EFFECT OF DURATION OF FEEDING DIETS RICH IN N-3 PUFA TO BELGIAN BLUE DOUBLE-MUSCLED YOUNG BULLS, ON THE INCORPORATION OF LONG-CHAIN N-3 AND N-6 PUFA IN THE PHOSPHOLIPIDS AND TRIGLYCERIDES OF THE *LONGISSIMUS THORACIS*

Raes, K., Balcaen, A., Claeys, E., De Smet, S, Demeyer, D.
Department of Animal Production, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Proefhoevestraat 10, 9090 Melle, Belgium

Background

Nutritional guidelines are recommending a higher intake of n-3 fatty acids instead of n-6 fatty acids. One of the possible ways to achieve this is to enrich the meat with those fatty acids by supplementation of animal diets with sources of n-3 fatty acids. Among the natural sources of linolenic acid, the most abundant ones are linseed, grass or grass silage. Besides, with respect to the EU policy towards extensification (using more grassland resources), it is worthwhile to study the effect of different n-3 sources in the diet of Belgian Blue animals, which are normally raised in high-concentrate feeding systems.

Objectives

The aim of this study was to examine the incorporation of n-3 fatty acids into the intramuscular fat of beef when using grass, grass silage and/or linseed in the diet. The effect of feeding different n-3 sources during different times was investigated. The incorporation of linoleic and linolenic acid, as well as the long-chain n-3 and n-6 PUFA were measured in the triglyceride and phospholipid fraction of the intramuscular fat of the *longissimus thoracis*.

Material and Methods

Three groups of 7 or 8 Belgian Blue young bulls were fed different rations during three different production stages: the final growing, the pre-fattening and the fattening stage. Depending on the stage and the group, the rations were formulated on an equal energy and protein basis. Concentrates were cereal and beet pulp based, with or without added linseed as n-3 source. During the final growing and pre-fattening stage, the concentrates were fed at low levels: 2 kg/day/animal and 1% of live weight respectively, whereas roughage was fed *ad libitum*. During the fattening phase, the concentrate/roughage ratio was fixed and diets were given *ad libitum*. The groups were divided as followed (Table 1): ① *Group* SC_1 shifted from a predominantly n-3 diet during the final growing and pre-fattening stage (linseed in the concentrates in combination with fresh grass (pasture) or triticale silage respectively) to a predominantly n-6 diet (no linseed in the concentrate and only straw as roughage) in the fattening stage; ② *Group* GC_2 received during the whole experimental period a predominantly n-3 diet (linseed in the concentrate in combination with fresh grass during the final growing stage and with grass silage in the pre-fattening and fattening period); ③ *Group* MC_3 shifted from a predominantly n-6 diet (no linseed in the concentrate and maize silage as roughage during the final growing and pre-fattening stage) to a predominantly n-3 diet in the fattening stage (linseed in the concentrate in combination with maize silage). Group MC_3 was kept indoors during the whole experimental period and represented mostly the normal practical conditions for fattening Belgian Blue double-muscled bulls. Group SC_1 and group GC_2 were put on pasture during the final growing phase and were kept indoors during the other periods. The bulls were slaughtered at an average live weight of 681 kg (sd 30 kg). One day post-mortem, a steak of the *longissimus thoracis* at the 11-12th rib was taken for fatty acid analysis. The steaks were vacuum packed and frozen (-18°C) until analysis. After mincing the sample, intramuscular fat was extracted using chloroform/methanol (2/1; v/v) (adapted from Folch et al, 1957). Part of the extract was used for the separation of the intramuscular fat in triglycerides and phospholipids using thin-layer chromatography. After separation of the classes, the fatty acids were methylated and analysed by gas chromatography, as described by Raes et al (2001). Concomitantly, fatty acid analysis was done on a total intramuscular fat extract. Statistical analysis was performed using One-Way Anova with Duncan as post-hoc test (SPSS version 9.0)

Results and discussion

Increased amounts of SFA and MUFA (mg/100g muscle) are observed with higher levels of intramuscular fat, while the total PUFA content remained almost constant (Table 2). This has also been observed by De Smet et al (2000) in a study with Belgian Blue double-muscled bulls. The amount of PUFA mainly reflects the phospholipid fraction, which remains fairly constant independent of the feeding of the animals. Because of the lean nature of the Belgian Blue animals, reflected in a very low total intramuscular fat content (< 1% fat), the phospholipid fraction makes up 40 to 50 % of the total intramuscular fat (Table 2). Such proportion corresponds to earlier reports on Belgian Blue double-muscled animals by Webb et al (1998), Nürnberg et al (1999) and De Smet et al (2000), and is much higher than found in fatter animals (Marmer et al, 1984; Scollan et al, 2001).

While the total PUFA content (mg/100g muscle) did not vary between the groups, there are clear differences in amounts of total and individual n-3 and n-6 fatty acids (Table 3). The amount of n-3 fatty acids in group GC_2 is twice as high than for group SC_1 and MC_3, in both the triglyceride and phospholipid fraction. Interestingly, the amount of total n-3 fatty acids is equal for group SC_1 (mainly n-3 fatty acids fed during the final growing and pre-fattening stage) and for group MC_3 (mainly n-3 fatty acids fed during fattening stage). However, these groups show a clearly different long-chain fatty acid profile in the phospholipids. Feeding n-3 fatty acids during the last stage of the experimental period resulted mainly in an increase of C18:3n-3 in the phospholipids, while the long- chain n-3 fatty acids (C20:5, C22:5) did not increase at the same rate. On the other hand, feeding n-3 fatty acids in the earlier periods of the experiment resulted in a higher deposition of C20:5n-3 and C22:5n-3 in the phospholipids, and no such differences were observed between group SC_1 and group GC_2. Further supplementation of n-3 fatty acids in the fattening period (group GC_2) resulted mainly in a further increase of C18:3n-3. The amounts of C22:6n-3 were low and did not differ much between the groups. Concomitantly, the animals with the highest n-3 content in the intramuscular fat showed the lowest n-6 fatty acid content (Table 3). The C18:2n-6/C20:4n-6 ratio was remarkably constant in the phospholipid fraction across the groups. These results suggest that the conversion of C18:2n-6 to C20:4n-6 is probably strongly regulated and is difficult to change by feeding, as suggested earlier by Whelan (1996). Incorporation of C18:2n-6 is mainly observed in the phospholipids whereas that of C18:3n-3 is more equally distributed between the phospholipid and the triglyceride fraction.

From a nutritional point of view, the P/S and n-6/n-3 ratio are important (Table 2) and for the total intramuscular fat, both parameters meet the nutritional recommendations (Nationale Raad voor Voeding, 1996). The P/S ratio of the phospholipids is about ten times higher than for the triglycerides, as the PUFA are preferentially incorporated into the phospholipids.

Conclusions

Feeding n-3 sources (linseed in combination with grass(silage) or not) results in an increased n-3 PUFA content in the intramuscular fat. A longer duration of feeding linolenic acid increases the content of the long-chain n-3 PUFA in the phospholipid fraction.

Literature

De Smet, S., Webb, E.C., Claeys, E., Uytterhaegen, L. & Demeyer, D. (2000). Meat Science, 56, 73-79.
Folch, J., Lee, M. & Sloane-Stanley, G.H.A. (1957). Journal of Biological Chemistry, 226, 497-509.
Marmer, W.N., Maxwell, R.J. & Williams, J.E. (1984). Journal of Animal Science, 59, 109-121.
Nurnberg, K., Ender, B., Papstein, H.J., Ender, K. & Nurnberg, G. (1999). Z. Lebensm. Unters. Fors. A, 208, 332-335.
Nationale Raad voor Voeding (1996). Belgium
Raes, K., De Smet, S. & Demeyer, D. (2001). Animal Science, 73, 253-260.
Scollan, N.D., Choi, N.J., Kurt, E., Fisher, A.V., Enser, M. & Wood, J.D. (2001). British Journal of Nutrition, 85, 115-124.
Webb, E.C., De Smet, S., Van Nevel, C., Martens, B. & Demeyer, D.I. (1998). Meat Science, 50, 45-53.
Whelan, J. (1996). Journal of Nutrition, 126, 1086S-1091S.

Acknowledgements

This research was partly supported by the Ministry of Small Enterprises, Traders and Agriculture and by the EU-community (project QLRT-2000-31423 ('Healthy Beef')). INVE Technologies (Belgium) are thanked for their advice in feed formulations and for supplying the n-3 premix.

Table 1 Experimental set-up and composition of the diets

	SC_1	GC_2	MC_3
Animals (n =)	7	8	8
Final growing phase (± 340-440 kg)[a] (t=[b])	Pasture (70)	Pasture (70)	Indoors (70)
Concentrate	+ linseed	+ linseed	- linseed
Roughage	n-3 / grass	n-3 / grass	n-6 / maize silage
Pre-fattening phase (± 440-520 kg) (t=)	Indoors (92)	Indoors (98)	Indoors (56)
Concentrate	+ linseed	+ linseed	- linseed
Roughage	n-6 / GPS[c]	n-3 / grass silage	n-6 / maize silage
Fattening phase (± 520-680 kg) (t=)	Indoors (139)	Indoors (134)	Indoors (83)
Concentrate	- linseed	+ linseed	+ linseed
Roughage	Straw	n-3 / grass silage	n-6 / maize silage
C/R[d]	100/0	70/30	80/20

[a] Mean live weight at start and end of the period; [b] Duration (days); [c]GPS = triticale silage; [d]C/R = Concentrate/Roughage ratio on DM basis mixed at feeding and fed ad libitum

Table 2. Fatty acid content and the P/S and n-6/n-3 ratio of total intramuscular fat, triglycerides and phospholipids of the *longissimus thoracis* of Belgian Blue double-muscled young bulls, depending on the feeding strategy

	Total intramuscular fat				Triglyceride fraction				Phospholipid fraction			
	SC_1	GC_2	MC_3	*P*	SC_1	GC_2	MC_3	*P*	SC_1	GC_2	MC_3	*P*
mg/100 g muscle												
Sum	674	838	651	*0.230*	368	532	359	*0.209*	361	347	326	*0.341*
SFA	266	355	255	*0.252*	183	273	164	*0.129*	114	115	115	*0.979*
MUFA	204	292	210	*0.224*	159	227	167	*0.299*	71.5	74.3	58.1	*0.106*
PUFA	207	189	188	*0.209*	20.5	24.2	21.8	*0.725*	181	161	157	*0.159*
n-6/n-3	5.08^{a}	2.14^{b}	4.57^{a}	*0.000*	3.90^{a}	1.56^{b}	3.68^{a}	*0.000*	5.57^{a}	2.46^{b}	4.72^{c}	*0.000*
P/S	0.65^{a}	0.42^{b}	0.58^{ab}	*0.043*	0.12^{ab}	0.09^{a}	0.12^{b}	*0.053*	1.17	1.03	1.05	*0.132*

[a,b,c] Means for each fraction with different superscript are significantly different ($P < 0.05$)

Table 3. Content of total and individual n-6 and n-3 long-chain fatty acids (mg/100 g muscle) of the triglyceride and phospholipid fraction of the *longissimus thoracis* of Belgian Blue double-muscled young bulls, depending on the feeding strategy

	Triglyceride fraction				Phospholipid fraction			
	SC_1	GC_2	MC_3	*P*	SC_1	GC_2	MC_3	*P*
Σn-6	15.9	14.4	16.6	*0.764*	152^{a}	113^{b}	129^{b}	*0.004*
C18:2 n-6	15.1	13.6	15.0	*0.859*	115^{a}	85.0^{b}	97.9^{b}	*0.005*
C20:4n-6	0.35^{a}	0.33^{a}	0.65^{b}	*0.000*	28.5^{a}	21.6^{b}	23.2^{b}	*0.018*
C22:4 n-6					1.99^{a}	1.35^{b}	2.05^{a}	*0.022*
Σn-3	4.33^{a}	9.36^{b}	4.63^{a}	*0.005*	27.4^{a}	46.4^{b}	27.5^{a}	*0.000*
C18:3n-3	3.57^{a}	8.19^{b}	3.56^{a}	*0.005*	8.33^{a}	23.8^{b}	13.4^{a}	*0.000*
C20:5 n-3					6.43^{a}	8.85^{b}	4.74^{c}	*0.000*
C22:5 n-3	0.45	0.52	0.50	*0.932*	11.6^{a}	12.7^{a}	8.66^{b}	*0.001*
C22:6 n-3					0.99^{a}	0.95^{a}	0.68^{b}	*0.031*

[a,b,c] Means for each fraction with different superscript are significantly different ($P < 0.05$)