bulgaria further investigations are needed with different diets and betaine concentrations as well as different durations

REFERENCES

Barber, C. M ., R. J. Ward, S. E. Richards, A. M. Salter, P. J. Buttery, R. G. Vernon, M. T. Travers, Ovine adipose tissue monounsaturated fat content is correlated to depot – specific expression of the stearoyl-CoA desaturase gene, *J. Anim. Sci.,* 78, 62–68 (2000).

Bligh E. G., W. Y. Dyer, A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol*., 37, 911–917 (1959).

Casarin A., M. Forat, B. J. Zabaras-Krick, Interrelationships between betaine (Batafin-BRC) and level of feed intake on the performance parameters and carcass of growing-finishing pigs. *J. Anim. Sci*., 75 (Suppl.1), 75 (Abstr.) (1997).

Christie W. W., *Lipid analysis.* Pergamon Press. Oxford (1973).

Clarke W. C., E. Vitranen, J. Blackburn, D. Higgs, Effects of dietary betaine / aminoacid active additive on growth and seawater adaption in yearling Chinook salmon. *Aquaculture*, 121 : 137–145 (1994).

Daniel Z. C. T., R. S. E. Richards, A. M. Salter, P. J. Buttery, Insulin and dexamethasone regulate stearoyl-CoA desaturase mRNA levels and fatty acid composition in sheep tissues. *Anim. Sci*., 78, 237–243 (2004a).

Fernandez-Figares I., J. M. Rodriguez-Lopez, L. Gonzalez-Valero, R. Nieto, M. Lachica, J. F. Aguilera, Effect of conjugated linoleic acid. betaine or both on fatty acid composition of growing Iberian gilts. *J. Anim. Sci.*, 86. E-Suppl. 2 (2008).

Harwood J. K., Lipid Metabolism. Pages 605–664 in *The Lipid Handbook*. 2nd ed. Chapman & Hall. London. UK (1994).

Huang Q. C., Z. R. Xu, X. Y. Han, W. F. Li, Changes in hormones. growth factor and lipid metabolism in finishing pigs fed betaine. *Livest. Sci.*, 105, 78–85 (2006).

Huang Q. C., Z. R. Xu, X. Y. Han, W. F. Li, Effect of dietary betaine supplementation on lipogenic enzyme activities and fatty acid synthase mRNA expression in finishing pigs. *Anim. Feed Sci. Tech.*, 140, 365–375 (2008).

Hur S. J., H. S. Yang, G. B. Park, S. T. Joo, Effects of Dietary Glycine Betaine on Pork Quality in Different Muscle Types. Asian-Aust. *J. Anim. Sci*., 20, 1754–1760 (2007).

Jakobsen K., Dietary modifications of animal fats: status and future perspectives. *Fett-Lipid,* 101, 475–483 (1999).

JMP. Version 7. SAS Institute Inc. Cary. NC, (2007).

Kidd M. T., P. R. Ferket, J. D. Garlich, Nutritional and osmoregulatory functions of betaine. *World's Poultry Sci. J.*, 53, 125–139 (1997).

Matthews J. O., L. L. Southern, T. D. Bidner, Estimation of the total sulfur amino acid requirement and the effect of betaine in diets deficient in total sulfur amino acids for the weanling pig. *J. Anim. Sci*., 79, 1557–1565 (2001a).

Lauridsen C., S. Hojsgaard, M. T. Sorensen, Influence of dietary rapeseed oil, vitamin E. and copper on the performance and the antioxidative and oxidative status of pigs. *J. Anim. Sci.*, 77, 906–916 (1999b).

Lauridsen C., J. H. Nielsen, P. Henckel, M. T. Sorensen, Antioxidative and oxidative status in muscles of pigs fed rapeseed oil, vitamin E and copper. *J. Anim. Sci.*, 77, 105–115 (1999c).

Lawrence B., A. Schinckel, O. Adeola, K. Cera, Impact of betain on pig finishing performance and carcass composition. *J. Anim. Sci.*, 80, 475–482 (2002).

Nurnberg K., U. Kuchenmeister K. Ender, G. Nurnberg, W. Hackl, Influence of dietary n-3 fatty acids on the membrane properties of skeletal muscle in pigs. *Fett-Lipid*, 100, 353–358 (1998).

Simon J., Choline, betaine and methionine interactions in chickens. pigs and fish (including crustaceans). *World's Poultry Sci. J*., 55, 353–374 (1999).

Vernon, R., D. Flint, Lipid metabolism in farm animals. *Proc. Nutr. Soc.*, 47, 287–293 (1988).

Warnants N., M. J. Van Oeckel, C. V. Boucqué, Incorporation of dietary polyunsaturated fatty acids in pork fatty tissues and its implication for the quality of the end products. *Meat Sci*., 44, 125–144 (1996).

Wood, J. D., M. Enser, Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *Br. J. Nutr*., 78, 49–60 (1997).

Zhang S., T. J. Knight, K. J. Stadler, R. N. Goodwin, S. M. Lonergan, D. C. Beitz, Effect of breed, sex and halothane genotype on fatty acid composition of pork longissimus muscle. *J. Anim. Sci.*, 85, 583–591 (2007).