Distinct fatty acid composition of some edible by-products from bovines fed high or low silage diets

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ABSTRACT

Fatty acid composition, including conjugated linoleic acid (CLA) isomers, of the most relevant beef by-products (brain, heart, kidney, liver, pancreas and tongue) from young bulls fed distinct silage levels was assessed. Data indicated a large variation on fatty acid profile and CLA composition among edible by-products. The most abundant fatty acids were C16:0 (kidney), C18:0 (heart and liver), and C18:1*c*9 (brain, pancreas and tongue)

followed by C20:4*n*-6, except in brain (C22:6*n*-3 predominates). Brain, as shown by Principal Components Analysis, presents a distinct fatty acid composition compared to the other beef by-products analysed. In addition, high silage diet relative to low silage diet promoted an increase of *n*-3 PUFA, *t*11,*t*13 and *t*11,*c*13 CLA in heart, kidney, liver and pancreas. Overall, data suggested that fatty acid composition, including CLA isomers, of beef by-products may contribute for their added-value. Nevertheless, from a nutritional point of view they are recommended only in small amounts as part of a balanced diet.

Keywords

Edible by-products, fatty acids, CLA isomers, bulls, diet, silage

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INTRODUCTION

Fatty acid (FA) content and composition of ruminant meat have been the focus of intensive research due to its impact on human nutrition and health. Notwithstanding the high concentration of saturated fatty acids (SFA) and *trans* fatty acids (TFA), which are associated with the development of several chronic diseases, ruminant edible fats contribute also with health beneficial nutrients, including *n*-3 long-chain polyunsaturated fatty acids (*n*-3 LC-PUFA) and conjugated linoleic acid (CLA) isomers. The *n*-3 LC-PUFA, eicosapentaenoic acid (EPA, C20:5*n*-3) and docosahexaenoic acid (DHA, C22:6*n*-3) are structural components of phospholipids in animal tissues, particularly in brain, heart, liver and kidney, and the precursors of eicosanoids with anti-inflammatory properties (Christie, 2003; Tinoco, 1982). In addition, the *cis*-9,*trans*-11 CLA (C18:2*c*9,*t*11) is naturally found at higher proportions in mammalian tissues with a multitude of biological actions on cancer, body fat, and immune response (Dilzer and Park, 2012). CLA isomers are produced in ruminant animals through ruminal microbial biohydrogenation of dietary polyunsaturated fatty acids (PUFA) and endogenously by Δ^9 desaturation of *trans* monoenes in the adipose tissue and mammary gland (Palmquist et al., 2004; Nuernberg et al., 2005). Beyond genetics, the FA composition of ruminant meat can be improved through animal nutrition with use of forages and dietary lipids (Scollan et al., 2006).

Edible animal by-products, in general, are defined as the edible part of carcass fit for human consumption, which includes internal organs. Even the consumption of organ meat can be more or less popular around the countries, which depends on the tradition, culture and religion, edible by-products may be used as value-added and functional

ingredients in the meat industry (Mora et al., 2014; Nollet and Toldrá, 2011; Toldrá et al., 2012). Moreover, edible meat by-products may provide valuable amounts of essential amino acids, fatty acids, minerals and vitamins (Aristoy and Toldrá, 2011; Honikel, 2011). However, to our best knowledge, the characterization of FA composition from the most consumed edible beef by-products is still somewhat scarce (Florek et al., 2012; Prates et al., 2011). The major aim of the present study was, therefore, to evaluate and compare the FA composition, including CLA isomeric profile, in the most consumed beef by-products (brain, heart, kidney, liver, pancreas and tongue) from young Barrosã bulls. In addition, we also investigated whether there is an organ specific response to different dietary silage /concentrate levels.

MATERIALS AND METHODS

Animals and management

Animal trial and the experimental procedures were carried out according to the recommendations of the Animal Care Committee of National Veterinary Authority (Direcção-Geral de Veterinária) following the appropriated European Union guidelines (Directive 86/609/EEC). Nine male bulls from autochthonous Barrosã breed were randomly assigned to either higher (70% silage/30% concentrate, n=4) or low (30% silage/70% concentrate, n=5) silage diets, with individual control of feed intake. During the experiment, animals were individually fed twice a day and had *ad libitum* access to water. The proximate and FA compositions of the experimental diets are displayed in Table 1. Animals were slaughtered at 18 month-old, with approximately 485 ± 31 kg of live body weight, at the experimental abattoir (UIPA-INIAV, Unidade de Investigação

em Produção Animal, Instituto Nacional de Investigação Agrária e Veterinária), after stunning with cartridge-fired captive bolt stunner and exsanguination. Then, carcasses were suspended from the Achilles tendon and chilled at 10 ºC. The internal organs (brain, heart, kidney, liver, pancreas and tongue) were collected, vacuum packed and frozen at –80 °C until analysis.

Please insert Table 1 here

Lipid extraction and methylation

Total lipids were extracted, in duplicate, from lyophilized samples (–60 °C and 2.0 hPa; Edwards High Vacuum International, UK) according to the method of Folch et al. (1957) modified by Carlson (1985) and gravimetrically measured by weighting the fatty residue after solvent evaporation.

Analysis of fatty acid methyl esters

Fatty acid methyl esters (FAME) were prepared from FA through a combined base/acid methylation method using 0.5 mol/L sodium methoxide in anhydrous methanol (Sigma-Aldrich Ltd., St. Louis, MO, USA) for 30 min at 50 ºC, followed by hydrochloric acid in methanol (1:1 v/v) for 10 min at 50 ºC (Raes et al., 2001). FAME were extracted with *n*-hexane and analysed by GC (chromatograph HP 6890; Hewlett–Packard, Avondale, PA, USA) fitted with a flame ionisation detector (FID). For the separation of FAME was used a long fused-capillary column (CP-Sil 88 100 m, 0.25 mm i.d., 0.20 µm film thickness; Chrompack, Varian Inc., Walnut Creek, CA, USA) according to Alves and Bessa (2009).

Nonadecanoic acid methyl ester (C19:0; Sigma) was added to the samples prior to methylation as an internal standard. FA identification was based on FAME standard (FAME mix with 37 components, Supelco, Inc., Bellefonte, PA) and confirmed by electron impact mass spectrometry using a Shimadzu GC-MS QP2010 Plus (Shimadzu). Each FA was expressed as g/100 g of total FA.

Analysis of individual CLA isomers

Geometric and positional CLA isomers were separated by silver ion high performance liquid chromatography (Ag^+-HPLC) using a chromatograph system (Agilent 1100 Series, Agilent Technologies Inc., Palo Alto, CA, USA) with three columns in series (ChromSpher 5 Lipids; 250 mm × 4.6 mm i.d., 5 μm particle size; Chrompack, Bridgewater, NJ, USA) and equipped with a diode array detector (DAD) set to 233 nm according to Cruz-Hernandez et al. (2006). Commercial standards (*c*9,*t*11, *t*10,*c*12, *c*11,*t*13, *c*9,*c*11 and *t*9,*t*11 from Matreya Inc., Pleasant Gap, PA, USA) were used to identify the individual CLA isomers. Quantitation of CLA isomers was based on their Ag^+-HPLC areas relative to the area of the main isomer *c*9,*t*11 determined by GC-FID (plus *t*7,*c*9 and *t*8,*c*10 isomers) as described by Kraft et al. (2003). Total CLA was expressed as gravimetric content (mg/g tissue) and the individual isomers as a percentage of the sum of CLA isomers (% total CLA).

Statistical analysis

Data was analyzed using the PROC MIXED and checked for variance heterogeneity using the software package (SAS, 2009). The statistical model included the feeding effect

as repeated measure. Data were reported as mean ± standard error (SE). Least squares means (LSMEANS), with the option PDIFF adjusted with Tukey–Kramer method, were determined to compare groups. The level of significance was set at *P*-value below 0.05.

The principal component analysis (PCA) of FA composition and CLA isomers (as a percentage of total FA) in beef by-products (brain, heart, kidney, liver, pancreas and tongue) were carried out using the STATISTICA software (StatSoft, Inc., 2004).

RESULTS AND DISCUSSION

Fatty acid composition

Total lipids of some edible by-products (brain, heart, kidney, liver, pancreas and tongue) from Barrosã bulls fed HS or LS diets are shown in Tables 2 and 3. Total lipids were higher in brain (6.8-7.0%), intermediate in pancreas (3.8-4.0%), tongue (2.9-3.6%) and liver (2.7%) and lower in kidney and heart (2.1% and 1.7%, respectively), although no differences were found between feeding strategies ($P>0.05$). The contents of total lipids in beef by-products were comparable to lean meat (<5%), except in brain. However, these results were relatively lower than those reported by Honikel (2011) for the same by-products, probably, due to differences in breed, gender, age and feeding regimens. Indeed, breed and diet are the main factors influencing lipid content and composition.

The cholesterol content (mg/100 g tissue) in edible by-products from Barrosã bulls is presented in Tables 2 and 3. Diet had no effect on cholesterol levels of beef-products. Total cholesterol was higher in brain (1640-2075 mg/100 g) of Barrosã bulls from both feeding regimens when compared to the remaining organs. Similar cholesterol levels in beef brain (1456-3010 mg/100 g) were reported elsewhere (Mustafa, 1988; USDA,

153 2009). In addition, our results of cholesterol contents in kidney (304-309 mg/100 g), liver
154 (154-158 mg/100 g) and heart (99-102 mg/100 g) agree with those found by Bragagnolo
155 (2011) in beef kidney (100-517 mg/100 g), liver (159-162 mg/100 g) and heart (72-150
156 mg/100 g). It has long been known that cholesterol plays an important role for body
157 function, as a main lipid constituent of the cell membranes and the precursor of steroid
158 hormones, vitamins and bile acids. Cholesterol can be synthesized endogenously, within
159 the liver and intestines, and obtained from the diet by animal-derived products. Yet, high
160 serum cholesterol levels have been associated with an increased risk of chronic diseases,
161 whereby the recommended maximum cholesterol intake should be less than 300 mg *per*
162 day (American Heart Association, 2008). Regarding cholesterol in organ meats, the
163 values of cholesterol can increase 3- to 5-fold up than in lean meat (Bragagnolo, 2011).
164 The FA composition (g/100 g of total FA), partial sums and ratios of FA in beef by-
165 products (brain, heart, kidney, liver, pancreas and tongue) from bulls fed HS or LS diets
166 are also presented in Tables 2 and 3. The most representative FA in beef by-products
167 were C18:1*c*9 (9–36% of total FAME), C18:0 (11–32 %) and C16:0 (8–22%). Our
168 research group has previously found a similar FA pattern in meat from Barrosã bulls
169 (Costa et al., 2013). Nevertheless, the order of appearance of the FA detected in beef by-
170 products was quite different. The main LC-PUFA in beef by-products was arachidonic
171 acid (ARA, 20:4*n*-6), except in brain where DHA (7%) and ARA (6%) predominates.
172 These data are in agreement with the study of Cordain et al. (2002), who also reported
173 higher DHA levels in brain of wild ruminant animals (8.9% in elk, 9.2% in antelope and
174 9.6% in deer) compared to other internal organs. DHA, EPA and ARA can be synthesized
175 by a series of reactions involving Δ^6 desaturation, elongation and Δ^5 desaturation from the

176 precursors substrates α-linolenic acid (C18:3*n*-3, ALA) and linoleic acid (C18:2*n*-6, LA),
177 respectively (Burdge and Calder, 2005). Thus, the conversion of these essential fatty
178 acids (ALA and LA) into more desaturated FA depends on the type of dietary PUFA
179 (Harnack et al., 2009). Diet influenced the percentages of minor FA in beef by-products,
180 except in brain. Bulls fed the HS diet had higher percentages of *a*-C15:0 ($P<0.05$), C15:0
181 ($P<0.01$), *i*-C16:0 ($P<0.05$), *i*-C17:0 ($P<0.05$), *a*-C17:0 ($P<0.001$), C17:0 ($P<0.05$),
182 C20:0 ($P<0.05$), C18:3*n*-3 ($P<0.01$) and C20:5*n*-3 ($P<0.05$) in heart than those fed the LS
183 diet. Moreover, the HS diet promoted the deposition of C14:1*c*9 ($P<0.01$), *i*-C15:0
184 ($P<0.05$), *i*-C16:0 ($P<0.01$), *i*-C17:0 ($P<0.01$), C18:3*n*-3, C20:5*n*-3 and C22:5*n*-3
185 ($P<0.05$) in kidney when compared to the LS diet. Only the proportion of C18:1*c*13
186 ($P<0.05$) was higher in the LS diet. A similar FA pattern was observed in pancreas,
187 except for C20:5*n*-3 and C22:5*n*-3 ($P>0.05$). Barrosã bulls fed the HS diet had the highest
188 percentages of C15:0, C18:3*n*-3 and C22:5*n*-3 in liver whereas the percentages of DMA-
189 C18:1, C18:1*t*9, C18:1*t*10 and C18:1*c*13 in those fed the LS diet. Feeding the HS diet
190 also promoted higher percentages of minor FA in tongue (*i*-C15:0 ($P<0.01$), *a*-C15:0
191 ($P<0.05$), *i*-C16:0 ($P<0.05$), *i*-C17:0 ($P<0.01$), *a*-C17:0 ($P<0.01$), C18:1*c*14+*t*16
192 ($P<0.05$) and C18:3*n*-3 ($P<0.05$)) compared to LS diet.

193 The pattern obtained for the partial sums of FA (Tables 2 and 3) reflected the values
194 described for individual FA. Besides genetic and nutritional factors, the fatty acid
195 composition depends largely on the fat level and tissue (Wood et al., 2008). Pancreas and
196 liver of Barrosã bulls had higher SFA and lower monounsaturated fatty acids (MUFA)
197 percentages. In contrast, tongue presented higher MUFA and lower SFA percentages.
198 However, no differential response to dietary silage level was detected for SFA and

MUFA contents (P>0.05). These results are in line with those reported by Florek et al. (2012), who observed distinctly higher SFA and MUFA percentages in offal of veal calves and suckler beef. The former authors found that liver presented the highest SFA and the lowest MUFA, and consequently the lowest SFA/MUFA ratio, while tongue showed the highest percentage of MUFA. Beef by-products, with exception of heart, are relatively saturated mainly due to the percentages of C16:0 and C18:0 (and C18:1*c*9), which suggest a strong contribution of *de novo* synthesis of FA in these tissues. The different rates of *de novo* FA biosynthesis have been ascribed to the activity of lipogenic enzymes. Steroyl-CoA desaturase (SCD) is a rate-limiting enzyme that catalyzes the MUFA biosynthesis, and therefore, with a specific role in lipid deposition (Taniguchi et al., 2004). In contrast to SFA and MUFA, the content of TFA in beef by-products is very low (ranging from 2-6% of the total FA) with vaccenic acid (C18:1*t*11) predominating, except in brain. However, feeding the LS diet, rich in starch, promoted the deposition of C18:1*t*10 in liver of Barrosã bulls in comparison to the HS diet (P<0.05). It has been suggested that the use of concentrate in ruminant diets induces changes in the rumen microbiota, which tend to shift the pattern of major biohydrogenation intermediates (C18:1*t*11 and CLA isomers) towards C18:1*t*10 production (Bessa et al., 2015; Glasser et al., 2008; Griinari and Bauman, 1999). Relatively to PUFA, as expected, heart (41%) kidney (38%) and liver (35%) had the highest proportions of these FA due to the lowest values of total lipids, although no variation between diets was observed (P>0.05). In general, the PUFA percentages of the *n*-3 family were relatively low in the beef by-products analysed, except in brain. These results are consistent with those described by Prates et al. (2011) for beef and pork by-products. Moreover, the HS diet (rich in C18:3*n*-

3) promoted higher *n*-3 PUFA percentages in liver, heart and kidney in comparison to the LS diet ($P<0.01$). Finally, the proportions of dimethylacetals (DMA) in beef by-products varied greatly contributing for total FA with 1-3% in pancreas, liver, tongue and kidney, 6-8% in brain and 11-13% in heart. In addition, Pérez-Palacios et al. (2007) have shown that DMA may be influenced by dietary FA composition. In the present study, the proportions of DMA in the beef by-products analysed were unaffected by diets ($P>0.05$) but, in contrast to our data, Aldai and co-workers (2011) found higher proportions of DMA in ruminant meat promoted by HS diet. Minor proportions of *iso*- and *anteiso*- of branched chain fatty acids (BCFA) were found in beef by-products. While feeding HS or LS diets had no influence on BCFA of brain and liver ($P>0.05$), the HS diet promoted the deposition of BCFA in heart ($P<0.001$), kidney and tongue ($P<0.01$) and pancreas ($P<0.05$) in comparison to the LS diet.

The FA ratios of beef by-products from Barrosã bulls fed HS or LS diets are also presented on Tables 2 and 3. According to nutritional guidelines, the PUFA/SFA ratio in the human diet should be above 0.45 and the *n*-6/*n*-3 ratio should not exceed 4.0 (Department of Health, 1994). The PUFA/SFA ratios in beef by-products were within the recommended values, except in pancreas and tongue. Florek et al. (2012) found similar PUFA/SFA ratios in offal from suckler beef and veal calves, however, the values were higher in offal from suckler beef compared with those from veal calves. In ruminants, a large amount of dietary PUFA is biohydrogenated by rumen microorganisms into high levels of SFA available for deposition in muscle tissue, and the net result is a lower PUFA/SFA ratio. Diet did not affect the PUFA/SFA ratio ($P>0.05$). Raes et al. (2004) suggested that the ratio between PUFA and SFA fatty acids is mainly influenced by

245 genetics rather than nutrition. In contrast, the *n*-6/*n*-3 ratios were above the recommended
246 values in all beef by-products. The values of *n*-6/*n*-3 ratios were higher in heart (16-22),
247 intermediate in pancreas and tongue (8-13) and lower in brain and liver (1-6). Moreover,
248 the *n*-6/*n*-3 ratio was consistently higher in LS diet compared to those from HS diet
249 ($P<0.05$), except in brain. It has been shown that the use of cereals-based diets (poor in *n*-
250 3 FA) changes the FA composition creating an unfavourable *n*-6/*n*-3 FA ratio (Garcia et
251 al., 2008: Wood et al., 2008).
252
253 Please insert Tables 2 and 3 here
254
255 **CLA isomeric profile**
256 Total CLA content (mg/100 g tissue) and CLA isomeric profile (% of total CLA) of beef
257 by-products are displayed in Table 4. Pancreas (10.9-11.6), tongue (11.9-15.1), kidney
258 (10.5) and liver (9.3-9.4) of Barrosã bulls showed the highest values of total CLA
259 whereas brain (1.2-1.6) and heart (2.4-2.6) the lowest ones. However, no differential
260 response to dietary silage level was observed for total CLA ($P>0.05$). The large variation
261 in total CLA contents among beef by-products may be explained by the distinct
262 concentration of triacylglycerols in these tissues. Florek et al. (2012) also found high
263 contents of CLA (C18:2*c*9*t*11/*t*10*c*12) in offal from veal calves (liver > tongue > heart >
264 kidney). CLA contents vary substantially between species as well as from animal to
265 animal and within different tissues (Prates and Bessa, 2009; Schmid et al., 2006). The
266 major CLA isomer (*c*9,*t*11) as well as the usually second most prevalent isomer (*t*7,*c*9)
267 are mainly produced in the tissues through Δ^9 desaturation of *trans* C18:1 and in the

268 rumen during microbial biohydrogenation of dietary C18 PUFA (Griinari and Bauman,
269 1999; Palmquist et al., 2004; Prates and Bessa, 2009). Even though the major dietary
270 sources of CLA are ruminant-derived products, the levels of intake remain to be
271 established. It has been recommended a daily intake of 0.8-3.0 g/day based on the anti-
272 cancer effects of CLA (Parish et al., 2003; Schmid et al., 2006). Specific physiological
273 effects have been linked to CLA isomers, the *t*10,*c*12 CLA isomer may play an important
274 role in lipid metabolism, while the c9,t11 and the *t*10,*c*12 isomers seem to be equally
275 effective in anticarcinogenesis (Pariza et al., 2001).

276 The most abundant CLA isomer in beef by-products was, as in ruminant meat, the
277 *c*9,*t*11 isomer (62-84% of total CLA) followed by *t*7,*c*9 (3.5-6.3%), except in brain.
278 *t*11,*c*13 (20%) was the second predominant isomer in brain. As far as we know, this
279 preferential deposition of *t*11,*c*13 CLA in brain remains to be established (Prates et al.,
280 2011). The *t*10,*c*12 CLA isomer was only residual in beef by-products. In addition,,
281 *c*9,*t*11 was more accumulated (84%) in liver and kidney while the *t*10,*c*12 was relatively
282 incorporated (up to 1%) into heart and tongue. The studies carried out by Kramer et al.
283 (1998) and Li and Warkins (1998) also reported a preferential accumulation of *c*9,*t*11
284 isomer in liver, serum, bone and marrow and a higher incorporation of the *t1*0,*c*12 into
285 spleen, muscle and heart.

286 Data in Table 4 also shows that bulls fed the HS diet had the highest percentages of
287 *t*11,*t*13 and *t*11,*c*13 in heart, kidney, liver and pancreas when compared to those fed the
288 LS diet. In contrast, feeding the LS diet to Barrosã bulls increases the proportions of
289 *t*10,*c*12 in liver and kidney. CLA composition of ruminant meat has been related to
290 pasture *vs.* cereal-based concentrate diet (Alfaia et al, 2009; Raes et al., 2004; Schmid et

al., 2006). Indeed, meat from grass-fed ruminants have been shown to had highest proportions of *t*11,*c*13, *t*11,*t*13, and *t*12,*t*14 isomers and lowest percentage of *t*7,*c*9 CLA isomer than meat from ruminants fed on high grain diets (Dannenberger et al., 2005).

Please insert Table 4 here

Principal component analysis

The projection of the first (PC1) and second (PC2) components in the plane using the percentage of FA and CLA isomers determined in the six meat organs (brain, heart, kidney, liver, pancreas and tongue) is displayed in Fig. 1A. The score plot of the first two principal components explained 58.2% of the total variance, with 39% for PC1 and 19.2% for PC2 (Table 5). In addition, the pattern in Fig. 1A suggests that PC1 differentiates the FA derived from the endogenous synthesis (positive PC1 loadings) and PC2 the FA derived from ruminal biohydrogenation (negative PC1 loadings). The PC1 was characterised by variables with positive loadings, such as C20:5*n*-3 (0.941), DMA-C18:1 (0.901), C22:6*n*-3 (0.855), C20:1*c*11 (0.841) and C22:5*n*-6 (0.836), and by variables with negative loadings, like *a*-C17:0 (-0.959), *i*-C17:0 (-0.897), C18:1*t*11 (-0.854), C18:1*t*9 (-0.820), *c*9,*t*11 (-0.815) and *t*7,*c*9 (-0.781). The PC2 was positively defined by C18:1*c*9:0 (0.870), C16:0 (0.852), C18:1*c*13 (0.799) and C14:0 (0.743), and negatively by C22:5*n*-3 (-0.841), C22:0 (-0.823), C20:4*n*-6 (-0.750), C18:3*n*-3 (-0.714), C18:0 (-0.657), C18:2*n*-6 (-0.634) and *t*11,*t*13 (-0.627). However, the score plot from PC1 × PC2 plane does not discriminates between HS and LS treatments (both C18:2*n*-6 and C18:3*n*-3 formed a cluster in quadrant *d*).

In addition, projection of scores of the first two PCs (Figure 1B) was arranged in four clusters, corresponding to the six organs. Tongue and pancreas were located in quadrant *a*, brain in quadrant *b*, heart in quadrant *c*, liver in quadrant *d* and heart was dispersed across all quadrants. Summing up, it is important to notice a clear separation of brain from the remaining organs, which may contribute the highest percentages of DHA in this tissue.

Please insert Table 5 and Figure 1 here

CONCLUSIONS

Beef by-products (brain, heart, kidney, liver, pancreas and tongue) from bulls fed HS and LS diets had, in general, higher contents of cholesterol, SFA, and TFA, as well as high levels of CLA. The major FA in beef by-products were oleic acid, stearic and palmitic acids, as well as arachidonic acid, except in brain. FA composition of brain, compared to the other internal organs, presented higher *n*-3 LC-PUFA, particularly DHA. In addition, heart, kidney, liver and pancreas of bulls fed HS diet, compared to those from bulls fed LS diet, had the most favourable FA profile, specially greater proportions of *n*-3 PUFA, and *t*11,*t*13 and *t*11,*c*13 CLA isomers, as a result of the beneficial effects of grass silage. Based on these nutritional characteristics, it is recommended to consume these beef by-products in small amounts and integrated in a balanced diet.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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474

475 **FIGURE CAPTION**

476 **Figure 1**. Loading plot of the first (PC1) and second (PC2) principal components of the
477 pooled data (A) and components score vectors (B) for the beef by-products (brain, heart,
478 kidney, liver, pancreas and tongue).

479

480 **Table 1.** Composition of the experimental diets.

	HS	LS
Proximate composition (%DM)		
Crude protein	14.2	12.5
Crude fat	2.87	3.17
Crude fibre	19.8	15.0
NDF	40.3	32.1
ADF	24.9	18.6
Ash	5.53	6.17
Starch	28.5	37.6
Gross energy (MJ/kg DM)	19.1	18.6
Fatty acid composition (mg/g DM)		
C16:0	16.1	16.5
C18:0	4.05	6.33
C20:0	5.22	2.61
C18:1*c*9	12.1	11.0
C18:2*n*-6	35.1	28.2
C18:3*n*-3	7.36	4.21

481 HS, silage diet based on 30/70% of concentrate and silage, respectively (n = 3); LS, low
482 silage diet based on 70/30% of concentrate and silage, respectively (n = 3); DM = dry
483 matter; NDF = neutral detergent fibre; ADF = acid detergent fibre.

484 **Table 2**. Total lipids (%), total cholesterol (mg/100 g) and fatty acid composition (g/100 g total fatty acids) of beef brain, heart and kidney from Barrosã bulls fed high (HS) or low

485 silage (LS) diets.

	Brain					**Heart**					**Kidney**				
	HS		LS			HS		LS			HS		LS		
	Mean	SE	Mean	SE	*P* value	Mean	SE	Mean	SE	*P* value	Mean	SE	Mean	SE	*P* value
Total lipids	6.98	0.626	6.84	0.463	ns	1.63	0.175	1.70	0.141	ns	2.09	0.191	2.06	0.131	ns
Total cholesterol	2075	339	1640	292	ns	102	1.94	99.0	1.30	ns	304	7.03	309	4.12	ns
Fatty acid composition															
C14:0	0.53	0.009	0.53	0.023	ns	0.44	0.075	0.40	0.051	ns	0.46	0.032	0.48	0.032	ns
C14:1*c*9	0.14	0.011	0.12	0.011	ns	0.27	0.016	0.22	0.013	ns	0.25	0.013	0.17	0.013	**
i-C15:0	nd	nd	nd	nd	-	0.07	0.004	0.06	0.004	ns	0.16	0.013	0.12	0.002	*
a-C15:0	nd	nd	nd	nd	-	0.19	0.017	0.11	0.011	*	0.11	0.008	0.08	0.010	ns
C15:0	0.12	0.011	0.13	0.007	ns	0.25	0.008	0.17	0.014	**	0.42	0.021	0.35	0.021	ns
DMA-C16:0	2.36	0.403	2.90	0.253	ns	6.71	0.332	8.29	0.204	**	2.53	0.116	2.43	0.107	ns
i-C16:0	0.05	0.007	0.04	0.007	ns	0.16	0.017	0.08	0.008	*	0.21	0.009	0.12	0.015	**
C16:0	16.1	0.386	16.0	0.248	ns	11.3	0.412	10.8	0.173	ns	15.7	0.369	15.8	0.207	ns
C16:1	0.11	0.008	0.10	0.005	ns	0.08	0.008	0.08	0.013	ns	0.23	0.036	0.21	0.040	ns
i-C17:0	nd	nd	nd	nd	-	0.28	0.003	0.21	0.010	**	0.33	0.014	0.26	0.011	**
C16:1*c*7	0.47	0.008	0.50	0.021	ns	0.11	0.011	0.11	0.011	ns	0.42	0.021	0.41	0.022	ns
C16:1*c*9	0.33	0.011	0.35	0.007	ns	0.48	0.094	0.55	0.059	ns	0.77	0.063	0.95	0.111	ns

a-C17:0	nd	nd	nd	nd	-	0.32	0.008	0.25	0.005	***	0.45	0.022	0.38	0.013	ns
C17:0	0.43	0.016	0.44	0.011	ns	0.48	0.030	0.39	0.024	*	0.66	0.019	0.63	0.021	ns
DMA-C18:0	2.55	0.433	3.26	0.250	ns	4.14	0.259	3.76	0.143	ns	0.78	0.052	0.67	0.043	ns
DMA-C18:1	1.37	0.245	1.71	0.135	ns	0.53	0.032	0.61	0.032	ns	0.12	0.017	0.13	0.010	ns
C18:0	17.5	0.569	16.8	0.368	ns	15.5	0.419	13.9	0.230	*	12.9	0.115	12.4	0.245	ns
C18:1*t*6+*t*8	0.03	0.001	0.04	0.008	ns	0.09	0.011	0.07	0.006	ns	0.15	0.019	0.13	0.006	ns
C18:1*t*9	0.04	0.004	0.05	0.004	ns	0.09	0.007	0.07	0.006	ns	0.13	0.023	0.12	0.007	ns
C18:1*t*10	0.07	0.004	0.06	0.014	ns	0.09	0.011	0.11	0.019	ns	0.13	0.034	0.16	0.022	ns
C18:1*t*11	0.04	0.005	0.05	0.002	ns	0.79	0.130	0.62	0.058	ns	0.57	0.096	0.59	0.057	ns
C18:1*t*12	0.39	0.008	0.40	0.016	ns	0.19	0.020	0.16	0.010	ns	0.28	0.021	0.30	0.020	ns
C18:1*c*9	20.5	0.418	20.2	0.404	ns	9.33	1.112	8.83	0.757	ns	15.5	0.443	16.1	0.505	ns
C18:1*c*11+*t*15	4.61	0.123	4.39	0.048	ns	1.99	0.025	2.29	0.176	ns	3.13	0.117	3.63	0.213	ns
C18:1*c*12	0.45	0.107	0.35	0.037	ns	0.62	0.030	0.62	0.039	ns	0.45	0.064	0.48	0.026	ns
C18:1*c*13	0.20	0.019	0.20	0.015	ns	0.08	0.006	0.07	0.003	ns	0.13	0.023	0.23	0.026	*
C18:1*c*14+*t*16	0.07	0.016	0.08	0.010	ns	0.10	0.010	0.08	0.004	ns	0.18	0.024	0.17	0.011	ns
C18:1*c*15	nd	nd	nd	nd	-	nd	nd	nd	nd	-	0.08	0.009	0.08	0.004	ns
C18:2*n*-6	0.42	0.024	0.42	0.020	ns	27.9	1.515	28.8	0.920	ns	20.5	0.644	20.4	0.754	ns
C20:0	0.19	0.012	0.20	0.010	ns	0.15	0.011	0.11	0.008	*	0.29	0.012	0.30	0.013	ns
C18:3*n*-3	0.11	0.013	0.10	0.006	ns	0.88	0.049	0.57	0.038	**	0.98	0.037	0.82	0.047	*
C20:1*c*11	2.22	0.223	2.18	0.044	ns	0.08	0.008	0.10	0.012	ns	0.33	0.022	0.37	0.008	ns

C18:2*c*9*t*11	0.04	0.006	0.03	0.002	ns	0.17	0.020	0.18	0.020	ns	0.33	0.040	0.42	0.050	ns
C18:4*n*-3	0.43	0.026	0.42	0.018	ns	nd	nd	nd	nd	-	nd	nd	nd	nd	-
C20:2*n*-6	0.09	0.002	0.09	0.005	ns	0.16	0.013	0.19	0.013	ns	0.55	0.054	0.50	0.032	ns
C22:0	0.47	0.018	0.42	0.028	ns	1.09	0.151	1.01	0.104	ns	0.82	0.127	0.73	0.053	ns
C20:3*n*-6	0.14	0.022	0.17	0.013	ns	0.08	0.008	0.08	0.007	ns	0.09	0.009	0.09	0.010	ns
C20:3*n*-3	0.08	0.005	0.09	0.006	ns	nd	nd	nd	nd	-	0.24	0.019	0.19	0.018	ns
C20:4*n*-6	6.27	0.159	5.86	0.278	ns	9.91	0.412	11.1	0.433	ns	12.7	0.688	12.8	0.573	ns
C20:5*n*-3	nd	nd	nd	nd	-	0.50	0.040	0.37	0.027	*	0.47	0.029	0.34	0.012	*
C22:4*n*-6	4.14	0.030	4.12	0.049	ns	0.35	0.010	0.44	0.046	ns	0.66	0.057	0.68	0.027	ns
C22:5*n*-6	2.28	0.302	1.95	0.106	ns	0.10	0.012	0.13	0.015	ns	0.13	0.017	0.14	0.024	ns
C22:5*n*-3	0.35	0.008	0.36	0.006	ns	0.82	0.028	0.74	0.046	ns	1.28	0.062	1.03	0.042	*
C22:6*n*-3	7.40	0.502	7.44	0.458	ns	0.13	0.011	0.14	0.007	ns	0.39	0.051	0.31	0.028	ns
Others	5.61	0.471	6.29	0.446	ns	3.05	0.149	3.17	0.059	ns	2.99	0.160	3.37	0.073	ns
Fatty acid partial sums															
∑ SFA	35.3	0.964	34.4	0.592	ns	29.2	0.926	26.8	0.458	ns	31.4	0.375	30.6	0.307	ns
∑ *cis* MUFA	24.4	0.613	23.8	0.452	ns	11.0	1.16	10.5	0.786	ns	17.9	0.582	18.7	0.655	ns
∑ TFA	5.40	0.158	5.20	0.071	ns	3.58	0.201	3.67	0.163	ns	5.34	0.241	5.80	0.228	ns
∑ PUFA	23.0	0.773	22.3	0.654	ns	40.8	1.74	42.5	0.929	ns	38.0	0.617	37.2	0.859	ns
∑ *n*-6 PUFA	13.3	0.361	12.6	0.296	ns	38.5	1.68	40.7	0.883	ns	34.6	0.529	34.5	0.850	ns

∑ *n*-3 PUFA	9.65	0.437	9.68	0.399	ns	2.33	0.069	1.82	0.078	**	3.37	0.110	2.70	0.062	**
∑ *n*-6 LC-PUFA	12.9	0.347	12.2	0.281	ns	10.6	0.410	11.9	0.473	ns	14.1	0.717	14.2	0.602	ns
∑ *n*-3 LC-PUFA	9.12	0.451	9.16	0.426	ns	1.45	0.068	1.25	0.072	ns	2.39	0.114	1.88	0.071	*
∑ DMA	6.28	1.06	7.87	0.586	ns	11.4	0.559	12.7	0.287	ns	3.43	0.178	3.23	0.150	ns
∑ BCFA	0.07	0.013	0.02	0.014	ns	1.02	0.031	0.72	0.019	***	1.25	0.046	0.97	0.036	**
Fatty acid ratios															
PUFA/SFA	0.65	0.007	0.65	0.009	ns	1.41	0.108	1.59	0.060	ns	1.21	0.025	1.22	0.032	ns
n-6/*n*-3	1.38	0.036	1.31	0.040	ns	16.5	0.370	22.5	0.869	**	10.3	0.241	12.8	0.343	***

Data are means ± standard error (SE). SFA = ∑ C14:0, C16:0, C18:0, C20:0 and C22:0; *cis* MUFA = ∑ C14:1*c*9, C16:1*c*7, C16:1*c*9, C18:1*c*9, C18:1*c*12, C18:1*c*13, C18:1*c*15 and C20:1*c*11; TFA = ∑ C18:1*t*6+*t*8-, C18:1*t*9, C18:1*t*10, C18:1*t*11, C18:1*c*11+*t*15, C18:1*t*12, C18:1*c*14+*t*16 and C18:2*c*9*t*11; PUFA = ∑ C18:2*n*-6, C18:3*n*-3, C18:4*n*-3, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-6, C20:5*n*-3, C22:4*n*-6, C22:5*n*-6, C22:5*n*-3 and C22:6*n*-3; *n*-6 PUFA = ∑ C18:2*n*-6, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-6, C22:4*n*-6 and C22:5*n*-6; *n*-3 PUFA = ∑ C18:3*n*-3, C18:4*n*-3, C20:3*n*-3, C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3; *n*-6 LC-PUFA = *n*-6 long chain-PUFA = ∑ C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-6, C22:4*n*-6 and C22:5*n*-6; *n*-3 LC-PUFA = *n*-3 long chain-PUFA = ∑ C20:3*n*-3, C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3; DMA = dimethylacetals = ∑ DMA-C16:0, DMA-C18:0 and DMA-C18:1; BCFA = branched chain fatty acids = ∑ *i*-C15:0, *a*-C15:0, *i*-C16:0, *i*-C17:0 and *a*-C17:0.

491 **Table 3**. Total lipids (%), total cholesterol (mg/100 g tissue), total fatty acids (g/100 g tissue) and composition (g/100 g total fatty acids) of beef liver, pancreas and tongue from

492 Barrosã bulls fed high (HS) or low silage (LS) diets.

493

	Liver					**Pancreas**					**Tongue**				
	HS		LS			HS		LS			HS		LS		
	Mean	SE	Mean	SE	*P* value	Mean	SE	Mean	SE	*P* value	Mean	SE	Mean	SE	*P* value
Total lipids	2.66	0.286	2.62	0.200	ns	3.77	0.226	4.03	0.202	ns	3.59	0.750	2.89	0.335	ns
Total cholesterol	154	10.2	158	2.47	ns	157	7.69	153	3.92	ns	65.6	3.98	68.1	2.37	ns
Fatty acid composition															
C14:0	0.30	0.025	0.33	0.065	ns	1.68	0.187	1.59	0.098	ns	2.59	0.329	2.62	0.067	ns
C14:1*c*9	0.25	0.026	0.15	0.033	ns	0.13	0.014	0.14	0.013	ns	0.76	0.096	0.83	0.039	ns
i-C15:0	0.13	0.013	0.09	0.012	ns	0.18	0.018	0.11	0.009	*	0.11	0.007	0.07	0.002	**
a-C15:0	0.14	0.016	0.11	0.014	ns	0.22	0.030	0.14	0.016	ns	0.15	0.018	0.09	0.002	*
C15:0	0.23	0.017	0.18	0.011	*	0.47	0.052	0.34	0.047	ns	0.39	0.021	0.34	0.025	ns
DMA-C16:0	0.91	0.078	1.06	0.127	ns	0.71	0.144	0.89	0.097	ns	1.54	0.476	1.67	0.260	ns
i-C16:0	0.14	0.019	0.11	0.012	ns	0.32	0.036	0.16	0.014	*	0.22	0.035	0.10	0.012	*
C16:0	8.51	0.338	8.83	1.01	ns	21.6	0.455	21.6	0.630	ns	18.9	1.43	19.7	0.568	ns
C16:1	0.08	0.012	0.08	0.007	ns	0.09	0.015	0.10	0.007	ns	0.06	0.005	0.06	0.007	ns
i-C17:0	0.23	0.022	0.19	0.010	ns	0.49	0.018	0.36	0.019	**	0.36	0.009	0.27	0.012	**

C16:1*c*7	0.18	0.006	0.21	0.019	ns	0.30	0.017	0.29	0.015	ns	0.32	0.012	0.32	0.014	ns
C16:1*c*9	0.53	0.048	0.68	0.152	ns	1.77	0.074	2.45	0.276	ns	3.74	0.363	4.46	0.330	ns
a-C17:0	0.42	0.056	0.38	0.021	ns	0.72	0.049	0.64	0.032	ns	0.67	0.022	0.53	0.022	**
C17:0	1.03	0.079	0.92	0.066	ns	0.91	0.079	0.68	0.057	ns	0.82	0.061	0.76	0.043	ns
DMA-C18:0	0.33	0.028	0.30	0.012	ns	0.26	0.057	0.26	0.035	ns	1.07	0.361	0.81	0.124	ns
DMA-C18:1	0.05	0.005	0.07	0.007	*	0.14	0.031	0.14	0.025	ns	0.06	0.015	0.08	0.020	ns
C18:0	31.5	0.367	31.3	0.710	ns	20.0	1.67	16.6	1.25	ns	13.1	0.591	11.2	0.443	ns
C18:1*t*6+*t*8	0.07	0.005	0.08	0.006	ns	0.31	0.018	0.29	0.024	ns	0.14	0.013	0.12	0.008	ns
C18:1*t*9	0.07	0.007	0.09	0.005	*	0.18	0.018	0.18	0.011	ns	0.21	0.015	0.19	0.011	ns
C18:1*t*10	0.08	0.004	0.16	0.019	*	0.24	0.023	0.33	0.048	ns	0.19	0.024	0.23	0.019	ns
C18:1*t*11	0.94	0.111	1.12	0.131	ns	1.37	0.133	1.09	0.142	ns	1.01	0.135	0.76	0.085	ns
C18:1*t*12	0.41	0.029	0.46	0.016	ns	0.22	0.019	0.24	0.019	ns	0.19	0.027	0.16	0.019	ns
C18:1*c*9	10.6	0.236	11.3	1.28	ns	27.3	0.655	28.9	1.41	ns	34.4	1.75	35.6	0.942	ns
C18:1*c*11+*t*15	1.13	0.053	1.40	0.117	ns	1.83	0.038	2.18	0.132	ns	2.57	0.054	2.99	0.146	ns
C18:1*c*12	0.25	0.013	0.31	0.031	ns	0.45	0.027	0.49	0.032	ns	0.40	0.027	0.34	0.028	ns
C18:1*c*13	0.07	0.007	0.11	0.011	*	0.14	0.008	0.22	0.021	*	0.30	0.021	0.42	0.042	ns
C18:1*c*14+*t*16	0.10	0.010	0.12	0.009	ns	0.32	0.008	0.27	0.027	ns	0.20	0.015	0.15	0.013	*
C18:1*c*15	0.05	0.004	0.06	0.004	ns	0.08	0.004	0.09	0.003	ns	0.10	0.018	0.09	0.006	ns
C18:2*n*-6	15.2	0.704	15.2	0.674	ns	9.48	1.34	10.7	0.775	ns	7.18	1.15	7.35	0.684	ns

C20:0	0.06	0.004	0.06	0.004	ns	0.14	0.011	0.11	0.010	ns	0.08	0.009	0.07	0.007	ns
C18:3*n*-3	1.18	0.100	0.87	0.032	*	0.76	0.047	0.61	0.040	*	0.59	0.048	0.39	0.034	*
C20:1*c*11	0.08	0.005	0.09	0.010	ns	0.16	0.013	0.21	0.018	ns	0.20	0.015	0.19	0.024	ns
C18:2*c*9*t*11	0.42	0.039	0.48	0.061	ns	0.35	0.031	0.43	0.042	ns	0.49	0.062	0.47	0.051	ns
C18:4*n*-3	0.06	0.006	0.05	0.003	ns	nd	nd	nd	nd	-	nd	nd	nd	nd	-
C20:2*n*-6	0.31	0.031	0.27	0.033	ns	0.06	0.006	0.07	0.002	ns	0.07	0.017	0.07	0.010	ns
C22:0	2.98	0.539	2.76	0.306	ns	0.22	0.067	0.22	0.034	ns	0.34	0.102	0.32	0.058	ns
C20:3*n*-6	nd	nd	nd	nd	-	0.05	0.005	0.05	0.003	ns	nd	nd	nd	nd	-
C20:3*n*-3	0.08	0.004	0.07	0.011	ns	0.04	0.006	0.05	0.003	ns	nd	nd	nd	nd	-
C20:4*n*-6	9.65	0.227	9.68	0.498	ns	2.81	0.680	3.04	0.240	ns	2.24	0.727	2.25	0.366	ns
C20:5*n*-3	0.40	0.035	0.34	0.023	ns	0.18	0.042	0.13	0.015	ns	0.11	0.031	0.09	0.015	ns
C22:4*n*-6	2.50	0.224	2.41	0.304	ns	0.19	0.038	0.27	0.019	ns	0.31	0.094	0.31	0.048	ns
C22:5*n*-6	0.52	0.066	0.56	0.067	ns	0.04	0.008	0.06	0.006	ns	0.06	0.018	0.09	0.021	ns
C22:5*n*-3	3.59	0.080	2.83	0.223	*	0.52	0.106	0.49	0.046	ns	0.46	0.132	0.36	0.060	ns
C22:6*n*-3	1.25	0.146	0.99	0.105	ns	0.07	0.013	0.08	0.008	ns	0.09	0.023	0.08	0.027	ns
Others	3.12	0.044	3.19	0.249	ns	2.49	0.167	2.72	0.192	ns	3.34	0.447	3.01	0.053	ns
Fatty acid partial sums															
∑ SFA	42.8	0.265	42.5	0.400	ns	45.4	2.32	41.5	1.74	ns	36.3	1.46	35.0	0.434	ns
∑ *cis* MUFA	11.9	0.258	12.9	1.42	ns	30.3	0.647	32.7	1.59	ns	40.2	1.97	42.2	1.25	ns

∑ TFA	2.17	0.175	2.60	0.144	ns	4.63	0.134	4.74	0.165	ns	4.64	0.242	4.72	0.199	ns
∑ PUFA	34.7	0.608	33.2	1.50	ns	14.2	2.27	15.5	1.03	ns	11.1	2.19	11.0	1.20	ns
∑ *n*-6 PUFA	28.1	0.407	28.1	1.28	ns	12.6	2.062	14.2	0.940	ns	9.86	1.97	10.1	1.10	ns
∑ *n*-3 PUFA	6.50	0.256	5.10	0.274	**	1.58	0.214	1.36	0.097	ns	1.25	0.221	0.93	0.112	ns
∑ *n*-6 LC-PUFA	13.0	0.334	12.9	0.812	ns	3.14	0.727	3.48	0.257	ns	2.68	0.851	2.72	0.432	ns
∑ *n*-3 LC-PUFA	5.33	0.228	4.23	0.278	*	0.81	0.167	0.75	0.069	ns	0.66	0.175	0.54	0.094	ns
∑ DMA	1.29	0.089	1.43	0.135	ns	1.11	0.231	1.29	0.154	ns	2.66	0.850	2.56	0.394	ns
∑ BCFA	1.06	0.122	0.88	0.051	ns	1.93	0.141	1.39	0.072	*	1.51	0.084	1.06	0.039	**
Fatty acid ratios															
PUFA/SFA	0.81	0.016	0.78	0.040	ns	0.32	0.073	0.38	0.036	ns	0.31	0.073	0.32	0.036	ns
n-6/*n*-3	4.34	0.148	5.53	0.181	**	7.95	0.261	10.4	0.264	***	7.76	0.237	10.9	0.611	**

Data are means ± standard error (SE). SFA = ∑ C14:0, C16:0, C18:0, C20:0 and C22:0; *cis* MUFA = ∑ C14:1*c*9, C16:1*c*7, C16:1*c*9, C18:1*c*9, C18:1*c*12, C18:1*c*13, C18:1*c*15 and C20:1*c*11; TFA = ∑ C18:1*t*6+*t*8-, C18:1*t*9, C18:1*t*10, C18:1*t*11, C18:1*c*11+*t*15, C18:1*t*12, C18:1*c*14+*t*16 and C18:2*c*9*t*11; PUFA = ∑ C18:2*n*-6, C18:3*n*-3, C18:4*n*-3, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-6, C20:5*n*-3, C22:4*n*-6, C22:5*n*-6, C22:5*n*-3 and C22:6*n*-3; *n*-6 PUFA = ∑ C18:2*n*-6, C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-6, C22:4*n*-6 and C22:5*n*-6; *n*-3 PUFA = ∑ C18:3*n*-3, C18:4*n*-3, C20:3*n*-3, C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3; *n*-6 LC-PUFA = *n*-6 long chain-PUFA = ∑ C20:2*n*-6, C20:3*n*-6, C20:3*n*-3, C20:4*n*-6, C22:4*n*-6 and C22:5*n*-6; *n*-3 LC-PUFA = *n*-3 long chain-PUFA = ∑ C20:3*n*-3, C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3; DMA = dimethylacetals = ∑ DMA-C16:0, DMA-C18:0 and DMA-C18:1; BCFA = branched chain fatty acids = ∑ *i*-C15:0, *a*-C15:0, *i*-C16:0, *i*-C17:0 and *a*-C17:0.

500 **Table 4**. Conjugated linoleic acid (CLA) content (mg/100 g tissue) and isomeric distribution (% CLA) of beef brain, heart and kidney from Barrosã bulls fed high (HS) or low
501 silage (LS) diets.
502

	Brain					**Heart**					**Kidney**				
	HS		LS			HS		LS			HS		LS		
	Mean	SE	Mean	SE	*P* value	Mean	SE	Mean	SE	*P* value	Mean	SE	Mean	SE	*P* value
Total CLA^{+}	1.56	0.258	1.23	0.137	ns	2.59	0.447	2.36	0.219	ns	10.5	1.20	10.5	1.90	ns
CLA profile															
*t*12,*t*14	1.65	0.380	0.92	0.195	ns	1.43	0.127	1.15	0.144	ns	0.87	0.199	0.67	0.079	ns
*t*11,*t*13	2.77	0.113	3.04	0.128	ns	3.85	0.182	2.17	0.161	***	2.36	0.145	1.50	0.121	**
*t*10,*t*12	0.36	0.039	0.44	0.055	ns	1.06	0.094	0.95	0.151	ns	0.23	0.060	0.32	0.035	ns
*t*9,*t*11	2.80	0.107	3.13	0.266	ns	1.66	0.166	1.90	0.190	ns	3.11	0.740	3.67	0.847	ns
*t*8,*t*7	0.90	0.093	0.71	0.139	ns	0.28	0.082	0.30	0.076	ns	0.24	0.026	0.38	0.056	ns
*t*7,*t*9	2.17	0.278	1.75	0.126	ns	1.21	0.317	0.78	0.165	ns	1.36	0.275	1.09	0.284	ns
*t*6,*t*8	0.66	0.384	1.34	0.095	ns	0.97	0.334	0.81	0.225	ns	0.43	0.134	0.59	0.071	ns
c/*t*12,14	1.25	0.099	1.48	0.096	ns	0.58	0.043	0.75	0.042	*	0.38	0.090	0.32	0.030	ns
*t*11,*c*13	20.0	1.04	19.7	0.244	ns	3.52	0.268	2.11	0.205	**	1.04	0.042	0.67	0.083	**
*c*11,*t*13	2.15	0.088	2.08	0.166	ns	2.00	0.157	1.95	0.090	ns	0.99	0.017	1.10	0.101	ns
*t*10,*c*12^{++}	n.d.	-	n.d.	-	-	1.12	0.075	1.31	0.187	ns	0.62	0.076	0.89	0.073	*

*c*9,*t*11^{+++}	61.9	0.780	62.1	0.691	ns	78.3	1.35	81.9	1.05	ns	84.2	1.05	84.1	1.29	ns
*t*7,*c*9	3.47	0.035	3.23	0.153	ns	4.00	0.547	4.00	0.440	ns	4.32	0.303	4.73	0.367	ns
Partial sums															
Total *trans*,*trans*	11.3	0.454	11.3	0.688	ns	10.5	0.541	8.0	0.627	*	8.49	0.879	8.22	1.233	ns
Total *cis*/*trans*	88.7	0.454	88.7	0.688	ns	89.5	0.541	92.0	0.627	*	91.5	0.879	91.8	1.23	ns

	Liver					**Pancreas**					**Tongue**				
	HS		LS			HS		LS			HS		LS		
	Mean	SE	Mean	SE	*P* value	Mean	SE	Mean	SE	*P* value	Mean	SE	Mean	SE	*P* value
Total CLA^{+}	9.40	1.10	9.34	1.73	ns	10.9	2.16	11.6	1.26	ns	15.1	6.19	11.9	3.01	ns
CLA profile															
*t*12,*t*14	0.87	0.199	0.67	0.079	ns	1.05	0.080	0.68	0.080	*	0.65	0.037	0.56	0.053	ns
*t*11,*t*13	2.36	0.145	1.50	0.121	**	2.30	0.315	1.05	0.181	*	0.96	0.118	0.62	0.074	ns
*t*10,*t*12	0.23	0.060	0.32	0.035	ns	0.59	0.120	0.52	0.076	ns	0.37	0.053	0.33	0.037	ns
*t*9,*t*11	3.11	0.740	3.67	0.847	ns	1.75	0.226	1.84	0.292	ns	1.12	0.122	1.06	0.081	ns
*t*8,*t*7	0.24	0.026	0.38	0.056	ns	0.65	0.061	0.43	0.078	ns	0.42	0.064	0.32	0.040	ns
*t*7,*t*9	1.36	0.275	1.09	0.284	ns	0.91	0.163	0.66	0.101	ns	0.86	0.177	0.60	0.069	ns
*t*6,*t*8	0.43	0.134	0.59	0.071	ns	0.75	0.055	0.48	0.108	ns	0.53	0.068	0.27	0.050	*
c/*t*12,14	0.38	0.090	0.32	0.030	ns	0.56	0.044	0.47	0.044	ns	0.68	0.064	0.99	0.143	ns
*t*11,*c*13	1.04	0.042	0.67	0.083	**	2.05	0.302	1.05	0.065	*	2.38	0.129	2.33	0.114	ns

*c*11,*t*13	0.99	0.017	1.10	0.101	ns	1.03	0.158	1.11	0.052	ns	0.68	0.152	1.02	0.132	ns
*t*10,*c*12	0.62	0.076	0.89	0.073	*	0.67	0.096	0.89	0.111	ns	1.27	0.243	1.71	0.331	ns
*c*9,*t*11[+++]	84.2	1.05	84.1	1.29	ns	81.4	1.02	84.2	1.05	ns	83.8	0.837	83.4	0.955	ns
*t*7,*c*9	4.32	0.303	4.73	0.367	ns	6.31	0.234	6.71	0.713	ns	6.28	0.286	6.78	0.508	ns
Partial sums															
Total *trans,trans*	8.49	0.879	8.22	1.23	ns	8.00	0.875	5.56	0.475	ns	4.92	0.537	3.74	0.261	ns
Total *cis/trans*	91.5	0.879	91.8	1.23	ns	92.0	0.875	94.4	0.475	ns	95.1	0.537	96.3	0.261	ns

503 [+] Total CLA was determined by the combination of GC-FID and Ag^+-HPLC techniques, as described in the text.

504 [++] In brain and kidney samples, this minor CLA isomer co-eluted with the major *c*9,*t*11 isomer.

505 [+++] This CLA isomer co-eluted with minor amounts of the *t*9,*c*11 isomer.

506 n.d., not detected.

507 **Table 5**. Loadings for the first two principal components[1].

508

Variable	**PC1**	**PC2**
C14:0	-0.591	0.743
C14:1*c*9	-0.394	0.427
i-C15:0	-0.785	-0.259
a-C15:0	-0.753	-0.253
C15:0	-0.745	0.250
DMA-C16:0	0.389	-0.210
i-C16:0	-0.689	0.001
C16:0	-0.314	0.852
C16:1*t*9	0.089	-0.130
i-C17:0	-0.897	0.027
C16:1*c*7	0.379	0.552
C16:1*c*9	-0.624	0.661
a-C17:0	-0.959	0.112
C17:0	-0.686	-0.239
DMA-C18:0	0.684	0.008
DMA-C18:1	0.901	0.253
C18:0	-0.069	-0.657
C18:1*t*6-*t*8	-0.703	0.273
C18:1*t*9	-0.820	0.488
C18:1*t*10	-0.692	0.376
C18:1*t*11	-0.854	-0.199
C18:1*t*12	0.383	-0.398

C18:1*c*9	-0.391	0.870
C18:1*c*11+*t*15	0.664	0.577
C18:1*c*12	0.037	0.030
C18:1*c*13	-0.187	0.799
C18:1*t*16+*c*14	-0.738	0.322
C18:1*c*15	-0.818	0.230
C18:2*n*-6	-0.207	-0.634
C20:0	0.363	0.081
C18:3*n*-3	-0.570	-0.714
C20:1*c*11	0.841	0.376
C20:2*n*-6	-0.027	-0.579
C22:0	-0.025	-0.823
C20:3*n*-6	0.798	0.207
C20:3*n*-3	0.158	-0.228
C20:4*n*-6	0.285	-0.750
C20:5*n*-3	0.941	0.073
C22:4*n*-6	0.773	-0.101
C22:5*n*-6	0.836	0.158
C22:5*n*-3	-0.155	-0.841
C22:6*n*-3	0.855	0.235
*t*12,*t*14	-0.704	-0.256
*t*11,*t*13	-0.556	-0.627
*t*10,*t*12	-0.735	-0.010
*t*9,*t*11	-0.419	-0.586
*t*8,*t*10	-0.779	0.126

*t*7,*t*9	-0.454	-0.443
*t*6,*t*8	-0.584	-0.258
c/*t*12,14	-0.666	0.413
*t*11,*c*13	0.013	0.279
*c*11,*t*13	-0.639	-0.230
*t*10,*c*12	-0.626	0.229
*c*9,*t*11	-0.815	-0.080
*t*7,*c*9	-0.781	0.135
Proportion of variance (%)	38.96	19.19
Cumulative variance (%)	38.96	58.15

509 [1]PC: principal component