Effects of growth and breed on the fatty acid composition of the muscle lipids in cattle

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Summary. The adipose tissue development in muscle and the fatty acid composition of intramuscular fat of 200 bulls from three different breeds during growth was investigated. There were significant differences in quantitative and qualitative fat deposition which were genetically caused. Muscle of Galloway and White-blue Belgian bulls contained a high n-3 fatty acid content which is positive for human nutrition. During growth an increased deposition of saturated fatty acids into the adipocytes of muscle was measured.

Zusammenfassung. An insgesamt 200 Bullen von drei Rassen wurden während des Wachstums die Veränderungen der Fettgewebeentwicklung im Muskel sowie deren Fettzusammensetzung während des Wachstums geprüft. Zwischen den untersuchten Rinderrassen gibt es deutliche Differenzen in der qualitativen und quantitativen Fettspeicherung, die auf genetische Ursachen hinweisen. Das Muskelfleisch der Galloway und der Weiß-Blauen Belgier ist aufgrund des n-3 Fettsäuregehaltes für die menschliche Ernährung positiv zu bewerten. Mit fortschreitender Fetteinlagerung im Muskel während des Wachstums werden vermehrt gesättigte Fettsäuren in den Adipozyten deponiert.

Key words: Fatty acid, muscle, cattle, growth, breed

Introduction

The fat fulfils important biological functions in the animal organism. It is energy storage, pressure cushion, membrane constituent, carrier of essential fatty acids and fat-soluble vitamins as well as biosynthetic intermediates in various life processes leading to the production of hormones and eicosanoids. At birth the adipose tissue proportion in cattle amounts to about 3 % of the body weight, whilst in mature animals it is up to 20 % [1]. The deposition of fat and the supply of energy substrates are regulated by hormones, secondary messengers and enzymes in different metabolic pathways. The fat deposition is the result of the intake of fatty acids by the diet, the *de novo* fatty acid biosynthesis, the formation of triglycerids and the degradation of triglycerids. Exogenous and endogenous factors influence these anabolic and catabolic processes [2, 3]. The relative proportion of nutrients and the fatty acid composition of adipose and muscle tissues can be affected by different factors like diet, species, fatness, age/weight, depot site, gender, breed, maintenance, environmental temperature, and hormones [4-10]. In ruminants, the potential for dietary variation of the fatty acid composition is limited because of the hydrogenation of the polyunsaturated fatty acids (PUFA) in the rumen.
Current dietary guidelines recommend reducing fat consumption to 25-30 % of daily caloric intake and a contribution of one third of saturated, monounsaturated and PUFA in dietary fat. The requirement of n-3 fatty acids for young adults is 1.5 g/day and for n-6 fatty acids 10 g/day [11]. The intramuscular fat content of pork and beef are very low with 1-2 % or 2-6 %, and thus the energy content of lean meat is small. Also the fatty acid profile of lean meat is evaluated positively from nutrition-physiological view [12, 13]. In this experiment the effects

of breed and age on the fat deposition in the muscle as well as its fatty acid composition should be examined in cattle. Beside the Frisian cattle (German Holstein = GH), which the type of milk represents, the robust cattle breed Galloway (Ga) and an extreme meat breed, the White-blue Belgians (WBB) were examined, so that a wide spectrum of fat deposition capacity in the skeletal muscle was guaranteed. The fat deposition in longissimus muscle and the fatty acid composition were analysed during growth (from birth up to 24th life month at different age levels). Results of carcass composition [14], development of adipocytes [15], and meat quality [16] of these bulls are already publicated.

Material and methods

Animal material. Altogether 200 bulls of the breed Galloway, German Holstein and White-blue Belgians in different age levels were included in the investigation (Table 1).

Table 1
Number of bulls investigated in different age groups

Breed	Number of animals							Total
Slaughter age (months)	0	2	4	6	12	18	24	
Galloway	6	7	6	11	10	14	14	68
GH	6	10	10	10	10	11	12	69
WBB	4	6	7	8	9	15	14	63

The animals were kept and fed semi ad libitum at single fodder workstations in institute-own stable systems. According to the development of bulls the ration was increased weekly. The energy level of the feed amounted to that 1.6 -1.7fold of the maintenance requirement (530 kJ/kg LM $^{0.75}$) for the WBB and GH, for Galloway 500 kJ/kg LM$^{0.75}$). Wilted silage, maize silage, concentrate, soybean meal, drying beet pulpe and hay as well as a mixture of minerals were constituents of the fodder ration. The details of the experiment and the composition of the feeds are described by Schmundt [17].
Methods. The animals at the different age levels were slaughtered in the slaughter house of the institute. The carcass was dissected into the constituents meat, fat and bone. Muscle samples for chemical analyses were taken 24 h post mortem. The methods for the chemical analysis of the muscle and carcass composition are described by Ender and Hartung [18], Ender [14] and Kuhn et al. [19]. The fatty acid profile of the longissimus muscle fat was analysed by capillary gas chromatography (KGC). The lipids of the muscle were extracted after addition of an internal standard (C19:0) with chloroform/methanol (2:1, volume/volume). All solvents contained 0.01 % (wt/vol) of butylhydroxytoluol, in order to avoid oxidative modifications of the poly-unsaturated fatty acids. The homogenisation took place with the Ultra Turrax (3 x 15 seconds, 12000 U / min). After 18 h (5 °C in the refrigerator) the extract was washed, with 0.02 % $CaCl_2$ water, and dried with Na_2SO_4 and $CaCO_3$ (10:1 wt/wt) and filtered. Under nitrogen the solvent was removed. Methyl esters of muscle lipids were prepared after saponification with 0.5 N methanolic NaOH (5 minutes at 60 °C) and acidifying by methylation with boron trifluoride/methanol (14 % wt/vol) at 60 °C for 5 minutes. The fatty acid composition of the intramuscular fat were then analysed with a Perkin Elmer gas chromatograph with a flame ionisation detector, on a 0.25µm DB 23 fused silica capillary column (JBW Scientific, Fisons), 30 m x 0.25 mm i.d. The methyl esters were separated using hydrogen as carrier gas initially at 170^{o} C for 5 minutes, followed by linear programming from 170^{o} C to 200^{o} C at 5^{o} C/min,

hold for 10 min, followed by linear programming from 200° C to 220° C at 5° C per min and the final temperature was kept constant for 14 min. Split ratio was 1:40, the injector temperature was 260° C and the detector temperature 280° C. Individual fatty acids were identified by means of purified standards (Sigma-Aldrich, Deisenhofen, Germany) and calculated with an internal standard C19:0. Peak areas and percentages were calculated using PC software Turbochrom 4 (Perkin Elmer, San Jose, USA). The sum of n-3 fatty acids was calculated as C18:3 n-3 + C18:4 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3 and the sum of n-6 fatty acids consists of C18:2 n-6+ C20:4 n-6 +C20:3 n-6+ C22:4 n-6.

Statistical analysis. The influence of breed and age was studied by a twofold analysis of covariance (covariate ' live weight '). The data were analysed by the least-squares method using the GLM procedures (SAS®). All tables contain the Least Squares Mean (LSM) adjusted by covariate 'live weight' from GLM-procedures. In advance the homogeneity of slopes b (of covariate `live weight´) was tested by a separate calculation. All statistical tests of LSMeans were performed for a significance level $\alpha = 0.05$.

Results and discussion

In cattle, the adipose tissue development already starts in the fetal stage. Wegner et al. [15] found already mature adipocytes in semitendinosus muscle of 6 months old cattle fetus. After 115-120 days of pregnancy in perirenal adipocytes of sheep fetus high enzyme activities of the lipoprotein lipase and lipogenic enzymes were determined [20]. Already with the birth differences can be recognised concerning the total fat content in the carcass, the edible fat and the intramuscular fat content between the three breeds, them manifest themselves with increasing age of the animals. With 18 months clear breed-specific differences are visible in the storage of subcutaneous and intramuscular fat (Table 2, Figure 1).

Table 2
Selected carcass characteristics and meat quality of 18months old bulls

	Ga		GH		WBB		Sign.
	LSM	SE	LSM	SE	LSM	SE	effect
Live weight[1)], kg	460	11	571	12	597	10	breed
Subcutaneous fat of carcass, %	3.7	0.3	4.5	0.3	1.4	0.3	breed
Meat of carcass, %	77.2	0.4	76.6	0.5	84.0	0.4	breed
Area of longissimus muscle, cm^2	79.3	2.7	80.1	3.0	137.8	2.5	breed
Intramuscular fat of longissimus muscle, %	1.7	0.3	3.0	0.3	0.5	0.3	breed

[1)] at the slaughterhouse measured

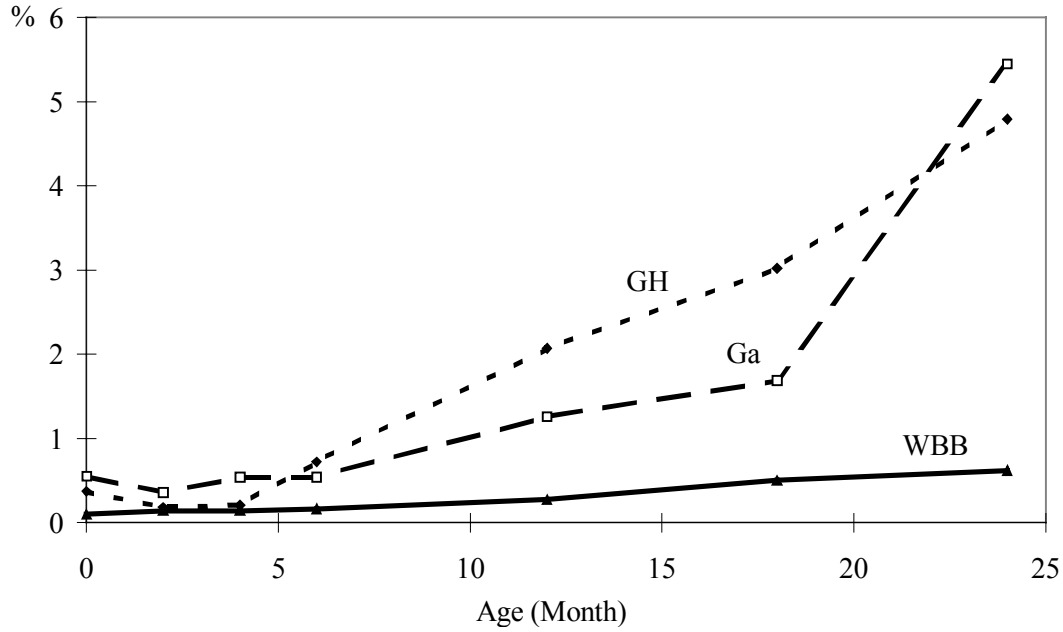

Figure 1 Modifications of the intramuscular fat content of longissimus muscle during growth

(4,5%) indicate a relatively strong greasing. The differences are still more drastic in the intramuscular fat content of the longissimus muscle (Figure 1).

The meat-richest and lowest fat carcass produced the White-blue Belgian bulls. With 1.4 % is the subcutaneous fat of carcass on a very low level, whilst Galloway bulls (3.7 %) and GH. The bandwidth reach here from only 0.5 % in the WBB up to 3.0 % in the GH [21]. The growth capacity of the robust breed Galloway is lower compared with the other breeds. Galloway bulls deposit early (12^{th} -14^{th} month) most fat [1]. The meat cattle breed White-blue Belgians produced the protein-richest and energy-poorest carcasses. At all age levels the WBB registered the highest protein gain and the smallest fat gain. Due to the lower adipose tissue proportion of the WBB the energy content of the meat is almost lower around half than in GH bulls. Additionally, it contains more water than the meat of the other breeds [22]. The differences of the quantitative fat deposition caused qualitative changes of the fatty acid profile in longissimus muscle (Table 3, Figure 2, 3). Already in the prenatal development the WBB showed a reduced fat synthesis compared with GH and Galloway, which reflects itself in the low total body fat and intramuscular fat content at birth (Figure 1). At birth the Galloway calves point the significantly highest n-3 fatty acid proportion and oleic acid content (C18:1) in the longissimus muscle (Table 3). Although the Galloway calves show the same fat content as the GH, differences are particularly observed here concerning individual fatty acids especially the n-3 fatty acids.

Genetic differences seem to exist in the deposition of certain fatty acids. The significantly increased proportional proportion of the polyunsaturated fatty acids in the muscle of the WBB male calves is caused by the very low muscle fat concentration. The muscle fat consist of the polar membrane lipids (phospholipids) and the neutral lipids in the adipocytes as well as a certain proportion of free fatty acids. The phospholipid concentration is a relatively constant size, so that an increase of the intramuscular fat content always is accompanied with an intensified storage of neutral lipids. In WBB at the age level 18 months the proportion of polar lipids was about 60 % of the intramuscular fat [13]. Already at birth 14.6 % polyunsaturated fatty acids are stored in the muscle fat of this meat-rich cattle breed.

Table 3
Fatty acid composition of longissimus muscle at birth

Fatty acids (mg-%)	Ga LSM	SE	GH LSM	SE	WBB LSM	SE	Significant effect
C18:0	13.9	0.8	13.0	0.8	13.4	1.0	breed
C18:1 cis 9	43.0	1.6	37.6	1.6	40.9	2.0	breed
C18:2 %	3.1	1.4	3.2	1.4	5.6	1.7	breed
C18:3 %	0.4	0.2	0.05	0.1	n.d.		breed
C20:3 n-6	1.3	0.2	2.2	0.2	2.1	0.2	breed
C20:4 n-6	3.2	0.5	4.2	0.5	4.7	0.6	breed
C20:5 n-3	0.3	0.2	0.17	0.2	n.d.		breed
C22:5 n-3	1.2	0.1	0.98	0.1	0.5	0.2	breed
C22:6 n-3	0.9	0.2	0.9	0.2	1.1	0.3	breed
UFA	63.2	1.4	68.6	1.4	68.4	1.7	breed
PUFA	10.8	2.1	13.2	2.1	14.6	2.6	breed
n-3 Fatty acids	2.8	0.5	2.1	0.5	1.6	0.6	breed
n-6 Fatty acids	7.9	1.9	11.1	1.9	13.0	2.4	breed

Table 4
Fatty acid composition of longissimus muscle at 18 months

Fatty acids (mg-%)	Ga LSM	SE	GH LSM	SE	WBB LSM	SE	Significant effect
C18:0	21.7	0.7	16.1	0.5	18.8	0.5	breed
C18:1 cis 9	32.5	1.3	38.8	1.0	20.7	1.0	breed
C18:2 n-6	6.7	1.1	4.6	0.9	17.2	0.8	breed
C18:3 n-3	1.3	0.1	0.7	0.1	1.5	0.1	breed
C20:4 n-6	2.1	0.4	1.5	0.3	4.8	0.3	breed
C20:5 n-3	0.3	0.1	0.1	0.1	0.4	0.1	breed
C22:6 n-3	0.8	0.2	0.01	0.1	n.d.		breed
UFA	49.7	1.1	54.8	0.9	59.9	0.9	breed
PUFA	11.8	1.7	8.3	1.3	26.8	1.3	breed
n-3 Fatty acids	2.6	0.4	1.5	0.3	2.9	0.3	breed
n-6 Fatty acids	9.2	1.6	6.8	1.2	23.9	1.2	breed

C22:5 not detectable

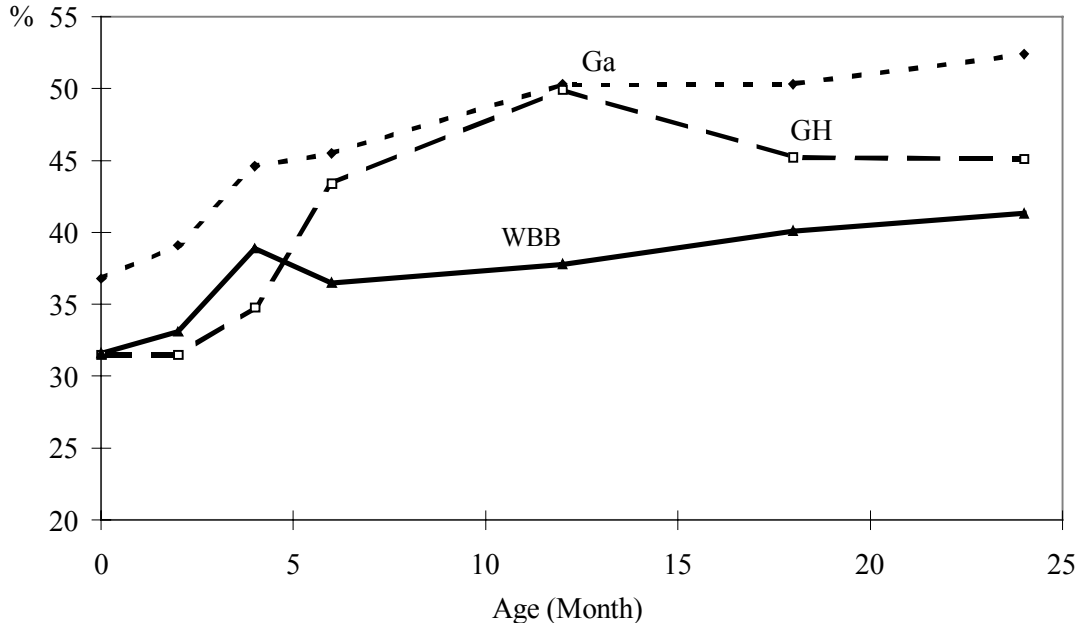

Figure 2 Modifications of the relative proportion of the saturated fatty acids in the muscle fat during growth

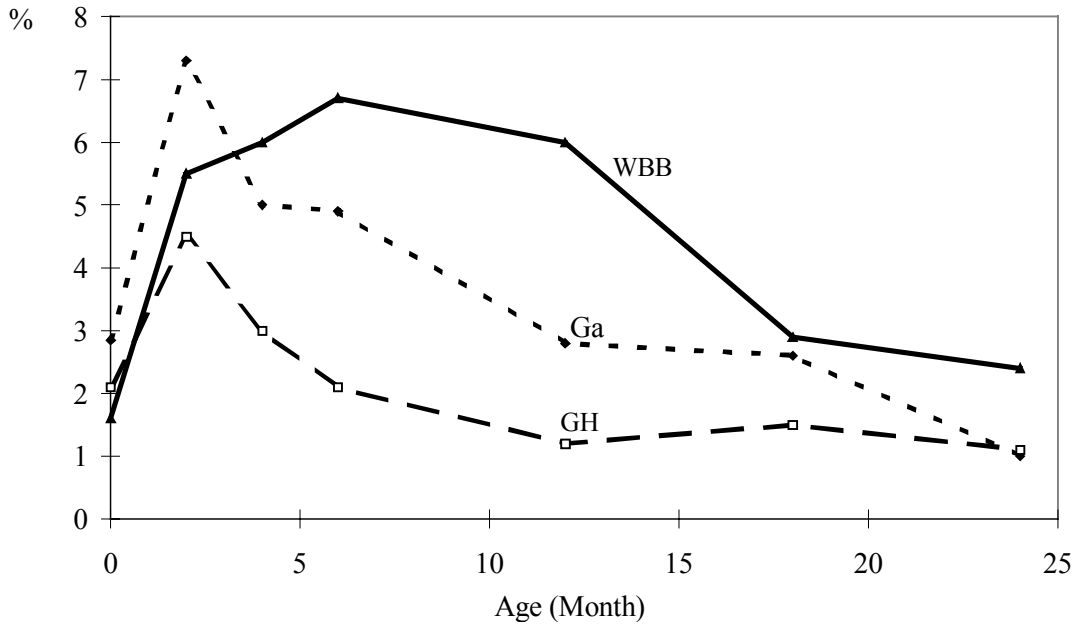

Figure 3 Modifications of the relative of n-3 fatty acid content in the muscle fat during growth

The typical fodder for cattle contains a low fat content, so that the exogenous fodder fat affects only slightly the fatty tissue storage. The deposited fat originates particularly from the de novo fatty acid biosynthesis [23]. Since the animals grew up under comparable feeding and

keeping conditions, can be suggested the differences genetically caused. The WBB show the highest lean meat proportion, the smallest fat content and a relatively high polyunsaturated fatty acid content in the muscle compared with the two breeds Ga and GH. These advantages are preserved up to the age from 24 months (Figure 1, 2 and 3).The nutrition-physiologically favourable n-3 fatty acid percentage is increased ($P<0.05$) in the WBB with starting at the age of 4 months (Fig. 3). The percentage of PUFA in longissimus muscle amounted to 27 % at the age of 24 months, while Galloways and GH indicated 4 and 7 %, respectively. In the typical battle age of 18 months the proportions of the n-3 fatty acids are higher ($P<0.05$) in Galloway and WBB bulls compared with the GH. The figure 2 shows the influence of age on the relative content of saturated fatty acids (SFA) in beef. At all three examined breeds a continuous increase of the saturated fatty acids was observed. The Ga-bulls indicated the highest content of saturated fatty acids in each age group. At 24 months it amounted to 52 %, while the WBB with 41.3 % indicated the smallest proportion of SFA. During the development of the adipocytes in the muscle the incorporation of saturated fatty acids was measured. In Galloway bulls, most adipocytes (7 x 10 4 in the muscle cross section M. semitendinosus) and the largest adipocyte diameter (91 mm) at the age of 24 months were found compared with WBB and GH. The low intramuscular fat content of the White-blue Belgians was caused by the reduced production of adipocytes and the reduced fat cell diameter [15].
The differences in the qualitative and quantitative fat deposition of the three cattle breeds investigated refer to genetic causes. The intramuscular n-3 fatty acid content of the Galloway and WBB bulls was still higly at the battle age of 18 months , respectively 2.65 % and 2.9 %, which is good for human nutrition. With progressive fat deposition into the muscle during growth saturated fatty acids were increasingly deposited into the adipocytes.

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