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Relationship between leptin content, metabolic hormones and fat deposition in three beef cattle breeds

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Abstract

The aim of the study was to determine if cattle breeds differing in their carcass characteristics also differ in the profiles of their leptin and metabolic hormones. Three breeds, Belgian Blue (BB) ($n = 12$), Limousin (L) ($n = 12$) and Aberdeen Angus (AA) ($n = 12$) with varying ability to deposit fat and protein were compared. Blood, muscle and subcutaneous (SC) adipose tissue were sampled. Animal performance, carcass and meat characteristics were determined as well as plasma leptin concentration, leptin gene expression in SC adipose tissue, leptin-receptor gene expression in SC adipose tissue and plasma concentration of insulin, tri-iodothyronin (T3), thyroxin (T4) and cortisol. The BB bulls showed the lowest values of leptin gene expression ($P < 0.05$). Values of plasma leptin concentration and of leptin-receptor gene expression tended to be lower in BB than in the other breeds. For a similar amount of adipose tissue (after normalisation), BB bulls showed a higher ratio of plasma leptin ($P < 0.05$), whereas normalised leptin gene and leptin-receptor gene expressions did not significantly differ between breeds. Belgian Blue bulls also differed in their metabolic hormone profile, tending to show lower values of insulin, T3 and T4 than the two other breeds. Cortisol levels were significantly lower ($P < 0.05$) in BB than in L and AA animals. © 2007 Elsevier Ltd. All rights reserved.

Keywords: Beef cattle; Breed; Carcass characteristics; Leptin; Metabolic hormones

Introduction

The word leptin comes from the Greek word *leptos*, meaning thin. The hormone leptin is the product of the *ob* gene and is a 16 kDa protein secreted mainly by white adipose tissue, and acts to regulate food intake, energy expenditure, homeostatic body weight, and consequently to influence fat deposition in both animals and humans (Considine et al., 1996; Yamada et al., 2003). Expression of the *ob* gene and circulating leptin concentrations are highly correlated with percentage of body fat in rodents (Soukas et al., 2000) and the degree of obesity in humans (Auwerx and Staels, 1998). Leptin is also involved in repro-

ductive function. Indeed, it has been shown in mice that leptin directly enhances insulin- and gonadotropin-stimulated ovarian steroidogenesis (Swain et al., 2004). Moreover, leptin modulates maternal nutrient partitioning in order to optimise the provision of nutrient for fetal growth and development (Holness et al., 1999).

Metabolic hormones such as insulin, cortisol or thyroid hormones also play a role in lipid metabolism. Insulin, for example, plays a major role by directing long chain fatty acids towards triglyceride storage rather than oxidation in muscle (Hocquette et al., 2000). Lents et al. (2005) showed that in swine insulin may be a potent regulator of leptin gene expression, and Ceddia et al. (2001) demonstrated that in the soleus and extensor digitorum longus muscles, leptin and insulin had opposing effects on lipid metabolism with leptin favouring lipid oxidation and insulin favouring lipid storage.

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The glucocorticoids, growth hormone, insulin, triiodothyronin (T3) and thyroxin (T4) are all factors that regulate leptin expression or its plasma level to some extent (Chilliard et al., 2001, 2005). In cattle, plasma leptin levels are closely correlated with adipose tissue cellularity, body condition score and nutritional status (Chilliard et al., 2001; Delavaud et al., 2002). Moreover, circulating leptin levels in beef cattle increase during fattening (Kawakita et al., 2001). *ob* mRNA levels are proportional to adipocyte size and increase with increasing body fat (Houseknecht and Portocarrero, 1998).

The mechanisms leading to differences in carcass characteristics, particularly fat accumulation, between cattle breeds are not well understood (Higashiyama et al., 2003). Chilliard et al. (2005) have shown that in rodents, through effects on either the central nervous system or endocrine glands, leptin decreases insulin and glucocorticoids and stimulates growth hormone, catecholamines and thyroid hormone secretions, thus increasing tissue energy expenditure and adipose tissue lipolysis, and decreasing adipose tissue and/or liver lipogenesis. Furthermore, leptin acts directly on peripheral tissues stimulating lipolysis and inhibiting lipogenesis in adipose tissue, increasing insulin sensitivity and glucose utilisation in muscle and boosting fatty acid oxidation in muscles, liver and adipose tissue.

Belgian Blue (BB) cattle are known for muscular hypertrophy, high killing out proportion (i.e., the ratio of the warm carcass weight to the slaughter weight) and very lean meat (Pagano Toscano et al., 1993). Intramuscular fat content is lower in BB in comparison to the Limousin (L) or Aberdeen Angus (AA). Comparing the metabolism in different breeds may help us to understand differences in fat deposition. The objective of the present study was to examine whether circulating levels and mRNA expression of leptin in subcutaneous (SC) adipose tissue were different between the three breeds. The metabolic hormone profiles of insulin, T3, T4 and cortisol were also investigated.

Materials and methods

Animals

The study was approved by the Animal Ethics Committee of the University of Liège. A total of 36 young growing fattening bulls aged 14–15 months were used: there were 12 BB, 12 L and 12 AA animals in the study. The bulls were fattened at the research farm of the University of Liège over a period of 3.5 months. During the experiment, the bulls were maintained in a free stanchion barn with straw as bedding. The mean live weight at the beginning of the trial was 351 ± 70 , 398 ± 65 and 345 ± 36 kg in the BB, L and AA groups, respectively. The animals were given ad libitum access to food. At the end of the finishing period, the bulls were slaughtered at an age ranging from 17.5 to 18.5 months.

Sample collection

Blood for leptin and metabolic hormone (insulin, T3, T4 and cortisol) determination was collected in the early morning. The animals were fasted from the evening before and samples were collected after shipping to the slaughterhouse, where the animals were killed immediately. Blood was

stored in K3EDTA-coated evacuated tubes (Vacutainer, VWR) and held on ice for approximately 1–2 h. Blood samples were subsequently centrifuged at 1200 g for 15 min and plasma was transferred to fresh tubes. Plasma samples were stored at -20°C .

Samples from SC adipose tissue and from the longissimus thoracis muscle were taken at slaughter, immediately cut up in pieces of approximately 5 mm^3 and snap frozen in liquid nitrogen. Tissue samples were further stored at -80°C in cryotubes.

Animal performance and carcass characteristics

Animal performance was calculated on the basis of daily feed intake and on live weight measurements taken every 4 weeks. The proportion of muscle, fat and bone in the carcass was assessed after dissection of the 7th to 9th rib joint.

The protein and lipid contents of the longissimus thoracis muscle samples were assessed on freeze dried samples according to official procedures (Association of Official Analytical Chemists, 1975).

Leptin protein assay

Plasma leptin concentration was determined using the commercially available multispecies I125-radioimmunoassay (RIA) kit (Linco Research). This assay is developed with human leptin as tracer and standard. It is recommended for various species including bovines and has been validated for use with bovine serum (Wegner et al., 2001). The lowest level of leptin that can be detected by this assay is 1 ng/mL (human equivalent) when using a 100 μL sample size. Its precision is estimated to be 3% (coefficient of variation: CV) within assays and 8% between assays.

Although the assay used here has been criticised in studies comparing this multispecies kit with species-specific assays (Delavaud et al., 2002), a preliminary study performed in our laboratory revealed a significant correlation ($r = 0.45$, $P = 0.0076$) between both assays and repeatable intra-assay results (CV: 3%).

Assessment of leptin expression

Total RNA was isolated from SC adipose tissue samples using the Tripure reagent (T9456 Production, Sigma), as recommended by the manufacturer. RNA yields and purities were assessed by absorbance at 260 and 280 nm in a RNA/DNA calculator (Biotech Photometer). Ratios of absorption (260/280 nm) of all preparations were 1.6–2.0. cDNA was obtained by reverse transcription of 2 μg total RNA with random sequence hexanucleotides (Cat RP-6, GeneWorks) using SuperScript Rnase II H⁻ (Cat 18125-019, Invitrogen). RT products were either stored at -20°C or used directly for PCR.

The cDNA of bovine leptin was obtained with the following primer pair: forward GACATCTCACACGCAG and reverse GAG-GTTCTCCAGTCATT. PCR was performed in a 20 μL reaction volume containing 3.0 μL cDNA, 2.0 mM of MgCl_2 , 2 U of *Taq* DNA polymerase (Amersham–Biosciences), 0.2 mM of dNTPs and 0.5 mM of each primer. This produced a fragment of 183 bp of bovine leptin. The optimal cycle used was: 120 s at 94°C , 35 cycles at 94°C for 30 s, 54°C for 30 s and 72°C for 30 s. The reaction was followed by 5 min at 72°C . For the amplification of leptin receptor (a fragment of 400-bp), the forward primer was GTGCCAGCAACTACAGATG and the reverse primer AATTCCTCAAGTTTCAA. The PCR was performed in a 20 μL reaction volume containing 2.0 μL cDNA, 1.5 mM of MgCl_2 , 1 U of *Taq* DNA polymerase (Amersham–Biosciences), 0.2 mM of dNTPs and 0.5 mM of each primer. A 243 bp of bovine β -actin cDNA was similarly amplified with TGAGTTCAGTGGCAACCT and GGACGACTG-GAAATATGA and used as internal standard.

Electrophoresis of the RT-PCR products was performed on a 1.5% agarose gel. This was stained in ethidium bromide, trans-illuminated with UV light and photographed (Nikon). Photography was transferred on the computer and the intensities of RT-PCR products were measured by Gene

Scan program. Molecular sizes of PCR products were estimated by comparing their electrophoretic migration pattern with this of a specific ladder (100 Base Pair Ladder, 27-4001 Amersham).

Assessment of metabolic hormones

Circulating levels of insulin, T3, T4 and cortisol were determined by RIA. Insulin plasma levels were measured by immunoradiometric assay kit for the quantitative measurement of human insulin in serum and plasma (kit INS-IRMA Biosource Europe). According to the manufacturer, this method is also validated for porcine and bovine insulin measurement and the test shows a detection limit of 0.1 μ UI/mL (within- and between-assay CV were 2.1% and 4.7%, respectively).

T3 circulating levels were measured by a solid-phase RIA for human serum or plasma (Orion Diagnostica). The detection limit was <0.2 nmol/L. Within- and between-assay CV were 3.3% and 4.5%, respectively. T4 circulating levels were also measured by a solid-phase RIA for human serum or plasma (Orion Diagnostica). This test shows a detection limit <5 nmol/L, and the within- and between-assay CV were both 3.3%.

Cortisol circulating levels were measured by a solid-phase RIA for human serum, plasma, urine or saliva (Orion Diagnostica). The detection limit of this test was <5 nmol/L and within- and between-assay CV of 2.6% and 6.5%, respectively.

Statistical analysis

Data were shown as means \pm standard deviation (SD) and were analysed using the general linear model (GLM) procedure of Statistical Analysis Systems (SAS) with a fixed effect of breed. The model used was: $Y = \mu + B + \varepsilon$, where B is the fixed effect of the breed and ε is the least square residual error. A P value ≤ 0.05 was considered as significant. Means were compared by Student's t test.

Results

Animal performances, carcass and meat characteristics

Animal performance, and the carcass and meat characteristics are summarised in Table 1. There was a large and significant breed effect except for the live weight gain, which averaged 1.62 kg/day. The largest feed intake was recorded in the AA animals and the lowest in BB bulls. Sig-

Table 1
Mean animal slaughtering performances, carcass and meat compositions recorded in Belgian Blue (BB, $n = 12$), Limousin (L, $n = 12$) and Aberdeen Angus (AA, $n = 12$) bulls

	BB	L	AA
<i>Animal performances</i>			
Average daily gain (kg/day)	1.59 \pm 0.20	1.62 \pm 0.16	1.66 \pm 0.23
Average feed intake (kg/day/animal)	9.0 ^a	9.6 ^b	9.9 ^c
<i>Carcass composition</i>			
Killing out proportion (%)	65.2 \pm 1.56 ^a	60.7 \pm 1.58 ^b	55.0 \pm 1.79 ^c
Muscle (%)	77.5 \pm 1.45 ^a	67.6 \pm 2.69 ^b	62.2 \pm 2.28 ^c
Adipose tissue (%)	10.2 \pm 0.82 ^a	18.7 \pm 1.88 ^b	23.6 \pm 1.37 ^c
Bone (%)	12.3 \pm 1.11 ^a	13.8 \pm 1.88 ^b	14.3 \pm 2.75 ^b
<i>Meat composition (in DM %)</i>			
Lipid (%)	2.7 \pm 0.63 ^a	6.5 \pm 1.59 ^b	9.3 \pm 3.50 ^c
Protein (%)	89.8 \pm 1.32 ^a	85.6 \pm 3.22 ^b	81.7 \pm 4.82 ^c

Data are presented as means \pm SD.

Data with different superscripts significantly differ, $P \leq 0.05$. DM, dry matter.

nificant differences were observed between the carcasses: BB carcasses were characterised by the largest killing-out and muscle proportions and the lowest adipose tissue and bone proportions, but the opposite was found for the AA bulls while intermediary data were observed in L animals. The breed also significantly affected the meat composition with BB meat characterised by the highest protein content and that of the AA bulls by the lowest. Opposite results were observed for fat content. The characteristics of the L animals lay in between those of BB and AA.

Metabolic hormones

Table 2 shows the mean values of plasma concentration of metabolic hormones (insulin, T3, T4 and cortisol) according to the breed. The concentration of all hormones except cortisol did not significantly differ between breeds although the values in BB tended to be lower than in the two other breeds. The cortisol plasma concentration was significantly lower in BB than in the two other breeds.

Plasma leptin concentration, leptin gene expression and leptin-receptor gene expression

Fig. 1 shows plasma leptin concentration (Fig. 1a), leptin gene expression in SC adipose tissue (Fig. 1b) and leptin-receptor gene expression in SC adipose tissue (Fig. 1c) according to breed. The plasma leptin concentration did not significantly differ between the three breeds. Leptin gene expression was significantly lower in BB than in the two other breeds. The expression of the leptin-receptor gene was not significantly different between the breeds.

The mean values of plasma leptin concentration, leptin gene expression in SC adipose tissue or leptin-receptor gene expression in SC adipose tissue, that were normalised by adipose tissue percentage are shown in Fig. 2. The mean ratio between plasma leptin concentration and adipose tissue percentage was significantly higher in BB than in the other breeds (Fig. 2a), while these mean ratio values were not significantly different between L and AA. The mean ratio between leptin gene expression (Fig. 2b) or leptin-receptor gene expression (Fig. 2c) and adipose tissue percentage did not significantly differ between breeds.

Table 2
Mean plasma concentrations of metabolic hormones recorded in Belgian Blue (BB, $n = 12$), Limousin (L, $n = 12$) and Aberdeen Angus (AA, $n = 12$)

Metabolic hormones	BB	L	AA
Insulin (μ UI/mL)	12.69 \pm 8.49	18.93 \pm 12.14	18.85 \pm 7.68
T3 (nmol/L)	0.88 \pm 0.21	1.05 \pm 0.40	1.16 \pm 0.22
T4 (nmol/L)	108.9 \pm 13.0	119.8 \pm 19.60	124.8 \pm 25.62
Cortisol (nmol/L)	89.64 \pm 30.45 ^a	119.83 \pm 27.20 ^b	116.36 \pm 17.67 ^b

Data are presented as means \pm SD.

Data with different superscripts significantly differ, $P \leq 0.05$. T3, tri-iodothyronin; T4, thyroxin.

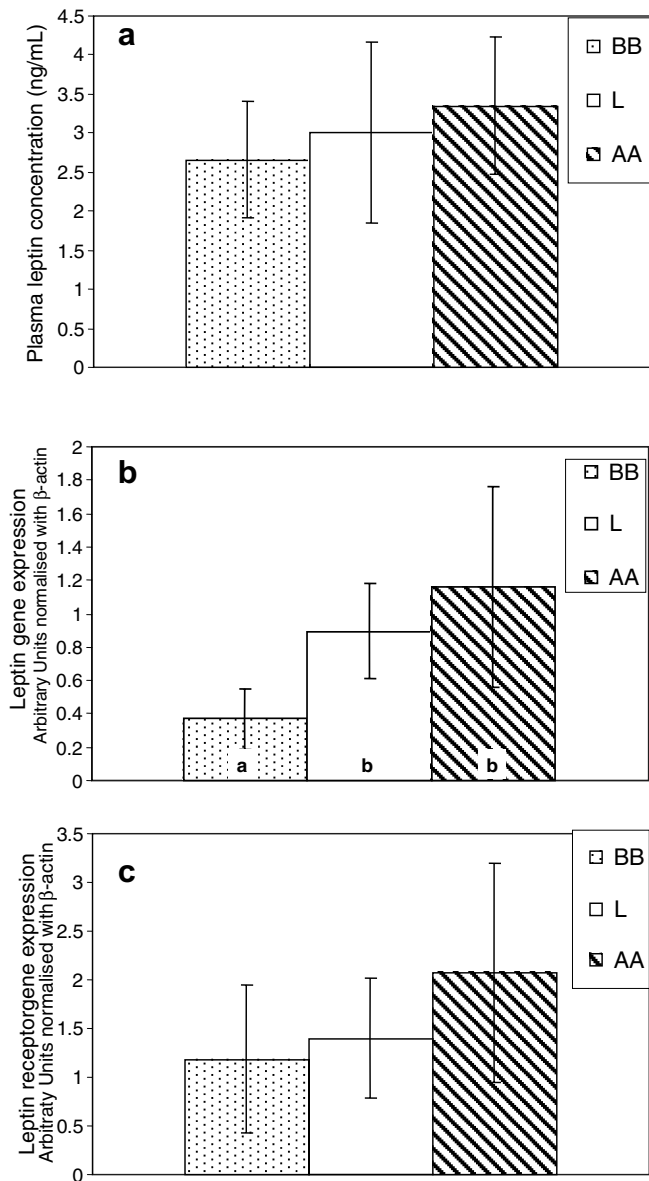


Fig. 1. Mean plasma leptin concentration (a), leptin gene expression in SC adipose tissue (b) and leptin-receptor gene expression in SC adipose tissue (c) recorded in BB ($n = 12$), L ($n = 12$) and AA ($n = 12$) bulls. Data are shown as means \pm SD. Data with different superscripts were significantly different at $P < 0.05$.

Discussion

The aim of the study was to determine if the differences observed between the three breeds (BB, L and AA) in terms of slaughter performances, carcass and meat characteristics were also present in the leptin and metabolic hormones profiles.

Metabolic hormones were measured in blood samples taken at slaughter and might have affected our results because some values could change when an animal is stressed (Chacon et al., 2005). Terlow (2002) has shown that cortisol plasma concentration increased within only 20 min of a stressor. In fact, the values of cortisol concen-

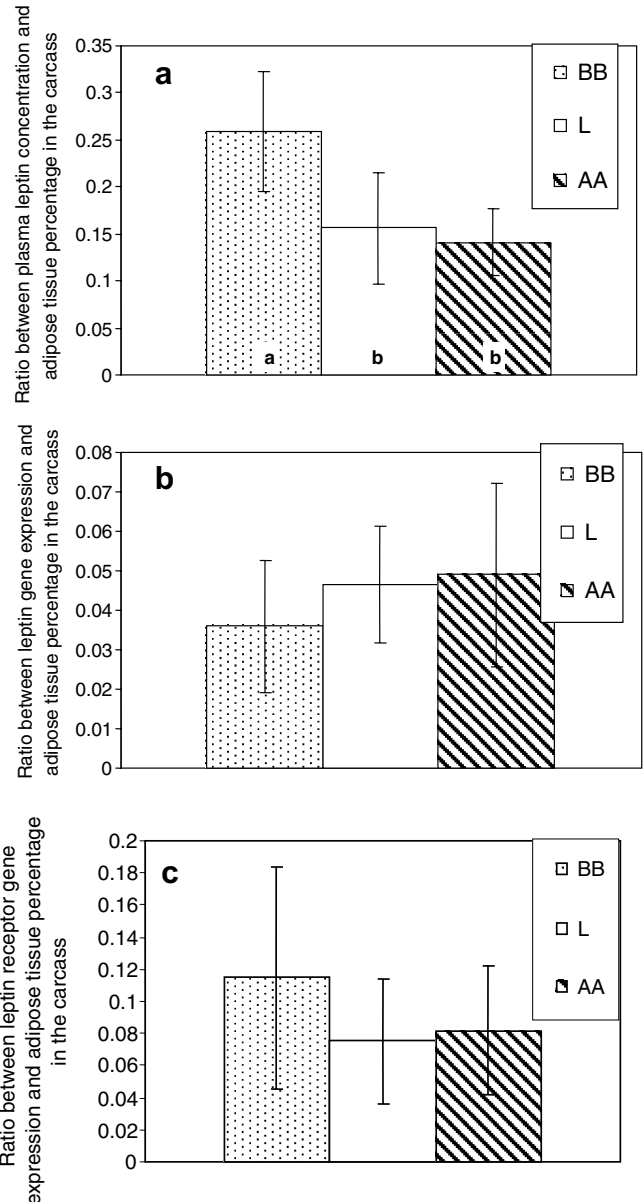


Fig. 2. Mean ratio between plasma leptin concentration (a), leptin gene expression in SC adipose tissue (b) or leptin-receptor gene expression in SC adipose tissue (c) and adipose tissue percentage recorded in BB ($n = 12$), L ($n = 12$) and AA ($n = 12$) bulls. Plasma leptin, leptin gene expression and leptin-receptor gene expression were normalised by adipose tissue percentage. Data are shown as means \pm SD. Data with different superscripts were significantly different at $P < 0.05$.

trations measured in our study were higher than basal values observed by Chastant-Maillard et al. (2003), which ranged from 15 to 20 nmol/L. The plasma concentrations of cortisol were in the same range as the concentrations found by Okeudo and Moss (2005) in freshly slaughtered lambs. In spite of a potential stress effect, it was nevertheless possible to compare the three breeds since all of the bulls were slaughtered and maintained in the same conditions during the whole study. With regard to the other metabolic hormones, the plasma concentration of insulin was in agreement with those measured by Hornick et al.

(1998) and results of plasma concentration in T3 and T4 were similar to those founded by Pagano Toscano et al. (1993).

In our study, the three breeds strongly differed with regard to slaughtering performance and carcass characteristics. In BB, the mean proportions of adipose tissue and muscle in carcass were 10.2% and 77.5%, respectively, and in agreement with results published by Hornick et al. (1998). The mean proportions of lipids and proteins in dry matter of the meat were 2.7% and 89.8%, respectively, which was in agreement with data published by Fiems et al. (2002). In AA, the mean proportions of adipose tissue and muscle in the carcass were 23.6% and 62.2%, respectively, and for lipid and protein percentages in the meat 9.3% and 81.7%, respectively. Values determined for L cattle lay between those for BB and those for AA. Thus BB had proportionally higher muscle and protein percentages, lower adipose tissue and lipid percentages and also a better killing out proportion than L and AA (Cuvelier et al., 2005).

Despite the lack of significance for certain variables, the results obtained for plasma leptin concentration, leptin gene expression and leptin-receptor gene expression suggest that a difference between breeds might exist. Only leptin gene expression was significantly lower in BB, but all leptin variables were lowest in BB and highest in AA. The values obtained for the L were intermediary. The plasma leptin concentrations measured in L and AA were in the same range as those observed by Ren et al. (2002). The difference between the breeds could be related to the lower adipose tissue percentage found in the BB. Effectively, leptin is synthesised by adipose tissue and its synthesis is proportional to abundance of adipose tissue (Chilliard et al., 1999, 2005). A high leptin concentration is associated with fatness in pigs, in growing cattle and other mammals (Ehrhardt et al., 2000).

Chilliard et al. (2005) compared the leptinemia found in some bovine breeds differing in their body fatness. They observed that the lean breeds showed lowest values for leptin. However, when leptin values were corrected for individual differences in SC adipocyte size, there was no longer any difference in leptinemia, suggesting that plasma leptin primarily reflects differences in body fatness.

Absolute values have been normalised in order to compare plasma leptin concentration, leptin gene expression and leptin-receptor gene expression synthesised by a similar amount of SC adipose tissue in the three breeds. The ratio obtained showed that, for the same SC adipose tissue percentage, BB had significantly higher plasma leptin concentrations. In contrast, there was no difference between the breeds in the ratio of leptin gene expression to leptin-receptor gene expression. This discrepancy could possibly be explained by a higher number of adipocytes for the same amount of adipose tissue in BB, or by an important source of leptin other than SC adipose tissue. In ruminants, leptin is synthesised in SC white adipose tissue as well as in visceral adipose tissue and brown adipose tissue (Auwerx

and Staels, 1998; Chilliard et al., 1999, 2005). In other species, leptin synthesis by the placenta, the skeletal muscle, the gastric and the mammary epithelium and the brain has also been reported (Ahima and Flier, 2000). It would therefore be interesting to determine whether leptin gene expression and leptin-receptor gene expression differ between adipose tissues of the body and whether breed-related differences exist.

Auwerx and Staels (1998) have shown that in humans leptin reflects the development of further body fat rather than a given body fatness level. They also observed differences in plasma leptin concentration in individuals with a similar weight and body fatness. Subjects with low plasma leptin concentrations stabilised or continued to enlarge, whereas those with high plasma leptin concentration stopped or even lost weight, thus the proportionally higher plasma leptin concentration (ratio) in BB bulls could perhaps be due to their different fattening capacities. Indeed, despite a similar daily weight gain, BB bulls showed the lowest daily feed intake among the three breeds, which could be a consequence of the inhibitory effect of leptin on appetite (Considine et al., 1996; Yamada et al., 2003). We could further speculate that the low fat deposition and high muscle development might be influenced by an increased plasma leptin/body fat ratio.

We also investigated the metabolic hormones insulin, T3, T4 and cortisol. Although no significant breed-related differences were detected, BB bulls showed the lowest values and the AA the highest. Similarly, Pagano Toscano et al. (2001) showed that plasma concentrations of some metabolic hormones (T3, T4, free T4 and insulin) were lower in cattle with muscular hypertrophy.

The relation between the hormones seems complex; insulin stimulates lipogenesis and fatty acid esterification while it inhibits their oxidation. This implies that a high insulin plasma level is apparently related to higher adiposity observed in some breeds (Ren et al., 2002) and positively correlated with plasma leptin concentration (Lents et al., 2005). Kadokawa et al. (2003) have shown that dexamethasone and insulin increase leptin gene expression *in vitro* but that an *in vivo* injection of dexamethasone did not affect plasma leptin concentration in Holstein multiparous non-lactating cows. In obese humans, leptin concentrations may be increased by simultaneous high cortisol and insulin levels (Robaczyk, 2002). Accordingly, the exact relation between leptin, cortisol and insulin remains unclear and species-related differences might exist. It would be interesting to analyse the correlations between metabolic hormones and leptin variables in animals with very different fattening states as well as to analyse the evolution of those correlations during the fattening period in individuals.

Conclusions

By comparing Belgian Blue cattle with Limousin and Aberdeen Angus, we have observed that Belgian Blues

had the lowest metabolic hormone plasma concentrations, associated with the highest muscle proportion in the carcass, the highest protein percentage in muscles, the lowest proportion of adipose tissue and lowest lipid percentages. For the same amount of SC adipose tissue, the Belgian Blue had significantly more plasma leptin. These results may reflect a lower basal metabolism, which could, at least in part, explain the better food conversion efficiency of these cattle.

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