Meat Science 82 (2009) 365–371

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Effects of diet and storage on fatty acid profile, micronutrients and quality of muscle from German Simmental bulls

L. Mahecha [a], K. Nuernberg [b], G. Nuernberg [c], K. Ender [1], E. Hagemann [d], D. Dannenberger [b,*]

[a] Grupo Grica, Facultad de Ciencias Agrarias, Universidad de Antioquia, AA 1226 Medellin, Colombia
[b] Research Unit of Muscle Biology and Growth, Research Institute for the Biology of Farm Animals, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
[c] Research Unit of Genetics and Biometry, Research Institute for the Biology of Farm Animals, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
[d] State Office for Agriculture, Food Safety and Fishery Mecklenburg-West Pomerania, Department of Contaminant and Residue Analysis, Thierfelderstraße 18, 18059 Rostock, Germany

ARTICLE INFO

Article history:
Received 8 December 2008
Received in revised form 6 February 2009
Accepted 9 February 2009

Keywords:
Beef
n-3 PUFA
Grass silage
Maize silage
Vitamins
TBARS
Storage

ABSTRACT

This study evaluated the effect of diet on fatty acid profile, vitamins, trace elements, lipid peroxidation, and quality of *longissimus* muscle of German Simmental bulls. The effect of storage on fatty acid profile and vitamins was also included. A control group was fed concentrate including soybean, and maize silage/grass silage. Treatment group I (unrestricted) was fed concentrate including rapeseed, and grass silage. Treatment group II (restricted) was fed like treatment group I with a feed restriction period. The treatment diet was not effective to give similar daily live weight gain to the control diet, but it was successful in improving beef fatty acid composition without affecting tenderness and colour (under unrestricted conditions). There were no differences in vitamins and cooking loss, but selenium decreased in treatment groups. Stimulated lipid peroxidation, in samples taken immediately post-mortem, was higher in treatment groups. Polyunsaturated fatty acids decreased, saturated fatty acids and intramuscular fat increased after 14 days of storage while vitamins had no significant reduction.

1. Introduction

A high incidence of mortality in Europe resulting from cardiovascular problems associated with the actual diet has been recently found by Müller-Nordhorn, Binting, Roll, and Willich (2008). Studies like this have increased the interest of consumers in healthy food. For beef, much attention has been given to lipids, nutrition being the major route for increasing the content of beneficial fatty acids in beef (Scollan et al., 2006). It is well known that part of dietary 18:2*n*-6 and 18:3*n*-3 fatty acids can escape ruminal biohydrogenation and go on to be deposited in the tissues. However, most of them are extensively metabolized and biohydrogenated in the rumen (Harfoot & Hazlewood, 1997). The rumen microbial ecosystem yields not only the final product: stearic acid (18:0), but a range of biohydrogenation intermediates such as monounsaturated *trans*, cis and conjugated fatty acids (Jouany, Lassalas, Doreau, & Glasser, 2007). Some of these intermediates are transported via duodenal digesta and deposited finally in the tissues (Scollan et al., 2006). Therefore, it is really important that producers, industry and researchers generate alternatives to improve beef quality under the actual beef production conditions. A common diet used for beef cattle production under indoor conditions in Europe consists of maize silage:grass silage with a higher proportion of maize silage, and a concentrate including soybean. A high inclusion of maize silage in beef cattle diets has been related to a high amount of linoleic acid (18:2*n*-6), high *n*-6/*n*-3 ratio and low vitamin E contents in beef (O'Sullivan et al., 2002). In the same way, the use of grain and soybean has also been related with an increase of *n*-6 PUFA and an unbalance in the *n*-6/*n*-3 ratio (Kim, Adesogan, Badinga, & Staples, 2007) which is not desired for human health. Hence, the first purpose of this study was to evaluate an alternative diet in order to increase beneficial fatty acids in beef, without affecting other parameters of beef quality. Likewise, it was considered that just as the improvement of beef quality using different feeding strategies is important for actual and future beef production systems, the maintenance of quality after slaughter is necessary in order to guarantee quality to the consumers. This study had two objectives: (i) to evaluate fatty acid profile, trace elements, fat soluble vitamins, lipid peroxidation, and quality of *longissimus* muscle of German Simmental bulls comparing control animals fed concentrate including soybean and a mixture of maize silage and grass silage (70:30), versus restricted and unrestricted animals fed concentrate, including rapeseed and only grass silage, (ii) to investigate the effects of storage (14 days of storage versus 24 h post-mortem) on fatty acid profile and fat soluble vitamins.

* Corresponding author. Tel.: +49 038208 68884; fax: +49 038208 68602.
E-mail address: dannenberger@fbn-dummerstorf.de (D. Dannenberger).
[1] Deceased author.

0309-1740/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2009.02.005

2. Materials and methods

2.1. Animals and experimental design

25 male German Simmental calves (3–4 months) were included in an indoor experiment. They were randomly assigned into three groups with different feeding regimes. The control group ($n = 9$) were daily fed maize silage/grass silage (70/30, *ad libitum*), 1 kg of molasses, 1 kg of hay and concentrate including soybean (2 kg the first 112 days, 2.5 kg the next 110 days and 3 kg the rest of the time). 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 in the same proportion as the control group. 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 (approximately 10 MJ/d of MEI). All groups included 5% of commercial mineral mix in the concentrate. Vitamins were not supplemented. Details of feeding have been compiled in Table 1. All bulls were slaughtered at approximately 635 kg live weight by captive bolt stunning followed by exsanguinations in the abattoir of the Research Institute for the Biology of Farm Animals in Dummerstorf (Germany). The slaughter and dressing procedures were in accordance with EU specifications. *Longissimus* muscles were taken immediately after slaughter for the thiobarbituric acid (TBARS) assay, and after 24 h chilling for meat quality assessment, fatty acid composition, vitamins, trace elements and cooking loss. Samples were taken at the 6th–13th rib of the right carcass side. One muscle slice (5 cm) was vacuum-packed and stored in the dark at 2 °C for 14 days for fatty acids and vitamins analysis. After this time, it was kept at −20 °C until the respective analysis.

Table 1
Composition of the diets.

	Concentrate control group%	Concentrate treatment groups%	Maize silage	Grass silage
Crude protein	18.90	19.60	9.60	20.10
Crude fat	2.80	4.90	3.10	4.10
Crude Ash	8.30	8.60	5.20	14.10
Acid detergent fibre (ADF)	3.20	5.80	18.20	22.30
ME (MJ/kg)	12.30	12.40	11.00	10.10

Fatty acids (%)	TMR control group	TMR treatment groups
C14:0	0.14	0.19
C16:0	12.06	7.97
C16:1*cis*-9	0.44	1.43
C18:0	3.03	1.56
C18:1*cis*-9	23.07	35.60
C18:2*n*-6	46.58	26.96
C18:3*n*-3	7.25	8.06
PUFA	54.10	35.31
n-3 FA	7.36	8.21
n-6 FA	46.66	27.02
Ratio *n*-6/*n*-3	6.34	3.29
Vitamins (mg/kg)[a]		
Retinol (A)	13.71	14.79
δ-Tocopherol	0.44	n.d.
γ-Tocopherol	1.80	0.73
α-Tocopherol	18.50	22.30
Trace elements (mg/kg)[a]		
Zn	24.70	25.50
Se	<0.01	<0.01
Fe	231.00	242.00
Cu	4.45	5.35

n.d.: Not detected.
[a] mg/kg fresh material.

2.2. Tenderness, cooking loss and colour

The samples for tenderness (2.5 cm) were cooked in a water bath until the internal temperature of each steak was 70 °C. After cooling, for 90 min at room temperature, five cores (1.2 cm diameter) were taken from the steaks parallel to the muscle fibre orientation. Each core was sheared using a Texture Analyser Winopal (Ahnbeck) with a Warner–Bratzler blade (1.0 mm wide). The Warner–Bratzler peak force was the measurement considered as tenderness. Cooking loss and colour were measured according to the methodology described by Nuernberg et al. (2005).

2.3. Fatty acid composition

The intramuscular fat (IMF) of 2 g muscle was extracted with chloroform/methanol (2:1, v/v) according to Folch, Lees, and Stanley (1956) by homogenisation (Ultraturrax, 3 × 15 s, 12 000 rpm) at room temperature. The fatty acid composition of muscle lipids and feed was determined using the methodology described by Nuernberg et al. (2002).

2.4. Lipid peroxidation

For evaluating the stability of the beef muscle samples, postmortem, against stimulated lipid peroxidation, the production of thiobarbituric acid reactive substances (TBARS) was used. TBARS's are expressed in terms of malondialdehyde (MDA), a breakdown product formed during lipid peroxidation. To stimulate lipid peroxidation 3 ml of the muscle homogenate were incubated with 0.1 mM ascorbate and 5 μM FeSO4. From this, 0.5 ml were immediately removed and pipetted into 0.25 ml of 20% trichloric acid (TCA) in 100 mM KCl. The remaining incubated homogenate was placed in a water bath of 37 °C and after 0, 15, 30, 60, and 120 min 0.5 ml each of this incubated homogenate were pipetted into the TCA medium as above. These samples were centrifuged at 10,000g for 10 min and 0.5 ml of the supernatants were mixed with 0.5 ml thiobarbituric acid (0.67%) and boiled for 15 min in a water bath. After immediate cooling, the absorbance at 535 nm was determined. Standard MDA solution was prepared by hydrolysis of 1,1,3,3-tetraethoxypropane, and the results were expressed as mg/kg of sample (Kuechenmeister et al., 1999).

2.5. Determination of fat soluble vitamins

Retinol (vitamin A), δ-tocopherol, γ-tocopherol, α-tocopherol, and β-carotene were analysed in the muscle samples according to the adaptation of the methodology described by Linden (2003) and Ryynänen, Lampi, Salo-Väänänen, Ollilainen, and Piironen (2004). Three sub-samples were prepared by homogenising tissue (4 g per sub-sample) in 6 ml of a mixture composed by potassium chloride 0.15 M and Tris buffer 0.05 M, using an Ultra-turrax (3 × 15 s, 34 000 revolutions per minute; at room temperature) with 50 ml tubes. Each sub-sample was transferred to another Pyrex-tube of 25 ml washing with 6 ml ethanol absolute (with 0.01% of 3,5-di-tert-butyl-4-hydroxytoluene (BHT)). In order to avoid early oxidation, 500 μl of pyrogallol was added. After that, each tube was closed and agitated by hand followed by the addition of nitrogen for 10 s. Next, the tubes were introduced in a water bath (70 °C) for 5 min. Then, nitrogen was added again for 10 s, followed by the addition of 5 ml potassium hydroxide solution (10 N, pH 7.4). Each tube was closed and agitated by hand. The tubes were again heated in a water bath (70 °C) for 30 min. Finally, the tubes were cooled in ice for 10 min. Thereafter, the mixture was extracted three times with 4 ml of *n*-hexane:ethyl acetate 2:1 (v/v with 0.01% BHT). The supernatants, containing fat soluble vitamins, were transferred into another tube and subsequently, solvents