Influence of vitamin A, D & E status on post-mortem meat quality in steers

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Abstract

To determine if removal of vitamin supplements would negatively impact meat quality, Swedish Red Milk Breed steers (n=27, BW=405kg, SD=34kg) were divided into two treatment groups (vitamin supplemented vs. non-supplemented). Half the animals from each group were slaughtered after ca. 155d indoor feeding. Remaining grazed wooded pasture ca. 120d. Individual animal was the experimental unit; common endpoint was 630kg LW. Indoor vitamin- A, D and E supplement added to the feed met Swedish recommendations, outdoor was *ad libitum* access to a vitamin/mineral mix. All analysis performed on *longissimus dorsi* sections. No effect of treatment was noted for shear force values. Shear force was similar between aging 2d and 7d post-slaughter, but significantly decreased by 14d. The μ- and m- calpain system did not show any influence from vitamin restriction, whereas calpastatin content showed some response. There was no indication that vitamin A or E content of muscle was affected by the vitamin treatment. The failure to detect differences due to lack of vitamin supplementation indicates requirements were met by the non-supplemented diet.

Introduction

Tenderness is the most important parameter when it comes to consumer preference (Koohmaraie and Geesink 2006). Post-slaughter microfibrillar proteolysis by the calpains has a significant influence on final tenderness of the muscle (Bergh Houbak et al. 2008). The calpain system is activated by Ca^{2+}, which is related to ante-mortem vitamin D intake. Calpastatin regulates μ-calpain activity through inhibition (Koohmaraie and Geesink 2006) being more active in young, faster growing animals (McDonagh et al. 2001).

Large seasonal variations in circannual sunlight and climate in Scandinavia can potentially affect the vitamin status of cattle. Readdressing the vitamin requirements for optimal growth under various finishing conditions will contribute to the animals' health and welfare. Impact of vitamin status on post-slaughter tenderizing will also need to be re-assessed to ensure quality meat is available to Swedish consumers. Our investigation will focus on the pre-slaughter vitamin status of the animals and how that has an influence on the Ca^{2+} driven μ- and m-calpain enzyme activity, and ultimate meat tenderness.

Material and methods

Swedish Red Milk Breed steers (n=27 BW=405kg, SD=34kg) were raised at the Götala Research Station (Skara, SE), pre-weaning management was unknown. Beginning of winter housing, steers were divided into vitamin-supplemented and non-supplemented treatment groups. Steers were fed *ad libitum* indoors a 55:45 DM barley:grass silage diet ca 155d. Half the animals from each group were slaughtered; remaining animals grazed wooded grassland by treatment ca. 120d. Common endpoint was 630kg LW for all animals. Vitamin supplementation (2:1 Effekt Normal, Lantmännen, SE) indoors included daily amounts in the feed that met Swedish recommendations and free-choice access to a vitamin/mineral mix outdoors. Vitamin supplementation per animal while indoors was as follows: Vitamin A 40 000 IU/d, Vitamin D_3 10 000 IU/d, Vitamin E 300 mg/d; free-choice supplement contained Vitamin A 4000 IU/g, Vitamin D_3 1 000 IU/g, Vitamin E 3 mg/g.

A core sample (10g) was removed from the left *longissimus dorsi* (LD) and frozen in liquid nitrogen 40 minutes post-exsanguination. Entire left side loins were collected from the steers 24h after slaughter, vacuum packed and transported at 5°C to Uppsala. 48h post-slaughter, pH was measured then a 2cm slice was collected for vitamin and lipid analysis. Remaining loin was divided into sequential 3cm sections, re-vacuum packed and aged at 5°C for 2, 7 or 14 days post-slaughter then frozen at -20°C until analysis.

Warner-Bratzler shear force (WB-SF) analysis of the aged samples was as described by Honikel (1998). Peak and total force were measured on 11 core samples (1x1x3cm) per animal, cut parallel to the muscle fibre. Blade dimensions were: cutting area of 11x15mm; blade thickness of 1mm; blade speed of 0.83mm s^{-1}.

Muscle lipid content was determined gravimetrically following the method of Hara and Radin (1978). Vitamin A and E content was determined using the extracted lipid as described by Högberg et al. (2002).

Calpains and calpastatin activity were measured as described by Pompomio et al (2008). µ- and m-calpains were measured by zymogram gels at 80V for 3h at 4°C. Quantitative comparisons were made using the density of a reference standard. Calpastatin was quantified by inhibition of m-calpain. Absorbance was measured at 278nm, one unit calpastatin activity equated to one unit m-calpain activity inhibition.

Statistics analysis used the Mixed Procedure ANOVA using treatment as the main effect (SASv9.1, Cary, NC, USA), with significance level of $P<0.05$. Individual animal was used as a random effect in the model.

Results

Maximum and total WB-SF of the LD were unaffected by treatment (Table 1). Tenderness was equivalent between 2d and 7d aging; but tenderness increased significantly by 14d. Treatment and aging interactions showed no significant effect (data not shown). Loins from non-supplemented pasture animals had the highest final pH, with supplemented pasture animals being intermediate; however no influence on tenderness was noted (Table 1). There were no differences for drip-loss or cooking-loss between treatments (data not shown).

Table 1. Carcass and meat quality measurements in *longissimus dorsi* of Swedish Red Milk Breed steers finished under four vitamin supplementation regimes; aged 2, 7 or 14 days in vacuum bags stored at 5°C

	Treatment[z]					Meat Aging			
	No-Vit IN n=8	Vit IN n=6	No-Vit OUT n=7	Vit OUT n=6	PSEM[y]	Day 2 n=27	Day 7 n=27	Day 14 n=27	PSEM[y]
Age (mo)	22.8	22.3	26.1	25.5					
HCW[x] (-2%)	324.3	322.5	323.4	321.8	5.36				
pH	5.45 b	5.49 b	5.58 a	5.53 ab	0.03				
Max. Force (N)	30.87	33.74	41.08	35.89	4.94	41.58 a	39.12 a	25.49 b	2.77
Total Energy (N)	189.81	200.69	234.41	211.12	23.55	228.0 a	233.0 a	166.0 b	12.90

[z]No-Vit IN-Finished indoors on 45:55 Sil:conc diet, no vitamin supplement,Vit IN- Finished indoors on 45:55 Sil:conc diet, vitamin supplement,
No-Vit OUT-Finished outdoors on pasture, no vitamins supplemented, Vit OUT-Finished outdoors on pasture, vitamins supplemented
[y] Pooled standard error of mean
[x] Hot carcass weight, -2% shrink
[a,b] Different letters within a row indicate significant differences ($p<0.05$)

Lipid content of the LD was unaffected by the treatments (Table 2). There was no significant difference between treatments for vitamin E content measured by either lipid or tissue content. Animals housed indoors had higher vitamin A content than those housed outdoors; however no differences were apparent when converted to proportion fresh tissue. Blood vitamin D content analysis is incomplete. Effects due to vitamin supplementation were not apparent.

Table 2. Vitamin content of *longissimus dorsi* of Swedish Red Milk Breed steers finished under four vitamin supplementation regimes

	No-Vit IN[z] n=8	Vit IN n=6	No-Vit OUT n=7	Vit OUT n=6	PSEM
Lipid %	5.55	4.99	5.68	5.96	0.65
Vit-E, mg/100g lipid	2.28	3.38	3.06	2.81	0.41
Vit-A, µg/100g lipid	0.48 a	0.55 a	0.37 b	0.38 b	0.03
Vit-D, mg/100ml blood	*Incomplete analysis of data*				
Vit-E, mg/g tissue	12.00	15.99	15.32	15.65	1.36
Vit-A, µg/g tissue	2.65	2.70	2.09	2.19	0.25

[z] *definitions see table 1*
[a,u] Different letters within a row indicate significant differences ($P<0.05$)

Both µ- and m-calpain content were unaffected by vitamin supplementation during either the indoor or outdoor treatments (Table 3). Calpastatin content was higher for indoor animals receiving vitamin supplements. No distinct pattern between calpain and calpastatin levels in relation to treatment was apparent. There were no significant correlations between calpain and calpastatin values (data not shown).

Table 3. µ-, m-calpain and calpastatin values of Swedish Red Milk Breed steers finished under four vitamin supplementation regimes

	No-Vit IN[z] n=8	Vit IN n=6	No-Vit OUT n=7	Vit OUT n=6	PSEM
µ-calpain[y]	0.98	0.96	0.90	0.92	0.03
m-calpain[y]	1.06	1.09	1.00	1.10	0.06
Calpastatin	29.2 b	36.9 a	31.8 b	33.8 b	1.79

[z] *definitions see table 1*

[y] calpain and calpastatin measurments in arbitrary Units/g tissue

[a,b] Different letters within a row indicate significant differences (p <0.05)

Discussion

Lack of distinction between treatments within this trial of measured quality parameters would suggest that the vitamins available within the diet were able to meet the animals' requirements without influencing meat quality; however trial design limitations prevent any definitive conclusions. Animal diet composition most likely led to the higher pH of pasture fed animals, though age and slaughter conditions may have been factors (Priolo et al. 2001). All animals were within acceptable pH ranges (5.40-5.60), thus should not have affected tenderness. There was no indication that vitamin treatment influenced either maximum or total WB-SF measurements. No influence of animal age on tenderness was noted in this trial. Although there was an indication that non-supplemented indoor fed animals had lower vitamin E content, there was no clear distinction between treatments for vitamin E content. The significance of animal housing and not supplementation would suggest that the animals had greater vitamin A stores prior to trial initiation, possibly due to a multi-vitamin injection at weaning. The lower vitamin A content of animals on pasture is confusing, possibly confounded by a dilution effect of body fat and/or poor pasture quality. Inclusion of vitamin D analysis should clarify the animals' status.

Post-mortem calpain activity of the LD is regulated by Ca^{2+} content, which in turn is regulated by available vitamin D within the living animal. Removal of dietary vitamin supplements did not seem to inhibit the animals' ability to sequester Ca^{2+}, suggesting that the indoor animals were receiving enough UV light to form vitamin D. Although the calpastatin content was greater for indoor vitamin supplemented animals, there was no perceivable effect to tissue tenderness. No direct correlation between increased vitamin supplementation and higher calpastatin activity has been reported in literature. However, a connection between greater average daily gain as a result of meeting the animals' vitamin requirements, and increased protein accretion may partially explain the increased calpastatin activity of the indoor supplemented animals (Morgan et al. 1993)

Conclusions

Absence of Swedish recommended vitamin supplementation to Swedish Red Milk Breed steers during the finishing period did not have an influence on measured meat quality parameters in this trial. Exact threshold parameters for the influence of the calpain proteolysis system should be further explored using scaled vitamin dosages under tightly controlled feeding systems before making any changes to current vitamin allowance recommendations. In this trial, vitamins supplied by the diet were probably sufficient to provide a product of equivalent quality to that from animals supplemented at current nutritional recommendations.

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