Differential cellularity and fatty acid profile in subcutaneous and mesenteric fat depots from Portuguese bovine breeds

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Abstract - This study focused on the effects of feeding high or low silage diets on the cellularity of subcutaneous and mesenteric fat depots of beef cattle and their implications on the fatty acid profile. Thirty nine bulls from two philogenetically distant Portuguese bovine breeds, Alentejana and Barrosã, were selected. Breed showed no influence on subcutaneous fat deposition and visceral fat partitioning. Plasma adipokines showed an unclear relationship with fatness, as leptin remained constant among experimental groups while interleukin-6 (IL-6) was breed-related. Adipocytes´ size and number were determined by the fat depot location, as larger but fewer cells were observed in subcutaneous fat in comparison to mesenteric fat. Breed, diet and fat depot location influenced the fatty acid profile. The incorporation of saturated (SFA), *trans*, polyunsaturated (PUFA) and branched chain fatty acids (BCFA) was higher in the mesenteric fat depot, whereas the subcutaneous fat depot contained more monounsaturated fatty acids (MUFA). SFA and MUFA proportions revealed the influence of breed, but diet influenced PUFA and BCFA proportions. These results suggest contrasting cellularity and fatty acid biosynthesis in bovine's subcutaneous and mesenteric fats, and reinforce the need to consider factors such as breed and, to a lesser extent, diet.

Keywords - Bovine adipose tissue, Cellularity, Fatty acid profile

I. INTRODUCTION

The amount, location and composition of fat in cattle are essential as subcutaneous and visceral fat depots are considered as "waste fat", whereas intramuscular fat is regarded as "taste fat" [1]. The development of strategies to manipulate adipose tissue deposition in farm animals has been one of the major breeding goals for many years [2].

The information available regarding the effects of genotype on adipose tissue cellularity and fatty acid composition is scarce and, thus, biochemical studies in this field should be encouraged to clarify the molecular mechanisms involved [2]. In addition, genetic distances have been already described for some Portuguese autochthonous bovine breeds, independently of their geographical location [3]. In this experiment, we aimed to assess breed- and diet-induced variations on adipose tissue cellularity of young bulls. For this purpose, two philogenetically distant autochthonous bovine breeds (Alentejana and Barrosã), two experimental diets (based on 30/70% and 70/30% of silage and concentrate, respectively) and two distinct fat depots (subcutaneous and mesenteric fats) were selected. Adipocytes' size and number (per area) of subcutaneous and mesenteric fat depots were evaluated, through histometrical analysis, in parallel with plasma determination of some adipokines (leptin and IL-6). To further characterize these effects upon fatty acid deposition in subcutaneous and mesenteric fats, the fatty acid composition was determined in both fat depots.

II. MATERIAL AND METHODS

The experiment was conducted under the guidelines for the care and use of experimental animals of Unidade de Produção Animal, L-INIA, INRB (Fonte Boa, Vale de Santarém, Portugal).Thirty-nine young bulls from Alentejana (large-framed) and Barrosã (small-framed) breeds, were assigned to either high silage (HS, 30% concentrate/70% silage) or low silage (LS, 30% silage/70% concentrate) diets. The initial average weight was 266 ± 10.5 kg for Alentejana and 213 ± 3.64 kg for Barrosã bulls.

One week prior to slaughter, blood samples were collected from the tail vein and centrifuged to harvest plasma. Triacylglycerols and glucose levels were determined in plasma through diagnostic test kits using a Modular Hitachi Analytical System. Plasma insulin and IL-6 were quantified using Bovine ELISA

kits, whereas leptin was determined through a Multi-Species RIA kit.

The amount of adipose tissue in the subcutaneous fat was assessed by dissecting the leg joint. Mesenteric, omental and kidney knob and channel fat (KKCF) depots were excised and weighted. For histometrical analyses, samples from subcutaneous and mesenteric fat depots were fixed in 10% neutral buffered formalin and processed for paraffin embedding. Tissue sections (10 μm thick) were stained with classical hematoxylin and eosin to assess morphology under a light microscope. The entire histological plan was followed as described by Corino *et al*. [4].

Total lipids from adipose tissue samples were extracted as described by Folch *et al*. [5]. Fatty acid methyl esters were extracted with *n*-hexane and analysed as described by Bessa *et al.* [6].

Values are presented as mean ± standard error of the mean (SEM). Data analysis concerning body composition parameters, plasma metabolites and adipokines was performed using the General Linear Model of SAS software package, v 9.1 [7]. The analysis of variance on histometrical data and lipid profile was performed using SAS PROC MIXED.

III. RESULTS AND DISCUSSION

The economical and physiological importance of fat deposition in meat animal production has long been recognized [8]. Nonetheless, scarce information on the biology and regulation of each fat depot is available. Subcutaneous fat, along with the intermuscular fat, is the largest adipose tissue depot [9] with the highest lipogenic activity [10], whereas mesenteric fat displays distinctive immune-response potential [11].

Alentejana and Barrosã bulls have quite distinct morphological characteristics [12] and, as expected, live slaughter, carcass and leg joint weights (Table 1) varied significantly between breeds. The dissection of the leg showed no differences among groups regarding the subcutaneous fat. Mesenteric and omental fats were higher in concentrate-fed animals.

Glucose levels in plasma were higher in Alentejana than in Barrosã bulls. It is well known that ruminants show typical insulin resistance compared to monogastrics. Insulin concentrations were highest in concentrate-fed bulls. In ruminants, dietary carbohydrates are fermented into volatile fatty acids by ruminal microorganisms, and the propionate formed is used as a primary precursor for gluconeogenesis [13]. Propionate from rumen fermentation is largely associated with body fat deposition, as it promotes lipogenesis through the secretion of insulin.

The area and number of adipocytes (Table 2) were considerably different between fat depots. Some authors [8, 14] observed that subcutaneous fat had smaller adipocytes than visceral fat depots. Nevertheless, these studies failed to characterize the mesenteric fat depot. In contrast, our results revealed that the subcutaneous fat had larger adipocytes than the mesenteric fat, and similar findings were reported by Pike and Roberts [15]. The increase of adipocytes area in the subcutaneous fat in comparison to the mesenteric fat might be an indicator of an apparent early differentiation of adipocytes in the case of subcutaneous fat. These findings point out to a differential cellular dynamics of mesenteric fat from other visceral fat depots, which can be a direct consequence of its lipogenic activity.

This study showed that fat depot location is of extreme importance when considering cellularity and fatty acid profile. The highest amount of fatty acids incorporation in the mesenteric fat of Alentejana breed might be due to differences in fat partitioning between breeds, with Alentejana breed accumulating higher amounts of fatty acids in internal fat depots (Table 3). In fact, all classes of fatty acids were determined by the fat depot location. There were also significant effects of breed, as well as its interaction with fat depot, on the sum of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). High MUFA content in the subcutaneous adipose tissue has been reported [1] as a consequence of elevated stearoyl-coA Δ-9 desaturase activity. Total trans fatty acids (TFA) were not affected by diet, in contrast to the fat depot and its interaction with breed. This sum was higher in mesenteric fat of Barrosã bulls and subcutaneous fat of Alentejana bulls. Polyunsaturated fatty acids (PUFA) proportions reached the highest values in low silage fed animals. The branched chain fatty acids (BCFA) levels in both fat depots were higher in high silage than in low silage fed animals. According to Aldai *et al*. [1], BCFA are higher in leaner animals and, in fact, no effect of breed was observed, which is concomitant with other parameters measured in this study, namely fat depots mass, cellularity and leptin levels.

The contrasting cellularity observed in subcutaneous and mesenteric fats from Alentejana and Barrosã bulls, under these experimental conditions, may reflect a differential dynamics between

hypertrophy and hyperplasia processes in these adipose tissue depots, thus reinforcing our previous knowledge of distinct metabolic activity.

IV. CONCLUSION

Herein, fat depot location has been shown as the major determinant of adipocytes' area and number, along with fatty acid profile, thus suggesting a contrasting cellular dynamics between subcutaneous and mesenteric fats in bovines.

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REFERENCES

[1] Aldai N, Nájera AI, Dugan MER, Celaya R, Osoro K (2007). Characterization of intramuscular, intermuscular and subcutaneous adipose tissues in yearling bulls of different genetic groups. Meat Sci 76: 682–691.

[2] De Smet S, Raes K, Demeyer D (2004). Meat fatty acid composition as affected by fatness and genetic factors: a review. Anim Res 53: 81–98.

[3] Beja-Pereira A, Alexandrino P, Bessa I, Carretero Y, Dunner S, Ferrand N, Jordana J, Laloe D, Moazami-Goudarzi K, Sanchez A, Cañon J (2003). Genetic characterization of Southwestern European bovine breeds: a historical and biogeographical reassessment with a set of 16 microsatellites. J Hered 94: 243–250.

[4] Corino C, Di Giancamillo A, Rossi R, Domeneghini C (2005). Dietary conjugated linoleic acid affects morphofunctional and chemical aspects of subcutaneous adipose tissue in heavy pigs. J Nutr 135: 1444–1450.

[5] Folch J, Lees M, Stanley GHS (1957). A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497-509.

[6] Bessa RJB, Alves SP, Jerónimo E, Alfaia CM, Prates JAM, Santos-Silva J. Effect of lipid supplements on ruminal biohydrogenation intermediates and muscle fatty acids in lambs (2007). Eur J Lipid Sci Tech 109: 868–878.

[7] Statistical Analysis Institute (SAS) (2004). SAS/STAT user's guide. Version 9.1. Cary; NC, USA.

[8] Robelin J 1981. Cellularity of bovine adipose tissues: developmental changes from 15 to 65 percent mature weight. J Lipid Res 22: 452–457.

[9] Cianzio DS, Topel DG, Whitehurst GB, Beitz DC, Self HL 1985. Adipose tissue growth and cellularity: changes in bovine adipocyte size and number. J Anim Sci 60: 970–976.

[10] Poulos SP, Hausman DB, Hausman GJ 2010. The development and endocrine function of adipose tissue. Mol Cel Endocrinol 323: 20–34.

[11] Mukesh M, Bionaz M, Graugnard DE, Drackley JK, Loor JJ 2010. Adipose tissue depots of Holstein cows are immune responsive: inflammatory gene expression in vitro. Domest Anim Endocrin 38: 168–178.

[12] Fraústo da Silva M, Cardoso Lemos JP, Monteiro LS, Vaz Portugual A (1998). Studies on growth and form: multivariate analysis of distribution of muscle and fat in Portuguese cattle breeds. Livest Prod Sci 55: 261–271.

[13] Wan R, Du J, Ren L, Meng Q (2009). Selective adipogenic effects of propionate in bovine intramuscular and subcutaneous preadipocytes. Meat Sci 82: 372–378.

[14] Mendizabal JA, Albertí P, Eguinoa P, Arana A, Soret B, Purroy A (1999). Adipocyte size and lipogenic enzyme activities in different adipose tissue depots in steers of local Spanish breeds. Anim Sci 69: 115–121.

[15] Pike BV and Roberts CJ (1984). Size and lipolytic capacity of bovine adipocytes from subcutaneous and internal adipose tissue. Vet Res Commun 8: 61–64.

Table 1. Body composition parameters, plasma metabolites and adipokines from Alentejana and Barrosã bulls fed high (HS) or low silage (LS) diets.

	Alentejana		Barrosã			Significance level		
	HS	LS	HS	LS	SEM	B	D	B×D
Body composition parameters								
Slaughter weight (kg)	622	636	457	497	22.3	***	ns	ns
Hot carcass weight (kg)	357	371	257	284	13.1	***	ns	ns
Leg joint weight (kg)	46.8	47.8	35.0	36.0	1.65	***	ns	ns
Subcutaneous fat (g/kg leg)	4.10	4.59	5.92	4.54	0.459	ns	ns	ns
Mesenteric fat (g/kg carcass)	15.5	16.8	15.2	20.9	1.44	ns	*	ns
Omental fat (g/kg carcass)	21.1	24.1	19.0	28.4	1.65	ns	***	ns
KKCF[a] (g/kg carcass)	23.5	20.7	22.5	23.8	1.92	ns	ns	ns
Plasma metabolites and adipokines								
Triacylglycerols (mg/dl)	17.5	17.6	17.0	18.4	1.58	ns	ns	ns
Glucose (mg/dl)	88.9	88.5	82.0	80.6	3.14	*	ns	ns
Insulin (mg/l)	0.884	1.80	1.28	2.12	0.359	ns	*	ns
Leptin (ng/ml)	3.99	3.82	3.89	5.04	0.451	ns	ns	ns
IL-6 (pg/ml)	11.2	8.88	18.4	17.8	3.21	*	ns	ns

B = breed; D = diet; FD = fat depot. Significance level: not significant (ns), $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Table 2. Effects of breed, diet and fat depots on the adipocytes area (μm^2) and number (in 560×10^3 μm^2) of subcutaneous (S) and mesenteric (M) fats from Alentejana and Barrosã bulls fed high (HS) or low silage (LS) diets.

	Alentejana				Barrosã											
	HS		LS		HS		LS			Significance level						
	S	M	S	M	S	M	S	M	SEM	B	D	FD	B×D	B×FD	D×FD	B×D×FD
Area	6759	5353	5931	5217	6842	6087	7177	5676	466	ns	ns	***	ns	ns	ns	ns
Number	76.3	94.3	86.9	100	79	89.4	70	92.1	6.24	ns	ns	***	ns	ns	ns	ns

B = breed; D = diet; FD = fat depot. Significance level: not significant (ns), $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Table 3. Total fatty acids content (mg/g fat) and partial sums of fatty acids (g/100 g total fatty acids) of subcutaneous (S) and mesenteric (M) fats from Alentejana and Barrosã bulls fed high (HS) or low silage (LS) diets.

	Alentejana				Barrosã					Significance Level						
	HS		LS		HS		LS									
	S	M	S	M	S	M	S	M	SEM	B	D	FD	B×D	D×FD	B×FD	B×D×FD
Total fatty acids	496	603	473	558	455	442	436	532	32.5	**	ns	**	ns	ns	ns	ns
Σ SFA	46.9^{a}	63.9^{c}	44.8^{a}	62.1^{cd}	39.6^{b}	61.3^{cd}	39.2^{b}	59.4^{d}	1.06	***	ns	***	ns	ns	**	ns
Σ MUFA	45.1^{a}	26.1^{c}	46.0^{a}	27.3^{c}	50.6^{b}	27.6^{cd}	51.5^{b}	30.3^{d}	1.06	***	ns	***	ns	ns	**	ns
Σ TFA	2.33^{a}	3.69^{c}	3.23^{bc}	4.32^{d}	2.95^{b}	4.51^{d}	2.98^{b}	4.52^{d}	0.219	ns	ns	***	ns	ns	*	ns
Σ PUFA	1.80^{a}	2.30^{ce}	2.58^{be}	3.09^{d}	2.07^{c}	2.43^{e}	2.11^{c}	2.49^{e}	0.109	ns	***	***	***	ns	*	ns
Σ BCFA	2.69^{a}	3.15^{c}	2.06^{b}	2.38^{d}	2.77^{a}	2.86^{a}	2.25^{bd}	2.24^{bd}	0.082	ns	***	***	ns	ns	***	ns

B = breed; D = diet; FD = fat depot. Significance level: not significant (ns), $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.