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THE EFFECT OF FEEDING CLOVER SILAGE ON POLYUNSATURATED FATTY ACID AND VITAMIN E CONTENT, SENSORY, COLOUR AND LIPID OXIDATIVE SHELF LIFE, OF BEEF LOIN STEAKS

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Introduction

Grass and legumes are important constituents of the forage-based feeding systems used for beef in the UK. European Union policy on sustainability of the environment discourages the use of inorganic fertilizers for grass production and encourages the use of legumes. Their inclusion in a sward also produces a higher economic benefit for the livestock sector compared to grass-only systems (Rochon *et al.*, 2004). The inclusion of white and red clovers in swards and silages has been shown to increase dry matter intake and liveweight gain for beef cattle (Yarrow & Penning, 2001). In addition, studies on beef finished on clover pastures/silages showed a beneficial contribution of red clover to higher polyunsaturated fatty acid (PUFA), especially contents of meat n-3 PUFA (Enser *et al.*, 2001a; Scollan *et al.*, 2002). Clover silage produced a lower biohydrogenation and higher flow of PUFA through the rumen (Lee et al., 2003) and this may have been due to higher concentrations of polyphenol oxidase activity in the red clover (Lee et al., 2004)

Green forage and other leafy materials, including good quality hay and silage, are not only good sources of vitamin E but also of other compounds with antioxidant activity such as carotenoids and flavonoids (McDowell *et al.*, 1996; Gatellier *et al*., 2005). The vitamin E content of fresh herbage is between 5 to 10 times as great as that in some cereals or their by-products, thus meat from grass-fed animals is able to delay lipid oxidation and discoloration thereby maintaining a better shelf-life during retail display compared to meat from standard commercial concentrate-fed beef cattle ((McDowell *et al.*, 1996; Faustman & Wang, 2000)

Whilst an increase in n-3 PUFA can be beneficial in terms of human health, they can put an oxidative stress on meat systems. In a previous trial it was noted that meat from animals that had grazed mixed grass/clover swards were more oxidatively unstable and had lower vitamin E concentrations in the meat than that from animals grazed on a grass-only sward. (Enser et al., 2001; Scollan et al., 2002) A recent study (Al-Mabruk *et al*., 2004) has shown that milk from cows fed legumes (alfafa, white clover and red clover) had milk with lower oxidative stability compared to that from grass-fed animals and that vitamin E supplementation prevented this increased oxidative deterioration

Objectives

The present study aimed to establish the effect of feeding graded amounts of red clover silage with grass silage on intramuscular fatty acid composition, shelf-life (lipid and colour stability) and eating quality of beef meat and the effect of a vitamin E supplement on these characteristics.

Methodology

Thirty-two (32) Charolais steers with a mean initial live weight of 490 kg (s.e. 6.7 kg) were randomly allocated to one of four treatments, resulting in eight animals per treatment. The animals were fed *ad libitum* on forage and standard commercial concentrates to achieve approximately 0.7 and 0.3 of the dietary dry matter intake arespectively. The forages were as follows: GS: 100% grass silage; GCS: grass and red clover silage mix (50:50 DM basis); CS: 100% Red clover silage; $CS^{+}E$: Red clover silage plus high vitamin E concentrate (500 IU/kg).

The grass silage used was first cut perennial ryegrass (*Lolium perenne*) harvested in May, and the red clover (*Trifolium pratense*) silage was harvested in July of 2003. A biological innoculant (Powerstart, Genus Ltd) was added during collection at the recommended rate to aid the fermentation process. The grass was ensiled in a concrete clamp, covered by plastic and weighted down using tyres and bales and the clover was wilted for a 24 period before ensiling in big bales.

Animals within a treatment were penned together and fed individually using roughage intake control feeders and concentrate feeders. They were accustomed to the diet during a 21-day preliminary period and remained on treatment for 100 days. Animals were transported on the day before slaughter and were kept in lairage overnight with access to water and slaughtered conventionally using captive bolt stunning. Carcasses were held in chill (1-2°C) for 48 h before butchering and sampling.

At 48h post-mortem, samples of m. *longissimus thoracis* at the 11th rib were removed and blast frozen for fatty acid and vitamin E analysis. An additional 180mm section of muscle was conditioned at 1°C for 12 days in vacuum pack. A 100mm section was then frozen at -20°C for sensory analysis. After overnight thawing at 1°C, 20mm thick steaks were cut and grilled to 74°C internal temperature. The meat was assessed by a 10 person trained taste panel using 100mm unstructured line scales (see Vatansever et al., 2000). Four steaks 20mm thick were cut from the remaining sample, packed in modified atmosphere trays (O_2:CO_2, 75:25) and subjected to simulated retail display (700lux lighting for 16h a day, 4ºC±1ºC). Colour (L*a*b*) was measured on the surface of two steaks at three points, daily with a Minolta Chromameter. The saturation (chroma), $\sqrt{a^{*2}+b^{*2}}$, describing the intensity of the colour was calculated (MacDougall and Rhodes, 1972). The remaining steaks were taken at 7d of display and analysed for lipid oxidation as thiobarbituric acid reacting substances (TBARS) by the methods of Tarladgis et al. (1960).

Lipid was extracted using chloroform/methanol as per Folch *et al.* (1957) and separated into neutral and phospholipid. Fatty acid methyl esters were prepared by alkaline hydrolysis followed by methylation with diazomethane and analysed on a CP Sil 88, 100m x 0.25mm ID column (Chrompack, UK) and individual fatty acids quantified,

as described by Demirel *et al*. (2004). Total fatty acid was taken as the sum of all the phospholipid and neutral lipid fatty acids quantified. The extraction and HPLC separation and quantification of vitamin E was essentially as described by Liu et al. (1996) using rac-5, 7-dimethyltocol as internal standard.

Results & Discussion

Carcass, fat and fatty acid composition

Increasing the amount of clover silage in the diet tended to increase the dry matter intake, liveweight gain and carcass conformation of the animals but not significantly so over the 100d feeding period. Total fat content of the muscle (TFA), amount of saturated fatty acids (SFA), and monounsaturated fatty acids (MUFA) tended to increase (non-significantly) with the proportion of clover in the diet (Table 1), whilst the amount of polyunsaturated fatty acids (PUFA) increased significantly ($p<0.001$) with each increment. This was less clear with the CS+E group, which had less total fat. The PUFA change was due to increase in 18:2n-6 and 18:3n-3 (significant at $p<0.001$) and long chain PUFA (non-significant). Overall there was a significant decline in the ratio of 18:2n-6:18:3n-3 and a small but significant ($p<0.01$)increase in the P:S ratio as clover increased. In the neutral lipid fraction (results not shown) there was a similar pattern to that for total lipid, with TFA, SFA and MUFA tending to increase with each increment in clover, whilst PUFA ($p<0.05$) and 18:3n-3 ($p<0.01$) increased significantly. There were more changes in the composition of phospholipid fatty acids and as these affect lipid stability, a fuller set of results is shown in Table 2. TFA, SFA and PUFA all increased significantly with red clover addition. The percentage of 18:2n-6 and 18:3n-3 increased significantly with each increment in red clover fed ($p<0.001$) replacing, in part, the MUFAs, 18:1 *cis*-9 and 20:1 and conjugated linoleic acid (CLA 9-*cis*,11-*trans* C18:2). The fatty acid composition of the muscle of animals fed the CS+E was essentially the same as that from animals without the supplement.

Shelf life

The values for vitamin E concentration, TBARs and colour saturation after 7 days simulated retail display, and selected sensory attributes are shown in Table 3. TBARS increased incrementally with increasing amount of clover in the diet, but the largest and most significant change ($p<0.001$) was from GCS to CS. This was mirrored by the change in colour saturation with the intensity of redness decreasing with increasing increments of clover in the diet (Figure 1). The amount of unsaturated fatty acids in the meat increased with increasing amounts of clover inclusion in the diet and would have placed a greater oxidative stress upon the system. However, it would appear that these results can be explained, at least in part, by the concentration of vitamin E found in the muscle. As the amount of clover in the silage increased so the concentration of vitamin E in the muscle decreased ($p<0.001$). Adding a supra-nutritional amount of vitamin E to the 100% red clover diet restored the concentration of muscle vitamin E to that found in meat from grass-fed animals, reduced the TBARS value and increased the colour stability (Figure 1). It has been suggested that optimum stability for meat is obtained when the

muscle concentration of m. <I>longissimus</I> is 3-3.5 mg/kg lean muscle (Liu et al., 1996) and this was reached in muscle from GS and CS+ animals.

There was no effect of diet on sensory characteristics, though the CS+E diet produced the toughest meat (which was least fat). There was a trend for the more unstable meat (that from CS) to have slightly more rancid and fishy notes.

Conclusions

Feeding red clover silage as 0.7 of the diet increased the content of the beneficial PUFA in meat, but this was at the expense of both lipid and colour stability. This instability was more easily explainable as being due to a low vitamin E content rather than the increased content of PUFA, since a supra-nutritional supplement of vitamin E in the diet restored the vitamin E concentration and stability of the meat to that seen with other diets.

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Tables and Figures

Table 1. Total fatty acid composition (mg/100g of lean tissue) and ratios in beef loin steak

Muscle	GS	GCS	CS	CS^{+}	sed	sig
Total FA	3081	3639	4001	3074	0.36	NS
SFA	1339	1584	1760	1322	276.9	NS
MUFA[b]	1300	1535	1643	1270	261.7	NS
PUFA	170.7^{a}	206.4^{b}	244.4^{c}	216.8^{bc}	13.44	***
18:2n-6	73.2^{a}	92.8^{b}	113.2^{c}	99.3^{b}	6.68	***
18:3n-3	22.5^{a}	34.1^{b}	50.7^{c}	37.5^{b}	3.83	***
P:S[†]	0.07^{a}	0.09^{ab}	0.10^{bc}	0.12^{c}	0.01	**
18:2n-6:18:3n-3	3.28^{c}	2.73^{b}	2.30^{a}	2.66^{b}	0.15	***

[a,b,c]means within a row with the same letter do not differ significantly (Fisher's least significant difference procedure, post hoc)

*** $p \leq 0.001$, NS - not significant

GS- grass silage; GCS- mixture (50/50) of grass and red clover silage; CS- red clover silage; CS^{+}- red clover silage plus vitamin E supplement (500 IU/kg concentrate)

P:S calculated as (18:2n-6 + 18:3n-3)/(12:0+14:0+16:0+18:0)

SFA calculated as (12:0+14:0+16:0+18:0)

MUFA calculated as (16:1+18:1trans+18:1 cis-9+18:1cis-11+20:1)

PUFA calculated as (18:2n-6+18:3n-3+20:3n-6+20:4n-6+20:5n-3+22:4n-6+22:5n-3+22:6n-3

Table 2. Fatty acid composition of the phospholipid fraction of lean tissue

% of fatty acids

	GS	GCS	CS	CS+E	sed	sig
14:0	0.28	0.29	0.39	0.35	0.06	ns
16:0	14.48	14.01	14.97	14.78	0.44	ns
18:0	9.76	10.00	10.09	9.74	0.02	ns
18:1 cis-9	22.55^{b}	20.98^{ab}	19.30^{a}	19.02^{a}	1.28	*
18:2n-6	10.54^{a}	12.07^{b}	13.38^{b}	13.57^{b}	0.74	***
18:3n-3	2.58^{a}	3.42^{b}	4.36^{c}	3.98^{c}	0.26	***
CLA	0.21^{b}	0.17^{a}	0.15^{a}	0.15^{a}	0.02	**

Table 3. Meat quality measurements, shelf life and sensory

	GS	GCS	CS	CS+E	sed	sig
TBARS d7	0.64^{a}	1.31^{b}	4.88^{c}	1.13^{b}	0.45	***
Colour saturation d7	22.9^{a}	21.5^{b}	20.3^{c}	22.6^{ab}	0.57	***
Vitamin E	3.47^{a}	2.92^{b}	1.80^{c}	3.32^{a}	0.18	***
Toughness	46.5^{ab}	41.6^{a}	42.0^{a}	48.2^{b}	2.8	*
Beef flavour	31.7	34.2	34.2	31.0	2.28	ns
Rancid	0.2	0.3	0.6	0.3	0.3	ns
Fishy	0.6	0.2	1.1	0.4	0.5	ns

Figure 1. Effect of diet and days displayed upon colour saturation of beef loin steaks during simulated retail display in MAP.