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## Effect of dietary vitamin E content on the CLA, cholesterol and triglycerides composition of Italian Mediterranean buffalo meat

## R. Romano, I. Borriello, L. Chianese, R. Sacchi

Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici, Naples, Italy

Corresponding author: R. Romano. Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (Na), Italy - Tel. +39-081-2539348 - Fax: 081-7762580 - Email:rafroman@unina.it

ABSTRACT: The composition of fatty acids, CLA, triglycerides and cholesterol in intramuscular fat depots of buffalo meat was determined using high-resolution gas chromatography to investigate the influence of dietary vitamin E content. Three groups of Italian Mediterranean buffalo calves were fed on three diets with high (H), low (L) and zero (Z) vitamin E contents. The animal were slaughtered at 15 months and three muscles were dissected on the half-carcass: Longissimus dorsi (LD), Tricipitis brachii (TB) and Semimembranosus (Sm). Lipid extracts from muscles (g/100g f.m.: 0.82 for LD, 0.66 for TB and 0.48 for Sm) were used to quantify the amount (mg/100g of lipids) of fatty acids, total conjugated linoleic acid (CLA) and cholesterol. The effects of dietary vitamin E content were significant (P<0.05) but marginal. Comparison of lipid extracts from muscles showed that C18:2 and total CLA were higher respectively in TB and Sm muscles when vitamin content was low. Also Cholesterol content variation was affected by low dietary vitamin E: LD muscle has a lower cholesterol concentration for diet L. The different vitamin content of two diets did not significantly influence the composition of triglycerides. Considering the low lipid concentrations (<1g/100 g of fresh muscle) none of the meat muscles should be considered a significant source of CLA.

**Key words:** CLA, Cholesterol, Triglyceride composition, Italian Mediterranean buffalo (*Bubalus bubalis*).

**INTRODUCTION** - Some studies comparing buffalo meat and beef have shown that the nutritional and organoleptic properties of buffalo meat are similar and in some respects (flavour, rheology, colour, etc.) superior to those of beef (Gigli et. al., 1993; Failla *et al.*, 1997; Ferrara and Infascelli, 1994). The fatty acids most represented in buffalo meat are palmitic (C 16:0), stearic (C18:0) and oleic (C18:1) (Cutrignelli *et al.*, 1996). The study of the fat depots and composition of fatty acids is of great importance in terms of flavour and nutrition for integrating buffalo meat into human diet. The main objectives of this research was to to explore the probable influence of antioxidant vitamin E on fatty acids, CLA, triglycerides and cholesterol composition in the intramuscolar fat of Italian Mediterranean buffalo.

**MATERIAL AND METHODS** - Animals and sample collection. The study was carried out on 18 male water buffalo, slaughtered at the age 15 months. The buffaloes were divided into three groups of 6, and fed three isoenergetic diets different for vitamin E supplementation: 1500 UI,600 UI and 0 UI respectively for diet H. L and Z. For brevity for the experimental design and characteristics of the diet see Zicarelli *et al.* (2005). After slaughter, the carcasses were refrigerated for 7 days at a temperature of 4°C.

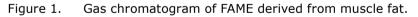
The chemical composition was determined on the following three muscles: *Longissimus dorsi* (LD), *Tricipitis brachii* (TB) and *Semimembranosus* (Sm). Sample were partially thawed, trimmed of all surrounding fat tissue, homogeneizad and packaged for frozen storage (-20°C) until final preparation for the chemical analyses. All the samples were analysed in triplicate.

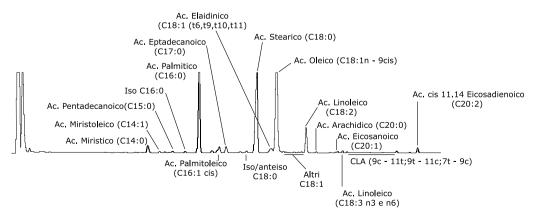
- *Chemical analysis.* Total lipid (g/100 g fresh muscle) were extracted from 50 g of muscle samples according to Folch *et al.* (1957). Analyses of the triglycerides and fatty acids of buffalo meat were carried out according to AOAC methods (1984).

- Determination of fatty acid and CLA. Methyl esters of fatty acids were prepared by base catalysed reaction (Gutnikov, 1995) and analysed by Perkin-Elmer gas chromatograph was equipped with PTV injector and fused silica capillary column SP 2560: 100m, 0.25mm ID; 0.20µm f.t., (Supelco Bellofonte, USA), FID Detector CLA's isomers were identified by comparing the retention times with those of pure reference standards obtained from NU-Chek Prep, Inc.

*Triacylglycerol and cholesterol analysis.* Lipid samples (50 mg) were dissolved in *n*-hexane (2ml) and 1 microL of the lipid solution was injected on a GC Trace 2000 ThermoFinnigan equipped with PTV injector and fused silica capillary column RTX 65 TG (7.5 mx0.25 mm i.d.; 0.10 f.t.; Restek Co.). Chromatograms were recorded, and the area calculated with Chrom Card for Windows after identification by comparison with external standard (Sigma-Aldrich, USA). *Statistical analysis.* For data set, analysis of variance (ANOVA), on diet and muscle effects, using the Statistical Analysis System (SAS, 1996) was performed.

**RESULTS AND CONCLUSIONS** - *Determination of fatty acids and CLA*. Mean fat concentration for three muscle was (g/100g f.m.): 0.82 for LD, 0.66 for TB and 0.48 for Sm. Figure 1 shows a typical fatty acids gas chromatogram of lipid sample.





23 fatty acids were identified by HRGC. Table 1 show the composition of fatty acids muscles in corresponding three diet. Vitamin E different diet intake had an influence on fatty acids composition of three muscles examined. Regarding CLA, there were significant differences for three diet in LD, TB and Sm muscles examined.

Table 1. Fe	edstuff	s and co	mpositio	ons of the	experii	mental d	iets.			
	LD diet				TB diet			Sm diet		
Fatty acids %	Н	L	Z	Н	L	Z	Н	L	Z	
C12:0	0,03	0,09	0,05	0,22	0,40	0,42	0,24	0,28	0,32	
C14:0	1,40	1,51	1,64	2,03a	1,8b	2,08a	1,26	1,35	1,49	
C14:1	1,48	1,16	1,29	0,6a	0,74b	0,59a	0,29a	0,62b	0,43c	
C15:0	0,29	0,26	0,31	0,29a	0,45b	0,54b	0,42	0,51	0,56	
Iso/ante C16:0	0,25	0,13	0,25	0,18	0,21	0,29	0,17	0,20	0,22	
C16:0	25,88a	23,13115b	24,44c	23,73	23,17	23,81	25,50	25,84	25,40	
C16:1cis	2,18a	2,68b	2,55c	2,91a	2,41b	2,4b	2,54	2,14	2,20	
C17:0	1,52	1,98	1,48	1,73	1,52	1,14	1,04	1,05	1,28	
iso/anteiso C18:0	0,33	0,49	0,36	0,36	0,77	0,69	1,58	1,45	1,40	
18:0	23,15a	22,31b	22,83b	20,03a	19,32b	20,32a	17,00	17,60	17,10	
C18:1t	0,70a	0,95b	1,09c	0,87a	1,13b	1,25c	1,24	1,13	1,26	
C18:1n-9cis	32,52a	34,76b	35,47c	32,88	31,26	32,00	33,76a	32,53b	32,80b	
others C18:1	0,97	0,62	0,54	0,72	0,25	0,63	1,20	1,24	1,36	
C18:2	5,28a	5,59b	4,86c	9,78a	10,17a	9,09b	8,86a	8,41a	7,43b	
C20:0	0,36a	0,24b	0,17c	1,18a	1,80b	0,88c	0,54	0,44	0,50	
C18:3 n3 e n6	0,85a	1,15b	0,76c	0,68a	1,59b	1,29c	1,24	1,26	1,51	
CLA 9c-11t;9t-11c;7	t-9c 0,45a	0,51b	0,32c	0,48a	0,46a	0,57b	0,91a	1,02b	1,57c	
cis 11,14 C20:2	0,42	0,25	0,21	0,44	0,28	0,27	0,16	0,45	0,67	
C22:0	0,51	0,64	0,45	0,32	0,29	0,44	0,55	0,88	0,80	
C22:1	0,72	0,56	0,61	0,54	1,13	0,79	1,27	1,19	1,28	
C24:0	0,74	0,46	0,38	0,23	0,53	0,38	0,26	0,48	0,54	
ΣCLA	0,45a	0,51b	0,32c	0,48a	0,46a	0,57b	0,91a	2,02b	1,58c	
SFA	54,46a	51,22b	52,37c	50,31	50,26	50,99	48,56a	50,08b	49,61b	
UFA	45,58a	48,27b	47,72c	49,90	49,42	48,89	51,49a	49,98b	50,52b	
SFA/UFA	1,19a	1,06b	1,09c	1,01	1,02	1,04	0,94a	1,00b	0,98b	

<sup>a</sup> Different letters indicate significant difference among the diet for P<0.05.

SFA: saturated fatty acids; UFA: unsaturated fatty acids.

The results can be summarized as follows:

diet H resulted in a higher percentage of C16:0, C18:0 for LD muscle. The same muscle has a significantly higher content (P<0.05) of SFA and SFA/UFA ratio indicating that the intramuscolar fat deposited has higher level of saturates;

Semimembranosus muscle, for diet L, has shown an higher content of CLA than TB and LD muscles. Sm has the highest level of UFA for diet H;

the differences observed are due essentially to the different ratio between saturated and unsaturated fatty acids.

- *Determination of Triacylglycerol.* As regard triacylglycerol composition, had been identified family from C 46 to C54. In Table 2 it is possible observed that the triglyceridic composition of buffalo intramuscolar lipids do not varies with diet.

Table 2. Me	an value c	of triacyg	lycerol i	n relatio	n to the	muscle a	and diet.		
		LD diet			TB diet			Sm diet	
Triacylglycero	I% H	L	Z	Н	L	Z	Н	L	Z
C46	1,32	0,94	1,31	1,12	1,45	1,56	1,18	1,43	1,24
C48	4,06	4,54	4,54	4,22	4,78	4,97	5,12	4,13	4,56
C50	14,08	14,73	15,81	16,02	16,46	16,84	17,55	16,98	16,82
C52	44,71	45,21	45,84	46,45	46,01	45,36	49,91	48,36	48,52
C54	35,41	34,86	33,44	32,27	31,78	30,42	30,03	29,31	29,54

ble 3. Mean value of Cholesterol in relation to the muscle and diet.				
Cholesterol mg/100g				
>Vit.E <vit.e absent</vit.e 	3,8 4,2 19,16			
>Vit.E <vit.e absent</vit.e 	16,7 7 11,4			
>Vit.E <vit.e absent</vit.e 	14,5 6,25 15,8			
	Cholesteror relation to muscle an Cholesterol mg/100g >Vit.E <vit.e absent &gt;Vit.E absent &gt;Vit.E absent &gt;Vit.E</vit.e 			

*Cholesterol quantitative level.* Table 3 shows a significant variation corresponding to the different feeding conditions (tab.3). In particular, LD muscle has a lower cholesterol concentration for diet L.

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