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Original article

Effect of diets rich in N-3 polyunsatured fatty acids on muscle lipids and fatty acids in Belgian Blue double-muscled young bulls

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Abstract — The present study was aimed at investigating the effect of duration and time of feeding n-3 fatty acids on the fatty acid composition of intramuscular fat and adipose tissue of bulls at slaughter. Four groups of bulls were given during three periods different diets, mainly differing in the presence of linseed as the predominant n-3 fatty acid source in the concentrate either or not in combination with grass (silage) as the roughage. The results show that the fatty acid composition of the feed during the earlier periods of life of the animal were important and influenced the final intramuscular fatty acid composition. Feeding n-3 PUFA during the phases before the finishing diet increased the long chain n-3 PUFA (C20:5n-3, C22:5n-3 and C22:6n-3) compared to animals which were fed only a C18:3n-3 rich concentrate in the finishing period. The cis-9,trans-11CLA content was increased by feeding linseed in the fattening period and was mainly deposited in the triacylglycerol fraction of the intramuscular fat.

fatty acids / n-3 PUFA / conjugated linoleic acid / beef / Belgian Blue bulls

1. INTRODUCTION

In rich, industrialised societies, consumers attach increasing importance on the diet as a major factor contributing to health. This has led to clear effects on consumer food demand [1]. Meat, and in particular beef, is often subject to a negative health image both because of its fat content and composition (i.e. its high saturated fatty acid content) [2]. However, it should now be generally recognised that the fat content of edible lean meat is mostly less than 5% on a fresh tissue basis [3], especially for meat from double-muscled Belgian Blue cattle [4, 5]. In more recent nutritional considerations, attention is directed to the importance of n-3 polyunsaturated fatty acids (PUFA) [6] and conjugated linoleic acid (CLA) [7, 8], since they possess several important metabolic effects,

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e.g. anti-atherogenic, anti-thrombotic, anti-carcinogenic, immunomodulating effects.

Reformulation of the fatty acid composition of meat is recognised as an important mean in the development of "functional meat products" [9], a process of considerable potential for product development in animal production [10]. Fatty acid reformulation or modification can be aimed at by either genetic or feeding strategies [2, 11, 12], or by technological interventions in the production of meat products [9]. For both social and ecological reasons, an intervention by the farmer rather than by the food technologist may be preferred since it could be associated with "healthy food" [2].

Several studies have already described the potential for increasing the beef n-3 fatty acid content through feeding, however using breeds characterised by intramuscular fat contents in excess of 3% and dietary interventions limited to the final production stages [13–15]. Limited data are available from studies aimed at increasing the n-3 PUFA content in beef from very lean animals like Belgian Blue double-muscled bulls by feeding strategies [16, 17]. Our experiment was aimed at increasing the n-3 PUFA and CLA content of the very lean Belgian Blue beef by modifying the animals diet at different stages of the production period, using both grass feeding and incorporation of linseed in the diet. Grass(silage) and linseed are the two major sources rich in C18:3n-3 available for ruminant feeding. By supplementing these sources in the diet during the different stages of fattening, the incorporation of long chain n-3 PUFA in muscle and fat tissue could be followed.

2. MATERIAL AND METHODS

2.1. Animals and experimental design

The experiment was carried out on the farm of the KTA school, Diksmuide (Belgium). In May 2000, thirty-two Belgian Blue double-muscled young bulls were divided on a live weight base (mean weight 339 ± 44 kg) into a group kept indoors (intensive) (maize silage-concentrate$_4$, MC$_4$; $n = 8$) and a group on pasture (extensive) ($n = 24$). The animals were kept at this regime for a period of 70 days (= last growing phase). During this period (May–July) one animal on the pasture died for an unknown reason. At the end of July, the animals of the pasture group were divided, based on their live weights, into three groups (concentrate$_1$, C$_1$, $n = 7$; grass silage-concentrate$_2$, GC$_2$, $n = 8$; and grass silage-concentrate$_3$, GC$_3$, $n = 8$) and were further raised indoors during the pre-fattening and fattening period. The animals were shifted from the pre-fattening to the fattening period per group based on their average live weight (around 550 kg). Due to differences in average daily gain, the length of the pre-fattening and fattening period differed between groups. During the experimental period, the animals were weighed individually each month. Slaughtering of the animals was performed on a live weight basis (approximately 650–700 kg) at the abattoir near the animal facilities. Due to practical constraints at the abattoir, the animals were slaughtered over an extended period and there was thus a quite large variability for age (mean age 22 ± 2 months) and to a minor extent live weight at slaughter (mean live weight 681 ± 28 kg). Slaughtering of the animals was performed after captive bolt stunning. Before sampling, carcasses were chilled at 2–3 °C for 24 h, weighed and graded according to the (S)EUROP classification system.

2.2. Diet composition

The composition of the diets of the four feeding groups differed between the three successive feeding periods, i.e. last growing, pre-fattening and fattening periods. Within the pre-fattening and fattening phase, diets of the different groups were

formulated on an equal protein and energy content. Based on the data matrix, the protein content of the concentrates, expressed as DVE [18], varied between 90–105 and between 90–100 for the pre-fattening and fattening phase respectively, while the energy content of the concentrates, expressed as VEVI [19] varied between 950–1100 and between 1050–1100 for the pre-fattening and fattening phase respectively. On the contrary, in the last growing phase the energy and protein levels in the diets were different for the animals of the MC_4 group versus the animals on pasture (C_1, GC_2 and GC_3 groups). All concentrates were cereal and beet pulp based, and crushed linseed (included in the commercial linseed based premix at a level of 75% (INVE, Baasrode, Belgium)) was used as the predominant n-3 PUFA source. During the last growing and pre-fattening periods, concentrates were fed to the animals of the MC_4 group at 1% of the live weight. For the other groups, concentrates were fed at 2 kg/day/animal and at 1% of the live weight during the last growing phase and pre-fattening phase respectively. Roughage was fed ad libitum during the last growing and pre-fattening stage. For animals at pasture, the concentrate composition was adapted after 40 days to balance the decrease in protein content of the pasture towards the end of the growing season. The botanical composition of the permanent pasture consisted of *Lolium perenne* (approximately 70–75%), *Poa trivalis* (approximately 10–15%), *Phleum pratense* (approximately 5%) and *Trifolium repens* (approximately 5%) (J. Latré, personal communication). Grass silage fed to the animals of group GC_2 and GC_3 had the same origin of the fresh grass and was of medium to high quality (J. Latré, personal communication). During the fattening phase, the concentrate/roughage ratio was fixed for all groups. The average daily intake of the animals was 6–8 kg concentrate (on a dry matter basis) during the fattening period. Based on the total daily intake of concentrate and roughage, an intake of approximately 40 g C18:3n-3·d^{-1} for animals of the C_1 group and 200 g C18:3n-3·d^{-1} for animals of the GC_2, GC_3 and MC_4 groups is estimated. During the periods at stable, straw was used as the litter and was also available on the rack as an additional roughage source for all groups. Composition and fatty acid profile of the diets, given to the animals of the 4 groups during the experimental period are given in Tables I and II. In brief the different groups were fed as follows:

(1) *C_1 group* shifted from a predominantly n-3 PUFA diet during the last growing and pre-fattening stages i.e., linseed in concentrates given respectively in combination with fresh grass (pasture) or whole triticale silage (GPS) to a predominantly n-6 PUFA diet without linseed;

(2) *GC_2 group* was fed a predominantly n-3 PUFA diet during the whole experimental period (linseed in all the concentrates in combination with fresh grass (pasture), GPS and grass silage during the last growing, pre-fattening and fattening periods respectively);

(3) *GC_3 group* received a predominantly n-3 PUFA diet during the whole experimental period (incorporation of linseed in the concentrate in combination with fresh grass during the last growing period or grass silage during the pre-fattening and fattening periods). The rations of the GC_2 and GC_3 groups differed in the use of triticale silage in the pre-fattening period in the GC_2 group and the higher level of grass silage in the fattening period in the GC_3 group. The former was not related to the aim of this study but was imposed by practical constraints on the experimental farm. The different levels of grass silage in the fattening period of the GC_2 and GC_3 groups were aimed at maximising the supply of C18:3n-3 from roughage for this type of animal;

(4) *MC_4 group* shifted from a predominantly n-6 PUFA diet (containing no linseed in the concentrates and maize silage as roughage during the last growing and pre-fattening period) to a predominantly n-3

PUFA diet in the fattening stage since linseed was included in the concentrate in combination with maize silage as the roughage.

The MC_4 group was kept indoors during the whole experimental period and represented the best, the normal practical conditions of fattening Belgian Blue double-muscled bulls in Belgium.

2.3. Feed and tissue samples

Feed samples were obtained at the beginning and at the end of each feeding period. Samples of the *longissimus thoracis* (7th rib) (LT), *semitendinosus* (ST) and *triceps brachii* (TB) were taken 24 h post-mortem on the left side of the carcass. Also subcutaneous fat, at the 7th rib, and perirenal fat

Table I. Diet composition for the experimental groups according to the feeding phase.

Last growing phase			
	$C_1 + GC_2 + GC_3$		MC_4
Roughage	Fresh grass		Maize silage
Composition of concentrates (%)			
	First 40 days	Last 30 days	
Barley	16	15	10
Maize	–	–	6.3
Wheat	25	25	18
Soybean meal	24	28	47
Beet pulp	16.2	13.2	10
Molasses	4.0	4.0	2.0
Limestone	4.0	4.0	0.5
Salt	–	–	0.2
Phosphate18/25[1]	1.3	1.3	1.0
Vitamin-Mineral premix[2]	2.5	2.5	5.0
Linseed based premix[3]	7.0	7.0	–
Pre-fattening phase			
	$C_1 + GC_2$	GC_3	MC_4
Roughage	Triticale silage	Grass silage	Maize silage
Composition of concentrates (%)			
Barley	–	25	10
Maize	–	–	6.3
Wheat	24	10	18
Soybean meal	55	27	47
Soybean oil	2.5	–	–
Beet pulp	–	13.2	10
Molasses	–	4.0	2.0
Limestone	2.8	4.4	0.5
Salt	–	–	0.2
Phosphate18/25	0.7	1.4	1.0
Vitamin-Mineral premix	–	–	5.0
Linseed based premix	15	15	–

Table I. Continued.

	Fattening phase			
	C_1	GC_2	GC_3	MC_4
Roughage		Grass silage	Grass silage	Maize silage
Concentrate/Roughage (on DM basis)		80/20	70/30	80/20
Composition of concentrates (%)				
Barley	13	28.8	27	12.5
Maize	2.0	–	–	–
Maize germ meal	11.5	–	–	18.8
Wheat	20	25	25	16.9
Soybean meal	17	16.3	15.7	17.5
Beet pulp	29.5	15.6	14.3	18.8
Molasses	2.0	1.25	1.4	1.3
Bypass fat 100%[4]	2.5	0.6	2.3	1.9
Vitamin-Mineral Premix	2.5	–	–	–
Linseed based premix	–	12.5	14.3	12.5

[1] Phosphate 18/25 consisted of CaH_2PO_4 with 18.2% total phosphorus and 24.5% calcium.
[2] The vitamin-mineral premix had the following characteristics: vitamin A = 400 000 IU·kg^{-1}; vitamin D_3 = 80 000 IU·kg^{-1}; vitamin E = 200 mg·kg^{-1}; Fe as ferric sulphate = 0.32%, Cu as copper sulphate = 0.04%; Mn as manganese oxyde = 0.32%; Co as cobalt carbonate = 0.004%; Zn as zinc oxyde = 0.4%, I as calcium iodate = 0.008%; selenium = 0.0016%; calcium = 20.4%; phosphorus = 2.7%; sodium = 7.8%; magnesium = 4.3%; ethoxyquine = 0.024%; propylgallate = 0.005%.
[3] The linseed based premix was a vitamin-mineral premix mixed with products and by-products of oil-rich grains (75% crushed linseed) with the following characteristics: crude ash = 25.6%; crude protein = 16.5%; crude fat = 26.0%, crude fibre = 7.5%; calcium = 5.6%; magnesium = 1.1%; Cu as copper sulphate = 100 mg·kg^{-1}; vitamin A = 90 000 IU·kg^{-1}; vitamin D_3 = 18 000 IU·kg^{-1}; vitamine E = 90 mg·kg^{-1}; Fe as ferric carbonate = 720 mg·kg^{-1}; Cu as copper oxyde = 90 mg·kg^{-1}; Zn as zinc oxyde = 750 mg·kg^{-1}; Mn as manganese oxyde = 600 mg·kg^{-1}; BHT = 305 ppm; BHA = 45 ppm; ethoxyquine = 675 ppm, propylgallate = 30 ppm.
[4] Bypass fat 100%: Free flowing, white spray dried powder containing 99.7% fat (palm kernel oil) with the main fatty acids C16:0 (28.0%) and C18:0 (64.0%); melting point = 60 °C; iodine value < 5; free fatty acids = max 2.0%.

was taken. All samples were immediately vacuum packed and frozen at –18 ºC until analysis.

2.4. Lipid and fatty acid analysis

Before extraction of the total lipids for fatty acid analysis, subcutaneous fat was separated from connective tissue and all samples were minced. Extraction of the total lipids was done using chloroform/methanol (2/1; v/v) according to the method of Folch et al. [20] and analysis of the lipids by gas-liquid chromatography as described by Raes et al. [21]. Nonadecanoic acid (C19:0) was used as an internal standard to quantify the fatty acids present in the feed and the different tissue samples. The lipids were transmethylated using NaOH/MeOH (0.5 N) (30 min at 50 ºC) followed by HCl/MeOH (1/1; v/v) (10 min at 50 ºC). The fatty acid methyl esters (FAME) were analysed by gas chromatography (HP 6890, Brussels, Belgium) using a CP-Sil88 column for FAME (100 m × 250 µm × 0.20 µm) (Chrompack, The Netherlands). The conditions were: injector: 250 ºC; detector: 280 ºC; H_2 as the carrier gas; temperature program: 150 ºC for 2 min, followed by an increase of 1.5 ºC·min^{-1} to 200 ºC, then 5 ºC·min^{-1} to 215 ºC. The peaks were identified by comparing the retention times with those of the corresponding standards (Sigma, Belgium; Nu-Chek Prep., Mn, USA).

Table II. Total fat contents (% of fresh material) and fatty acid composition of the concentrate and roughage components in the three feeding phases (weight % of total fatty acid methyl esters).

Last growing phase						
	$C_1 + GC_2 + GC_3$				MC_4	
	First 40 days		Last 30 days			
	C[1]	R[2]	C	R	C	R
Total fat	3.2	0.8	2.4	0.7	1.3	1.2
C14:0	0.1	0.8	0.1	0.9	0.6	0.4
C16:0	10.1	12.4	10.6	13.8	14.6	18.9
C18:0	2.8	1.6	2.8	1.6	2.6	1.0
C18:1	16.2	2.7	16.6	3.1	18.2	26.7
C18:2 n-6	32.4	14.5	33.5	15.5	52.4	44.2
C18:3 n-3	36.7	57.8	34.0	56.0	7.9	3.2
Pre-fattening phase						
	$C_1 + GC_2$		GC_3		MC_4	
	C	R	C	R	C	R
Total fat	6.8	0.4	6.2	0.5	1.3	1.2
C14:0	0.4	1.2	0.1	0.2	0.6	0.4
C16:0	10.7	18.7	8.9	17.7	14.6	18.9
C18:0	8.3	0.6	3.3	1.6	2.6	1.0
C18:1	15.1	12.2	17.4	3.6	18.2	26.7
C18:2 n-6	25.3	31.5	26.6	15.2	52.4	44.2
C18:3 n-3	37.1	19.0	42.1	43.4	7.9	3.2
Fattening phase						
	C_1	GC_2		GC_3		MC_4	
	C	C	R	C	R	C	R
Total fat	3.5	5.3	0.8	6.1	0.8	6.7	2.3
C14:0	0.3	0.3	0.9	0.1	0.9	0.5	0.3
C16:0	14.4	9.1	26.8	8.7	26.8	12.5	16.6
C18:0	4.6	5.7	4.4	3.3	4.4	10.8	1.5
C18:1	16.3	16.5	5.3	17.0	5.3	15.8	22.9
C18:2 n-6	47.1	23.2	12.6	24.7	12.6	28.2	41.4
C18:3 n-3	12.3	42.2	33.5	43.2	33.5	28.8	3.1

[1] C: concentrate component; [2] R: roughage component.

The separation of muscle total lipids into phospholipid and triacylglycerol fractions was only performed on LT muscle samples. This was done by a preseparation of the fatty acids into the different lipid classes using thin layer chromatography. Aliquot volumes of the extracts were evaporated under N_2 and redissolved in 150 µL chloroform. After activating the plates covered with Kieselgel (Fluka, Belgium) (1 h at 103 °C), the total lipids were spotted on the plates. The plates were developed in closed tanks saturated with hexane/diethylether/acetic acid (70/30/2; v/v/v) (until the top of the

plate) to separate triacylglycerol, free fatty acid, diacylglycerol, cholesteryl ester and the free cholesterol fraction. Further separation of the phospholipids and monoacylglycerols was performed using a second developing solvent hexane/diethylether/acetic acid (40/60/1.3; v/v/v). After spraying the plates with dichlorofluoresceine (0.1% in 2-propanol), the bands were visualised under UV and those corresponding to triacylglycerol (TAG) and phospholipid (PL) fractions were scraped off. TAG and PL were extracted from the kieselgel with chloroform/methanol (1/2; v/v) followed twice by chloroform/methanol (1/1; v/v), transmethylated and analysed by gas-liquid chromatography as described by Raes et al. [21].

2.5. Statistical analysis

Animal performance data and fatty acid composition of subcutaneous and perirenal fat, and of the triacylglycerol and phospholipid fraction of LT muscle, were analysed with a linear model including only the fixed effect of the feeding group. Fatty acid composition data from muscle tissues were analysed with a linear model including the fixed effects of muscle type, feeding treatment and the interaction term muscle type × feeding treatment. Data were analysed with the General Linear Model Univariate procedure and using the Bonferonni post-hoc test for comparison of the mean values using SPSS (SPSS software for windows, release 9.0, SPSS inc., USA).

3. RESULTS

3.1. Zootechnical performances

The last growing period consisted of 70 days for all animals (Tab. III). Since the animals of the MC_4 group had a higher average daily weight gain during the whole experimental period, these animals were kept for a shorter time in the pre-fattening and fattening period compared to the other three groups. Despite the fact that the diets were formulated to supply equal energy and protein for all four groups in the pre-fattening and fattening period respectively, the animals of the MC_4 group grew faster. The age at slaughter of animals of the MC_4 group was lower than for the other groups ($P < 0.05$), i.e. approximately 20 months versus 23–24 months. No significant differences in dressing yield (67–68.5%) were observed between the groups (Tab. III).

Table III. Zootechnical data and animal performances of the different groups.

	C_1	GC_2	GC_3	MC_4	RSE
Number of animals	7	8	8	8	
Length of time per period (days)					
Last growing	70	70	70	70	
Pre-fattening	92	98	98	56	
Fattening[1]	139	141	134	83	
	(98–168)	(92–190)	(85–183)	(42–120)	
Animal performances					
Mean average daily gain ($g \cdot day^{-1}$)	1133	1099	1146	1674	165
Age at slaughter (months)	23.5[a]	23.7[a]	22.9[a]	19.8[b]	1.7
Live weight at slaughter (kg)	686	682	694	662	26.8
Cold carcass weight (kg)	470	459	470	447	17.5
Dressing yield (%)	68.5	67.3	67.7	67.6	1.58

[1] Mean (minimum – maximum); [ab] within a row, means without common superscript letter differ ($P < 0.05$).

Table IV. Amounts of the different fatty acids (mg·100 g^{-1} fresh muscle tissue) in total lipids of *longissimus thoracis* (LT), *semitendinosus* (ST) and *triceps brachii* (TB) muscles of bulls given C_1, GC_2, GC_3 and MC_4 treatments ($n = 7$ for C_1, $n = 8$ for GC_2, GC_3 and MC_4).

	LT				ST				TB				P1		
	C1	GC2	GC3	MC4	C1	GC2	GC3	MC4	C1	GC2	GC3	MC4	Muscle	Diet	RSE
C14:0	8.8	8.9	12.4	8.7	5.5	6.1	6. 9	6.1	5.6^{a}	9.6^{b}	10.0^{b}	7.5^{ab}	0.001	0.032	3.7
C16:0	134	128	177	129	113	111	122	115	105^{a}	138^{ab}	146^{b}	118^{ab}	0.024	0.022	37.1
C18:0	105^{ab}	116^{ab}	149^{a}	103^{b}	88.3	100	104	86.2	85.4^{a}	125^{b}	122^{b}	93.7^{b}	0.008	0.000	29.1
ΣC16:1	14.1	13.6	18.6	14.8	11.8	11.0	11.3	12.7	10.9	15.6	15.3	13.7	0.060	0.461	5.9
ΣC18:1	180	200	262	183	149	170	173	155	131^{a}	226^{c}	211^{bc}	168^{ab}	0.032	0.008	65.6
c9,t11 CLA	2.5^{a}	3.8^{ab}	5.0^{b}	3.5^{ab}	2.3	3.4	3.3	2.9	1.8^{a}	4.0^{b}	3.9^{b}	3.1^{ab}	0.194	0.001	1.6
C18:2n-6	132^{a}	97.3^{b}	97.7^{b}	116^{c}	136^{a}	111^{bc}	101^{c}	124^{ab}	130^{a}	109^{b}	103^{b}	129^{a}	0.067	0.000	13.9
C20:3n-6	7.6^{a}	4.5^{b}	4.6^{b}	6.7^{a}	8.4^{a}	5.9^{ab}	5.0^{b}	8.3^{a}	7.0^{ab}	3.26^{a}	5.1^{ab}	8.0^{b}	0.177	0.000	2.5
C20:4n-6	29.2^{a}	22.4^{b}	22.5^{b}	26.2^{a}	35.0^{a}	28.5^{bc}	25.8^{c}	31.7^{ab}	32.7^{a}	26.1^{b}	25.6^{b}	31.0^{a}	0.000	0.000	3.5
C18:3n-3	12.7^{a}	27.9^{b}	33.2^{c}	17.1^{a}	12.6^{a}	31.7^{b}	33.2^{b}	19.8^{c}	11.4^{a}	32.8^{b}	35.2^{b}	20.1^{c}	0.093	0.000	4.0
C20:5n-3	6.8^{a}	8.8^{b}	10.3^{b}	5.6^{a}	9.8^{a}	12.6^{b}	12.4^{b}	7.7^{c}	7.9^{a}	11.2^{b}	12.3^{b}	7.1^{a}	0.000	0.000	1.6
C22:5n-3	12.9^{a}	12.7^{a}	14.5^{a}	10.1^{b}	13.9^{ab}	15.1^{bc}	16.4^{c}	12.2^{a}	13.1^{ab}	14.2^{b}	17.4^{c}	11.6^{a}	0.001	0.000	1.9
C22:6n-3	1.29	1.0	1.2	0.8	1.1	1.2	1.3	0.9	1.2^{ab}	1.1^{ab}	1.4^{a}	0.9^{b}	0.532	0.001	0.3
Total FA	674	670	838	651	614	631	640	607	572^{a}	744^{b}	737^{b}	638^{ab}	0.095	0.040	153
SFA[2]	259	263	352	250	217	227	243	215	207^{a}	286^{b}	290^{b}	227^{a}	0.009	0.004	69.3
MUFA[3]	204	224	292	210	173	190	195	179	154^{a}	252^{b}	237^{b}	194^{ab}	0.038	0.019	72.9
PUFA[4]	207^{a}	179^{b}	189^{ab}	188^{ab}	222	210	199	211	209	202	205	214	0.002	0.070	22.5
Σn-6 PUFA[5]	172^{a}	126^{b}	127^{b}	153^{c}	183^{a}	127^{b}	139^{b}	168^{a}	174^{a}	141^{b}	137^{b}	173^{a}	0.010	0.000	18.2
Σn-3 PUFA[6]	33.8^{a}	51.0^{b}	60.2^{c}	34.0^{a}	37.6^{a}	61.0^{b}	63.7^{b}	40.8^{a}	33.8^{a}	69.7^{b}	66.9^{c}	40.1^{a}	0.001	0.000	6.5
P/S[7]	0.65^{a}	0.5_{1}	0.42^{b}	0.58^{ab}	0.74^{a}	0.67^{ab}	0.58^{b}	0.72^{a}	0.76^{a}	0.55^{b}	0.51^{b}	0.71^{a}	0.001	0.000	0.14
C18:2n-6/C18:3n-3	10.5^{a}	3.50^{b}	2.99^{b}	7.12^{c}	10.8^{a}	3.50^{b}	3.08^{b}	6.51^{c}	11.5^{a}	3.34^{b}	2.94^{b}	6.71^{c}	0.812	0.000	0.99
n-6/n-3[8]	5.08^{a}	2.49^{b}	2.14^{b}	4.57^{c}	4.86^{a}	2.42^{b}	2.12^{b}	4.17^{c}	5.18^{a}	2.35^{b}	2.06^{b}	4.37^{c}	0.317	0.000	0.45

[1] The muscle × diet interaction term was never significant and is not mentioned here.
[2] SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C22:0.
[3] MUFA = C14:1 + C15:1 + ΣC16:1 + C17:1 + ΣC18:1 + C22:1.
[4] PUFA = C20:2n-6 + Σn-6 PUFA + Σn-3 PUFA. C20:2n-6 is not reported and represented 1.0–1.7 mg·100 g^{-1} fresh muscle tissue.
[5] Σn-6 PUFA= C18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6. C18:3n-6, C22:4n-6 are not reported and respectively 0.45–1.40 mg·100 g^{-1} fresh muscle tissue, and 1.4–2.9 mg·100 g^{-1} fresh muscle tissue.
[6] Σn-3 PUFA= C18:3n-3 + C18:4n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3. C18:4n-3 is not reported and represented 0.2–0.9 mg·100 g^{-1} fresh muscle tissue.
[7] P/S = (C18:2n-6 + C18:3n-3)/(C14:0 + C16:0 + C18:0).
[8] n-6/n-3 = Σn-6 PUFA / Σn-3 PUFA.
[abc] Within a row, means within a muscle without a common superscript letter differ ($P < 0.05$).

3.2. Fatty acid composition of total muscle lipids

Detailed fatty acid composition (mg·100 g^{-1} fresh muscle tissue) of total lipids from LT, ST and TB muscles are presented for each feeding group in Table IV. The interaction term muscle × diet was not significant for any of the variables and is not discussed further. Despite the absence of the interaction, the fatty acid composition data are presented separately for the muscles to allow comparison with other literature data.

The overall intramuscular total FA content was very low (570–840 mg·100 g^{-1} muscle). The higher intramuscular FA content of the GC_2 and GC_3 groups ($P = 0.04$) was mainly due to the higher amounts of saturated fatty acid (SFA) ($P = 0.004$) and monounsaturated fatty acid (MUFA) ($P = 0.019$) for both of these groups compared to the other two groups (Tab. IV). The PUFA content varied between 180 and 220 mg·100 g^{-1} muscle for the different feeding groups ($P = 0.07$) and different muscles ($P = 0.002$).

Compared with ST and TB muscles, LT muscle had the highest intramuscular TFA content ($P > 0.05$), caused by a significantly higher absolute amount of SFA ($P = 0.009$) and MUFA ($P = 0.038$) and a lower content of PUFA ($P = 0.002$) (Tab. IV). The amount of n-3 PUFA was twice as high for the GC_2 and GC_3 groups compared with the C_1 and MC_4 groups for all three muscles (Tab. IV). Concomitantly with the highest n-3 PUFA content observed in the GC_2 and GC_3 groups, these groups showed the lowest n-6 PUFA contents (Tab. IV). Similar n-3 PUFA contents were found for the C_1 group (mainly C18:3n-3 during the last growing and pre-fattening period) and the MC_4 group (mainly C18:3n-3 during the fattening phase) (Tab. IV). Although the total n-3 PUFA content did not differ between these two groups, a significantly higher content of C18:3n-3 and a significantly lower content of C20:5n-3 and C22:5n-3 fatty acids were measured in the MC_4 group compared to the C_1 group. The amounts of C22:6n-3 were generally low and were slightly different between the MC_4 group and the other groups ($P = 0.001$). The amount of cis-9,trans-11 CLA was two times lower in the C_1 group compared with the other groups (Tab. IV). The P/S ratio in the total intramuscular fat ranged between 0.40 and 0.75 depending on the feeding group (Tab. IV), with a significantly higher ratio observed for groups C_1 and MC_4. The n-6/n-3 ratio was less than 5 for all of the groups and was further reduced to a value of 2.0–2.5 by continuously feeding the animals C18:3n-3 (groups GC_2 and GC_3) (Tab. IV).

3.3. Fatty acid composition of the triacylglycerol and phospholipid fraction for the LT muscle

The detailed fatty acid composition (mg·100 g^{-1} fresh muscle tissue) of the TAG and PL fractions for the LT muscle is given in Table V. The phospholipid content did not differ significantly between the 4 treatments (Tab. V). The higher intramuscular fatty acid content for the LT muscle of the GC_3 group was accompanied by a non-significantly higher amount of TAG (Tab. V). The effects of feeding on the incorporation and deposition of the n-3 and n-6 PUFA in the total intramuscular FA (as described above) were also observed in the PL (Tab. V). The separation of the total lipids in the intramuscular fat into the PL and TAG showed that the cis-9,trans-11 CLA was mainly incorporated into the TAG (75–80% of total intramuscular CLA) (Tab. V). The n-6/n-3 ratio in the phospholipid and in the intramuscular TFA fraction were similar, while the n-6/n-3 ratio of the TAG fraction was mainly a reflection of the C18:2n-6/C18:3n-3 ratio (Tabs. IV and V).

3.4. Fatty acid composition of subcutaneous and perirenal fat

The total FA composition of the subcutaneous and perirenal fat is given in Table VI,

Table V. Amounts of the different fatty acids (mg·100 g^{-1} fresh muscle tissue) of the triacylglycerol and phospholipid fractions of the *longissimus thoracis* of bulls given C_1, GC_2, GC_3 and MC_4 treatments (n = 7 for C_1, n = 8 for GC_2, GC_3 and MC_4).

	Triacylglycerol fraction						Phospholipid fraction					
	C_1	GC_2	GC_3	MC_4	P	RSE	C_1	GC_2	GC_3	MC_4	P	RSE
C14:0	8.7	8.3	11.9	8.4	0.335	4.5	0.4	0.4	0.4	0.5	0.234	0.1
C16:0	89.8	86.1	128	82.4	0.222	48.2	69.5	59.6	68.5	73.8	0.009	5.9
C18:0	75.4	86.7	124	66.3	0.078	45.2	35.2	32.1	38.4	32.7	0.215	6.4
ΣC16:1	11.7	10.7	15.0	12.4	0.531	5.9	1.7	1.5	1.7	2.0	0.807	0.95
ΣC18:1	143	167	207	149	0.336	73.9	62.8^{a}	56.0^{ab}	66.8^{a}	44.6^{b}	0.011	10.8
c9,t11 CLA	1.6	2.5	3.1	2.3	0.243	1.4	0.5^{ab}	0.49^{ab}	0.70^{b}	0.29^{ab}	0.121	0.3
C18:2n-6	15.1	11.8	13.6	15.0	0.584	5.2	115^{a}	83.7^{b}	85.0^{b}	97.9^{b}	0.001	14.2
C20:3n-6	–	–	–	–	–	–	6.2^{a}	4.5^{b}	4.8^{b}	5.3^{ab}	0.033	0.5
C20:4n-6	0.4^{a}	0.3^{a}	0.3^{a}	0.7^{b}	0.000	0.1	28.5^{a}	21.1^{b}	21.6^{b}	23.2^{b}	0.008	4.2
C18:3n-3	3.6^{a}	6.0^{b}	8.2^{b}	3.6^{a}	0.004	2.6	8.3^{a}	20.2^{b}	23.8^{c}	13.4^{d}	0.000	3.0
C20:3n-3	–	–	–	–	–	–	0.2^{a}	0.3^{ab}	0.3^{b}	0.01^{c}	0.000	0.1
C20:5n-3	–	–	–	–	–	–	6.4^{a}	8.2^{b}	8.9^{b}	4.7^{c}	0.000	1.7
C22:5n-3	0.45	0.36	0.52	0.50	0.784	0.3	11.6^{a}	11.2^{a}	12.7^{a}	8.7^{b}	0.003	2.0
C22:6n-3	–	–	–	–	–	–	1.0^{a}	0.9^{ab}	1.0^{a}	0.7^{b}	0.065	0.2
Total FA	368	395	532	359	0.254	188	361	311	347	326	0.118	36.5
SFA[1]	183	189	273	164	0.145	98.2	114	98.8	115	115	0.088	12.3
MUFA[2]	159	181	227	167	0.387	82.5	71.5	63.5	74.3	58.1	0.096	11.5
PUFA[3]	20.5	19.4	24.2	21.8	0.678	8.0	181	152	161	157	0.118	23.0
Σn-6 PUFA[4]	15.9	12.5	14.4	16.6	0.456	5.4	152^{a}	111^{b}	113^{b}	129^{b}	0.001	18.7
Σn-3 PUFA[5]	4.3^{a}	6.8^{ab}	9.4^{b}	4.6^{a}	0.004	2.7	27.4^{a}	40.5^{b}	46.4^{b}	27.5^{a}	0.000	6.0
P/S[6]	3.90^{a}	1.93^{b}	1.56^{b}	3.68^{a}	0.000	0.0	5.57^{a}	2.76^{b}	2.46^{b}	4.72^{c}	0.000	0.2
C18:2n-6/C18:3n-3	4.70^{a}	2.05^{b}	1.69^{b}	4.32^{a}	0.000	0.8	13.9^{a}	4.15^{b}	3.60^{b}	7.52^{c}	0.000	1.4
n-6/n-3[7]	0.12^{ab}	0.11^{ab}	0.09^{a}	0.12^{b}	0.077	0.5	1.17	1.13	1.03	1.05	0.120	0.5

[1] SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C22:0.
[2] MUFA = C14:1 + C15:1 + ΣC16:1 + C17:1 + ΣC18:1 + C22:1.
[3] PUFA = C20:2n-6 + Σn-6 PUFA + Σn-3 PUFA. C20:2n-6 is not reported and represented in phospholipid fraction 0.7–1.0 mg·100 g^{-1} fresh muscle tissue.
[4] Σn-6 PUFA = C18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6 + C22:4 n-6. C18:3n-6, C22:4n-6. C18:3n-6, C22:4n-6 are not reported and respectively represented in phospholipid fraction 0.3–0.7% and 1.1–2.1 mg·100 g^{-1} fresh muscle tissue.
[5] Σn-3 PUFA = C18:3n-3 + C18:4n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3. C18:4n-3 is not reported and represented in phospholipid fraction 0.04–0.1 mg·100 g^{-1} fresh muscle tissue.
[6] P/S = (C18:2n-6 + C18:3n-3)/(C14:0 + C16:0 + C18:0).
[7] n-6/n-3 = Σn-6 PUFA / Σn-3 PUFA.
[abc] Within a row, means per fraction without a common superscript letter differ ($P < 0.05$).

Table VI. Amounts of the different fatty acids (mg·g^{-1} fresh fat tissue) in total lipids of subcutaneous and perirenal fat of bulls given C_1, GC_2, GC_3 and MC_4 treatments (n = 7 for C_1, n = 8 for GC_2, GC_3 and MC_4).

	Subcutaneous fat						Perirenal fat					
	C_1	GC_2	GC_3	MC_4	P	RSE	C_1	GC_2	GC_3	MC_4	P	RSE
C14:0	26.7	23.0	25.4	24.4	0.373	4.0	23.7^{a}	19.1^{b}	20.9^{ab}	23.9^{a}	0.022	3.3
C16:0	206	177	207	193	0.091	25.0	205^{a}	169^{b}	190	201^{a}	0.009	20.8
C18:0	124	118	128	120	0.805	22.0	304	303	336	300	0.106	32.3
ΣC16:1	36.8	28.2	38.5	35.2	0.102	8.3	14.7	10.3	11.5	7.8	0.102	5.2
ΣC18:1	335	339	340	308	0.233	34.3	277	291	267	300	0.181	31.3
c9,t11 CLA	5.5	7.2	8.0	6.6	0.116	1.9	3.5	4.2	4.0	3.9	0.538	1.0
C18:2n-6	28.1^{ab}	21.8^{a}	20.5^{a}	30.6^{b}	0.029	7.2	40.4^{a}	32.0^{bc}	27.9^{b}	38.9^{ac}	0.007	7.4
C18:3n-3	7.9^{a}	12.4^{b}	12.6^{b}	7.2^{a}	0.000	2.0	9.9^{a}	15.3^{b}	13.9^{b}	10.1^{a}	0.000	2.6
Total FA	828	776	811	754	0.253	75.1	915	874	904	919	0.347	54.0
SFA[1]	372	330	372	351	0.129	38.7	559	512	570	546	0.055	41.9
MUFA[2]	386	380	392	356	0.321	40.2	299	305	282	315	0.247	32.3
PUFA[3]	42.4	39.8	39.3	40.9	0.940	10.0	53.9	51.2	46.5	53.8	0.384	9.5
P/S[4]	0.10	0.11	0.09	0.11	0.380	0.0	0.09	0.10	0.08	0.09	0.163	0.0
C18:2n-6/C18:3n-3	3.58^{a}	1.74^{b}	1.62^{b}	4.34^{c}	0.000	0.5	4.17^{a}	2.13^{b}	2.05^{b}	3.98^{a}	0.000	0.6

[1] SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0.
[2] MUFA = C14:1 + ΣC16:1 + C17:1 + ΣC18:1.
[3] PUFA = C18:2n-6 + C18:3n-3 .
[4] P/S = (C18:2n-6 + C18:3n-3)/(C14:0 + C16:0 + C18:0).
[abc] Within a row, means per fat type without a common superscript letter differ ($P < 0.05$).

expressed as $mg \cdot g^{-1}$ fat. For both adipose tissues, the main FA are SFA and MUFA, while the PUFA content was low and consisted mainly of C18:2n-6 and C18:3n-3 (Tab. VI). The effect of feeding was also reflected in the FA profile of both fat depots, in the same way as that observed for the intramuscular fat. A higher incorporation of C18:3n-3 in the GC_2 and GC_3 groups compared with the C_1 and MC_4 groups was measured ($P < 0.001$), while the cis-9,trans-11 CLA content was higher for the groups which received a C18:3n-3 source in the finishing diet ($P > 0.05$).

4. DISCUSSION

The objective of this study was to examine the effect of different feeding strategies applicable to double-muscled animals aimed at improving the meat fatty acid composition. Belgian Blue double-muscled animals are generally fattened indoors on high-concentrate, maize silage diets. Occasionally, they have access to pasture in the summer period. Therefore, the combination of grass (silage) and linseed as major C18:3n-3 sources in the diet was investigated. It is generally accepted that growth performances of double-muscled animals are lower on pasture or on grass silage, irrespective of the concentrate supply. This appears to be due to a reduced intake capacity compared to normal animals [22]. This explains that in this study, daily weight gains were substantially higher for the MC_4 group raised continuously indoors and receiving maize silage in the diet. This study was aimed at comparing two relatively low levels of grass silage in combination with linseed in the concentrates in the diet to allow a maximum supply of C18:3n-3 without compromising animal performances. However, this seemed difficult.

4.1. Fatty acid composition of muscles

The Belgian Blue double-muscled young bulls of this study showed an intramuscular TFA content lower than 1%, confirming previous work from our and other laboratories [5, 21, 23]. Across feeding treatments and muscle types, a strong linear relationship was found between the intramuscular total FA content and the SFA and MUFA content ($r = 0.979$ and 0.970 respectively). The absence of a linear relation between intramuscular total FA and PUFA contents ($r = 0.258$) reflects the fairly constant PUFA content and the PUFA enrichment of the PL. The absolute amount of PL was not significantly affected by the diets, and the value is in agreement with our earlier work using Belgian Blue double-muscled animals [5, 24]. Because of the low fat content, the PL fatty acids represented 40 to 50% of the total FA, similar to data reported by Nürnberg et al. [25] in 18 month old Belgian Blue double-muscled animals.

Clear differences between groups in relation to the feeding strategies were observed on the PUFA composition, especially with regards to the amount of total and individual n-3 and n-6 fatty acids. These results suggest that feeding linseed only during the fattening period results in an increase in the n-3 PUFA content but mainly as C18:3n-3 and not as C20:5n-3 and C22:5n-3. On the contrary, feeding the animals during the previous periods (last growing and pre-fattening) with an n-3 PUFA rich diet followed by a shift in the fattening period to a predominantly n-6 PUFA diet resulted in an equal amount of the long chain C20:5n-3 and C22:5n-3 fatty acids, and a clearly lower deposition of C18:3n-3. Therefore, the supplementation of the n-3 PUFA during the earlier stages of the experimental period led to the elongation of C18:3n-3 into its longer metabolites (C20:5n-3 and C22:5n-3) and to the incorporation of these fatty acids into the phospholipid fraction.

The C18:3n-3 supply in the fattening diets by linseed, combined or not with grass silage, resulted in a higher deposition of cis-9,trans-11 CLA in the tissues. This confirmed earlier studies using grass(silage)

either or not in combination with linseed [13, 26, 27]. However, the deposition of cis-9,trans-11 CLA in the adipose tissue seems to be less influenced by the fatty acid composition (mainly n-3 or n-6) of the diet of the previous stages. It appears that feeding n-3 fatty acids in the fattening period results in a higher ruminal production of trans-11 C18:1 [28], compared to the feeding of n-6 rich diets. Trans-11 C18:1 is the precursor for the formation of cis-9,trans-11 CLA by the action of the Δ^9-desaturation in the mammary gland [29] and in adipose tissue [30]. The proportion of intramuscular cis-9,trans-11 CLA is similar to values reported in other studies [13, 26], however the absolute amounts in our study were lower due to the lower fat content of the Belgian Blue double-muscled animals. In combination with the predominant deposition of cis-9,trans-11 CLA in the triacylglycerols, it can be concluded that the absolute content of cis-9,trans-11 CLA would be influenced by the degree of fatness of the animals, as confirmed by the strong linear correlation between the content of cis-9,trans-11CLA and the TFA or TAG contents ($r = 0.899$ and 0.905 respectively).

4.2. Nutritional considerations

Concerning the nutritional value of beef, an n-6/n-3 and PUFA/SFA ratio of respectively 5 or lower and 0.7 or lower has been recommended [31]. The values obtained for the different groups in this study are meeting these nutritional recommendations. The n-6/n-3 ratio is much better for the animals in this study, which were fattened on a high concentrate diet (even without an n-3 source), than for the animals of a breed or genotype with higher fat deposition fattened on high concentrate diets [13, 15, 32]. Combining the high concentrate diet with grass(silage) results in a further decrease of the n-6/n-3 ratio to a value of 2.0 to 2.5. These results are comparable with fatter animals raised on a grass diet without concentrates [13, 32]. Also, the P/S ratio nearly meets the nutritional recommendations [31], mainly due to the relatively high proportions of phospholipids in this lean breed.

It is not only important to focus on the recommended P/S and n-6/n-3 ratios, it is also worthwhile examining if this meat can supply the recommended intakes of C18:3n-3, C20:5n-3, C22:6n-3 and CLA. Extrapolated from animal studies, an intake of 3 g CLA (active isomers) per day is recommended to profit for mammary cancer prevention [33]. Consuming 100 g beef·day^{-1} only supplies 3 to 5 mg cis-9,trans-11 CLA, which represents 1% of the recommended value. However supplementing n-3 fatty acids in the fattening diet resulted in a doubling of the cis-9,trans-11 CLA content in the intramuscular fat in this study, an effect that should not be neglected.

Concerning the n-3 PUFA, Bjerve et al. [34] has proposed an optimal intake of 860–990 mg C18:3n-3/day and 350–400 mg (C20:5n-3 + C22:6n-3)·day^{-1} for adults. A 100 g beef portion from group GC_2 or GC_3 could provide 30 mg C18:3n-3 and 11 mg C20:5n-3 + C22:6n-3, and from group GC_4 15 mg C18:3n-3 and 6 mg C20:5n-3 + C22:6n-3. By the action of desaturase and elongase enzymes, only 15% of the dietary C18:3n-3 intake [35] can be converted to C20:5n-3 and C22:6n-3. Thus, a consumption of 100 g beef can only result in a supplementary provision of 2 to 5 mg long chain C20:5n-3 and C22:6n-3. This suggests that beef can only provide a small amount of the recommended intake of the n-3 fatty acids as well as of the recommended CLA intake, especially if the estimated daily intake of beef is taken into account (on average 35–50 g beef/day/person). However, this small contribution of beef, and meat in general, in the supply of essential fatty acids is important for most people in Western countries. A survey study in Australia revealed that the intake of long chain n-3 fatty acids is approximately 0.1–0.18 g·day^{-1} [36, 37], of which one third

of the long chain n-3 fatty acids was provided by meat [36]. Further increasing the content of the n-3 fatty acids and CLA in bovine meat, is advisable since many people in Western societies have a dislike to eat fatty fish. Finally, it should be clear that optimisation of meat fatty acid composition is a very limited approach within the concept of "functional meat" production. Besides the important effects of fatty acids, meat contains many other molecules e.g. anti-oxidants, vitamins, selenium, which can contribute to the aspects of "functional meat", and will be important to investigate in future research. Indeed, the relationships between consumer's health, meat consumption and meat production are many and complex [37].

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ABBREVIATIONS USED

CLA, conjugated linoleic acid; DVE, true protein digested in the small intestine;. GC_2, grass silage-concentrate$_2$; GC_3, grass silage-concentrate$_3$; GPS, whole triticale silage; LT, *longissimus thoracis*; MC_4, maize silage-concentrate$_4$; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; C_1, concentrate$_1$; SFA, saturated fatty acids; ST, *semitendinosus*; TB, *triceps brachii*; TFA, total fatty acids; VEVI, feed unit beef cattle intensive.

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