EFFECT OF COTTONSEED WITH OR WITHOUT VITAMIN E AND LENGTH OF FINISHING IN NELLORE BEEF CATTLE: FATTY ACID COMPOSITION OF MEAT

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Abstract – Beef fatty acid (FA) composition can be modified by the diet, specifically with the addition of different oilseeds rich in polyunsatured FA (PUFA). The aim of this study was to evaluate the effect of replacing corn with whole cottonseed (30%) with or without vitamin E (500 IU vitamin E/kg of DM) and the length of finishing (83, 104 and 111 days) on the meat fatty acid composition of Nellore bulls (3x3 factorial design, n=54). In general, inclusion of cottonseed in the diet of Nellore bulls increased the content of several PUFAs while the inclusion of vitamin E increased total *trans*-MUFA, and the main isomer 10*t*-18:2. The SFA content increased over the finishing days, while in general the PUFA content decreased. Inclusion of cottonseed appears to be a good option to improve the FA profile of beef, but not the addition of vitamin E.

Key Words – *trans*-fatty acid, CLA, n-3 PUFA.

I. INTRODUCTION

Ruminant fats are high in saturated fatty acids (SFA) that are regarded as a risk factor for atherosclerosis, obesity and certain types of cancer [1]. Studies have shown that the beef fatty acid (FA) composition of ruminant can be modified by diet [2], specifically oilseeds rich in polyunsaturated FAs (PUFA) that can increase the accumulation of unsaturated FAs [3-4] even though a considerable portion of PUFA will be biohydrogenated in the rumen [5]. In addition, several PUFA intermediates produced in the rumen accumulate in the muscle and adipose tissues [6]. It has been suggested that unsaturated FAs in oilseeds are partially protected from biohydrogenation when fed with the intact seed coat [7]. Among the oilseeds, whole cottonseed (*Gossypium hirsutum*) could be an interesting option for feedlot cattle due to its high content of energy in the form of oil, moderate level of crude protein, and high quality fiber [8]. The meat produced could be more unsaturated [3-4] and provide a healthier profile for consumers. Up to date there are few studies on nutritional strategies to improve the lipid composition of *Bos indicus* (i.e., Nellore) [9] compared to *Bos taurus* breeds. On the other hand, higher levels of PUFA in meat could accelerate lipid oxidation processes. This would lead to meat discoloration, odor and flavor development, and accumulation of components in meat that lower consumer acceptability [10].
Our hypothesis was to include whole cottonseed in the finishing diets to improve the PUFA profile of beef, and by addition of vitamin E protect the PUFA content in meat. To monitor the FA changes in meat from Nellore bulls, a serial slaughter at different ages was conducted.

II. MATERIALS AND METHODS

The feeding study was conducted at the Campus of Pirassununga (Brazil) and the FA analyses were performed at Vitoria-Gasteiz (Spain). A total of 54 Nellore bulls were selected with an initial weight of about 350 kg and 24 months of age. Bulls were fed one of three diets during the finishing period: **1)** control without cottonseed (C), **2)** a diet containing cottonseed at 30% of DM (WCS) and **3)** the WCS diet 2 plus 500 IU vitamin E/kg of DM (WCSE). The ingredients of the finishing diet were cottonseed (0%, 30.5%, 30.5%), corn (57.2%, 28.1%, 28.1%), citrus pulp

(18.3%), sugarcane bagasse (13.4%), soybean meal (8.2%) plus mineral supplement, for C, WCS and WCSE diets, respectively. Bulls were kept in pens of three animals and fed once a day for 83, 104 and 111 days until slaughter. A randomized block design was used with factorial arrangement of treatments (3x3); 9 treatments with 6 animals in each one. Bulls were slaughtered at the Teaching Abattoir of the University of Sao Paulo following the recommendations of Regulation of Industrial Inspection and Safety of Animal Products (Decree 2244). After carcasses were cooled for 24h at 0-2ºC, a 2.5cm thick steak was removed between the 12th-13th ribs, *Longissimus thoracis et lumborum* (LTL) muscle was isolated, vacuum packed, and frozen at -18°C. Total lipids were extracted from LTL using a mixture of chloroform-methanol (2:1, v/v) [11]. Lipid aliquots (10 mg) were methylated separately using acid and base reagents [12]. For quantitative purposes, internal standard (23:0ME) was added prior to methylation. Fatty acid methyl esters (FAMEs) were analyzed by GC/FID using a 100 m SP2560 column and two temperature programs [13]. To determine CLA isomers, samples were subjected to a second GC/FID analysis using a 100 m ionic liquid SLB-IL111 [14]. For identification purposes, several reference standards (NuCheck, Supelco, Matreya, Larodan), fractions of FAMEs obtained by Ag^{+}-SPE [13], and retention times and elution patterns reported in the literature [6] were used. Statistical analysis was carried out using IBM SPSS Statistics 20.

III. RESULTS AND DISCUSSION

For all traits, the interaction between diet and length of finishing was not significant, therefore each factor was studied separately. The type of diet had no effect on the total SFAs, but significant differences were observed for branched-chain FAs (BCFA), *cis*- and *trans*-monounsaturated FAs (MUFA), non-conjugated (nc) dienes and some individual PUFAs. BCFA and *cis*-MUFA contents were higher in meat from control diet fed bulls in comparison to WCS, but vitamin E addition had no effect (Table 1). This was evident in the major individual FAs within each group; i.e., *iso*- and *anteiso*-17:0 in BCFAs, and 9*c*-16:1 and 9*c*-18:1 in the *cis*-MUFA group. These results were in agreement with other studies [9, 15]. In contrast, *trans*-MUFA content and its major isomer 10*t*-18:1 was higher in muscle from bulls fed WCSE (4.5%) compared to the WCS diet (2.6%), while the relative amounts of vaccenic acid (11*t*-18:1) in meat were opposite in these two diets. Kay et al. [16], Pottier et al. [17] and Juarez et al. [18] found a decrease in total *trans*-MUFA content and a prevention of the '11*t*- to 10*t*-18:1 shift' in plasma, milk and beef backfat, respectively, by supplementing diets with vitamin E even in the presence of high levels of PUFA in the concentrate. The mechanisms of these findings have not been clarified. These results are different from those generally reported that vitamin E would alleviate oxidative stress on bacteria under ruminal conditions resulting in a lower production of *trans* FAs.

Table 1. Least square means for FA (%) of muscle from Nellore bulls finished with different diets

	Diet			
	C	**WCS**	**WCSE**	**SEM**
SFA	39.8	40.6	41.1	0.508
16:0	22.5	22.6	22.9	0.278
18:0	12.5	13.5	13.5	0.239
BCFA	1.52^{a}	1.18^{b}	1.22^{b}	0.0365
***cis*-MUFA**	33.3^{a}	30.0^{b}	27.7^{b}	0.437
9*c*-16:1	2.34^{a}	1.91^{b}	1.77^{b}	0.0581
9*c*-18:1	26.9^{a}	23.7^{b}	21.8^{b}	0.377
***trans*-MUFA**	5.15^{b}	6.20^{b}	7.78^{a}	0.254
10*t*-18:1	2.77^{b}	2.61^{b}	4.48^{a}	0.211
11*t*-18:1	0.970^{b}	1.71^{a}	1.39^{a,b}	0.106
PUFA	11.6	13.8	13.9	0.541
18:2n-6	5.67^{b}	8.49^{a}	8.58^{a}	0.275
20:4n-6	2.52	2.13	2.11	0.123
18:3n-3	0.335^{b}	0.401^{a,b}	0.426^{a}	0.0198
20:5n-3	0.499	0.441	0.474	0.0336
22:5n-3	1.14	0.936	0.938	0.0563
22:6n-3	0.112	0.126	0.111	0.00664
20:3n-9	0.299^{a}	0.223^{a,b}	0.209^{b}	0.0138
CLA	0.543	0.573	0.526	0.0164
9*c*,11*t*-	0.362	0.399	0.323	0.0173
7*t*,9*c*-	0.0695	0.0724	0.0769	0.00283
nc-18:2	0.587^{b}	0.826^{a}	0.847^{a}	0.0125
9*c*,13*t*-/8*t*,12*c*-	0.113^{b}	0.192^{a}	0.168^{a}	0.00565
11*t*,15*c*-	0.0717^{b}	0.112^{a}	0.117^{a}	0.00305
10*t*,15*c*-^{X}	0.0691	0.0628	0.0758	0.00238

C: control; WCS: whole cottonseed; WCSE: WCS plus vitamin E; ^{X}coelution with a non-conjugated 18:2. SFA: saturated FA; BCFA: branched-chain FA; MUFA, monounsatured FA, nc-18:2: non-conjugated dienes;

PUFA, polyunsatured FA; CLA, conjugated linoleic acid. [a-c]within a row, means without a common superscript differ $P<0.05$)

The essential FAs (18:2n-6, 18:3n-3) were significantly higher in WCS compared to control animals reflecting the higher content of these FAs in WCS diets [3], and possibly the result of their protection by the seed coat [7]. In contrast, 20:3n-9 was lower in muscle tissue of bulls fed the C diet. No differences were found in the content of conjugated linoleic acid (CLA) as reported by others [9], the accumulation of major nc-dienes (9*c*,13*t*-/8*t*,12*c*-, 11*t*,15*c*-) was highest in the WCS.

Table 2. Least square means for FA (%) of muscle from Nellore bulls at different length of finishing

	Length of finishing			Contrasts
	83	**104**	**111**	
SFA	38.5	41.1	41.9	L**
16:0	21.6	23.5	23.0	L*, Q*
18:0	12.6	12.7	14.2	L**
BCFA	1.38	1.24	1.29	
***cis*-MUFA**	29.5	30.5	31.0	
9*c*-16:1	1.92	2.11	1.99	
9*c*-18:1	23.4	24.1	24.9	
***trans*-MUFA**	6.00	6.85	6.28	
10*t*-18:1	2.93	3.80	3.14	
11*t*-18:1	1.411	1.25	1.40	
PUFA	15.1	12.3	11.9	L*
18:2n-6	8.36	7.32	7.08	
20:4n-6	2.68	2.08	2.00	
18:3n-3	0.517	0.327	0.318	L***, Q**
20:5n-3	0.626	0.404	0.383	L**
22:5n-3	1.25	0.893	0.868	L**
22:6n-3	0.142	0.112	0.0951	L**
20:3n-9	0.2969	0.2188	0.2160	L*
CLA	0.568	0.546	0.527	
9*c*,11*t*-	0.392	0.342	0.350	
7*t*,9*c*-	0.0641	0.0853	0.0695	Q**
nc-18:2	0.742	0.745	0.774	
9*c*,13*t*-/8*t*,12*c*-	0.149	0.156	0.168	
11*t*,15*c*-	0.108	0.0929	0.100	
10*t*,5*c*-[X]	0.0718	0.0692	0.0666	

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. Contrasts: L, linear; Q, quadratic. Other abbreviations as in Table 1.

The effect of length of finishing on the FA composition of muscle is presented in Table 2. Orthogonal contrasts were used to test for linear and quadratic effects of days on test (83, 104 and 111 days). In general, few significant differences were found. Total SFA content, including major FAs, increased linearly while PUFAs decreased linearly over the finishing period. No change was observed in n-6 PUFAs but a linear decrease was observed in most of n-3 PUFAs and 20:3n-9. No differences in MUFA, CLA or nc-dienes were observed but the second most abundant CLA (13.8%, 7*t*,9*c*-18:2) responded quadratically over time by first increasing and then decreasing. This CLA isomer is related to high contents of 10*t*-18:1 [19] and the highest levels of both of these isomers occurred after 104 days of feeding.

Another interesting observation was the higher content of 20:5n-3 (21.4%) and 22:5n-3 (47.2%) compared to their precursor 18:3n-3 which was only present at 18.8% of total n-3 PUFA content. The high content of these long-chain n-3 PUFA metabolites evidenced that some cattle breeds [20-21] show a potential to elongate 18:3n-3 including the Nellore breed.

IV. CONCLUSION

The inclusion of cottonseed in the diet of Nellore bulls increased the content of several PUFAs while the inclusion of vitamin E increased total *trans*-MUFA and specifically 10*t*-18:2. The SFA content increased over the finishing days, while in general the PUFA content decreased. The inclusion of cottonseed appears to be a good alternative to improve the beef FA profile, but not the inclusion of vitamin E.

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