Differential mesenteric fat deposition in bovines fed on silage or concentrate is independent of glycerol membrane permeability

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Membrane permeability in bovines´mesenteric fat

Abstract

In the meat industry, the manipulation of fat deposition in cattle is of pivotal importance to improve production efficiency, carcass composition and ultimately meat quality. There is an increasing interest in the identification of key factors and molecular mechanisms responsible for the development of specific fat depots. This study aimed at elucidating the influence of breed and diet on adipose tissue membrane permeability and fluidity and their interplay on fat deposition in bovines. Two Portuguese autochthonous breeds, Alentejana and Barrosã, recognized as late and early maturing breeds, respectively, were chosen to examine the effects of breed and diet on fat deposition and on adipose membrane composition and permeability. Twenty-four male bovines from these breeds were fed on silage- or concentrate-based diets for 11 months. Animals were slaughtered to determine their live slaughter and hot carcass weights, as well as weights of subcutaneous and visceral adipose depots. Mesenteric fat depots were excised and used to isolate adipocyte membrane vesicles where cholesterol content, fatty acid profile as well as permeability and fluidity were determined. Total accumulation of neither subcutaneous nor visceral fat was influenced by breed. In contrast, mesenteric and omental fat depots weights were higher in concentrate-fed bulls relative to silage-fed animals. Membrane fluidity and permeability to water and glycerol in mesenteric adipose tissue were found to be independent of breed and diet. Moreover, the deposition of cholesterol and unsaturated fatty acids, which may influence membrane properties, were unchanged among experimental groups. Adipose membrane lipids from the mesenteric fat depot of ruminants were rich

in saturated fatty acids, and unaffected by polyunsaturated fatty acids (PUFA) dietary levels. Our results provide evidence against the involvement of cellular membrane permeability to glycerol on fat accumulation in mesenteric fat tissue of concentrate-fed bovines, which is consistent with the unchanged membrane lipid profile found among experimental groups.

Keywords: adipose membrane, glycerol permeability, membrane fluidity, lipid composition, bovine breeds

Implications

Visceral fat deposition has been considered an important factor in cattle finishing. As maturing proceeds, large amounts of mesenteric fat can be deposited leading to production inefficiencies, which can jeopardize meat quality. Yet, no studies addressing fatty acid incorporation at the cell membrane and its outcome on membrane physical properties, including rigidity and permeability to water and solutes, are available for ruminants. This study exploits the membrane fluidity and permeability to glycerol, a key substrate involved in lipogenesis, while an underlying mechanism for differential visceral fat deposition in bovines as influenced by breed or diet.

Introduction

Traditional meats with Protected Designation of Origin (PDO), derived from local extensive production systems and autochthonous breeds, have the certification of EU legislation due to their supposed quality and sensory traits, which have been associated with their specific lipid fraction properties (Council Regulation nº 2081/92 of 14/7, EEC). Curiously, the scientific information available to sustain the claimed quality, mainly dependent on its lipid composition, is scarce. On the other hand, the manipulation of adipose tissue deposition in cattle has represented for many years a major breeding goal as a future guarantee for the improvement of production efficiency, visceral fat partitioning, carcass composition and meat quality (De Smet *et al.*, 2004). The identification of key factors and molecular mechanisms responsible for the development of specific fat depots (Azain, 2004) in autochthonous bovine breeds is necessary, in particular mechanisms underlying visceral fat accumulation in young bulls with distinct precociousness, Alentejana and Barrosã, known as late and early maturing breeds, respectively (da Silva *et al.*, 1998). Precociousness is intimately related to adipose tissue deposition in meat-producing animals, as early-maturing breeds deposit noticeable amounts of marbling fat before late-maturing breeds (Hocquette *et al.*, 2010).

The degree of saturation of plasma membrane acyl chains might be among the primary events in adipocyte differentiation (Stubbs and Smith, 1984). Nevertheless, literature addressing bovines´ fatty acid deposition at the cell membrane level and its outcome on membrane physical properties is unavailable. Given that the membrane bilayer permeability to water and solutes

99 is tightly related to phospholipid composition and membrane fluidity (Lande *et*
100 *al.*, 1995), it seems reasonable that distinct fat depots with specific metabolic
101 characteristics would affect membrane physical properties. Fatty acid
102 incorporation into cellular membranes is known to affect permeability to water
103 and, most importantly, to glycerol (Soveral *et al.*, 2009; Martins *et al.*, 2010). It is
104 well established that glycerol is a key substrate for lipogenesis and lipolysis in
105 adipose tissues of ruminants. The concept of membrane permeability and
106 fluidity could therefore be critical for understanding membrane structure-
107 function.
108 Specific genetic characteristics have been also described, with the purebred
109 Alentejana breed considered phylogenetically distant from the purebred Barrosã
110 breed (Beja-Pereira *et al.*, 2003). Large differences on the levels of
111 intramuscular fat in Alentejana and Barrosã bovines were previously reported
112 by our group, with values of Alentejana breed (1.2%) nearly half of the other
113 (2.3%) (Alfaia *et al.*, 2007; Alfaia *et al.*, 2009). Studies in humans and mice
114 (Field and Clandinin, 1984; Field *et al.*, 1988) demonstrated that dietary fat can
115 alter adipose cell membrane composition. Thus, the structure and physiological
116 properties of the adipocyte membrane may be changed. The goal of the present
117 study was to assess the effect of breed and diet on fat deposition, as well as on
118 the lipid composition, permeability and fluidity of adipocyte membranes.
119
120 **Material and Methods**
121 ***Experimental design: animals and diets***
122 The experimental design included 24 male bovines from two philogenetically
123 distant autochthonous breeds, the late-maturing breed, Alentejana, and the

124 early-maturing breed, Barrosã, allocated to silage- or concentrate-based diets
125 (four experimental groups of 6 animals each) from January to November 2009.
126 At the beginning of the experiment, Alentejana bulls were 332 ± 10.2 days old
127 (initial weight of 275 ± 15.6 kg) and Barrosã bulls were 268 ± 2.96 days old
128 (initial weight of 217 ± 4.57 kg). Bulls were fed two experimental diets
129 composed by 70/30% and 30/70% of corn silage and concentrate, respectively.
130 The proximate composition and fatty acid profile in both experimental diets (*n* =
131 3) are shown in Table 1.
132 Bulls were slaughtered at 18 months of age at the INRB Experimental Abattoir
133 by exsanguination after stunning with a cartridge-fired captive bolt stunner. The
134 amount of subcutaneous fat was determined by dissection of the leg joint. The
135 former has been suggested to be representative of the overall bovine carcass
136 composition in these particular breeds (Simões and Mendes, 2003). Mesenteric,
137 omental and kidney knob and channel fat (KKCF) depots were excised and
138 weighed. Samples from the mesenteric fat were collected, flash-frozen in liquid
139 nitrogen, and stored at -80 ºC for subsequent analysis.
140 The entire experiment was conducted under the guidelines for the care and use
141 of experimental animals in the Unidade de Produção Animal, L-INIA, INRB
142 (Fonte Boa, Vale de Santarém, Portugal).
143
144 ***Preparation of membrane vesicles from bovine´s mesenteric fat***
145 Membrane vesicles from bovine´s mesenteric fat were prepared by differential
146 centrifugation with buffer without detergents, according to (Martins *et al.*, 2010).
147 Briefly, approximately 20 g of fat tissue from each bovine was chopped into
148 small pieces, removing visible blood vessels, and homogenized in 200 ml

mannitol-Hepes buffer (100 mM mannitol, 10 mM Tris-Hepes, pH 7.4) in a Warring blender for 2 min. The homogenate was filtered through a 70 μm nylon mesh to separate the vascular stroma and intracellular fat retained in the filter. The filtrate was centrifuged at 46000 × *g* for 45 min at 10 ºC to obtain a pellet of crude membranes and further washed in the same buffer. The membrane pellet was then resuspended in mannitol-Hepes buffer, transferred to a syringe and sheared by vigorously passing it 10 times through a 21-gauge needle and immediately used for transport experiments. Protein content was determined by the Bradford method (Bradford, 1976). The vesicle size of all membrane preparations was determined by Dynamic Light Scattering (DLS) (Brookhaven Inst. BI-90).

Fatty acid composition and cholesterol concentration of adipose membranes

After membrane vesicle lyophilisation (at -60 °C and 2.0 hPa), fatty acids were converted to methyl esters (FAME) (Raes *et al.*, 2001; Christie *et al.*, 2007). The resulting FAME were then analyzed by gas chromatography (GC), using a capillary column (Omegawax 250; 30 m × 0.25 mm i.d., 0.25 mm film thickness; Supelco, Bellefont, CA, USA), equipped with a flame-ionization detector. The chromatographic conditions were described in detail elsewhere (Alves and Bessa, 2009). The fatty acid composition was expressed as g/100 g of total fatty acids identified.

Total cholesterol was extracted from lyophilised adipose membrane vesicles through a direct saponification with saturated methanolic KOH solution (Naeemi *et al.*, 1995). Cholesterol was separated and identified using high performance

liquid chromatography (HPLC) equipment (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) by normal phase (Zorbax Rx-Sil column, 250 mm × 4.6 mm i.d., 5 µm particle size, Agilent Technologies Inc.). HPLC was equipped with a diode array detector set at 206 nm and the solvent (30 ml/l isopropanol in *n*-hexane) flowed at 1 ml/min. Total cholesterol concentration was calculated, in duplicate, based on the external standard technique, from a standard curve for peak area *versus* cholesterol concentration and expressed as mg/mg vesicles.

Water and glycerol permeability experiments

Stopped-flow experiments were performed on a HI-TECH Scientific PQ/SF-53 apparatus with 2 ms dead time, temperature controlled and interfaced with a PC microcomputer. Experiments were performed at temperatures ranging from 14 ºC to 37 ºC. Typically, five runs were stored and analysed in each experimental condition. For the measurement of osmotic water permeability, membrane vesicles (0.3 mg protein/ml) resuspended in mannitol-Hepes buffer (120 mOsM) were mixed with an equal amount of isosmotic or hyperosmotic (240 mOsM) mannitol solutions to reach an inwardly directed gradient of the impermeant solute. The kinetics of vesicle shrinkage was monitored from the time course of scattered light intensity at 400 nm until a stable light scatter signal was attained. The osmotic water permeability coefficient (P_f) was estimated by fitting the light scatter signal to a single exponential curve and using the linear relation between *Pf* and the exponential time constant *k* (van Heeswijk and van Os, 1986), $P_f = k\ (V_o/A)(1/V_w(osm_{out})_\infty)$, where V_w is the molar volume of water, V_o/A is the initial volume to area ratio of the vesicles, and $(osm_{out})_\infty$ is the final

199 medium osmolarity after the applied osmotic gradient. For glycerol permeability,
200 membrane vesicles equilibrated in 120 mOsM mannitol-Hepes buffer were
201 exposed to an external solution where the impermeant solute was partially
202 replaced by glycerol (60 mOsM mannitol plus 180 mOsM glycerol, creating an
203 inwardly directed glycerol gradient). After the first fast vesicle shrinkage due to
204 water outflow, glycerol influx in response to its chemical gradient was followed
205 by water influx with subsequent vesicle reswelling. Glycerol permeability was
206 calculated as $P_{gly} = k\ (V_o/A)$, where k is the single exponential time constant
207 fitted to the light scatter signal of glycerol influx (Dix *et al.*, 1985). All solution
208 osmolarities were determined from freezing point depression on a semi-micro
209 osmometer (Knauer GmbH, Germany) using standards of 100 and 400 mOsM.
210 The activation energy E_a of water and glycerol transport was calculated from the
211 slope of the Arrhenius plot ($\ln P_f$ or $\ln P_{gly}$ as a function of $1/T$) multiplied by the
212 gas constant R.
213
214 ***Membrane fluidity measurements***
215 Membrane fluidity was studied by a fluorescence polarization method, which
216 measures the fluorescence anisotropy (r) of two probes incorporated in the
217 membrane: 1,6-diphenyl-1,3,5-hexatriene (DPH), or 1-(4-(trimethylamino)-
218 phenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) (Molecular Probes, USA), as
219 previously described (Martins *et al.*, 2010). Membrane fluidity was assessed
220 based on the fluorescence anisotropy values, calculated by the equation
221 $r = (I_{VV} - GI_{VH}) / (I_{VV} + 2\,GI_{VH})$, where I_{VV} and I_{VH} are the fluorescence intensities and the
222 subscripts indicate the vertical (V) or horizontal (H) orientations of the excitation
223 and emission polarizers, and $G = I_{HV}/I_{HH}$ is the instrumental factor (Lakowicz,

1999). DPH fluorescence measurements were performed with an excitation wavelength (λ_{exc}) of 357 nm and an emission wavelength (λ_{em}) of 428 nm. For TMA-DPH, λ_{exc} = 343 nm and λ_{em} = 427 nm. The fluorescence intensity data points used for calculations were the average of three replicate aliquots (after blank subtraction) measured on a Varian Cary Eclipse fluorescence spectrophotometer (Mulgrave, Australia).

Statistics

Statistical analysis was performed using the Statistical Analysis System (SAS) software package, version 9.1 (SAS Institute, USA). Data were expressed as mean and standard error of the mean (SEM). The GLM procedure was used to perform a 2 × 2 factorial analysis to determine significant main effects of breed (Alentejana or Barrosã), diet (silage or concentrate) and their respective interaction (breed × diet). In the case of interaction, significant differences between groups were identified using Fisher´s *post-hoc* test at $P<0.05$.

Results

Mesenteric and omental fat weights are affected by diet but not by breed

Some of the growth performance parameters, including live slaughter weight, hot carcass weight, and subcutaneous and visceral fat depots weights are shown in Table 2. An effect of breed was observed for live slaughter and hot carcass weights, as Alentejana bulls had higher values of both variables in relation to Barrosã bulls ($P<0.0001$).

The subcutaneous fat weight obtained through the dissection of the leg was unchanged across the experimental groups ($P>0.05$). The same occurred for

249 total visceral fat (P>0.05). Mesenteric and omental fats weights were increased
250 (P<0.05 and P<0.01, respectively), by feeding a concentrate based-diet. An
251 interaction between breed and diet was observed for KKCF depot weight
252 (P<0.05). Regarding this adipose depot weight, and for concentrate-fed
253 bovines, Alentejana bulls had lower values whereas Barrosã bulls had the
254 opposite.
255
256 ***Membrane saturated fatty acids but not cholesterol concentration is***
257 ***influenced by diet and not by breed***
258 Table 3 depicts the lipid composition of membrane vesicles from mesenteric fat
259 from the four experimental groups. Total cholesterol concentration was
260 unaffected by breed or diet (P>0.05). The distribution of the main fatty acid
261 classes showed a highest occurrence of saturated fatty acids (SFA), followed by
262 monounsaturated fatty acids (MUFA), then polyunsaturated fatty acids (PUFA)
263 and lastly, *trans* fatty acids (TFA). The diet affected the sum of SFA (P<0.05),
264 including the 18:0 fatty acid, being the values higher in silage fed animals than
265 in concentrate fed bulls (P<0.05). For this former fatty acid, a breed effect was
266 also observed, as overall Barrosã breed showed a lower concentration
267 (P<0.05). In contrast, the 22:0 fatty acid was enhanced in Barrosã bulls,
268 regardless the diet (P<0.05). Even if the sum of MUFA was not influenced by
269 the factors under study (P>0.05), 16:1*c*9 and 17:1*c*9 fatty acids were affected
270 by diet, showing consistently higher concentrations in concentrate fed bulls
271 (P<0.05). Moreover, the breed had a notorious effect in the 14:1*c*9 fatty acid
272 concentration, with Barrosã bulls showing higher values than Alentejana
273 (P<0.05). The same effect was observed for 18:1*t*11 fatty acid (P<0.05). In

addition, an interaction between breed and diet was found for 18:1*c*11 and 18:1*c*12 fatty acids ($P<0.05$), because significant differences on these concentrations were observed for the diet factor for Alentejana breed, but not for Barrosã. As so, for Alentejana bulls, 18:1*c*11 fatty acid concentration was higher in concentrate- than in silage-based diets; the opposite was observed for 18:1*c*12 fatty acid. Apart from these changes, no other significant variations concerning the sum or the individual fatty acids promoted by breed, diet or their respective interaction were observed.

Permeability and fluidity of adipose membranes are independent of breed or diet

Membrane vesicles obtained from mesenteric fat revealed a unimodal size distribution, showing a mean diameter of 371 ± 57 nm for all tested groups. These membrane preparations were subsequently used to assess water and glycerol permeability by stopped flow spectroscopy, as well as membrane fluidity by fluorescence anisotropy.

Figure 1 depicts typical stopped flow light scatter signals of vesicle volume changes when gradients of mannitol (water permeability, panel A) and glycerol (panel B) were imposed. The time courses of vesicle volume changes are used to calculate P_f and P_{gly}, as described in Material and Methods.

The permeability values obtained for the two bovine breeds fed silage- or concentrate-based diets are shown in Table 4. Neither breed nor diet affected the permeability of adipose membrane vesicles to water (P_f) and glycerol (P_{gly}) ($P>0.05$). Accordingly, the activation energy values (E_a) for water and glycerol permeation were similar among experimental groups, ranging from 14.5 ± 0.3 to

299 15.0 ± 0.2 kcal/mol (60.7 ± 1.3 to 62.8 ± 0.8 kJ/mol) for water (P>0.05) and 23.7
300 ± 0.3 to 24.3 ± 0.2 kcal/mol (99.2 ± 1.4 to 102 ± 1.0 kJ/mol) for glycerol
301 (P>0.05). These relative high E_a values suggest that permeation occurs mainly
302 through the lipid bilayer with no contribution of specific protein channels for
303 transport.
304 The fluorescence anisotropy of DPH and TMA-DPH in membrane vesicles from
305 each experimental group are also shown in Table 4. Following the same
306 pattern, no significant effects of breed or diet were detected among
307 experimental groups (*P*>0.05) for both fluorescence probes.
308
309
310

Discussion

The manipulation of adipose tissue growth, deposition and metabolism has important economic implications for the livestock industry, because it can improve production efficiency, carcass composition and meat quality.

Two underlying processes are responsible for increased adiposity in beef cattle: hypertrophy (larger adipocyte size) and hyperplasia (larger number of adipocytes) (Novakofski, 2004), which are affected by factors such as genetics, sex, age, feeding regimen, food supply and the specific adipose tissue depot (Vernon and Houseknecht, 1991). However, an understanding of the mechanisms of body fat deposition in farm animals and its outcome in adipocyte physiology is far from well established. Consequently, this study was designed to elucidate the contribution of breed and diet to adipose tissue membrane permeability and fluidity as possible key players on fat deposition in bovines. The morphological features between these breeds reflect differences in mature size and, consequently, fat accumulation (da Silva *et al.*, 1998). Following on our previous results, in which the mesenteric fat depot had smaller adipocytes but a greater number of cells than subcutaneous fat (Costa *et al.*, unpublished data), the aforementioned visceral fat was selected for analysis due to its unique properties, regarding lipogenic activity and immune-response potential (Mukesh *et al.*, 2010).

As expected, a clear effect of breed was observed for some growth performance parameters, with Alentejana displaying higher values for live slaughter and hot carcass weights (da Silva *et al.*, 1998; Reis *et al.*, 2001).

335 Regarding lipid deposition, all fat depots under study were unaffected by breed.
336 However, diet appeared to play a significant role in determining mesenteric and
337 omental fat depots weight, which were consistently higher in concentrate fed
338 bovines. The chosen silage- and concentrate-based diets provided significant
339 differences in their composition. The concentrate-based diet exhibited higher fat
340 and starch concentrations while the silage-based diet exhibited higher fibre
341 concentration. These differences extend to the detailed fatty acid composition,
342 in particular to the sums of SFA and PUFA classes. The proportions for SFA
343 were higher in concentrate-based diets (silage 31.9 *versus* concentrate 37.2) at
344 the expenses of 16:0, 18:0 and 20:0 fatty acids. The inverse trend was
345 observed for PUFA (silage 53.0 *versus* concentrate 46.8), determined by 18:2*n*-
346 6 and 18:3*n*-3 fatty acids.
347 There is convincing evidence in animal models that dietary fat influences cell
348 membrane phospholipid composition (Clandinin *et al.*, 1985; Jenkins, 1994).
349 Typically ruminant diets are low in fat but high in PUFA contents. Nevertheless,
350 unsaturated fatty acids in the diet undergo an extensive biohydrogenation in the
351 rumen, with consequently high levels of SFA being absorbed in the intestine
352 and deposited in the tissues (Wachira *et al.*, 2002). In agreement, adipose
353 membranes from visceral fat were found to be richer in SFA, displaying
354 concomitantly a lower proportion of PUFA.
355 Cholesterol is a biomembrane-rigidifying component. When cholesterol is
356 aligned in parallel array with the phospholipid fatty acyl chains it reduces
357 membrane fluidity (Stubbs and Smith, 1984; Onuki *et al.*, 2008), but inversely,
358 increases rigidity. Our results indicate that total cholesterol concentration was
359 unchanged by any factor under study. Accordingly, neither breed nor diet

affected the permeability of adipose membranes to water or glycerol. The activation energy for both water and glycerol transport was high and relatively stable in all cases, thus indicating that permeation is not protein-mediated and occurs mainly via the lipid bilayer where permeability correlates with fluidity. Accordingly, no variations for adipose membrane fluidity were found with DPH or TMA-DPH probes, which assess fluidity at different depths in the bilayer (TMA-DPH assessing a region closer to the lipid-water interface). Altogether, these results corroborate the stability found in permeability data.

Besides cholesterol, fatty acids strongly influence membrane fluidity. With an increase in unsaturated fatty acids concentration, membrane fluidity increases because PUFA acyl chains are extremely flexible and can rapidly change conformational states. The fatty acid profile in adipose membranes from mesenteric fat of Alentejana and Barrosã bovines fed silage- and concentrate-based diets showed no variations in PUFA sum and, foremost important, included none of the *n*-3 fatty acids, EPA (20:5*n*-3) and DHA (22:6*n*-3), well known for their impressive range of health benefits, being the later recognized as a potent membrane fluidizer agent (Stillwell and Wassall, 2003). These results are in accordance to Wachira *et al.* (Wachira *et al.*, 2002), who found residual concentration of both *n*-3 fatty acids in subcutaneous fat of sheep, even after the intake of feeding regimens enriched in linseed and fish oils. Dietary lipids do not directly affect the fatty acid composition of ruminant adipose tissues, as they do in non-ruminants (Sarkkinen *et al.*, 1994). Raising the PUFA content of ruminant tissues by PUFA feeding is rather complex due to the extensive hydrogenation of dietary unsaturated fatty acids by rumen microorganisms (Pond, 1999; Jambrenghi *et al.*, 2007). Nevertheless, the few

385 changes observed for the general fatty acid profile in adipose membranes
386 appear to reflect the dietary treatment imposed, instead of a breed-related
387 effect. The same pattern had already been observed for the fatty acids profile in
388 mesenteric fat, with diet determining the proportions of the major fatty acids as
389 well as their partial sums (Costa et al., unpublished data). Although the sum of
390 MUFA was kept similar across experimental groups, the 16:1*c*9 and 17:1*c*9 fatty
391 acids were under the influence of diet, with higher concentration in concentrate
392 fed bulls. This is in line with previous reports stating that concentrate promotes
393 higher expression or activity levels of delta-9 desaturase enzyme, responsible
394 for the conversion of SFA to MUFA (Daniel *et al.*, 2004). Nevertheless, these
395 differences relate to residual concentration of these fatty acids, ranging from
396 0.48% to 1.82%, and therefore, we believe play an irrelevant physiological role.
397 Similarly, total SFA was affected by the diet factor, being the difference
398 observed largely determined by stearic acid (18:0) concentration in Alentejana
399 bulls fed on silage. More 18:2*n*-6 fatty acid in the silage feeding regimen results
400 in more 18:0 in adipose plasma membranes, as reported formerly (Jenkins,
401 1994). High stearic levels are also in accordance to the reported values in the
402 adipose tissue of lambs (Enser *et al.*, 1996).
403 Finally, a breed effect was observed for 22:0, 14:1*c*9 and 18:1*t*11. All three fatty
404 acids concentration were higher in Barrosã bulls when compared to Alentejana
405 bulls. The long-chain fatty acids (LCFA) occurred at very low levels in the
406 adipose membranes of bovines, either Alentejana or Barrosã, fed any of the
407 dietary treatments. Low levels of LCFA in ruminant's adipose tissue have
408 already been reported and were attributed to the low incorporation of these fatty
409 acids into the triacylglycerol fraction, as well as to the low proportion of

410 phospholipid in the adipose tissue (Enser *et al.*, 1996; Wachira *et al.*, 2002).
411 Concerning the difference observed between breeds for the 22:0 fatty acid, it
412 might be due to a higher elongase expression, or enzymatic activity, in the
413 Barrosã than in the Alentejana breed. Genetic background also appeared to
414 dictate a differential expression, or activity, of delta-9 desaturase enzyme,
415 responsible for the conversion of 14:0 to 14:1*c*9 (Keating *et al.*, 2006). These
416 hypotheses remain to be tested. In relation to the 18:1*t*11 fatty acid, commonly
417 known as vaccenic acid, it is metabolized into the *c*9,*t*11 conjugated linoleic acid
418 (CLA) isomer (Lock *et al.*, 2004), to which numerous health claims have been
419 attributed (Bhattacharya *et al.*, 2006), and for this reason has been considered
420 as beneficial or neutral.
421
422 **Conclusions**
423 This study reports that adipose membranes from ruminant´s mesenteric fat
424 depot were rich in SFA due to ruminal biohydrogenation of dietary PUFA.
425 Membrane fluidity and permeability to glycerol were found to be independent of
426 breed (Alentejana or Barrosã) and diet (based on 70/30% or 30/70% of corn
427 silage and concentrate, respectively). Re-enforcing these findings, cholesterol,
428 the main biomembrane-rigidifying component, and in particular, unsaturated
429 fatty acids concentration were unchanged among experimental groups.
430

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572 FIGURE CAPTIONS

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574 **Figure 1** – Representative stopped flow light scatter of adipose membrane
575 vesicles permeability to water and glycerol at 23 ºC. Time course of (**A**) vesicle
576 shrinkage due to water outflow after an hyperosmotic shock with 120 mOsM
577 mannitol gradient or (**B**) vesicle reswelling due to glycerol uptake after an
578 inwardly directed 180 mOsM glycerol gradient.

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582 **Table 1.** Diet composition.

	Silage[†]	Concentrate[‡]	SEM	Significance level
Gross energy (MJ/kg DM)	19.1	18.6	0.417	0.391
Proximate composition (g/kg DM)				
Crude protein	14.2	12.5	0.632	0.130
Crude fat	2.87	3.17	0.033	0.003
Crude fibre	19.8	15.0	1.14	0.041
Ash	5.53	6.17	0.307	0.219
Starch	28.5	37.6	1.51	0.013
Fatty acid composition (g/100 g fatty acids)				
16:0	20.2	24.1	0.677	0.016
18:0	5.11	9.44	1.05	0.043
20:0	6.51	3.66	0.567	0.024
18:1*c*9	15.1	16.0	0.345	0.154
18:2*n*-6	43.9	40.9	0.399	0.006
18:3*n*-3	9.16	5.96	0.716	0.034

583 [†]Silage-based diet = 30/70% of concentrate and silage, respectively; [‡]Concentrate-
584 based diet = 70/30% of concentrate and silage, respectively. *n* = 3; Values are mean ±
585 SEM.
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590 **Table 2.** Slaughter weight, hot carcass weight, subcutaneous and visceral
591 adipose depots weights from Alentejana and Barrosã bulls fed silage and
592 concentrate based diets.

	Alentejana		Barrosã			Significance level		
	Sil	Conc	Sil	Conc	SEM	Breed	Diet	Breed × Diet
Performance traits								
Live slaughter weight (kg)	640	655	447	485	24.4	<0.0001	0.286	0.640
Hot carcass weight (kg)	368	382	252	275	14.2	<0.0001	0.213	0.774
Carcass traits								
Subcutaneous fat (g/kg leg)	41.3	49.5	45.8	50.7	6.47	0.663	0.325	0.800
Visceral fat (g/kg carcass)	62.6	61.5	53.7	77.5	6.19	0.576	0.082	0.058
Mesenteric fat (g/kg carcass)	16.2	17.7	14.7	22.7	2.03	0.396	0.028	0.121
Omental (g/kg carcass)	21.7	24.4	18.2	29.4	2.35	0.747	0.008	0.087
KKCF (g/kg carcass)	24.8	19.4	20.8	25.3	2.28	0.666	0.852	0.042

593 Dietary treatments: Sil = silage diet based on 30/70% of concentrate and silage,
594 respectively; Conc = concentrate diet based on 70/30% of concentrate and silage,
595 respectively. Values are mean ± SEM.
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599 **Table 3.** Cholesterol concentration (mg/mg vesicles) and fatty acid profile
600 (g/100 g fatty acids) in mesenteric adipose tissue membrane vesicles from
601 Alentejana and Barrosã bulls fed silage and concentrate based diets.

	Alentejana		Barrosã			Significance level		
	Sil	Conc	Sil	Conc	SEM	Breed	Diet	Breed × Diet
Cholesterol	0.014	0.014	0.011	0.017	0.004	0.922	0.380	0.345
Fatty acid profile								
14:0	2.23	2.22	2.48	2.20	2.74	0.508	0.399	0.280
14:1*c*9	0.265	0.212	0.325	0.513	0.092	0.034	0.404	0.143
15:0	0.579	0.405	0.614	0.530	0.073	0.222	0.056	0.4883
16:0	18.5	18.7	19.1	19.4	0.668	0.251	0.607	0.975
16:1*c*7	0.387	0.290	0.362	0.383	0.163	0.507	0.463	0.252
16:1*c*9	1.32	1.68	1.57	1.82	0.185	0.124	0.023	0.679
17:0	1.09	1.06	1.01	0.950	0.152	0.501	0.737	0.891
17:1*c*9	0.476	0.680	0.485	0.535	1.68	0.219	0.028	0.163
18:0	25.5	22.7	23.0	21.5	2.11	0.030	0.014	0.437
18:1*t*6+*t*8	0.408	0.288	0.382	0.440	0.168	0.428	0.699	0.269
18:1*t*9	0.335	0.243	0.258	0.462	0.092	0.423	0.526	0.105
18:1*t*10	0.222	0.618	0.275	0.399	0.134	0.521	0.054	0.296
18:1*t*11	1.44	1.00	2.00	1.56	1.91	0.018	0.055	0.988
18:1*t*12	0.548	0.428	0.475	0.535	0.083	0.828	0.703	0.253
18:1*c*9	24.0	26.5	26.0	25.9	2.67	0.593	0.345	0.325
18:1*c*11	1.16[a]	1.63[b]	1.40[ab]	1.44[ab]	0.154	0.803	0.018	0.045
18:1*c*12	0.529[a]	0.332[b]	0.383[ab]	0.493[ab]	0.114	0.912	0.517	0.030
18:1*t*16+*c*14	0.321	0.176	0.302	0.252	0.055	0.577	0.072	0.365
18:2*n*-6	3.72	3.82	3.76	4.17	2.28	0.719	0.629	0.775
18:3*n*-3	0.551	0.396	0.520	0.504	0.071	0.529	0.173	0.265
20:0	0.509	0.501	0.491	0.500	0.116	0.924	0.996	0.928
20:2*n*-6	0.134	0.130	0.219	0.128	0.046	0.324	0.259	0.303
20:4*n*-6	3.49	3.42	3.54	3.29	0.701	0.951	0.792	0.884
22:0	1.14	1.13	1.32	1.77	0.279	0.018	0.187	0.170
22:5*n*-3	0.464	0.332	0.550	0.562	0.120	0.174	0.597	0.526
Σ SFA	49.5	46.8	48.0	46.9	4.19	0.449	0.041	0.394
Σ MUFA	28.1	31.4	30.5	31.1	2.90	0.448	0.182	0.356
Σ TFA	3.28	2.75	3.69	3.65	0.625	0.138	0.517	0.576
Σ PUFA	8.36	8.10	8.59	8.65	1.32	0.706	0.924	0.876
Σ *n*-3	1.02	0.728	1.07	1.07	4.21	0.199	0.336	0.350
Σ *n*-6	7.34	7.37	7.52	7.59	2.53	0.840	0.962	0.984
Σ Unidentified	10.8	11.0	9.17	9.70	1.42	0.179	0.718	0.888

602 Dietary treatments: Sil = silage diet based on 30/70% of concentrate and silage,
603 respectively; Conc = concentrate diet based on 70/30% of concentrate and silage,
604 respectively. Values are mean ± SEM. [a-b]Mean values within the same row with
605 different superscript letters are significantly different (Fisher´s *post-hoc* test, $P<0.05$).
606 FAME = fatty acid methyl esters. Σ SFA = sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 and
607 22:0; Σ MUFA = sum of 14:1*c*9, 16:1*c*7, 16:1*c*9, 17:1*c*9, 18:1*c*9, 18:1*c*11 and 18:1*c*12;
608 Σ TFA = sum of 18:1*t*6+*t*8, 18:1*t*9, 18:1*t*10, 18:1*t*11, 18:1*t*12 and 18:1*t*16+*c*14; Σ PUFA
609 = sum of 18:2*n*-6, 18:3*n*-3, 20:2*n*-6, 20:4*n*-6 and 22:5*n*-3; Σ *n*-6 = sum of 18:2*n*-6,
610 20:2*n*-6 and 20:4*n*-6; Σ *n*-3 = sum of 18:3*n*-3 and 22:5*n*-3.
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Table 4. Permeability values for water (P_f) and glycerol (P_{gly}), and fluorescence anisotropy of DPH and TMA-DPH in mesenteric adipose tissue membrane vesicles from Alentejana and Barrosã bulls fed silage and concentrate based diets.

	Alentejana		Barrosã			Significance level		
	Sil	Conc	Sil	Conc	SEM	Breed	Diet	Breed × Diet
Permeability								
$Pf \times 10^{-4}$ cm/s	1.42	1.41	1.45	1.42	0.053	0.766	0.681	0.894
$Pgly \times 10^{-7}$ cm/s	4.47	4.57	4.26	4.55	0.213	0.593	0.370	0.673
Fluorescence anisotropy								
DPH	0.159	0.148	0.168	0.169	0.010	0.118	0.617	0.524
TMA-DPH	0.274	0.266	0.271	0.270	0.005	0.943	0.364	0.525

Dietary treatments: Sil = silage diet based on 30/70% of concentrate and silage, respectively; Conc = concentrate diet based on 70/30% of concentrate and silage, respectively. Values are mean ± SEM.