Antioxidant status and lipid peroxidation in the muscle of German Simmental and German Holstein bulls fed *n*-3 and *n*-6 PUFA-based diets

Dannenberger D.[1], Nuernberg K.[1], Mahecha L.[2], Nuernberg G.[3]

[1] Research Unit of Muscle Biology and Growth, FBN Leibniz Institute for Farm Animal Biology, 18196 Dummerstorf, Germany
[2] Group Grica, Faculty of Agricultural Science, University of Antioquia, AA 1226 Medellin, Colombia
[3] Research Unit of Genetics and Biometry, FBN Leibniz Institute for Farm Animal Biology, 18196 Dummerstorf, Germany

***Abstract*— The studies were established to investigate effects of *n*-3 and *n*-6 PUFA-based diets on the relationship between antioxidant status and lipid peroxidation in beef muscle. Two indoor feeding experiments with German Simmental and German Holstein bulls were carried out premised on *n*-3 PUFA based (grass silage) and *n*-6 PUFA based diets (maize silage) with supplements. Antioxidant capacity (TEAC, FRAP), antioxidant enzymes, TBARS and fatty acid profiles were investigated. The antioxidant capacity of muscle of both breeds using TEAC and FRAP assay was not affected by different PUFA-based diets, however *n*-3 PUFA based diet caused a significantly higher extent of lipid peroxidation using TBARS assay in muscle of German Simmental- and German Holstein bulls compared to *n*-6 PUFA based diets. Muscle of the *n*-3 PUFA based diets showed higher activity of catalase of both breeds, and additional elevated superoxide dismutase activity in German Simmental bulls compared to muscle of bulls fed *n*-6 PUFA based diets. *N*-3 PUFA based diet caused increased level of all single *n*-3 fatty acids resulting in decreased *n*-6/*n*-3 PUFA ratio in muscle of both breeds.**

Keywords— **antioxidant capacity, beef, muscle**

I. INTRODUCTION

A variety of polyunsaturated fatty acids (PUFA) differing in their chemical structures, such as *n*-6 and *n*-3 PUFA, play essential roles in many biological functions. In ruminants, the dietary fatty acids are extensively metabolized and biohydrogenated in the rumen, resulting in a broad range of monounsaturated fatty acids (MUFA), PUFA isomers, and saturated fatty acids (SFA). The MUFA intermediates are transformed to longer chain PUFA in muscle by lipogenic enzymes—e.g., stearoyl-CoA-desaturase (SCD), Δ6-desaturase (Δ6d), elongase and Δ5-desaturase (Δ5d) [1-3]. Since the last decade research is focused on improving the nutritional and health value of beef. Much attention has been given to strategies for increasing the content of *n*-3 PUFA [4, 5]. Additional interest is focused on the conservation of these PUFA for human consumption because PUFA are highly susceptible to lipid peroxidation by highly reactive species. Under balanced conditions, the body cells can minimize this oxidative damage due to their antioxidant defense conformed by non-enzymatic hydrophilic and lipophilic compounds and by endogenous enzymes like catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). There is limited research comparing the overall antioxidant capacity (AOC), antioxidant enzyme activities and lipid peroxidation of beef cattle fed different diets based on *n*-3 and *n*-6 PUFA.

Two feeding experiments with German Simmental and German Holstein bulls premised on *n*-3 PUFA based (grass silage) and *n*-6 PUFA based diets (maize silage) with supplements were carried out to study antioxidant capacity (TEAC, FRAP), antioxidant enzymes, lipid peroxidation (TBARS) and fatty acid profiles.

A. Material and methods

Experiment 1:

25 male German Simmental calves (3–4 months) were included in an indoor experiment comparing enriched *n*-6 and *n*-3 PUFA diets previously described in detail [4]. The animals were randomly assigned into three groups. The control group (n = 9) was daily fed maize silage/grass silage (70/30, ad libitum), 1 kg of molasses, 1 kg of hay, and concentrate including soybean meal and oil. Treatment group I, consisted of unrestricted

animals (n = 7), fed grass silage (ad libitum), 1 kg of molasses, 1 kg of hay, and concentrate including rapeseed cake. Treatment group II, consisted of restricted animals (n = 9), fed as treatment group I with a restriction of 1 kg of concentrate (50%) per day during the first 112 days of the fattening period.

Experiment 2:

29 German Holstein bulls were randomly selected and assigned one of the test diets: a control diet (n=15) containing maize silage with soybean-based concentrate (*n*-6 FA) and an experimental diet (n=14) containing grass silage with linseed oil and rapeseed cake (*n*-3 FA). The experiment details were described before [6]. German Simmental and German Holstein bulls were slaughtered at approximately 630 kg live-weight by captive bolt stunning followed by exsanguinations in the abattoir of the Leibniz Institute for Farm Animal Biology in Dummerstorf (Germany). *Longissimus* muscles were taken immediately after slaughter to test for thiobarbituric acid reactive substances (TBARS), enzyme activities, AOC and fatty acid profile and stored at -70°C until the respective analysis. AOC of muscle samples was determined using two different assays: Ferric Reducing Antioxidant Power (FRAP) and Trolox Equivalent Antioxidant Capacity (TEAC). Details of the methodologies were recently described by Mahecha et al. [7, 8]. The FA profile of muscle lipids was determined using the methodology described by Nuernberg et al. [9], the activity of antioxidant enzymes and lipid peroxidation (TBARS) were measured as previously described by Machecha et al. [7, 8]. All data were analysed by the least-squares means method using GLM procedures of SAS.

B. Results

Two assays (FRAP and TEAC) carried out using both extraction systems (hydrophilic and lipophilic) did not show significant diet differences in muscle of German Simmental (Experiment 1) and German Holstein bulls (Experiment 2). Hydrophilic values were higher than lipophilic values according to both AOC assays (Tables 1 and 2). AOC values increased significantly over reaction time with all assays (Tables 1 and 2). There was no significant relationship between diet and reaction time.

Table 1 Antioxidant capacity in l*ongissimus* muscle of German Simmental bulls (Experiment 1)

	Diets			Reaction times			P values		
	Control	Treat. I	Treat. II	5 min	30 min	60 min	D	T	D*T
	LSM_{SEM}	LSM_{SEM}	LSM_{SEM}	LSM_{SEM}	LSM_{SEM}	LSM_{SEM}			
$FRAP_{water}$	$2.23_{0.08}$	$2.22_{0.09}$	$2.34_{0.1}$	$0.71_{0.06}^{a}$	$2.38_{0.06}^{b}$	$3.70_{0.06}^{c}$	0.52	*	0.96
$FRAP_{Lipid}$	$0.89_{0.06}$	$0.90_{0.08}$	$0.93_{0.06}$	$0.18_{0.05}^{a}$	$0.90_{0.05}^{b}$	$1.64_{0.05}^{c}$	0.89	*	0.99
$TEAC_{water}$	$11.2_{0.16}$	$10.7_{0.19}$	$10.8_{0.16}$	$7.14_{0.11}^{a}$	$11.8_{0.11}^{b}$	$13.8_{0.11}^{c}$	0.11	*	0.97
$TEAC_{Lipid}$	$4.99_{0.16}$	$4.86_{0.19}$	$4.98_{0.16}$	$1.49_{0.12}^{a}$	$4.76_{0.12}^{b}$	$8.57_{0.12}^{c}$	0.85	*	0.98

Means with different letters are significantly different at P<0.05, FRAP values are expressed as Fe^{II} equivalent in mmol/g sample and TEAC values are expressed as trolox equivalents in mmol/g sample, D: diet, T: time, D*T: interaction diet * time, * p <0.0001

Table 2 Antioxidant capacity in *longissimus* muscle of German Holstein bulls (Experiment 2)

	Diets		Reaction times			P values		
	Control	Treatment	5 min	30 min	60 min	D	T	D*T
	LSM_{SEM}	LSM_{SEM}	LSM_{SEM}	LSM_{SEM}	LSM_{SEM}			
$FRAP_{water}$	$1.75_{0.09}$	$1.83_{0.1}$	$0.86_{0.07}^{a}$	$1.98_{0.07}^{b}$	$2.53_{0.07}^{c}$	0.55	*	0.60
$FRAP_{Lipid}$	$0.38_{0.02}$	$0.35_{0.02}$	$0.24_{0.02}^{a}$	$0.38_{0.02}^{b}$	$0.48_{0.02}^{c}$	0.49	*	0.55
$TEAC_{water}$	$9.40_{0.19}$	$9.43_{0.20}$	$5.62_{0.14}^{a}$	$10.23_{0.14}^{b}$	$12.39_{0.14}^{c}$	0.90	*	0.84
$TEAC_{Lipid}$	$3.71_{0.23}$	$3.46_{0.23}$	$1.63_{0.19}^{a}$	$3.83_{0.19}^{b}$	$5.30_{0.19}^{c}$	0.44	*	0.87

Means with different letters are significantly different at P<0.05, FRAP values are expressed as Fe^{II} equivalent in mmol/g sample and TEAC values are expressed as trolox equivalents in mmol/g sample, D: diet, T: time, D*T: interaction diet * time, * p <0.0001

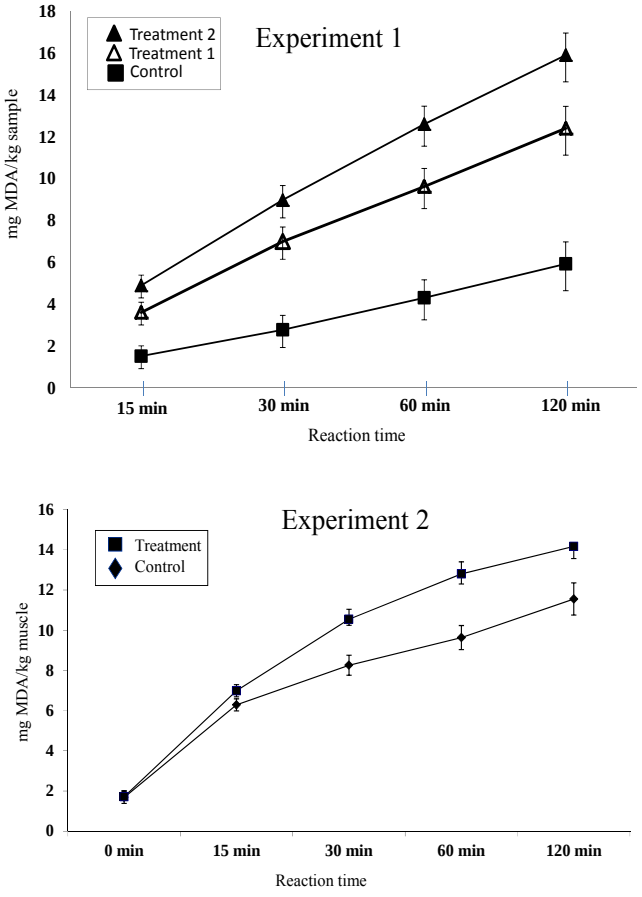
Table 3 Activity of endogenous enzymes in *longissimus* muscle of German Simmental- and Holstein bulls (U/g)

	Experiment 1		
	Control	Treat. I	Treat. II
	LSM_{SEM}	LSM_{SEM}	LSM_{SEM}
CAT	$76.7_{4.8}^{a}$	$107.9_{5.5}^{b}$	$98.9_{4.8}^{b}$
SOD	$8.15_{0.6}$	$11.5_{0.7}^{b}$	$10.9_{0.6}^{b}$
GSH-Px	$1.66_{0.1}$	$1.47_{0.1}$	$1.30_{0.1}$
	Experiment 2		
	Control	Treat.	
	LSM_{SEM}	LSM_{SEM}	
CAT	$45.5_{7.4}^{a}$	$79.7_{7.6}^{b}$	
SOD	$5.6_{0.2}$	$5.4_{0.2}$	
GSH-Px	$1.39_{0.1}$	$1.60_{0.1}$	

Means with different letters are significantly different (p< 0.05)

The activity of the endogenous enzymes CAT, GSH-Px, and SOD in *longissimus* muscle of German Simmental bulls (Experiment 1) and German Holstein bulls (Experiment 2) is presented in Table 3. Diet caused significant changes on CAT (Exp. 1 and 2) and SOD (Exp. 1) activity with higher values in the treatment groups fed with grass silage and concentrate including rapeseed, however no diet effect on GSH-Px activity.

Figure 1 Lipid peroxidation of *longissimus* muscle of German Simmental (Exp. 1) and German Holstein bulls (Exp. 2), measured as TBARS at different times



N-3 PUFA based diets caused significantly higher extent of lipid peroxidation using TBARS assay in muscle of German Simmental and German Holstein bulls compared to *n*-6 PUFA based diets.

C Discussion

The results of the AOC measurements showed that diet did not affect the antioxidant capacity of the *longissimus* muscle from Simmental - and Holstein bulls using both assays (FRAP and TEAC), two extraction systems, and different reaction times (5, 30, and 60 minutes) [7, 8]. Our results confirm the results of Gatellier et al. [10] and Descalzo et al [11] with regard to TEAC assay. These authors found that AOC measured by TEAC and ABTS assays was similar in beef muscle from steers kept on pasture compared with animals fed grain or grain silage, respectively. In contrast, higher AOC values were found by Descalzo et al. [11, 12] in the muscle of animals kept on pastures when AOC was measured by FRAP assay. Wu et al. [13] also found significant differences between AOC values of muscle from beef cattle produced under different finishing systems, using ORAC assay with lipophilic extracts. However, they did not find differences with hydrophilic extracts. Wu et al. [13] only found differences in AOC of muscle from beef cattle finished in a system based on alfalfa compared to a system based on high concentrate diets, but not between a system based on high concentrate diets and native pastures.

For endogenous enzyme activities in beef muscle, the effect of diet is controversial. Some studies have found a clear significant effect on CAT, SOD, and GSH-Px [10], while others have only found effects on some of these enzymes [11, 14] or no effects at all [15]. In the present studies, the activity of CAT (Exp. 1 and 2) and SOD (Exp. 1) in the muscle of bulls was up to 1.7-fold higher in treatment animals compared to control animals (Table 3) [7, 8]. The effect of diet on GSH-Px activity in beef of both breeds was not obvious; however the values did not reach statistically relevance. In Experiment 1 the GSH-Px activity tended to lower values, and in experiment 2 the enzyme activity tended to higher values. In the literature, lower values in muscle of pasture-fed animals were found by Gatellier et al. [10], Insani et al. [14] and Mercier et al. [16]. However, Descalzo et al. [11] and Santé-Lhoutellier et al. [15] did not detect differences between diets. The absence of any quantitative appreciable differences of diets on GSH-Px activity in both bull experiments has

been attributed to high variation among groups [11, 17]. The different effects of diet on each endogenous antioxidant enzyme could be related to unique mechanisms of action and the specific conditions generated by diets in each study. SOD is a potent protective enzyme that can selectively scavenge the superoxide radical (O_2•-) by catalysing its dismutation to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). The other antioxidant enzymes, CAT and GSH-Px, act to decompose H_2O_2 to water. On the other side, lipid peroxidation investigations (TBARS) revealed that the muscle of animals in the treatment groups fed *n*-3 PUFA based diets of both experiments was higher compared the *n*-6 PUFA based diet groups (Figure 1) [7, 8]. Our studies of indoor-fed grass silage-based bulls are in contrast to other studies of grass-fed animals, where lower lipid peroxidation compared to *n*-6 PUFA based diets was measured [18, 19]. The concentration of *n*-3 PUFA - high susceptible to lipid peroxidation - in beef muscle in the treatment groups of both experiments was 61 and 56 mg/100g fresh muscle, respectively [4, 7]. It seems that the increase in some endogenous enzyme activities and the significantly higher concentration of some lipophilic vitamins found in the present studies could not enough balance the extent of lipid peroxidation [7].

Finally, the significant increase of some antioxidants in *n*-3 PUFA based fed animals from both experiments was not reflected on higher total antioxidant capacity (AOC). AOC refers to a full spectrum of each component which shows antioxidant activity against reactive radicals; it gives a general idea of the quantitative contribution of antioxidants to the antioxidant defence in beef under the evaluated conditions. According to this, it looks like that the balance in the quantitative contribution of antioxidant substances to the antioxidant defence in muscle German Simmental- and German Holstein bulls was similar between control and treatment diets (Exp. 1 and 2). Furthermore, results from these two bull studies also confirmed that the reaction between antioxidants present in the muscle samples and the reactive medium during the AOC measurements continues even to 60 minutes after beginning. Then, a minimum reaction time of 30 minutes has been suggested for AOC measurements in beef muscle by using TEAC and FRAP assays.

CONCLUSIONS

Present results suggest that the antioxidant capacity in muscle of both breeds fed *n*-3 PUFA-based diets could not balance the higher extent of lipid peroxidation compared to the muscle of *n*-6 PUFA-based fed bulls, despite partly higher antioxidant enzyme activities.

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