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THE EFFECT OF THE SECOND GRAZING PERIOD ON THE FATTY ACID COMPOSITION IN MEAT OF INDIGENOUS CIKA AND SIMMENTAL BULLS

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SUMMARY

*The aim of the study was to determine fatty acid composition in meat of Cika and Simmental bulls from two different fattening technologies. The herd of 39 young bulls was housed during the winter time and fed the same total mixed ration diet (TMR) based on corn and grass silage with a limited amount of concentrates. In the spring bulls of both breeds were divided into two subgroups. Bulls in the first subgroup (10 Cika, 9 Simmental; S-INT) were fattened indoors with the semi-intensive TMR. Bulls in the second subgroup (10 Cika, 10 Simmental; G+S-INT) were put on all-day grazing in the pasture. After grazing period bulls were housed under the same conditions as the first subgroup. Samples of *M. longissimus dorsi* were collected from the right carcass side to determine the total fat content and the fatty acid composition. The breed significantly influenced fatty acid composition in meat. The beef of Simmental bulls resulted in higher percentage of PUFA and lower percentage of SFA and MUFA. Higher percentage of n-3 and n-6 PUFA was determined in meat of Simmental bulls but the n-6/n-3 ratio was lower in Cika bulls meat. The fattening technology had less effect on FA composition in meat. The second grazing period produced higher percentage of SFA, beneficially lower values of n-6/n-3 ratio and higher values of long-chain C20-22n-3 PUFA. Higher CLA percentage was determined in beef from S-INT group.*

Key-words: *beef meat, fatty acids, breed, grazing*

INTRODUCTION

In the past, the indigenous Cika cattle was used mainly for the milk production. Nowadays it is considered a low milk productivity breed compared to popular commercial breeds and it is mainly reared in herds with cow-calf system (Simčič et al., 2013a). On the other hand, most widespread breed in Slovenia is a dual purpose Simmental breed. The young growing fattening bulls are usually maintained indoors but a grazing period could be set up in the growing-fattening scheme, e.g., the first grazing season as calves in the suckler herds, the first indoor period as young stock, the second grazing season starting at 300-350 kg and a final finishing period indoors (Dieuguz Cameroni et al., 2006). Regarding natural conditions in Slovenia where cattle diet is based on the forage, the second grazing period for bulls could be easily adapted to the previously mentioned technology. Fats from animal origin have been

a subject of many debates because they increase the risk of some diseases when they consumed in excess (Salobir, 2001). Besides concentrated source of energy for the body, fat is also a source of fat soluble vitamins A, D, E and K and essential fatty acids (FA) important for normal growth and play a role in maintaining many body functions. Nutritional guidelines advocate reduction in the intake of total fat, saturated (SFA) and *trans* FA (TFA) and increased intake of n-3 polyunsaturated FA (PUFA), especially long chain PUFA (Shinfield et al., 2008). Fatty acid profile of meat shows a wide variability depending on several factors such as breed, diet, age and sex (Daley et al., 2010). Many studies have confirmed an effect of cattle breed on the FA composition

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in beef (Warren et al., 2008; Brugiapaglia et al., 2014). Manipulating fatty acid profile in ruminant fat by changing their diet is far more difficult than in monogastric animals. That is due to the biohydrogenation process in the rumen. Nevertheless, the results of many studies confirmed that fatty acid composition in ruminant fat can be influenced by different feeding systems (De la Fuente et al., 2009; Humanda et al., 2012). The objective of this study was to investigate the effect of breed and fattening technology on the FA composition in meat, in particular, to examine the difference in the intramuscular fatty acid composition of indigenous Cika and Simmental young bulls.

MATERIAL AND METHODS

Animals

The study was conducted at the Educational and Research Animal Husbandry Centre Logatec (Slovenia) and included 20 Cika and 19 Simmental young bulls bought from farms throughout Slovenia in November 2010. The herd was housed in a feedlot with a closed barn with multiple pens for the winter time (178 days in average) and fed the same total mixed ration (TMR) diet based on maize and grass silage with a limited amount of concentrates. The experimental period started in May 2011 when bulls were divided into two subgroups by their live weight. The first subgroup consisted of 10 Cika and 9 Simmental bulls with initial body weight 445.7 kg and 392.4 kg, respectively. Bulls in the first subgroup (S-INT) were fattened indoors in four pens with a fully slatted floor. They were fed semi-intensive TMR consisted of maize silage (66.0%), grass silage (16.5%), corn grain (9.5%), sunflower meal (7.1%) and mineral-vitamin premix (0.9%) that is commonly used for bulls fattening. The second subgroup (G+S-INT) included 10 Cika and 10 Simmental bulls with initial body weight 339.7 kg and 312.5 kg, respectively. Bulls in the second subgroup were put on all-day grazing in the pasture. After grazing period (131 days in total) bulls were housed in the same conditions as the first subgroup. They were fed with the same semi-intensive diet as bulls in the first subgroup. This period lasted for 233 days on the average. Bulls were slaughtered when they achieved appropriate commercial requirements according by the Slovenian market. The average age of bulls at slaughter for S-INT group was 703 days and for G+S-INT group 740 days. Samples of *M. longissimus dorsi* located between the 7th and 8th rib were collected from the right carcass side to determine total fat content and FA composition. The carcass traits of bulls from this experiment were presented by Simčič et al. (2014).

Total fat and fatty acid composition

Intramuscular fat content was determined using petroleum ether extraction after hydrolysis of sample in 4 M HCl solution by the manufacturer's applica-

tion notes (Foss, Application note AN 3904). Total fat determination includes acid hydrolysis step, in which fat bound polar components are separated and later extracted. In our case, samples were hydrolysed in 4 M HCl in SoxCap 2047 (Soxtec 2050, Foss system, Höganäs, Sweden). After drying, fat from hydrolysed samples was extracted using petroleum ether in Soxtec 2050 (Foss system, Höganäs, Sweden).

The fatty acid composition of meat samples was analysed using a gas chromatographic method following transesterification of lipids. Fatty acid methyl esters (FAME) were prepared according to Park and Goins (1994) using gas chromatograph (6890 series, Agilent, Santa Clara, CA, USA). FAME were separated using a capillary column (Varian CP 4720, length 100 m, internal diameter 250 μ m, film thickness 0.25 μ m). Agilent GC ChemStation was used for data acquisition and processing. Separated FAME were identified by retention time comparison and results were calculated using response factors derived from chromatographic standards of known fatty acid composition (Nu Chek Prep). The exactness and reliability of the method used was assessed by the certified reference material NIST SRM 1546 Meat Homogenate. The fatty acid composition was expressed as a percentage by weight (wt%) of the total identified fatty acids.

Statistical analysis

Data were analysed using the GLM procedure in the statistical package SAS/STAT (SAS Institute Inc., 2001). The effect of breed and fattening technology were included in the model. The interaction between these effects was eliminated from the model because it was not significant. The results in the table are presented as Least square means \pm SE and they were considered to be significantly different when $P < 0.05$.

RESULTS AND DISCUSSION

Nutrition is the major factor influencing FA composition in beef whereas both nutrition and genetics affect the level of fat (Kamihiro et al., 2015). In this study, the breed significantly influenced total fat content in meat. Cika bulls compared to Simmental bulls had higher total fat content in meat. Regarding total fat content in meat, no difference was observed between fattening technologies (Table 1). This is in contrary with Simčič et al. (2013) who reported significantly higher intramuscular fat content in semi-intensively Cika bulls compared to grazed Cika bulls. This might be due to the fact that all bulls in our experiment were put on the same TMR diet in the finishing period.

Breed significantly affected percentage by weight of total SFA, MUFA and PUFA. Cika meat had higher percentage of SFA (46.94 wt%) and MUFA (40.16 wt%) thus consequently lower percentage of PUFA (12.91 wt%) compared to Simmental meat (44.35 wt%, 35.55 wt%, 20.09 wt%), respectively. This might be due to the fact that meat of Simmental bulls contained

considerably lower proportion of fat and consequently higher proportion of phospholipids in total lipid content. According to many studies, the wt% of PUFA in meat is correlated with the level of intramuscular fat. Aldai et al. (2007) found out that the lowest percentage of SFA and MUFA, and the highest percentage of PUFA were observed in meat from the leanest animals. Likewise, the high level of PUFA (23.2 wt%) and the low level of intramuscular fat in the *Longissimus thoracis* muscle in Maremmana bulls was reported by Sargentini et al. (2010). Percentage of major hypercholesterolaemic FA, C14:0 and C16:0 was higher in the meat of Cika bulls, but there was no influence of breed found in the percentage of C18:0 (Table 1). The meat of Simmental bulls contained more n-6 PUFA (10.88 wt%) and n-3 PUFA (1.88 wt%) compared to Cika bulls (17.40 wt%, 2.46 wt%), respectively. The higher percentage of both essential FA, linoleic (C18:2n-6) and α -linolenic acid (C18:3n-3), was established in meat of Simmental bulls. There was no difference between these two breeds found out in the proportion of CLA (C18:2cis-9, trans-11). Ruminant dairy and meat products are the principal sources of cis-9, trans-11 CLA isomer in the human diet. Due to its biological benefits, e.g. reduction of carcinogenesis, atherosclerosis, inflammation, obesity, diabetes, the CLA has received much attention in recent years (Yang et al., 2015). Likewise, Brugiapaglia et al. (2014) found no effect of breed (Piemontese, Limousine, Friesian) on the CLA content in beef. On the other hand, Warren et al. (2008) established the significant effect of breed (Aberdeen Angus cross, Holstein-Friesian) on the CLA proportion in steers, but only when animals were 14 or 19 months old at slaughter. When animals were slaughtered at 24 months no effect of breed was detected. Meat from Simmental bulls contained significantly higher wt% of AA (arachidonic acid), EPA (eicosapentaenoic acid), DPA (docosapentaenoic acid) and DHA (docosahexaenoic acid) compared to Cika bulls meat. These long chain PUFAs are in meat presented in low but important concentrations due to increasing evidence from animal and *in vitro* studies which indicates anticarcinogenic properties of n-3 PUFA, especially EPA and DHA. Concerning n-6/n-3 PUFA ratio, the values in Cika bulls meat (5.82) were lower and thus preferential as in Simmental bulls (7.29). According to DACH (2008) nutrition guidelines the recommended n-6/n-3 ratio is 5:1, but in human diets this ratio is often above 10:1 and connected with many chronic diseases.

Manipulating FA composition in ruminant meat has been extensively studied with purpose to elevate n-3 PUFA and reduce SFA concentrations. SFA tends to dominate in ruminant fat, as a consequence of dietary PUFA being hydrogenated by microbial population in the rumen. Nutritional guidelines recommend a reduction in total fat intake, particularly of SFA (DACH, 2008). In this study fattening technology had no impact on percentage by weight of PUFA, but SFA and MUFA were significantly affected. Higher percentages of SFA were found out in G+S-INT group. That is in agreement with

Alfaia et al. (2009) who demonstrated higher percentage of SFA and no difference in PUFA percentage in meat from Alentejano bulls fed concentrate diet after grazing period compared to bulls fed concentrate diet entire experimental period. The wt% of C14:0 and C16:0 was not affected by fattening technology, but the proportion of C18:0 was significantly higher in G+S-INT group. There was also no effect of fattening technology on wt% of linoleic and α -linolenic acid observed. Regarding CLA, the higher percentage by weight was discovered in S-INT group (0.27 wt%) compared to the G+S-INT group (0.20 wt%). This is in the contrary with many studies which demonstrated a positive effect of grazing on CLA concentration in meat (De la Fuente et al., 2009; Humanda et al., 2012). This is probably due to the fact that all bulls were put on the same TMR diet in the finishing period. Percentages of EPA, DPA and DHA, which are of special interest in the human diet, were influenced by the fattening technology. The higher values of these long chain PUFAs were in meat of bulls from G+S-INT group. The fattening technology significantly affected the percentage by weight of n-3 PUFA and consequently n-6/n-3 PUFA ratio being lower in meat of grazing bulls (5.86) compared to meat from bulls that were fattened indoors (7.25).

Table 1. Total fat content (%) and fatty acid composition (percentage by weight of total identified fatty acids; wt%) in *M. longissimus dorsi* of Cika and Simmental young bulls from different fattening technologies (LSM±SE)

	Breed		P-value	Fattening technology		P-value
	Cika	Simmental		S-INT	G+S-INT	
Total fat	2.03±0.16	1.28±0.16	**	1.81±0.16	1.50±0.16	ns
C14:0	2.42±0.08	1.74±0.08	***	2.12±0.09	2.06±0.08	ns
C16:0	24.40±0.39	21.49±0.40	***	22.50±0.40	23.39±0.38	ns
C18:0	15.64±0.30	15.68±0.31	ns	15.19±0.31	16.14±0.30	*
C18:1	35.63±0.80	31.44±0.82	**	34.67±0.82	32.40±0.80	ns
C18:2n-6	7.95±0.67	12.77±0.68	***	10.17±0.68	10.56±0.67	ns
C18:3n-3	0.77±0.04	0.88±0.04	ns	0.81±0.04	0.85±0.04	ns
C18:2c9,t11(CLA)	0.23±0.01	0.23±0.01	ns	0.27±0.01	0.20±0.01	***
C20:4n-6 (AA)	2.08±0.21	3.51±0.22	***	2.72±0.22	2.87±0.21	ns
C20:5n-3 (EPA)	0.34±0.04	0.40±0.04	*	0.31±0.04	0.52±0.04	**
C22:5n-3 (DPA)	0.62±0.05	0.88±0.06	**	0.65±0.06	0.85±0.05	*
C22:6n-3 (DHA)	0.08±0.00	0.12±0.01	**	0.08±0.01	0.12±0.01	*
SFA	46.94±0.42	44.35±0.44	***	44.71±0.44	46.58±0.42	**
MUFA	40.16±0.89	35.55±0.92	**	39.29±0.92	36.42±0.89	*
PUFA	12.91±1.03	20.09±1.06	***	16.00±1.06	17.00±1.03	ns
n-6 PUFA	10.80±0.91	17.40±0.94	***	13.81±0.94	14.38±0.91	ns
n-3 PUFA	1.88±0.14	2.46±0.14	**	1.92±0.14	2.42±0.14	*
n-6/n-3	5.82±0.21	7.29±0.21	***	7.25±0.21	5.86±0.21	***

*P<0.05; **P<0.001; ***P<0.0001; ns P>0.05; LSM - Least square means; SE - standard error; CLA-C18:2*cis*-9,*trans*-11; AA-arachidonic acid, EPA-eicosapentaenoic acid, DPA-docosapentaenoic acid, DHA-docosahexaenoic acid, SFA-saturated FA, MUFA-monounsaturated FA; PUFA-polyunsaturated FA

CONCLUSION

Data reported here demonstrate considerable differences in beef FA profiles between breeds, in this Cika and Simmental case. The beef from Simmental bulls resulted in higher percentage of PUFA and lower percentage of SFA and MUFA. Higher percentage of n-3 and n-6 PUFA was determined in meat of Simmental bulls but the n-6/n-3 ratio was lower in meat of Cika bulls. On the other hand, the second grazing period had less effect on the fatty acid composition. That is probably due to the fact that bulls were fattened under the same conditions in the last period before the slaughter. Nevertheless, the second grazing period produced higher values for SFA, beneficially lower values for n-6/n-3 ratio and higher values for long-chain C20-22n-3 PUFA.

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