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INTRAMUSCULAR FATTY ACID PROFILE OF INTENSIVELY REARED SIMMENTAL BEEF-BULLS

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Summary

Beef intramuscular fatty acid (FA) profile was investigated on a sample of Simmental beef bulls from an intensive rearing system in Croatia. Animals were housed indoor in common pens on a concrete slatted floor (SF group, n=15) or a full floor with straw bedding (FF group, n=15). Diet in both groups was given as total mixture ration composed from maize grain and stalk silage, super-concentrate and hay (average composition per kg: 599 g of DM, 76 g of crude protein and 4.59 MJ of ME as feed). Bulls were slaughter at similar age (494±17 days) and body weight (597.5±56.4 kg) and the intramuscular fat (IMF) content (g/kg) and FA composition (g/100g of total FA) of the M. longissimus thoracis were determined. The average IMF content was 25.49±9.21 with C18:1 (36.28±3.99), C16:0 (22.61±1.81), C18:0 (17.18±1.71) and C18:2n-6 (9.15±3.08) as the most represented FAs. The average proportions of total saturated FA (SFA) and monounsaturated FA (MUFA) were 44.33±1.89 and 41.78±4.31, while the share of total polyunsaturated FA (PUFA) was 13.89±4.72, with the averages of n-6 and n-3 PUFA totals of 12.93±4.52 and 0.68±0.24, respectively. The PUFA/SFA and n-6/n-3 ratios were out of the nutritionally recommended limits; while the proportion of beneficial conjugated linoleic acid (CLA) averaged 0.285±0.082 g/100g of FA. Floor type had no major effect on FA profile, except for slightly higher CLA and C20:1 and lower C12 and n-6/n-3 in SF group. It is concluded that the obtained results imply a need for feeding strategy that could enhance the nutritional quality of beef FA under the intensive production systems.

Key words: beef, Simmental breed, intramuscular fat, fatty acids, floor type.

Introduction

Meat is traditionally considered as highly nutritious and valued food associated with good health and prosperity. However, that positive image was disturbed when high consumption of red meat has been linked with increased frequency of chronic diseases such as coronary heart disease and some types of cancer (Higgs, 2002; Wood et al., 2003; Biesalski, 2005). That was mainly due to its fat content and fatty acid (FA) composition and in particular beef has been often criticized for being too high in saturated FA (SFA) and low in polyunsaturated FA (PUFA) and thus potentially unhealthy for consumers (Scollan et al., 2006). Moreover, in grain-fed beef obtained under intensive production systems the concentration of PUFA and beneficial n-3 PUFA tends to be lower than in grass-fed beef from grazing cattle (Moloney, 2002). In Croatia, beef cattle are generally rear under intensive production systems (indoor, high stocking density) and usually feed with corn-based concentrates and corn grain silage with addition of some fodder, typically in the form of corn stalk silage and hay or straw. It could be assumed that such predominantly grain-based diet may results in nutritive less valuable FA composition of beef fats. Thus, this study aimed to investigate the FA composition of intramuscular fat (IMF) of Simmental bulls from intensive beef production system in Croatia. As beef cattle under intensive farming are mainly kept indoors either on a concrete slatted floor or on a full floor with straw bedding, the potential influence of floor type (slatted vs. bedding) on intramuscular FA profile was additionally investigated.

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Material and methods

Animals, housing and diets

The investigation was conducted on 30 Simmental bulls produced under an intensive rearing system at two commercial beef farms with a different type of animals' housing: a) at full concrete floor with straw bedding (Farm A, FF group, n=15) and b) at fully slatted concrete floor (Farm B, SF group, n=15). The FF bulls were kept loosely at common pen inside the barn with closed walls on three sides and an open section toward the outdoor feeding corridor. The pen box had a length of 6.3 m at the lying area and 2.7 m at excrementary corridor, with a width of 10 m. The lying area in relation to the excrementary corridor was lower for 50 cm. The average space allowance was 6 m^2 per bull. The utilization of straw for floor bedding was around 5-6 kg per bull daily. The dirty corridor is cleaned 2 to 3 times a week, while the lying area was not cleaned during fattening. The SF bulls were housed inside the barn on a fully slatted concrete floor (15.5 cm width of beams, with the distance between beams of 35 mm). The animals were also kept loosely in a common pen with the dimensions 7x10 m. The average space allowance in SF group was 4.7 m^2 per bull. The fattening and feeding technique were similar in both FF and SF groups; given that both investigated farms are operated under the same management system (*Belje d.d., AGROKOR*). In brief, bulls are fed with diets provided as total mixture ration (TMR) consisted of high moisture corn, corn silage, hay, and protein rich supplement (34 % of crude protein) based on soybean meal and rapeseed meal with a mineral and vitamin additive (30 000 U of vitamin A, 3 300 U of vitamin D3, 120 mg of vitamin E/kg and 37.5 mg Cu/kg of DM). The TMR was given in a single daily distribution at morning (average composition per kg: 599 g of DM, 76 g of crude protein and 4.59 MJ of ME as feed). The final age and live weight (mean ± standard deviation) of bulls were 491.2 ± 17.7 days and 596.7 ± 54.4 kg in FF group, and 495.8 ± 17.5 d and 598.3 ± 60.0 kg in SF group.

Slaughtering and muscle sampling

All animals were slaughtered on the same day in one commercial slaughterhouse (PIK Vrbovec) using the standard procedure and in accordance with established regulations (Anon., 2004 and 2006). The samples of *M. longissimus thoracis* were taken 24 hours *post mortem* on the right side of cooled carcass at the level of 8th rib and stored frozen (-20 ºC) until analyses of intramuscular fat content and FA composition.

Chemical analysis

The muscle total fat content was determined by standard method for meat and meat products group (Anon., 2001). The FA composition was determined by gas liquid chromatography (GLC) using *in situ* transesterification method (Park and Goins, 1994). The content of FA methyl esters (FAME) was determined using Agilent Technologies 6890 N (USA) gas chromatograph equipped with a flame ionisation detector and the capillary column Supelco Omegawax™ 320 (length 30 m, internal diameter 0.32 mm and film thickness 0.25 μm) for FAME separation. Separated FAMEs were identified by the comparison with the retention times of the FAMEs in a standard mixture (Nu-Check Prep, Inc, Elysian, USA). The same standard mixture was used to determine the response factor (Rf) for each FA. The mass portion of each FA in the sample was determined using the Rf and the factor of conversion of FA content from the FAME content.

Data analysis

For all analysed variables the descriptive statistic (mean, standard deviation, minimum, maximum, coefficient of variation) was calculated. Group means were compared by Student's t – test using PROC TTEST procedure of SAS (S A S , 2002).

Results and discussion

Descriptive statistics of intramuscular fat content and FA composition of Simmental bulls and comparison of FF and SF groups are given in Tables 1 and 2, respectively.

Table 1. – DESCRIPTIVE STATISTICS FOR TOTAL FAT AND FATTY ACID COMPOSITION OF SIMMENTAL BULLS (n=30) LONGISSIMUS MUSCLE (g/100g of total FA)
Tablica 1. – OPISNA STATISTIKA SADRŽAJA UKUPNE MASTI I SASTAVA MASNIH KISELINA (g/100g ukupnih MK) LEĐNOG MIŠIĆA SIMENTALSKIH BIKOVA (n=30)

Fatty acids		Mean	SD	Min	Max	CV (%)
Intramuscular fat (g/kg)		25.49	9.21	10.9	50.2	36.14
C10:0	Capric	0.115	0.027	0.071	0.181	23.16
C12:0	Lauric	0.102	0.019	0.073	0.159	18.66
C14:0	Myristic	2.63	0.573	1.61	4.20	21.83
C14:1	Myristoleic	0.553	0.188	0.264	0.972	33.98
C15:0	Pentadecanoic	0.375	0.074	0.258	0.577	19.67
C16:0	Palmitic	22.61	1.81	17.82	26.21	8.02
C16:1	Palmitoleic	2.90	0.630	1.63	4.14	21.76
C17:0	Heptadecanoic	1.09	0.182	0.767	1.50	16.73
C18:0	Stearic	17.18	1.71	14.07	20.52	9.97
C18:1	Oleic	36.28	3.99	27.90	42.67	11.01
C18:2n-6	Linoleic	9.15	3.08	4.29	16.45	33.64
C18:2 (c+t)	CLA	0.285	0.082	0.167	0.526	28.81
C18:3n-3	α-linolenic	0.248	0.060	0.151	0.388	24.09
C20:0	Arachidic	0.096	0.014	0.073	0.130	14.55
C20:1	Eicosaenoic	0.262	0.048	0.174	0.386	18.15
C20:2n-6	Eicosadienoic	0.197	0.063	0.108	0.357	31.90
C20:3n-6	Eicosatrienoic	0.512	0.201	0.286	1.1	39.21
C20:4n-6	Arachidonic	2.63	1.13	1.17	5.93	42.96
C20:5n-3	Eicosapentaenoic	0.091	0.043	0.036	0.203	46.79
C22:4n-6	Adrenic	0.391	0.141	0.200	0.720	36.21
C22:5n-3	Docosapentaenoic	0.305	0.128	0.144	0.593	41.94
C22:6n-3	Docosahexaenoic	0.031	0.015	0.007	0.067	48.98
Σn-6		12.93	4.52	6.08	24.61	34.94
Σn-3		0.678	0.24	0.35	1.25	35.10
n-6/n-3		19.23	2.24	15.01	24.65	11.65
ΣSFA		44.33	1.89	39.13	48.65	4.26
ΣMUFA		41.78	4.37	32.22	49.93	10.47
ΣPUFA		13.89	4.72	6.65	26.01	33.96
PUFA/SFA		0.31	0.12	0.15	0.62	36.79

SD - standard deviation; Min - minimum; Max - maximum; CV - coefficient of variability; CLA - Conjugated linoleic acid (c9,t11+t10,c11); SFA - saturated FA (10:0+11:0+12:0+13:0+14:0+15:0+16:0+17:0+18:0+19:0+20:0+21:0+22:0+24:0); MUFA - monounsaturated FA (12:1+13:1+14:1+16:1+17:1+18:1+19:1+20:1+22:1+24:1); PUFA - polyunsaturated FA (18:2n-6+CLA+18:3n-6+18:3n-3+20:2n-6+20:3n-6+20:4n-6+ 20:3n-3+20:5n-3+22:4n-6+22:5n-3+22:6n-3).

Table 2. – FATTY ACID PROFILE (g/100g of total FA) OF LONGISSIMUS MUSCLE OF SIMMENTAL BULLS KEPT ON BEDDED FULL FLOOR (FF, n=15) OR SLATTED FLOOR (SF, n=15)
Tablica 2. – SASTAV MASNIH KISELINA (g/100g ukupnih MK) LEĐNOG MIŠIĆA SIMENTALSKIH BIKOVA DRŽANIH NA STELJENOM PUNOM PODU (FF, n=15) ILI REŠETKASTOM PODU (SF, n=15)

Fatty acids		Floor type: FF	Floor type: SF	*P*-value
Intramuscular fat (g/kg)		25.11 ± 2.62	25.87 ± 2.20	0.8258
C10:0	Capric	0.123 ± 0.007	0.108 ± 0.007	0.1109
C12:0	Lauric	0.110 ± 0.005	0.093 ± 0.003	**0.0153**
C14:0	Myristic	2.72 ± 0.187	2.53 ± 0.096	0.3723†
C14:1	Myristoleic	0.542 ± 0.055	0.564 ± 0.043	0.7524
C15:0	Pentadecanoic	0.379 ± 0.019	0.371 ± 0.020	0.7594
C16:0	Palmitic	22.78 ± 0.598	22.44 ± 0.303	0.6156†
C16:1	Palmitoleic	2.93 ± 0.174	2.86 ± 0.157	0.7827
C17:0	Heptadecanoic	1.04 ± 0.037	1.13 ± 0.054	0.1727
C18:0	Stearic	17.15 ± 0.462	17.22 ± 0.437	0.9164
C18:1	Oleic	35.22 ± 1.02	37.34 ± 1.00	0.1486
C18:2n-6	Linoleic	9.58 ± 0.884	8.71 ± 0.706	0.4469
C18:2 (c+t)	CLA	0.253 ± 0.015	0.318 ± 0.023	**0.0275**
C18:3n-3	α-linolenic	0.245 ± 0.016	0.251 ± 0.015	0.7679
C20:0	Arachidic	0.095 ± 0.004	0.097 ± 0.004	0.6455
C20:1	Eicosaenoic	0.245 ± 0.010	0.279 ± 0.014	**0.0462**
C20:2n-6	Eicosadienoic	0.209 ± 0.020	0.185 ± 0.011	0.2885†
C20:3n-6	Eicosatrienoic	0.564 ± 0.066	0.460 ± 0.029	0.1623†
C20:4n-6	Arachidonic	2.92 ± 0.361	2.35 ± 0.186	0.1739†
C20:5n-3	Eicosapentaenoic	0.093 ± 0.013	0.089 ± 0.010	0.8215
C22:4n-6	Adrenic	0.423 ± 0.046	0.358 ± 0.022	0.2168†
C22:5n-3	Docosapentaenoic	0.313 ± 0.039	0.296 ± 0.027	0.7257
C22:6n-3	Docosahexaenoic	0.031 ± 0.004	0.030 ± 0.004	0.8595
Σn-6		13.75 ± 1.36	12.10 ± 0.922	0.3262
Σn-3		0.686 ± 0.071	0.670 ± 0.053	0.8641
n-6/n-3		20.25 ± 0.560	18.21 ± 0.481	**0.0102**
ΣSFA		44.53 ± 0.584	44.12 ± 0.380	0.5588
ΣMUFA		40.78 ± 1.11	42.79 ± 1.13	0.2137
ΣPUFA		14.69 ± 1.44	13.09 ± 0.960	0.3625
PUFA/SFA		0.34 ± 0.037	0.30 ± 0.021	0.3717†

Mean ± standard error; Student t-test (two-sided); †Satterthwaite approximation

The average intramuscular fat content established in present study (25.49 g/kg, CV=36.14%) was lower than that reported by Štoković et al. (2009) in local Simmental bulls (n=698) aged about 420 days (31.4 g/kg), but comparable with results of Bureš et al. (2006) for Czech Simmental bulls (n=21) aged 508 days (24.1 g/kg) and Dannenberger et al. (2006) for concentrate-fed German Simmental bulls (n=16) weighted 620 kg at slaughter (2.61 %). Compared with yearling Simmental "baby-beef" bulls (Karolyi et al., 2011) the average intramuscular fat content found in present study was expectedly higher, roughly twofold.

Regarding the average muscle FA composition (%) the most abundant and consistent FAs were C18:1 (36.28, CV=11.01%), C16:0 (22.61, CV=8.02%) and C18:0 (17.18; CV=9.97%), followed by more variable C18:2n-6 (9.15, CV=33.64%). The proportion of C17:0, C14:0, C20:4n-6 and

C16:1 was between 1.0 and 3 %, while the percentage of other FAs was below 1 %. Conjugated linoleic acid (CLA) refers to a mixture of positional and geometric isomers of linoleic acid with two conjugated double bonds, of which the *cis*9, *trans*11 isomer is the most common natural isomer with biological activity, representing 75-90% of total CLA in ruminant meat (Moreno et al., 2008). The CLA, which may have a potential human health benefits, is mainly deposited in triacylglycerols and its amounts in beef lipids can vary with feeding conditions (e.g. pasture and supplementation with oil seeds, vegetable oils and fish oil have proved to be enhancing factors) and intrinsic factors such as breed, sex, age, level of fatness and type of the muscle (De La Torre et al., 2006; Scollan et al., 2006). In present study, the CLA content in intramuscular fat, determined by GLC as a mixture of c9, t11 and t10,c11, averaged 0.29 g/100 g of FA (CV=28,8%), what is comparable with the results for maize silage/concentrate fed Slovenian Simmental bulls reported by Petrič et al. (2005). The average shares of total saturated and monounsaturated FA, i.e. SFA and MUFA, were 44.33 (CV=4.26%) and 41.78 (CV=10.47%), while the share of total polyunsaturated FA (PUFA) was 13.89 (CV=33.86%), with the averages of n-6 and n-3 PUFA totals of 12.93 (CV=34.94%) and 0.68 (CV=35.10%), respectively. In comparison with beef FA profile reported in literature (e.g. review of Muchenje et al., 2009) the observed FA values in present study chiefly correspond with reported ranges for grain-fed cattle.

The PUFA/SFA ratio has been widely used as an indicator of nutritional quality of fat for human consumption, particularly in the view of diet-related chronic diseases such as atherosclerosis. The recommended value for this ratio is ≥0.4 (Higgs, 2002). In beef the PUFA/SFA ratio is generally low due to ruminal biohydrogenation of unsaturated FAs from feeds and it decreases with increase in age/weight and level of fatness (De Smet et al., 2004). The average PUFA/SFA ratio of 0.31 (CV=36.79%) founded in present study was lower than the recommended threshold for human diet but more favourable than PUFA/SFA ratio (~0.2) in concentrate-fed Simmental cattle of similar age but higher live weight reported by Nuernberg et al. (2005). More nutritionally beneficial PUFA/SFA beef ratios were reported in intensively reared Simmental bulls slaughtered at lower age and weight (e.g. Petrič et al., 2005; Karolyi et al., 2009). Contrary to these results, Štoković et al. (2013) recently reported a very low PUFA content and PUFA/SFA ratio in young Simmental bulls (4.16 % and 0.09, respectively). For the prevention of diet-related chronic diseases, it is also suggested that intake of n-3 PUFA should be increased relative to n-6 PUFA, with optimal n-6/n-3 ratio in the diet less than 4 (Higgs, 2002). As shown above, the proportion of total n-3 PUFA relative to n-6 PUFA in longissimus muscle of intensively reared Simmental beef-bulls was low and corresponding result for n-6/n-3 ratio of 19.23 (CV=11.65%) was well beyond the dietary recommendations. Grass-feeding can increase the n-3 PUFA and other beneficial FA like CLA in beef, as it was also shown in Simmental bulls (Nuernberg et al., 2005; Petrič et al., 2005).

Considering the effects of floor type on investigated variables, the results in Table 2 show that neither the content nor the FA profile of IMF differed significantly (P>0.05) between FF and SF bulls, except in terms of CLA and C20:1, which where slightly higher in SF group (0.253 vs. 0.318 and 0.245 vs. 0.279 g/100g of total FA, respectively) and C12:0 and n-6/n-3 ratio, which were slightly higher in FF group (0.110 vs. 0.093 g/100g of total FA and 20.25 vs. 18.21, respectively).

Conclusions

This study showed that typical meat from Simmental beef bulls produced under the intensive production system in Croatia may have a less favourable FA composition for human health. That was particularly noted through the low proportion of PUFA in general and n-3 PUFA in particular and hence too low PUFA/SFA and too high n-6/n-3 ratios in regard to the current nutritional recommendations. The potentially positive aspect of beef FA profile is its CLA content, which amount in present study was slightly higher in bulls raised on slatted floor. However, this single finding should be additionally explored, as floor type in present study generally had no major influence on FA profile. It could be concluded that the obtained results imply a need for feeding strategy that could enhance the nutritional quality of beef FA under the intensive production systems.

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PROFIL INTRAMUSKULARNIH MASNIH KISELINA TOVNIH BIKOVA IZ INTENZIVNOG UZGOJA

Sažetak

Profil intramuskularnih masnih kiselina (MK) mesa goveda istraživan je na uzorku simentalskih tovnih bikova iz intenzivnog sustava uzgoja u Hrvatskoj. Smještaj životinje bio je unutar objekata u zajedničkim betonskim boksovim na rešetkastom podu (RP grupa, n=15) ili na punom podu uz steljenje (PP grupa, n=15). Hranidba u obje grupe bila je u obliku potpuno izmiješanog obroka sastavljenog iz silaže kukuruznog zrna i stabljike, super koncentrata i sijena (prosječni sastav kg obroka: 599 g ST, 76 g sirovog proteina i 4,59 MJ ME). Bikovi su zaklani pri sličnoj dobi (494±17 dana) i tjelesnoj masi (597,5±56,4 kg) te je određen sadržaj intramuskularne masti (IMM, g/kg) i sastav MK (g/100g of total FA) M. longissimus thoracis-a. Prosječan sadržaj IMM iznosio je 25,49±9,21, dok su najzastupljenije MK bile C18:1 (36,28±3,99), C16:0 (22,61±1,81), C18:0 (17,18±1,71) i C18:2n-6 (9,15±3,08). Prosječni sadržaji ukupnih zasićenih MK (ZMK) i mono-nezasićenih MK (MNK) iznosili su 44,33±1,89 i 41,78±4,31, dok je sadržaj ukupnih poli-nezasićenih MK (PNMK) bio 13,89±4,72, s prosjecima ukupnih n-6 i n-3 PNMK od 12,93±4,52, odnosno 0,68±0,24. Omjeri PNMK/ZMK i n-6/n-3 bili su izvan nutritivno preporučenih vrijednosti, dok je sadržaj korisne konjugirane linolne kiseline (KLK) u prosjeku iznosio 0,285±0,082 g/100g ukupnih MK. Tip poda nije imao značajniji utjecaj na MK profil, osim nešto višeg sadržaja KLK i C20:1 te nižeg sadržaja C12:0 i n-6/n-3 omjera u RP grupi. Zaključeno je da dobiveni rezultati ukazuju na potrebu za hranidbenom strategijom koja može poboljšati nutritivnu kakvoću MK u goveđem mesu iz intenzivnog sustava proizvodnje.

Ključne riječi: govedina, simentalska pasmina, intramuskularna mast, masne kiseline, tip poda.

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