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Meat quality of buffalo young bulls fed faba bean as protein source

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ABSTRACT

Sixteen Italian Mediterranean Buffalo young bulls were divided into two groups fed isoprotein and isoenergy diets and only differing for protein source of concentrate: faba bean (FB) vs soybean (SB). Animals were slaughtered at 350 kg BW. Meat from FB group showed significantly lower fat, protein, cholesterol and saturated fatty acids than SB group. Significant differences were also found between the three muscles analysed [*Longissimus thoracis* (LT), *Semitendinosus* (ST) and *Iliopsoas* plus *Psoas minor* (IP)]. ST showed the most favourable fatty acids profile: lower SFA, higher PUFA, MUFA, ω -3, ω -6, CLA and, consequently, lower values for both atherogenic and thrombogenic indexes. Results showed that faba bean can be used as a protein source alternative to soybean in the diet of young buffalo bulls for the production of high quality meat.

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1. Introduction

The Italian Mediterranean Buffalo (*Bubalus bubalis*) amounts to about 380000 heads and it is mainly bred for milk production in order to produce Mozzarella, the well known fresh cheese which is highly appreciated both in national and international markets. In recent years, an increasing interest for buffalo meat was seen in Italy. Up to few years ago, buffalo meat was little appreciated since only low quality meat from females with productive or reproductive problems was marketed for human consumption (Borghese et al., 2010). More recently, it has been demonstrated that using adequate feeding systems, favourable nutritional characteristics can be achieved (Infascelli, Gigli, & Campanile, 2004). The fatty acids profile and the cholesterol content of buffalo meat appears particularly interesting (Cutrignelli, Calabrò, Laudadio, Grasso, & Di Lella, 1996; Di Luccia et al., 2003; Infascelli et al., 2003, 2005; Spanghero, Gracco, Valusso, & Piasentier, 2004). In a recent cross-sectional study, Giordano et al. (2010), demonstrated that buffalo meat can confer significant benefits in terms of cardiovascular risk profile, including lower carotid atherosclerotic burden and susceptibility to oxidative stress.

In recent years in order to reduce feeding costs, several farmers attempt protein sources alternative to soybean in buffalo diet (Cutrignelli, Calabrò, Tudisco, Infascelli, & Piccolo, 2011) and tried to increase the domestic production of GMO-free plant proteins. Indeed, large part of the available soybean is genetically modified thus still determining public concerns due its health potential risks (Tudisco, Ma stellone, et al., 2010).

Faba bean (*Vicia faba* var. *minor*) is relatively cheap in spite of its high nutritional value (Larralde & Martinez, 1991) traditionally attributed to the high protein content (25–35%) and to the high lysine content despite the imbalance in sulphur aminoacids. Additionally, Faba bean as grain legume increases the sustainability of crop-livestock systems through the safeguarding of soil fertility. The occurrence of some anti-nutritional factors (ANF) has hampered a wider nutritional utilization of faba bean (Calabrò et al., 2009). However, the considerable progress in plant breeding notably decreased the presence of secondary plant metabolites (Duc, Marget, Esnault, Le Guen, & Bastianelli, 1999). Recent studies performed in other species (Cutrignelli, Calabrò, et al., 2008; Cutrignelli, Piccolo, et al., 2008; Lanza et al., 2011; Scerra et al., 2011) showed that the use of faba bean as the main protein source did not negatively affect animal performance and meat fatty acids profile and cholesterol content. Because few data are available on buffalo species, the aim of this study was to evaluate the influence of a diet containing faba bean on the nutritional characteristics of meat from young buffalo bulls.

2. Material and methods

2.1. Animals and diets

The trial was carried out in a farm situated at 47 m a.s.l. in Southern Italy (Cassino, FR), where about 200 Italian Mediterranean buffaloes are bred. All experiments were authorized by the Animal Welfare Commission of the Department of Veterinary Medicine and Animal Production, University of Naples Federico II.

Sixteen eight days buffalo males were equally divided into two groups (FB, faba bean and SB, soybean) and received 6.0 L/head/d of

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acidified milk replacer (180 g/L of water) until 56 days of age. Therefore, the replacer amount was gradually decreased, whilst administering the same volume, in order to obtain a complete weaning at the age of 80 days. Starting from the 70th day, corn silage was administered, whereas roughly chopped mixed hay and weaning concentrate were added from the fifth week. After weaning until the beginning of trial, the animals were fed mixed hay and corn silage *ad libitum*, whereas concentrate was fed in the amount of 2.0 kg/d

At 84 days, each animal was placed in individual box up to the slaughtering weight and fed (2.7% BW) isoprotein (CP: 15.2% DM) and isoenergy (0.91 veal forage unit_VFU/kg DM) diets, characterized by 39% of NDF and differing in protein source of concentrate: faba bean tannin-free variety (*Vicia faba minor* L.) vs. whole seed soybean (*Soja hispida*). As forage, a mixed hay and a corn silage produced by the farm were administered to both groups. Every two months, samples of each feed were collected to determine the chemical composition (AOAC, 2000; Van Soest, Robertson, & Lewis, 1991) and to estimate the nutritive value as net energy for meat production (VFU/kg DM; INRA, 1988). In Table 1, percentages (%DM of diet), crude protein and nutritive values of diet ingredients are depicted.

Faba bean, soybean and the other ingredients of concentrates were also analysed for the fatty acid profile. For this purpose, the total fat was extracted from 0.5 g of each samples into 20 × 150 mm test tubes with Teflonlined screw caps (Folch, Lees, & Sloane Stanley, 1957). Fatty acids were methylated using HCl in methanol prepared by adding 20 mL of acetyl chloride to 100 mL of methanol (Christie, 1993). Fatty acid methyl esters were analysed by a gas-chromatography (GC) apparatus (Focus ThermoQuest equipped a computer integration system Chrome-card.), equipped with a CP-SIL 88 fused silica capillary column [30 m × 0.25 mm (internal diameter) with 0.2-µm film thickness; Varian, Walnut Creek, CA, USA]. The GC conditions were the following: the oven temperature was programmed at 160 °C and held for 1 min; then increased up to 230 °C at a rate of 1 °C/min and held for 3 min. The injector and detector temperatures were set up at 220 °C and 250 °C, respectively. Helium was used as carrier gas with flow of 1.5 mL/min. Fatty acids were identified using a mixture of standard fatty acids (Larodan Fine Chemical AB, Malmo, Sweden) and quantified using the peak areas, and were expressed as mg fatty acid/g dry matter of feed sample (Antongiovanni, Lercker, & Serchiari, 2007; D'Urso et al., 2008; Tudisco, Cutrignelli, et al., 2010).

2.2. Meat nutritional characteristics

When animals reached about 350 kg BW (429.7 ± 57.6 days of age) they were slaughtered in an authorized slaughterhouse (1.5 km from the farm) according to EU legislation (EU Regulation 882/2004). After 15 days of ageing at 4 ± 1 °C, the right side of each animal was dissected. The pH (Hanna pH-meter, mod. HI 9025, equipped with an electrode FC 230C) was measured in *Longissimus thoracis* (LT), *Semitenidinosus* (ST) and *Iliopsoas plus Psoas minor* (IP) muscles within 1 h from death and at the end of ageing.

Samples of LT, ST and IP muscles were collected and rapidly transported, upon refrigeration temperature, to the laboratories for the chemical analysis. The muscles were homogenised and divided into three different aliquots: two were vacuum-packed and frozen (−20 °C)

Table 1
Diet ingredients: percentage (% of DM diet), crude protein and nutritive value.

	Corn silage	Mixed hay	FB concentrate ^a	SB concentrate ^b
% of DM diet	30.0	20.0	50.0	50.0
Crude protein, % DM	7.60	7.80	22.8	22.8
Energy, VFU/kg DM	0.80	0.60	1.10	1.12

DM: dry matter; VFU: veal forage unit.

FB: faba bean; SB: soybean.

^a corn (10%), barley (11%), wheat bran (23%) faba bean 29% CP (56%).

^b corn (15%), barley (15%), wheat bran (26%) and soybean 35% CP (44%).

until the analysis (cholesterol content and fatty acid composition). The last one was analysed for moisture, crude fat, ash and protein by using a food analyser (FoodScan Lab, FOSS Electric, Denmark). The cholesterol extraction was made according to Naeemi, Ahmad, Al-Sharrah, and Behbahani (1995) and for its quantification a GC was utilized. The GC (Focus ThermoQuest) was equipped with a split/splitless injector, a flame ionization detector, a fused silica capillary column (SACTM-5, Supelco, 30 m × 0.25 mm × 0.25 µm film thickness) and computer integration system Chrome-card. The operative conditions were the following: oven temperature isothermal 285 °C; injector and detector temperature 300 °C; injection volume 1.0 µL; carrier gas: helium (25 cm/s); split ratio: 1:100. The identification of cholesterol was made by comparing the peak retention time of samples with an external standard (C-8667, SIGMA). The concentration was expressed as g/100 g of edible part, each chromatographic analysis was replicated three times.

To determine the fatty acids profile, total fat was previously extracted (Folch et al., 1957) and subsequently turned into methyl esters (FAMES) by direct transesterification (Christie, 1993) according to Chiofalo et al. (2010) and Chiofalo et al. (2011). The oil extracted from each sample was suspended in a mixture of sulphuric acid/methanol (1:9, mL/mL) and heated for three h. The FAMES were isolated by adding 1.0 mL of n-hexane. The mixture was shaken and, after two min, the formed top-layer with n-hexane was transferred into the vial for the GC injection. The FAMES were analyzed by GC-FID (Agilent Technologies 6890 N, Palo Alto, CA, U.S.A.) with a split/splitless injector, a flame ionization detector and fused silica capillary column Omegawax 250 (Supelco, Bellefonte, PA, U.S.A.; 30 m × 0.25 mm I.D., 0.25 µm film thickness). Column temperature was programmed: initial isotherm of 160 °C (6 min.), increment of 3 °C/min and final isotherm of 250 °C (30 min.). Temperature of the injector and detector: 250 °C. Injection volume: 1.0 µL. Carrier gas: helium (1 mL/min). Split ratio: 1:50. Identification of fatty acids was made by comparing the relative retention times of FAME peaks from samples with standards from Supelco (Bellefonte, PA, U.S.A.). Chromatogram peak areas were acquired and calculated by Chemstation software (Agilent, Palo Alto, CA, U.S.A.). The concentration of each fatty acid was expressed as g/100 g, considering 100 g the summation of the areas of all fatty acid methyl esters identified. For each sample the chromatographic analysis was replicated three times.

On the basis of the identified fatty acids, the Atherogenic Index (AI) and the Thrombogenic Index (TI) were estimated using the following equations proposed by Ulbricht and Southgate (1991):

$$AI = \frac{C12 : 0 + (4 \cdot C14 : 0) + C16 : 0}{n-6 \text{ PUFA} + n-3 \text{ PUFA} + MUFA}$$

$$TI = \frac{C14 : 0 + C16 : 0 + C18 : 0}{(0.5 \cdot MUFA) + (0.5 \cdot n-6 \text{ PUFA}) + (3 \cdot n-3 \text{ PUFA}) + (n-3 \text{ PUFA}/n-6 \text{ PUFA})}$$

2.3. Statistical analysis

The statistical analysis was performed (Proc GLM, SAS, 2000) with the following model: $y_{ijk} = \mu + D_i + M_j + (D^*M)_{ij} + \varepsilon_{ijk}$ where: y = dependent variable, μ = mean; D = dietary effect (i = FB, SB), M = muscle effect (j = LT, ST, IP), D^*M = interaction diet × muscle and ε = error. The mean was statistically compared using the Least Significant Differences (LSD) test.

3. Results

3.1. Fatty acid profile of the diet ingredient

The fatty acid profile of faba bean, soybean and concentrates is depicted in Table 2. Soybean showed higher concentration of palmitic (C16:0), oleic (C18:1 n-9) and linoleic (C18:2 n-6) acids than faba bean. Conversely, the levels of stearic (C18:0) and linolenic (C18:3 n-3) acids were higher in faba bean than soybean. These results

reflected the fatty acid profile of FB and SB concentrates despite a lower difference was recorded in that case.

3.2. Meat pH, chemical composition, cholesterol and collagen

The pH of *Longissimus thoracis*, *Semitendinosus* and *Iliopsoas* plus *Psoas minor*, despite significant differences were recorded between muscles, generally indicated a correct glycolytic process in muscle (Fig. 1): indeed, pH values at slaughter were always lower than 7.0 and at dissection always higher than 5.5.

Dietary treatments affected some qualitative parameters of meat (Table 3). In particular, FB group showed significantly ($P < 0.05$) lower value for fat, protein and cholesterol if compared with SB group.

The muscle type highly affected all the parameters ($P < 0.001$) except cholesterol content. In particular, ST showed the lowest fat ($P < 0.01$) and IP the highest protein content ($P < 0.01$).

3.3. Meat fatty acids profile

The dietary treatment significantly influenced the proportion of C17:1-cis10, C20:0, C22:0, C20:4 n-6, C24:0, C24:1-cis15, C22:5 n-3, C22:6 n-3 ($P < 0.05$), C20:3 n-6 ($P < 0.01$) and C1:0-anteiso, C15:0, C17:0, C23:0 ($P < 0.001$). The large part of fatty acids was significantly ($P < 0.01$) affected by muscle type, except C18:1-trans10 and C18:1-trans11, trans10-trans12 CLA, C24:1-cis15 and C22:6 n-3 (Table 4).

The great number of analysed fatty acids was divided in classes according to a particular nutritional interest (Table 5). It can be observed that saturated fatty acids (SFA) were significantly ($P > 0.05$) higher in SB than FB group (50.3 vs. 49.9 g/100 g). By contrast, monounsaturated (MUFA), polyunsaturated (PUFA), ω -6, ω -3, CLA were not affected by dietary treatment, as well as PUFA/SFA and both ω -6/ ω -3 ratios and quality indexes (AI, TI).

Significant ($P < 0.01$) differences were found comparing the three muscles, and ST showed the most favourable fatty acids profile: lower SFA, higher PUFA, MUFA, ω -3, ω -6, CLA and, consequently, lower values for both quality indexes.

The interactions dietary treatment \times muscle was never significant.

4. Discussion

4.1. Fatty acid profile of the diets ingredients

The results concerning fatty acids profile of faba bean and soybean are in agreement with those reported by Lanza et al. (2011) and in contrast with those of Grela and Günther (1995) who found lower linoleic (C18:2) acid content in faba bean. As reported by Lanza et al. (2011) legume seeds are characterized by a high content of linoleic and palmitic acids (C16:0), while level of linolenic acid (C18:3) is low and variable among varieties (Grela & Günther, 1995). Some authors (Priolo, Lanza, Galofaro, Fasone, & Bella, 2003; Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007; Yoshida, Saiki, Yoshida, Tomiyama, & Mizushina, 2008; Yoshida, Tomiyama, Saiki, & Mizushina, 2007) reported low and similar

Table 2

Fatty acid profile of faba bean, soybean and concentrates.

mg FA/g DM	Faba bean	Soybean	FB concentrate	SB concentrate
C16:0	2.874	3.987	3.256	3.720
C18:0	0.933	0.642	0.655	0.444
C18:1 n-9	2.376	4.754	2.773	3.881
C18:2 n-6	12.33	14.57	12.49	13.13
C18:3 n-3	2.145	1.628	1.928	1.569

FA: fatty acids.

FB: faba bean; SB: soybean.

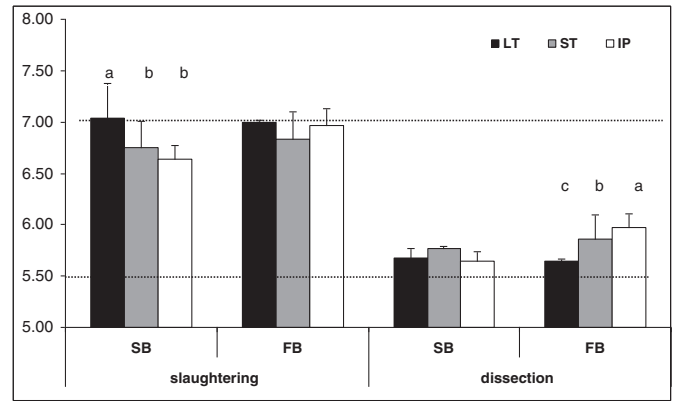


Fig. 1. pH values at slaughtering and dissection in the soybean and faba bean groups and in the three tested muscles. FB: faba bean group; SB: soybean group. LT: *Longissimus thoracis*; ST: *Semitendinosus*; IP: *Iliopsoas* plus *Psoas minor* muscles. a,b,c: $P < 0.05$.

linolenic content in peas (3.8 – 9.3% total FA), faba bean (3.8 – 5.1% total FA) and chickpeas (2.4 – 3.2% total FA) legume seeds. However, the favourable level of C18:3 n-3 in faba bean was lower when compared with linseed or algae (Cooper et al., 2004), but higher when compared with chickpeas (Priolo et al., 2003).

4.2. Meat quality

In Italy, the consumers' growing interest for buffalo meat is due to its nutritional characteristics (Cutrignelli et al., 1996; Di Luccia et al., 2003; Infascelli et al., 2003, 2004, 2005; Spanghero et al., 2004). Particularly, buffalo meat seems to be highly suitable for patients who need dietetical foods. Therefore, the significant lower fat and cholesterol content found in meat from animals fed diet including faba bean come to have a great appeal. In each case, the average level of cholesterol found in this study (33.3 mg/100 g), although sensibly higher than that reported for buffalo by Samoggia, Lo Fiego, De Grossi, and Bergonzoni (1993) on gracilis muscle (27.7 mg/100 g) and on the diaphragm muscle (31.4 mg/100 g) is lower than that obtained by Cutrignelli et al. (1996) in *Longissimus dorsi* muscle of young buffalo bulls (41.1 mg/100 g). In the literature, the content of cholesterol in buffalo meat is rather disomogeneous: Kesava Rao, Kowale, Murthy, and Sharma (1992) found 83 mg/100 g in the *Longissimus dorsi* muscle, Yadav and Singh (1974) indicated a sensibly lower content (32 mg/100 g), Sinclair, Slattery, and O'Dea (1982) found 46 mg/100 g in Australian buffalo meat. Some authors found no differences in cholesterol content among muscles (Bohac & Rhee, 1988; Cifuni, Napolitano, Riviezzi, Braghieri, & Girolami, 2004) but, again, in contrast with others (Browning, Huffman, Egbert, & Jungst, 1990; Eichhorn et al., 1986; Rusman, Soeparano, Setiyono, & Suzuki, 2003). This contradiction is probably due to the different muscles analysed in each

Table 3

Chemical composition and cholesterol content.

	Groups		Muscles			Prob.	MSE
	FB	SB	LT	ST	IP		
Fat, %	1.82 ^b	1.98 ^a	2.07 ^A	1.47 ^B	2.17 ^A	***	*
Moisture, %	75.9	75.7	75.5 ^B	75.5 ^B	76.5 ^A	***	NS
Protein, %	21.0 ^b	21.3 ^a	21.3 ^{Ab}	21.6 ^{Aa}	20.6 ^B	***	NS
Ash, %	0.76	0.57	0.72 ^A	0.94 ^A	0.33 ^B	***	NS
Cholesterol, mg/100 g	32.2 ^b	33.7 ^a	32.4	33.7	32.8	NS	*

FB: faba bean; SB: soybean.

LT: *Longissimus thoracis*; ST: *Semitendinosus*; IP: *Iliopsoas* plus *Psoas minor*.

MSE: mean square error; along the column A,B: $P < 0.01$. a,b: $P < 0.05$.

***, *, NS: $P < 0.001$, $P < 0.05$, not significant, respectively.

Table 4
Fatty acids classes (g/100 g), ratios and quality indexes in the soybean and faba bean groups and in the three tested muscles.

	Groups		Muscles			Prob.		MSE
	FB	SB	LT	ST	IP	Muscle	Group	
SFA	49.9 ^b	50.3 ^a	52.5 ^A	44.2 ^B	53.5 ^A	***	*	9.40
MUFA	36.3	36.6	36.7 ^B	39.9 ^A	32.8 ^C	***	NS	10.29
PUFA	13.4	12.8	10.5 ^C	16.1 ^a	12.91 ^b	***	NS	6.38
ω-3	1.79	1.74	1.52 ^A	2.17 ^A	1.61 ^B	***	NS	4.36
ω-6	11.4	10.9	8.76 ^C	13.7 ^A	11.1 ^B	***	NS	0.0042
CLAs	0.21	0.23	0.21 ^b	0.25 ^{Aa}	0.20 ^{Bb}	***	NS	0.012
PUFA/SFA	0.26	0.28	0.20 ^{Bb}	0.36 ^A	0.24 ^a	***	NS	0.011
ω-6/ω-3	6.44	6.24	5.90 ^b	6.26 ^a	6.95 ^a	*	NS	1.53
AI	0.53	0.53	0.57 ^a	0.42 ^b	0.60 ^a	***	NS	0.0033
TI	1.34	1.35	1.51 ^a	1.02 ^b	1.50 ^a	***	NS	0.027

FB: faba bean; SB: soybean.

LT: *Longissimus thoracis*; ST: *Semitendinosus*; IP: *Iliopsoas plus Psoas minor*.

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CLA: conjugated linoleic acids; AI: Atherogenic Index; TI: Thrombogenic index. MSE: mean square error. Along the row: A,B,C: $P < 0.01$ and a,b,c: $P < 0.05$. ***, *, NS: $P < 0.001$, $P < 0.05$, not significant, respectively.

experiment: indeed Bohac and Rhee (1988) and Cifuni et al. (2004) utilised *Longissimus thoracis*, *Semimembranosus* and *Semitendinosus*, while Eichhorn et al. (1986) and Browning et al. (1990) analysed *Biceps femoris* and *Longissimus thoracis*. As theorised by Wheeler, Davis, Stoeker, and Hatmon (1987), the cholesterol content may be affected by the different physiological function of each muscle.

Table 5
Fatty acid profile in the soybean and faba bean groups and in the three tested muscles.

g/100 g FA	Groups		Muscles			Prob.		MSE
	FB	SB	LT	ST	IP	Muscle	Group	
C14:0	1.18	1.23	1.31 ^A	0.91 ^B	1.40 ^A	***	NS	0.53
C15:0-iso	0.15 ^b	0.17 ^a	0.18 ^A	0.12 ^B	0.19 ^A	***	NS	0.00096
C15:0-anteiso	0.20 ^B	0.25 ^A	0.22 ^{ABb}	0.18 ^B	0.27 ^{Aa}	***	***	0.081
C14:1-cis9	0.48	0.46	0.35 ^B	0.73 ^A	0.32 ^B	***	NS	0.0276
C15:0	0.29 ^B	0.36 ^A	0.31 ^{ABb}	0.27 ^{Bc}	0.36 ^{Aa}	***	***	0.00036
C16:0	21.0	20.8	21.1 ^{AB}	20.4 ^B	21.6 ^A	***	NS	2.43
C17:0-iso	0.50	0.53	0.47 ^a	0.57 ^a	0.50 ^b	*	NS	0.192
C16:1n-7	1.18	1.14	1.10 ^B	1.38 ^A	1.00 ^B	***	NS	0.614
C17:0	1.10 ^B	1.22 ^A	1.23 ^A	0.97 ^B	1.24 ^A	***	***	0.0011
C17:1-cis10	0.40 ^b	0.45 ^a	0.41 ^{ABb}	0.48 ^{Aa}	0.36 ^B	***	*	0.0089
C18:0	24.6	25.0	27.2 ^A	19.8 ^B	27.6 ^A	***	NS	6.062
C18:1-trans10	0.56	0.64	0.61	0.63	0.52	NS	NS	0.039
C18:1-trans11	1.30	1.31	1.28	1.24	1.41	NS	NS	0.165
C18:1n-9	31.0	30.8	31.2 ^{Ab}	33.2 ^{Aa}	27.8 ^B	***	NS	8.70
C18:1-cis11	1.29	1.26	1.17 ^B	1.49 ^A	1.17 ^B	***	NS	0.024
C18:2n-6	7.91	7.87	6.49 ^B	9.13 ^{Aa}	8.10 ^{Ab}	***	NS	2.77
C20:0	0.19 ^b	0.21 ^a	0.21 ^A	0.17 ^B	0.21 ^A	***	*	0.0023
C18:3n-6	0.11	0.10	0.09 ^B	0.14 ^A	0.09 ^B	***	NS	0.00088
C20:1-cis11	0.10	0.11	0.10 ^{ABb}	0.13 ^{Aa}	0.08 ^B	***	NS	0.0013
C18:3n-3	0.57	0.59	0.51 ^b	0.65 ^a	0.56 ^b	*	NS	0.015
cis9-trans 11 CLA	0.05	0.05	0.04 ^b	0.06 ^a	0.05 ^b	*	NS	0.0007
trans10-trans12 CLA	0.17	0.18	0.17 ^{ab}	0.19 ^a	0.15 ^b	*	NS	0.0032
C20:2-cis11,14	0.21	0.20	0.18 ^B	0.26 ^A	0.18 ^B	***	NS	0.0039
C22:0	0.17 ^b	0.21 ^a	0.17 ^b	0.21 ^a	0.17 ^b	*	*	0.0026
C20:3n-6	0.63 ^A	0.53 ^B	0.42 ^C	0.80 ^A	0.56 ^B	***	**	0.015
C20:3n-3	0.29	0.28	0.23 ^{Bb}	0.33 ^{Aa}	0.28 ^b	**	NS	0.0078
C20:4n-6	2.43 ^a	2.05 ^b	1.56 ^B	3.19 ^{Ab}	2.05 ^{Ba}	***	*	0.555
C23:0	0.17 ^B	0.21 ^A	0.16 ^B	0.22 ^A	0.18 ^B	***	***	0.0245
C20:5n-3	0.22 ^B	0.19 ^A	0.17 ^B	0.29 ^A	0.16 ^B	***	***	0.0034
C24:0	0.16 ^b	0.20 ^a	0.16 ^B	0.21 ^A	0.15 ^B	**	*	0.0017
C24:1-cis15	0.39 ^b	0.49 ^a	0.44	0.41	0.43	NS	*	0.0065
C22:4n-6	0.37	0.32	0.26 ^{Ba}	0.46 ^A	0.33 ^{Ba}	***	NS	0.021
C22:5n-3	0.54 ^a	0.45 ^b	0.38 ^B	0.69 ^A	0.44 ^B	***	*	0.039
C22:6n-3	0.20 ^b	0.24 ^a	0.22	0.22	0.21	NS	*	0.010

FB: faba bean group; SB: soybean group.

LT: *Longissimus thoracis*; ST: *Semitendinosus*; IP: *Iliopsoas plus Psoas minor*.

MSE: mean square error. Along the row: A,B,C: $P < 0.01$ and a,b,c: $P < 0.05$. ***, **, *, NS: $P < 0.001$, $P < 0.01$, $P < 0.05$, not significant, respectively.

Concerning the protein content of meat, the differences between groups (21.0% vs 21.3%, for FB and SB respectively) even if significant, appear too little to have any importance from the nutritional point of view.

4.3. Fatty acid profile

Meat from buffaloes fed faba bean showed significantly lower levels of total saturated fatty acids (SFA). This result is very interesting and it is mainly due to the differences found for some long-chain SFA (C15:0, C17:0, C20:0 – C24:0) which were significantly higher in SB group. According to Hornstra and Lussenberg (1975) and Renaud et al. (1986) long chain SFA are involved in thrombus formation. Differences in fatty acid profile using faba bean as protein source have been already found by other authors. Cutrignelli, Calabrò, et al. (2008) reported a significantly higher value for stearic acid in young Marchigiana bulls fed faba bean respect to those fed soybean meal solvent extract. Similarly, Scerra et al. (2011) found a higher palmitic acid value in meat from lambs fed soybean respect to those fed faba bean.

In the current trial, the contents of myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids were always lower than those recorded by Poli et al. (1996) for Chianina young bulls, thus confirming the favourable characteristics of buffalo meat.

The dietary treatment did not affect the level of most unsaturated fatty acids which are believed to be important for human health. Interestingly, the arachidonic acid (C20:4 ω-6), derived from C18:2 ω-6 through the action of Δ5 and Δ6 desaturase enzymes and elongase

(Gurr & Harwood, 1996), was significantly higher in FB group while its precursor was not found higher in meat from buffaloes fed faba bean. Similarly, C20:5 ω -3 was significantly higher in FB than in SB group, while its precursor C18:3 ω -3 wasn't different between groups. The ω -3 fatty acids play a very important physiological role, especially during fetal and infant growth, in the formation of the central nervous system and retina (Bourre, 2003; Bowen & Clandinin, 2005). Being antithrombotic, anti-inflammatory, antiarrhythmic and favoring plaque stabilization (Galli & Marangoni, 2006; Hu et al., 1999; Simon, Pong, Bernert, & Browner, 1995), they are also important for the prevention of cardiovascular diseases. Many significant differences were found between the three muscles studied concerning the FA profile, this was probably due to two main reasons: the sampling of the edible part of the muscle that also includes the infiltration fat, and the different type of muscle that, having a different role, favours the accumulation of different nutrients.

These results are very interesting from the nutritional point of view. Muscles with a high percentage of unsaturated fatty acids (UFA) generally scored higher in taste panel evaluation and foods with high UFA, especially PUFA, are good for human health (Westerling & Hedrick, 1979). Conjugated linoleic acid (CLA) has been shown to have immunomodulating, anticarcinogenic and antiatherosclerosis properties (Pastuschenko, Matthes, Hein, & Holzer, 2000; Whigham, Cook, & Atkinson, 2000). Additionally, high prominence is attributed to the MUFA, and particularly by the oleic acids that, reducing the oxidation of the cholesterol LDL, may slow the progression of atherosclerosis (Parthasarathy et al., 1990). Concerning the ω -6/ ω -3 ratio, if we compare the results obtained in this trial with those cited by Daley, Abbott, Doyle, Nader, and Larson (2010), we can notice that it is higher than the value reported for grass-fed beef, but lower than that reported for grain-fed beef. Nevertheless, the optimal nutritional value of the ω -6/ ω -3 ratio has still not been completely assessed both for human and animals. The studies on the relationship between ω -6/ ω -3 ratio and the pathogenesis of many diseases, including cancer, cardiovascular, inflammatory and autoimmune diseases, indicate that the optimal ratio may vary with the disease or condition under consideration. This is consistent with the fact that chronic diseases are multi-genetic and multifactorial. On the whole, the health authorities of many countries promote the intake of foods containing high levels of ω -6/ ω -3 ratio (West Sutor & Meyers, 2006) which seems to reduce the risk of many of the chronic diseases (Simopoulos, 2003).

According to Ulbricht and Southgate (1991), atherogenic and thrombogenic indexes might better characterize the health properties of vegetables or animal food respect to a simple approach based on total saturated fatty acids or on the ratio polyunsaturated/saturated fatty acids (Burr, 1989; Fehily, Pickering, Yarnell, & Elwood, 1994). Both the indexes were not affected by dietary treatment but were different between muscles. The AI for buffalo meat recorded in this trial was lower than those reported by Ulbricht and Southgate (1991) for raw minced beef and by Badiani et al. (2002) for cooked beef (0.72 and 0.77, respectively) while thrombogenic TI was lower if compared to that reported by these authors.

5. Conclusions

The results show that faba bean, that leads, by itself, to agronomical and economical advantages, can be used as a protein source alternative to soybean in the diet of young buffalo bulls for the production of high quality meat. In particular, fat percentage, cholesterol content and total saturated fatty acids decreased in meat from animals fed faba bean.

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References

- A.O.A.C. (2000). *Official methods of analysis* (17th ed.) Arlington, V.A., USA: Association of Official Analytical Chemists, Inc.
- Antongiovanni, M., Lercker, G., & Serchiari, P. (2007). *Metodi di analisi per lo studio della frazione lipidica del latte, dei prodotti di origine animale e degli alimenti zootecnici*. Pisa: Plus.
- Badiani, A., Stipa, S., Bitossi, F., Gatta, P. P., Vignola, G., & Chizzolini, R. (2002). Lipid composition, retention and oxidation in fresh and completely trimmed beef muscles as affected by common culinary practices. *Meat Science*, *60*, 169–186.
- Bohac, C. E., & Rhee, K. S. (1988). Influence of animal and diet and muscle on cholesterol content of beef and pork muscle. *Meat Science*, *23*, 71–75.
- Borghese, A., Terzano, G. M., Mazzi, M., Razzano, M., Sabia, E., & Pacelli, C. (2010). Fattening of buffalo young bulls with different diets. *Proceeding of IX World Buffalo Congress, Meat Production 05* (pp. 511–516).
- Bourre, J. (2003). Relations of omega-3, omega-9 fatty acids and brain structure and functions. A review on recent data. *Food financial cost of omega-3. Oleagineux, Corps Gras, Lipides*, *10*, 165–174.
- Bowen, R. A.R., & Clandinin, M. T. (2005). Maternal dietary 22:6n3 is more effective than 18:3n3 in increasing content in phospholipids of glial cells from neonatal rat brain. *British Journal of Nutrition*, *93*, 601–611.
- Browning, M.A., Huffman, D. L., Egbert, W. R., & Jungst, S. B. (1990). Physical and compositional characteristics of beef carcasses selected for leanness. *Journal of Food Science*, *55*, 9–14.
- Burr, M. L. (1989). Fish and the cardiovascular system. *Progress in Food & Nutrition Science*, *13*, 291–316.
- Calabrò, S., Tudisco, R., Balestrieri, A., Piccolo, G., Infascelli, F., & Cutrignelli, M. I. (2009). Fermentation characteristics of different grain cultivars with the in vitro gas production technique. *Italian Journal of Animal Science*, *8*(Suppl. 2), 280–282.
- Chiofalo, B., Lo Presti, V., Savoini, G., D'Alessandro, E., Chiofalo, V., & Liotta, L. (2011). Nucleotides administration in broiler chicken diet: Effect on breast muscles quality. *Czech Journal of Food Sciences*, *29*(4), 308–317.
- Chiofalo, B., Simonella, S., Di Grigoli, A., Liotta, L., Frenda, A. S., Lo Presti, V., Bonanno, A., & Chiofalo, V. (2010). Chemical and acidic composition of Longissimus dorsi muscle of Comisana lambs fed with Trifolium subterraneum and Lolium multiflorum. *Small Ruminant Research*, *88*, 89–96.
- Christie, W. W. (1993). Preparation of ester derivatives of fatty acids for chromatographic analysis. In W. W. Christie (Ed.), *Advances in lipid methodology* (pp. 69–111) (2nd ed.). Dundee, UK: Oily Press.
- Cifuni, G. F., Napolitano, F., Riviezz, A.M., Braghieri, A., & Girolami, A. (2004). Fatty acid profile, cholesterol content and tenderness of meat from Podolian young bulls. *Meat Science*, *67*, 289–297.
- Cooper, S. L., Sinclair, L. A., Wilkinson, R. G., Hallett, K., Enser, M., & Wood, J.D. (2004). Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. *Journal of Animal Science*, *82*, 1461–1470.
- Cutrignelli, M. I., Calabrò, S., Bovera, F., Tudisco, R., D'Urso, S., Marchiello, M., Piccolo, V., & Infascelli, F. (2008). Effects of protein source and energy value of diet on the performance of young Marchigiana bulls: 2. Meat quality. *Italian Journal of Animal Science*, *7*, 271–285.
- Cutrignelli, M. I., Calabrò, S., Laudadio, P., Grasso, F., & Di Lella, T. (1996). Chemical nutritional characteristics of meat produced by young buffalo bulls. *Proceeding of XXXI International Symposium of Zoothecny "Food and health: role of animal products"* (pp. 101–105).
- Cutrignelli, M. I., Calabrò, S., Tudisco, R., Infascelli, F., & Piccolo, V. (2011). Protein sources in ruminant nutrition. *Soybean/Book 2. 9* (pp. 191–214) (InTech open).
- Cutrignelli, M. I., Piccolo, G., Bovera, F., Calabrò, S., D'Urso, S., Tudisco, R., & Infascelli, F. (2008). Effects of protein source and energy value of diet on the performance of young Marchigiana bulls: 1 Infra vitam performance and carcass quality. *Italian Journal of Animal Science*, *7*, 259–270.
- D'Urso, S., Cutrignelli, M. I., Calabrò, S., Bovera, F., Tudisco, R., Piccolo, V., & Infascelli, F. (2008). Influence of pasture on fatty acid profile of goat milk. *Journal of Animal Physiology and Animal Nutrition*, *92*, 405–410.
- Daley, C. A., Abbott, A., Doyle, P.S., Nader, G. A., & Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal*, *9*, 10.
- Di Luccia, A., Satriani, A., Barone, C. M.A., Colatruglio, P., Gigli, S., Occidente, M., Trivellone, E., Zullo, A., & Matassino, D. (2003). Effect of dietary energy content on the intramuscular fat depots and triglyceride composition of river buffalo meat. *Meat Science*, *65*(4), 1379–1389.
- Duc, G., Marget, P., Esnault, R., Le Guen, J., & Bastianelli, D. (1999). Genetic variability for feeding value of faba bean seeds (*Vicia faba* L.). Comparative chemical composition of isogenes involving zero-tannin and zero-vicine genes. *Journal of Agriculture Science*, *133*, 185–196.
- Eichhorn, J. M., Coleman, L. J., Wakayama, E. J., Blomquist, G. J., Bailey, C. M., & Jenkins, T. G. (1986). Effects of breed and restricted versus ad libitum feeding on fatty acid composition and cholesterol content of muscle and adipose tissue from mature bovine females. *Journal of Animal Science*, *63*, 781–794.
- European Parliament and Council Regulation n. 882/2004 (29 April 2004) on "The official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules". *Official Journal, L 165*. (2004, April 30), 1–141.
- Fehily, A.M., Pickering, J. E., Yarnell, J. W. G., & Elwood, P. C. (1994). Dietary indices of atherogenicity and thrombogenicity and ischemic heart disease risk: The Caerphilly Prospective Study. *British Journal of Nutrition*, *71*, 249–257.
- Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal Biological Chemistry*, *226*(1), 497–509.

- Galli, C., & Marangoni, F. (2006). N-3 fatty acids in the Mediterranean diet. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, 75, 129–133.
- Giordano, G., Guarini, P., Ferrari, P., Biondi-Zoccai, G., Schiavone, B., & Giordano, A. (2010). Beneficial impact on cardiovascular risk profile of water buffalo meat consumption. *European Journal of Clinical Nutrition*, 64, 1000–1006.
- Grela, E. R., & Günther, K. D. (1995). Fatty acid composition and tocopherol content of some legume seeds. *Animal Feed Science and Technology*, 52, 325–331.
- Gurr, M. L., & Harwood, J. L. (1996). *Lipid biochemistry and introduction* (4th ed.). London: Chapman & Hall.
- Hornstra, G., & Lussenberg, R. N. (1975). Relationship between the type of dietary fatty acid and the arterial thrombus tendency in rats. *Atherosclerosis*, 22, 499–516.
- Hu, F. B., Stampfer, M. J., Manson, J. E., Rimm, E. B., Wolk, A., & Colditz, G. A. (1999). Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. *American Journal of Clinical Nutrition*, 69, 890–897.
- I.N.R.A. (1988). In R. Jarrige (Ed.), *Alimentation des bovins, ovins et caprins* (Paris).
- Infascelli, F., Cutrignelli, M. I., Bovera, F., Piccolo, G., Tudisco, R., Calabrò, S., Zicarelli, F., & Piccolo, V. (2003). Nutritional characteristics of buffalo meat: Cholesterol content and fatty acid composition. *Bubalus Bubalis*, 4, 51–57.
- Infascelli, F., Cutrignelli, M. I., Bovera, F., Tudisco, R., Calabrò, S., Zicarelli, F., D'Urso, S., & Piccolo, V. (2005). Cholesterol content and fatty acids composition of meat from buffalo and Marchigiana young bulls. *Proceeding of 1st Buffalo Symposium of Europe and the Americas* (pp. 146).
- Infascelli, F., Gigli, S., & Campanile, G. (2004). Buffalo meat production: Performance *intra vitam* and quality of meat. *Veterinary Research Communications*, 28, 143–148.
- Kesava Rao, V., Kowale, B. N., Murthy, T. R. K., & Sharma, N. (1992). Effect of processing and storage on neutral lipids of buffalo meat. *Meat Science*, 31, 25–34.
- Lanza, M., Fabro, C., Scerra, M., Bella, M., Pagano, R., Brogna, D.M. R., & Pennini, P. (2011). Lamb meat quality and intramuscular fatty acid composition as affected by concentrates including different legume seeds. *Italian Journal of Animal Science*, 10, E4.
- Larralde, J., & Martinez, J. A. (1991). Nutritional value of faba bean: Effects on nutrient utilization, protein turnover and immunity. *CIHEAM Options Méditerranéennes - Série Siminaires*, 10, 111–117.
- Naeemi, E. D., Ahmad, N., Al-Sharrah, T. K., & Behbahani, M. (1995). Rapid and simple method for determination of cholesterol in processed food. *Journal of AOAC International*, 78(6), 1522–1525.
- Parthasarathy, S., Khoo, C., Miller, E., Barnett, J., Witztum, J. L., & Steinberg, D. (1990). Low density lipoprotein rich in oleic acid is protected against oxidative modification: Implications for dietary prevention of atherosclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, 87(10), 3894–3898.
- Pastuschenko, V., Matthes, H. D., Hein, T., & Holzer, Z. (2000). Impact of cattle grazing on meat fatty acid composition in relation to human nutrition. In T. Alföldi, W. Lockeretz, & U. Niggli (Eds.), *Proceeding of 13th International IFOAM Scientific Conference* (pp. 293–296).
- Poli, B.M., Giorgetti, A., Bozzi, R., Funghi, R., Balò, F., & Lucifero, M. (1996). Quantity and quality of lipid fractions for human nutrition in Chianina muscles as influenced by age and nutritive level. *Proceeding of XXXI International Symposium of Zootechny "Food and health: role of animal products"* (pp. 199–204).
- Priolo, A., Lanza, M., Galofaro, V., Fasone, V., & Bella, M. (2003). Partially or totally replacing soybean meal and maize by chickpeas in lamb diets: Intramuscular fatty acid composition. *Animal Feed Science and Technology*, 108, 215–221.
- Renaud, S., Morazin, R., Godsey, F., Thevenon, C., Martin, J. L., & Mendy, F. (1986). Nutrients, platelet function and composition in nine groups of French and British farmers. *Atherosclerosis*, 60, 37–48.
- Rusman, Soeparno, Setiyono, & Suzuki, A. (2003). Characteristics of Biceps femoris and Longissimus thoracis muscles of five cattle breeds grown in a feedlot system. *Animal Science Journal*, 74, 59–65.
- Ryan, E., Galvin, K., O'Connor, T. P., Maguire, A.R., & O'Brien, N. M. (2007). Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains and legumes. *Plant Foods for Human Nutrition*, 62, 85–91.
- S.A.S. (2000). (4th ed.) *SAS/STAT User's Guide, Version 8.2, vol. 2*. Cary, NC: SAS Institute Inc.
- Samoggia, G., Lo Fiego, D. P., De Grossi, A., & Bergonzoni, M. L. (1993). Indagini sul tasso di colesterolo in alcuni tessuti di bovini e di bufali. *Agricoltura e Ricerca*, 149, 89–94.
- Scerra, M., Caparra, P., Foti, F., Cilione, C., Zappia, G., Motta, C., & Scerra, V. (2011). Intramuscular fatty acid composition of lambs fed diets containing alternative protein sources. *Meat Science*, 87, 229–233.
- Simon, J. A., Pong, J., Bernert, J., & Browner, W. (1995). Serum fatty acids and the risk of stroke. *Stroke*, 26, 778–782.
- Simopoulos, A. P. (2003). Importance of the ratio of omega 6/omega 3 essential fatty acids: Evolutionary aspects. *World Review of Nutrition and Dietetics*, 92, 1–22.
- Sinclair, A. J., Slattery, W. J., & O'Dea, K. (1982). The analysis of polyunsaturated fatty acids in meat by capillary gas chromatography. *Journal of the Science of Food and Agriculture*, 33, 771–776.
- Spanghero, M., Gracco, L., Valusso, R., & Piasentier, E. (2004). In vivo performance, slaughtering traits and meat quality of bovine (Italian Simmental) and buffalo (Italian Mediterranean) bulls. *Livestock Production Science*, 91, 129–141.
- Tudisco, R., Cutrignelli, M. I., Calabrò, S., Piccolo, G., Bovera, F., Guglielmelli, A., & Infascelli, F. (2010b). Influence of organic systems on milk fatty acid profile and CLA in goats. *Small Ruminant Research*, 88, 151–155.
- Tudisco, R., Ma stellone, V., Cutrignelli, M. I., Lombardi, P., Bovera, F., Mirabella, N., Piccolo, G., Calabrò, S., Avallone, L., & Infascelli, F. (2010a). Fate of transgenic DNA and metabolic effects in goats fed genetically modified soybean and in their offsprings. *Animal*, 4, 1662–1671.
- Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: Seven dietary factors. *Lancet*, 338, 985–992.
- Van Soest, P. J., Robertson, J. B., & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583–3597.
- West Sutor, C., & Meyers, L. D. (2006). *Dietary reference intakes research synthesis workshop summary*. Washington: The National Academies Press.
- Westerling, D. B., & Hedrick, H. B. (1979). Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. *Journal of Animal Science*, 48, 1343–1348.
- Wheeler, T. L., Davis, G. W., Stoeker, B. J., & Hatmon, C. J. (1987). Cholesterol concentration of Longissimus muscle, subcutaneous fat and serum of two beef cattle breed type. *Journal of Animal Science*, 65, 1531–1537.
- Whigham, L. D., Cook, M. E., & Atkinson, R. L. (2000). Conjugated linoleic acid: Implications for human health. *Pharmacological Research*, 42, 503–510.
- Yadav, B.S., & Singh, L. N. (1974). Chemical composition of buffalo meat available from the local slaughterhouse. *Indian Journal of Animal Science*, 44, 746–749.
- Yoshida, H., Saiki, M., Yoshida, N., Tomiyama, Y., & Mizushima, Y. (2008). Fatty acid distribution in triacylglycerols and phospholipids of broad beans (*Vicia faba*). *Food Chemistry*, 112, 924–928.
- Yoshida, H., Tomiyama, Y., Saiki, M., & Mizushima, Y. (2007). Tocopherol content and fatty acid distribution of peas (*Pisum sativum* L.). *Journal of the American Oil Chemists' Society*, 84, 1031–1038.