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Effect of linseed diet on intramuscular fatty acid profile of bulls slaughtered at different ages

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Background

- Beef
  - Meat wholesomeness an increasingly important aspect for consumers (Becker, 2002)
  - Highly nutritious and valued food, but positive perception disrupted when consumption is linked with cardiovascular disease and some types of cancer (e.g.,Biesalski, 2005; McAfee et al., 2010)
  - Negative *image* related to its fat content and fatty acid (FA) composition
  - In particular beef is criticized for being too high in saturated FA (SFA) and low in polyunsaturated FA (PUFA) and potentially unhealthy for consumers
  - Increase in PUFA/SFA and reduce in n-6/n-3 PUFA ratio are priorities (Scollan et al., 2006)

Background

- Grain-fed beef
  - Concentration of PUFA and beneficial n-3 PUFA, i.e. α-linolenic (ALA,18:3n-3), eicosapentaenoic (EPA,20:5n-3) and docosahexaenoic acid (DHA,22:6n-3), as well as conjugated linoleic acid (CLA) is generally lower than in grass-fed beef

    (e.g., Enser et al 1998; French et al., 2003; Petrič et al., 2005; Dannenberger et al., 2006)

  - To increase n-3 PUFA in grain-feed beef, n-3 sources like oilseeds should be include in rations, but result depends on part of n-3 supply that escapes rumen biohydrogenation (converts unsaturated FA to SFA) before it is absorbed and deposited in meat
  - Feeding whole oilseeds may increase the post-ruminal delivery of n-3 PUFA, because the seed coat prevents the access of rumen microorganisms to the unsaturated FA *(Aldrich et al., 2006)*

Background

- Linseed
  - Highest ALA source of any oilseed (≥ 50 %, n-6/n-3 = 0.2 - 0.3)
  - It is generally demonstrated to be highly effective in the enhancement of non-ruminant meats, such as pork and poultry with n-3 PUFA
  - Various effects of different forms/concentrations of linseed supplementation in diet on performance and tissue FA composition in beef cattle has been reported

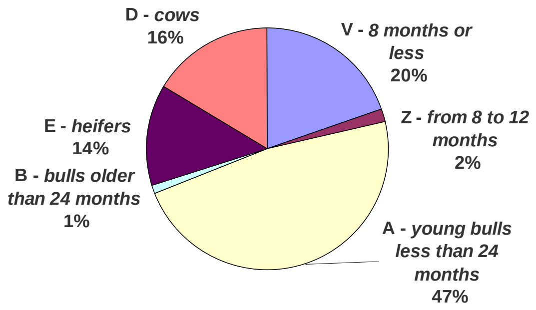
    (e.g., Raes et al., 2004; Mach et al., 2006; Holló et al., 2008; Corazzin et al.,2012)

- Weight/age at slaughter
  - Affects FA composition throughout its effect on muscle and carcass fattening

    (e.g., Smith et al., 2006; Wood et al., Moreno et al., 2008; Bartoň et al., 2011)

Background

- Beef production in Croatia
  - Under intensive systems of housing (indoors, common boxes) and feeding (total mixture ration: corn silage, concentrate and hay)
  - In 2011, the total number of beef carcasses classified was 245.944, of which about 85 % were of domestic origin (structure by category):



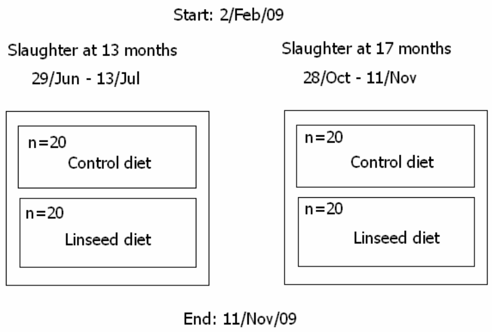
(Croatian Agricultural Agency, 2012)

Aim of the work

- **To study the effect of whole linseed diet and two slaughter age (13 vs. 17 months) in young bulls on:**
  - Carcass and meat quality traits
  - Intramuscular fatty acid (FA) profile
  - Beef oxidative stability

Material and methods

- **Animals and experimental protocol**
  - 80 Simmental bulls reared under intensive fattening conditions
  - At age of 221±9 d assigned to feeding groups and fed with linseed (L) or control diet until slaughter at about 13 or 17 months of age:



  - Kept freely in separated pens on straw bedded concrete floor (2.8-4.3 m^2/head)

Material and methods

- **Diets**
  - Total mixture ration (TMR) consisted of high moisture corn (HMC), corn silage (CS), hay (H), and protein supplement (PS) with a minerals and vitamins
  - In L diets, HMC was partly replaced by the whole linseed (WL)
  - Up to age of 13 months HMC and CS were gradually increased from 4.4 to 7.0 kg (or from 4.2 to 6.8 kg + 0.13 kg of WL in L diet) and from 6.0 to 8.5 kg per head and day, respectively
  - From 13 to 17 months of age, HMC and CS were additionally gradually increased to 8.0 kg (or to 7.7 kg + 0.16 kg of WL in L diet) and to 9.0 kg/day, respectively
  - PS and H were constant (1.5 kg and 0.2 kg per head and day, respectively)

Material and methods

Average chemical and FA composition of feedstuffs and diets provided to bulls

	HMC	CS	PS[a]	H	WL	TMR-C	TMR-L
Chemical composition (g/kg DM):							
Dry matter (DM)	745	416	925	935	929	599	585
Crude protein	79	69	366	67	192	128	126
Ether extract	45	32	19	19	237	36	41
Crude fiber	19	189	78	332	50	87	102
Ash	15	45	181	60	48	43	44
Fatty acid composition (%)[b]:							
C12:0	0.02	0.27	0.04	0.76	0.01	0.09	0.09
C12:1	0.25	0.41	0.40	1.36	0.04	0.30	0.34
C14:0	0.07	0.34	0.22	1.28	0.05	0.16	0.16
C16:0	12.41	13.74	11.58	27.02	5.38	12.86	11.36
C16:1	0.21	0.59	1.02	0.47	0.07	0.31	0.23
C18:0	2.74	3.52	4.30	8.13	3.01	15.19	13.88
C18:1	28.22	25.76	36.73	4.82	20.83	13.29	12.51
C18:2n-6	52.60	47.09	37.55	17.74	17.26	51.20	45.03
C18:3n-3	1.56	5.04	5.62	26.70	51.88	2.89	12.95
C20:0	0.44	0.61	0.33	1.55	0.10	0.46	0.40
Total SFA	16.50	20.20	17.55	46.24	9.39	17.81	15.96
Total MUFA	29.25	27.30	39.14	7.62	21.22	27.97	25.78
Total PUFA	54.25	52.50	43.31	46.13	69.39	54.23	58.26

HMC: high moisture corn, CS: corn silage, PS: protein supplement, H: hay, WL: whole linseed, TMR-C: total mixture ration-control diet, TMR-L: total mixture ration-linseed diet.

[a] Supplied (on DM basis): 30 000 U of vitamin A, 3 300 U of vitamin D3, 120 mg of vitamin E/kg, 37.5 mg Cu/kg.

[b] Percentage of total fatty acids quantified.

Material and methods

- **Carcass and meat quality**
  - Animals were transported ~10 km and slaughtered at either 13 or 17 months of age in commercial slaughterhouse using standard procedure
  - Weights (kg) at slaughter and of hot carcasses, dressing-out %, as well as EUROP conformation and fatness scores were recorded
  - *M. longissimus thoracis* pH and colour (CIE *Lab*) measured at 24h *post mortem,* sampled (at 8th rib level) and stored frozen (-20 ºC) until chemical analyses
- **Intramuscular fat (IMF)**
  - Soxhlet extraction with hydrolysis

(SIST ISO, 2001)

Material and methods

- **Fatty acid analysis**
  - FA composition (of total lipids) was determined by gas liquid chromatography using *in situ* transesterification method

(Park and Goins, 1994)

- **Oxidative stability**
  - Samples analyzed after 0, 3 and 6 days of cold (4 ºC) storage, measured by assaying 2-thiobarbituric acid-reactive substances (TBARS), expressed as mg of malonaldehyde (MDA) per kg of tissue

(Botsoglou et al, 1994)

- **Statistical analysis**
  - Analysis of variance by GLM procedure, with the model including slaughter age, diet, and their interaction as fixed effects

(SAS, 2002)

Results – Carcass traits

Traits	13 months		17 months			Significance [a]		
	Control	Linseed	Control	Linseed	RMSE	SA	D	SAxD
n	*20*	*20*	*20*	*20*				
Initial age (days)	222.0	222.3	218.2	221.1	8.5	ns	ns	ns
Slaughter age (days)	388.0	388.3	508.7	515.1	10.9	***	ns	ns
Initial weight (kg)	311.6	310.1	282.6	274.5	13.4	***	ns	ns
Final weight (kg)	552.5	556.5	632.1	626.7	40.7	***	ns	ns
Hot carcass weight (kg)	317.3	327.7	374.8	376.1	25.6	***	ns	ns
Dressing out (%)	57.4	58.9	59.3	60.0	1.39	***	**	ns
Trimmed fat [b] (kg)	5.3	5.1	7.2	7.5	2.8	***	ns	ns
Conformation score [c]	3.85[b]	4.35[a]	4.70[a]	4.70[a]	0.56	na	na	*
Fatness score [d]	2.95	2.90	3.05	3.10	0.31	*	ns	ns

[a] Significance of main effects (SA-slaughter age, D-diet) and their interaction (SAxD): *P<0.05; **P<0.01; ***P<0.001; ns - not significant; na – not applicable due to SAxD interaction ([a,b] within a row, least-square means lacking a common superscript letters significantly differ at P<0.05)
RMSE – root mean squared error
[b] excessive covering fat from round and groin area and internal fat depots (kidney and pelvic fat)
[c] 1= poor to 5= excellent
[d] 1= minimum to 5= maximum

Results – Meat quality traits

Traits	13 months		17 months			Significance [a]		
	Control	Linseed	Control	Linseed	RMSE	SA	D	SAxD
n	*20*	*20*	*20*	*20*				
pH24	5.73	5.70	5.68	5.87	0.34	ns	ns	ns
CIE L* (lightness)	41.20	41.45	39.22	38.67	2.43	***	ns	ns
CIE a* (redness)	23.48	23.50	23.65	23.23	1.31	ns	ns	ns
CIE b* (yellowness)	8.64	8.88	8.44	7.78	1.16	*	ns	†
Intramuscular fat (g/kg)	23.4	20.4	28.0	28.3	9.47	**	ns	ns

[a] Significance of main effects (SA-slaughter age, D-diet) and their interaction (SAxD): †P<0.1; *P<0.05; **P<0.01; ***P<0.001; ns - not significant; RMSE – root mean squared error

Results – Intramuscular SFA profile

g/100 g of total FA	13 months		17 months			Significance [a]		
	Control	Linseed	Control	Linseed	RMSE	SA	D	SAxD
n	*20*	*20*	*20*	*20*				
C14:0	2.94^{a}	2.52^{b}	2.89^{ab}	2.93^{a}	0.50	na	na	*
C15:0	0.43^{a}	0.36^{b}	0.36^{b}	0.39^{ab}	0.08	na	na	*
C16:0	23.72	22.63	24.22	23.78	2.00	†	†	ns
C17:0	1.11	0.99	0.95	0.99	0.20	†	ns	†
C18:0	17.46	16.64	16.87	16.72	1.60	ns	ns	ns
Σ SFA	45.87	43.42	45.44	44.95	2.33	ns	**	†

[a] Significance of main effects (SA-slaughter age, D-diet) and their interaction (SAxD): †P<0.1; *P<0.05; **P<0.01; ns - not significant; na – not applicable due to SAxD interaction ([a,b] within a row, least-square means lacking a common superscript letters significantly differ at P<0.05)
RMSE – root mean squared error
Σ SFA - saturated fatty acids = (C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C22:0 + C24:0).

Results – Intramuscular MUFA profile

g/100 g of total FA	13 months		17 months		RMSE	Significance [a]		
	Control	Linseed	Control	Linseed		SA	D	SAxD
n	*20*	*20*	*20*	*20*				
C12:1	0.67	0.55	0.59	0.57	0.27	ns	ns	ns
C14:1	0.45	0.39	0.41	0.41	0.17	ns	ns	ns
C16:1	2.85	3.01	2.84	2.82	0.54	ns	ns	ns
C17:1	1.00	0.92	0.85	0.88	0.18	*	ns	ns
C18:1	37.55	37.28	37.76	38.46	3.36	ns	ns	ns
C20:1	0.26	0.25	0.27	0.27	0.04	ns	ns	ns
Σ MUFA	42.89	42.55	42.51	43.41	3.62	ns	ns	ns

[a] Significance of main effects (SA-slaughter age, D-diet) and their interaction (SAxD): *P<0.05; ns - not significant; RMSE – root mean squared error
Σ MUFA - monounsaturated fatty acids = (C12:1 + C13:1 + C14:1 + C15:1 + C16:1 + C17:1 + C18:1 + C19:1 + C20:1 + C22:1 + C24:1)

Results – Intramuscular PUFA profile

g/100 g of total FA	13 months		17 months		RMSE	Significance [a]		
	Control	Linseed	Control	Linseed		SA	D	SAxD
n	*20*	*20*	*20*	*20*				
C18:2 n-6	7.05	8.63	8.07	7.76	2.99	ns	ns	ns
C18:3 n-3	0.28^{b}	0.56^{a}	0.31^{b}	0.44^{a}	0.14	na	na	*
CLA	0.21	0.22	0.29	0.33	0.07	***	†	ns
C20:2 n-6	0.17	0.13	0.17	0.13	0.08	ns	†	ns
C20:3 n-6	0.44	0.51	0.44	0.39	0.20	ns	ns	ns
C20:4 n-6	2.18	2.70	2.11	1.93	1.00	†	ns	ns
C20:5 n-3	0.10	0.17	0.06	0.09	0.08	**	*	ns
C22:4 n-6	0.40	0.45	0.38	0.31	0.14	**	ns	†
C22:5 n-3	0.25^{b}	0.48^{a}	0.21^{b}	0.29^{b}	0.15	na	na	*
Σ PUFA	11.24	14.03	12.05	11.64	4.61	ns	ns	ns
Σ n-3 PUFA	0.67^{b}	1.27^{a}	0.56^{b}	0.80^{b}	0.33	na	na	*
Σ n-6 PUFA	10.36	12.54	11.20	10.51	4.35	ns	ns	ns

[a] Significance of main effects (SA-slaughter age, D-diet) and their interaction: †P<0.1; *P<0.05; **P<0.01; ***P<0.001; ns - not significant; na – not applicable due to SAxD interaction (a,b within a row, least-square means lacking a common superscript letters significantly differ at P<0.05); RMSE – root mean squared error; Σ PUFA - polyunsaturated fatty acids = (C18:2n–6 + C18:3n–6 + C18:2n–7 + C20:2n–6 + C20:3n–6 + C20:4n–6 + C22:2n-6 + C22:4n–6 + C22:5n-6 + C18:3n–3 + C20:3n–3 + C20:5n–3 + C22:5n–3 + C22:6n–3)

Results – Nutritional indices

	13 months		17 months			Significance [a]		
	Control	Linseed	Control	Linseed	RMSE	SA	D	SAxD
n	*20*	*20*	*20*	*20*				
n-6/n-3 PUFA [b]	15.64	9.80	20.96	13.02	3.01	***	***	ns
PUFA/SFA [c]	0.25	0.33	0.27	0.26	0.12	ns	ns	†
AI [d]	0.66	0.57	0.66	0.65	0.09	†	*	ns

[a] Significance of main effects (SA-slaughter age, D-diet) and their interaction (SAxD): †P<0.1; *P<0.05; ***P<0.001; ns - not significant;
RMSE – root mean squared error
[b] Recommendation for human diet < 4
[c] Recommendation for human diet ≥ 0.4
[d] Atherogenicity index = (C12:0 + 4 x C14:0 + C16:0) / (n-6 PUFA + n-3 PUFA + MUFA)
(Ulbricht and Southgate, 1991)

Results – Oxidative stability

TBARS (mg MDA/kg tissue)	13 months		17 months			Significance [a]		
	Control	Linseed	Control	Linseed	RMSE	SA	D	SAxD
n	*20*	*20*	*20*	*20*				
Day 0	0.24	0.35	0.06	0.12	0.10	***	**	ns
Day 3	0.74	1.18	0.13	0.36	0.55	***	*	ns
Day 6	1.04	1.68	0.22	0.52	0.73	***	*	ns

[a] Significance of main effects (SA-slaughter age, D-diet) and their interaction (SAxD): *P<0.05; **P<0.01; ***P<0.001; ns - not significant;
RMSE – root mean squared error
TBARS - 2-thiobarbituric acid-reactive substances, MDA - malonaldehide

Conclusions

- Both, slaughter age and diet influenced the intramuscular FA profile of young bulls
- Bulls slaughtered at an older age, besides better slaughter performance, had darker and fatter meat with more CLA, but also higher n-6/n-3 PUFA ratio
- Diet had no influence on carcass and meat quality, except for slightly higher dressing-out % in linseed-fed animals
- Feeding whole linseed improved the nutritional value of beef in terms of FA composition, e.g., more n-3 PUFA, less SFA and lower n-6/n-3 ratio and AI
- Results of linseed supplementation were more pronounced in yearling bulls, while effects in more grown-up animals, particularly regard increasing the n-3 PUFA, were less clear
- Higher dietary levels of linseed (and antioxidant) should be investigated

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Thanks for your attention!