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Effect of intramuscular injections of DL-α-tocopheryl acetate on growth performance and extracellular matrix of growing lambs

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The effect of intramuscular injections of vitamin E on growth, carcass traits, intramuscular collagen (IMC) characteristics and decorin of growing lambs was studied. A total of 24 15-day-old Ile de France suckling male lambs were divided into two groups and weekly intramuscular injections of DL-α-tocopheryl acetate (control group, 0 IU; Vitamin E treatment, 150 IU) were given until the lambs were 64 days old. Lambs were individually weighted at 15, 29, 43, 57 days of age and at slaughter (71 days old). Dry matter intake and average daily weight gain were recorded. Hot and cold carcass weights were recorded and dressing percentages were calculated after dressing and chilling (2°C to 4°C for 24 h). Carcass shrink losses were calculated as well. Longissimus *muscle (LM) pH and area were measured. The pelvic limb was removed and its percentage was calculated based on cold carcass weight. IMC and decorin analyses were assessed on LM and* semimembranosus *muscle (SM). DL-α-tocopheryl acetate treatment reduced (*$P < 0.05$*) collagen maturity and increased (*$P < 0.05$*) decorin in both LM and SM muscles of growing lambs, while it did not affect IMC content. In addition, vitamin E did not influence growth, carcass weight, dressing percentage, carcass shrink losses and area of LM but decreased (*$P < 0.05$*) the pelvic limb percentage. The LM pH values were higher (*$P < 0.05$*) in vitamin group than in control group. Furthermore, different IMC characteristics between the muscles (*$P < 0.01$*) were apparent. Multiple intramuscular injections of DL-α-tocopheryl acetate influence extracellular matrix in lambs, which could affect meat tenderness.*

Keywords: decorin, growth, intramuscular collagen, lambs, vitamin E

Implications

Growth, carcass characteristics and meat quality are important components of an efficient lamb production system. Vitamin E plays an important role in enhancing meat stability and improving meat quality. This study shows that intramuscular injections of vitamin E, given to growing lambs, reduced collagen maturity, which could increase meat tenderness, but decreased the percentage of pelvic limb.

Introduction

Vitamin E is the generic term used for all tocol and tocotrienol derivatives that exhibit the activity of α-tocopherol. Vitamin E has been known for its roles as the principal lipid-soluble chain-breaking antioxidant in biological membranes (Chan, 1993) and in improving the quality characteristics of meat such as color, flavor, texture and nutritional value, and also extending its shelf-life (Morrisey *et al*., 1994; Kasapidou *et al*., 2012). Contradictory data regarding the effect of vitamin E on growth traits and collagen synthesis in lambs (reviewed in the study by Maiorano *et al*., 2007) and pigs (reviewed in the study by Maiorano *et al*., 1999) exist. Some works have demonstrated a beneficial effect of vitamin E treatment on growth traits in lambs (900 IU DL-α-tocopheryl acetate, Gentry *et al*., 1992; 15 mg vitamin E/lamb per day, Macit *et al*., 2003a). Conversely, other authors (1500 IU α-tocopherol, Birch *et al*., 1994; 1200 IU DL-α-tocopheryl Maiorano *et al*., 2007) observed a lower carcass weight and a negative effect on carcass wholesale cut weights in lambs injected with vitamin E. Several other studies provide evidence that vitamin E influences intramuscular collagen (IMC) characteristics, improving collagen solubility (50 or 100 μM α-tocopherol *in vitro*, Archile-Contreras *et al*., 2011) or reducing IMC maturity (Maiorano *et al*., 2007), which could affect meat tenderness. On the other hand, it has been reported that the use of 1000 IU of vitamin E, administered daily for 104 days before slaughter, effectively decreased

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toughness of *longissimus* steaks from heifers (Carnagey *et al*., 2008). However, according to the available information, no research has yet been conducted to study the effect of vitamin E on proteoglycan decorin. The extracellular matrix of muscle is composed mostly of the protein collagen with lesser quantities of other constituents such as proteoglycans also present (McCormick, 1999). It has been suggested a potential role for the proteoglycan decorin (a small dermatan sulfate) in regulating collagen fibrillogenesis, ordering the spatial arrangement of collagen molecules and, thus, influencing crosslinking patterns (McCormick, 1999).

The objective of the present study was to determine the effect of DL-α-tocopheryl acetate injections on growth, carcass traits and extracellular matrix in growing lambs.

Material and methods

Animals

Animal handling followed the recommendations of European Union directive 86/609/EEC and Italian law 116/92 concerning animal care. Lambs were reared in Molise, on a farm situated near the Abruzzi, Lazio and Molise National Park (Italy) at 560 m above sea level (latitude: 41°29'12"12 N, longitude: 14°28'25"68 E).

The trial was carried out on 24 15-day-old Ile de France suckling male lambs (average BW of 7.20 kg), born as singles in late February from 3-year-old dams of the same weight (58 ± 1.8 kg). The ewes selected for the experiment were homogeneous in terms of parity and of milk yield and milk protein and fat contents of previous lactations. During the experiment, all dams, reared indoors, were fed with 1.4 to 1.7 kg vetch/oat alfalfa and polyphitic hay and 0.5 to 0.7 kg of concentrate and had free access to water. The lambs were randomly allotted to two groups: control (C, $n = 12$) or vitamin E-treated (V, $n = 12$). From the beginning of the study (15 days of age) until day 64 of age, each lamb of the V group received intramuscular injections of DL-α-tocopheryl acetate (left gluteus, 150 IU/week) in aqueous solution (Vitalene E; Fatro, Bologna, Italy) for 8 weeks for a total dose of 1200 IU. The C group lambs received injections of physiological saline. All animals received only maternal milk until the 21th day of age and, until weaned at 28th day of age, they had free access to a starter feed (18% CP and 6.89 MJ/kg dry matter) for an adaptation period of 7 days. The lambs stayed with their respective mothers from 1800 to 0700 h. From weaning, lambs were housed into six pens of 4 m^2 with four animals per pen (three pens per treatment) and concentrate (Table 1) was offered *ad libitum* (in two daily meals at 0800 and 1600 h). The pens were cleaned weekly. Lambs had free access to water during the experiment. To calculate average daily weight gain (ADG), lambs were individually weighed at 15, 29, 43 and 57 days of age (in the morning after an overnight fast), and at slaughter. Moreover, average feed consumption for each experimental group was calculated; amounts of feed offered were recorded and refused feed was weighed daily.

Table 1 *Chemical composition of concentrate fed to lambs*

DM (%)	88.42
CP (% DM)	18.02
Diethyl ether extract (% DM)	4.95
Ash (% DM)	7.56
Nitrogen-free extract (% DM)	57.15
ADL (% DM)	3.20
ADF (% DM)	18.36
NDF (% DM)	29.11
Starch and sugar (mg/kg DM)	3113.92
DL-α-tocopheryl acetate (mg/kg DM)[1]	17.22
Net energy (MJ/kg DM)	7.64

DM = dry matter.
[1]17.22 IU DL-α-tocopheryl acetate/kg DM.

Slaughter surveys

At 71 days of age, after 12 h fasting, lambs were electrically stunned, exsanguinated and processed at a local slaughterhouse. Hot and cold carcass weights were recorded and dressing percentages were calculated after dressing and chilling at 2°C to 4°C for 24 h. Carcass shrink losses, calculated as the difference between hot and cold carcass weights, were expressed as a percentage of hot carcass weight. *Longissimus* muscle (LM) pH was measured between the 12th and 13th ribs at 45 min (pH_1) and 24 h (pH_u) *post-mortem* using a portable HI 9625 pH meter (Hanna Instruments, Padova, Italy) equipped with a penetrating glass electrode. In addition, LM area was measured, between the 12th and 13th ribs, by manually tracing muscles outlines onto acetate sheets and measuring areas by planimeter (Haff-Planimeter no. 317E). After the refrigeration period (24 h at 2°C to 4°C), from the right side of the carcass the pelvic limb was removed and its percentage was calculated based on cold carcass weight.

Collagen analysis

The LM, between the 11th to 12th thoracic vertebrae and the 4th to 5th lumbar vertebrae, and *semimembranosus* muscle (SM) were removed from the carcass (after 24 h at 2°C to 4°C); ~150 g of each muscle (wet weight) was collected, vacuum packaged and stored frozen (−40°C) until IMC and decorin analyses.

For IMC analyses, muscle samples were thawed, trimmed of fat and epimysium, lyophilized for 48 h, weighed and hydrolyzed in Duran tubes in 6 N HCl at 110°C for 18 to 20 h for determination of hydroxyproline (Woessner, 1961) and crosslinking. IMC concentration was expressed as μg hydroxyproline/mg of lyophilized sample. Hydroxylysylpyridinoline (HLP) concentration, the principal non-reducible crosslink of muscle collagen (McCormick, 1999), was determined using a modified HPLC procedure developed by Eyre *et al.* (1984). A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a Luna C18 column (250 × 4.6 mm × 5 μm; Phenomenex, Torrance, CA, USA) was used. The concentration of HLP residues in the samples was calculated based on the concentration of collagen in each hydrolyzate, assuming that the molecular weight of collagen was 300 000 Da and the

molar fluorescence yield of pyridoxamine (internal standard) was 3.1 times that of HLP (Eyre *et al.*, 1984). The HLP was expressed as moles of HLP per mole of collagen.

Decorin analysis

Decorin blots were determined by the method of Velleman (1995). Muscle portions were homogenized in Tris-EDTA buffer (10 mM Tris and 1 mM EDTA; pH 8.0; Sigma-Aldrich, St. Louis, MO, USA). Total protein concentrations were assessed using the Bio-Rad protein assay kit II (Bio-Rad Laboratories, Hercules, CA, USA). Protein (10 mg) from each sample was spotted onto nitrocellulose (Trans-blot transfer medium; Bio-Rad Laboratories) using the mini-Protean system (Bio-Rad, Hercules, CA, USA). Blots were exposed to the rabbit anti-decorin polyclonal antibody and to the goat anti-rabbit IgG with an alkaline phosphatase conjugate. Blots were then exposed to the 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium color system, and decorin concentrations within each blot were quantified to a relative scale by color intensity after scanning with a digital color scanner (GS-800 Calibrated Densitometer; Bio-Rad) (Velleman, 1995).

Statistics analyses

Growth, slaughter performance and pH data were analyzed by one-way ANOVA using the SPSS package (SPSS/PC1Statistics 18.0; SPSS Inc., Chicago, IL, USA, 2010). IMC and decorin data were evaluated by ANOVA, in a 2×2 factorial design. The model included DL-α-tocopheryl acetate treatment (C *v.* V) and muscle (LM *v.* SM) and their interaction as fixed effects and individual animal as a random effect. Scheffé's test was used for comparing mean values. BW at slaughter was included as a covariant. Pen was the experimental unit for the analysis of dry matter intake data and each individual lamb was considered as the experimental unit for growth, slaughter performance and pH.

Results and discussion

In the present study, α-tocopherol content was not measured in the muscle. However, it has been reported that there is a higher α-tocopherol accumulation in skeletal muscle of lambs intramuscular injected (Salvatori *et al.*, 2004) or supplemented (Ripoll *et al.,* 2013) with vitamin E when compared with control lambs. Lamb growth performance are presented in Table 2. Neither lamb growth nor amount of DMI was affected by DL-α-tocopheryl acetate administration, a finding in accordance with the results of Maiorano *et al.* (1999) who reported no effect of vitamin E administration on weight of lambs slaughtered at 40 days. Conversely, Maiorano *et al.* (2007) observed that 1200 IU of DL-α-tocopheryl acetate intramuscular injected in Ile de France lambs, slaughtered at 71 days, did not influence final live weight, but increased ADG significantly during suckling (day 15 to 22), whereas it did not affect the DMI. Similarly, Gentry *et al.* (1992) observed increased gains from birth to 30 days of age in Suffolk lambs injected with 900 IU DL-α-tocopheryl acetate. Our results are also in accordance with other studies conducted in different breeds, with vitamin E supplemented in the concentrate (Álvarez *et al.*, 2008; Ripoll *et al.*, 2013) or in grass silage-based feed (Kasapidou *et al.*, 2012). However, several other studies have demonstrated a beneficial effect of vitamin E treatment on growth traits in lambs (Macit *et al.*, 2003a).

The treatment with vitamin E did not affect ($P > 0.05$) the hot and cold carcass weight, the dressing percentage or LM area (Table 3). A similar result was obtained in other studies involving lambs of the same breed (Maiorano *et al.*, 1999, 2007) and on Awassi breed (Macit *et al.*, 2003b). On the contrary, Birch *et al.* (1994) observed a lower carcass weight and a negative effect on carcass wholesale cut weights in fine-wool wether lambs injected with a single-dose of vitamin E (1500 IU); while Njeru *et al.* (1992) did not find any effect of different doses (ranging from 125 to 1000 IU) of DL-α-tocopherol injection.

Table 2 *Mean values for growth performance of Ile de France lambs*

Group	C	V		
Lambs (*n*)	12	12	SEM	*P*-value
Live weight (kg)				
15 days	7.21	7.17	0.08	0.833
29 days	10.05	10.19	0.16	0.712
43 days	12.92	13.16	0.30	0.454
57 days	17.42	17.77	0.46	0.562
Slaughter	22.95	23.17	0.59	0.446
DMI (g/day)	895	915	0.87	0.236
ADG (g/day)				
15 to 29 days	204.0	214.6	7.8	0.312
30 to 43 days	205.0	208.0	11.8	0.781
44 to 57 days	321.5	333.0	14.8	0.346
58 days to slaughter	395.5	385.0	11.3	0.199

C = intramuscular injection of physiological saline; V = intramuscular injection of 1200 IU DL-α-tocopheryl acetate; DMI = dry matter intake; ADG = average daily weight gain.

Table 3 *Mean values for slaughter performance and pH of Ile de France lambs*

Group	C	V		
Lambs (*n*)	12	12	SEM	*P*-value
Hot carcass weight (kg)	14.12	14.75	0.40	0.781
Hot dressing (%)	61.52	63.65	0.50	0.523
Cold carcass weight (kg)	13.44	14.04	0.37	0.411
Cold dressing (%)	59.34	60.11	0.49	0.732
Carcass shrink losses (%)[1]	4.75	4.86	0.23	0.643
Pelvic limb (%)[2]	11.93	10.54	0.24	0.034
Longissimus muscle area (cm^2)	11.70	12.16	0.54	0.664
pH_1	6.00	6.55	0.10	0.022
pH_u	5.63	5.70	0.01	0.010

C = intramuscular injection of physiological saline; V = intramuscular injection of 1200 IU DL-α-tocopheryl acetate.
[1]Calculated on hot carcass weight.
[2]Calculated on cold carcass weight.

Table 4 *Mean values for collagen and decorin of LM and SM muscles of Ile de France lambs*

Group	C	V	LM	SM		*P*-value		
Lambs (*n*)	12	12	12	12	SEM	T	M	T × M
IMC (μg/mg)[1]	24.73	25.02	23.58	26.17	0.89	0.512	0.003	0.563
HLP/IMC (mol/mol)	0.121	0.099	0.071	0.110	0.005	0.049	0.001	0.452
Decorin (DO/mg)[2]	1.11	1.43	1.51	1.04	0.06	0.032	0.003	0.641

LM = *longissimus* muscle; SM = *semimembranosus* muscle; C = intramuscular injection of physiological saline; V = intramuscular injection of 1200 IU DL-α-tocopheryl acetate; T = treatment; M = muscle; IMC = intramuscular collagen; HLP = hydroxylysylpyridinoline.
[1]Of lyophilized tissue.
[2]Of fresh muscle tissue.

Carcass shrink losses were not influenced ($P > 0.05$) by the treatment (Table 3). However, contrasting findings are reported in literature. Maiorano *et al.* (2007), noted a higher value in the control group than in DL-α-tocopheryl acetate-treated lambs; they attributed this results to the treatment with vitamin E and its antioxidant effect on muscle cell membranes. Differently, Macit *et al.* (2003b) found a higher value (+1.9%) of carcass shrink losses of lamb receiving a supplement of 45 mg vitamin E/lamb per day for a 75 days fattening period when compared with control animals.

The treatment with vitamin E reduced (−1.39%; $P < 0.05$) the pelvic limb percentage (Table 3), with a potential negative impact on lamb market weight. This finding is in agreement with an earlier study on the same breed (Maiorano *et al.*, 2007), but in contrast with the result of Macit *et al.* (2003b). However, it has also been reported that vitamin E treated lambs had lower leg and shoulder weights (Birch *et al.*, 1994) compared with control lambs. Hatfield *et al.* (2000) suggested that this might be due to vitamin E stimulation of the immune system that, in turn, caused a partitioning of energy away from growth and promoted muscle catabolism.

Vitamin E treatment clearly influenced both pH_1 and pH_u in LM, which were higher ($P < 0.05$) in treated animals compared with control ones (Table 3). These findings are consistent with study involving injected lambs (Maiorano *et al.*, 2007). As reported by Cheah *et al.* (1995) and Castellini *et al.* (1999), the capability of vitamin E to stabilize membranes is presumably achieved by decreasing Ca^{++} level release (either mitochondria or the sarcoplasmic reticulum) that reduces phospholipase A2 activity and, hence, phospholipid hydrolysis. Decreased levels of cytosolic Ca^{++} reduce the rate of *postmortem* glycolysis resulting in a higher *postmortem* pH. However, the pH_u values observed in this study (mean 5.66; SEM 0.01) varied within the pH range accepted for commercial meats, with no evidence of pre-slaughter stress and meat defect.

IMC and decorin properties of LM and SM muscles are shown in Table 4. Administration of vitamin E did not affect ($P > 0.05$) IMC amount but slowed ($P < 0.05$) collagen maturation (HLP/collagen). These results may have implications on meat tenderness since it is reported that an increase in collagen maturation leads to an increased IMC thermal stability, which has been related to shear force (Monin and Ouali, 1991; Hopkins *et al.*, 2013) and with an undesirable changes in eating quality of meat (McCormick, 1999; Purslow, 2005; Lepetit, 2008).

A marked muscle effect on IMC properties was apparent (Table 4). The SM muscle had higher ($P < 0.01$) values of IMC amount as well as collagen maturation (HLP/collagen). Differences in IMC properties between muscles could be due to functional and structural differences between locomotor and postural muscles (Maiorano *et al.*, 2009). In fact, it has been documented that locomotor muscles possess more cross-linking than postural muscles (McCormick, 1999). The difference between the two studied muscles evidenced, in agreement with the literature (McCormick, 1999), that variation in IMC properties with muscle type and function leads to the well-known differences in background toughness among meat cuts.

Compared with lambs of the C group, those of the V group had higher decorin concentration (+28.8%; $P < 0.05$). Furthermore, it was recorded a marked difference in decorin concentration between muscles: LM had higher (+45.2%) values than that of SM ($P < 0.01$). To our knowledge, no studies are available on the effect of vitamin E on decorin concentration in lamb muscles. Fewer studies have focused on how α-tocopherol might regulate the extracellular matrix. Villacorta *et al.* (2007) recently proposed that vitamin E is able to protect the damaged vascular wall, not only by limiting cell proliferation, but also by mediating the process that leads to the stabilization of a fibrous cap by influencing components of the extracellular matrix. In previous study, Schwartz (1979) reported that a supplementation of α-tocopherol, in human articular cartilage, stimulated sulfate proteoglycan biosynthesis and, in addition, it inhibited the degradation activity of lysosomal enzymes (arylsulfatase A and acid phosphatase) on proteoglycans. However, as already observed, the proteoglycan decorin plays a key role in regulating cell proliferation, growth factor activity and collagen organization (Velleman *et al.*, 1999). Albrecht *et al.* (2011) suggested that an increase in decorin level could affect the formation of collagen fibers and therefore negatively influence meat quality. However, McCormick (1999) reported that the temporal gap between increased decorin expression and the measured levels of elevated crosslinking is consistent with the concept that development of cross-linking is a time-dependent process. As fibrillogenesis

proceeds, crosslinks form as reactive residues align; initial reducible crosslinks condense, ultimately forming the mature trivalent crosslinks (McCormick, 1999).

Conclusion

The results of the present study indicate that the DL-α-tocopheryl acetate treatment did not affect growth, hot and cold carcass weights, carcass shrink losses and area of LM, but increased pH values. However, it should be noted that intramuscular vitamin E administration has the potential of impacting negatively on pelvic limb mass, in agreement with some previous findings. Vitamin E treatment reduced collagen maturity and increased decorin in the LM and SM muscles of growing lambs. Although the treatment effects noted in this study were subtle, further research is warranted to elucidate, in particular, the effect of vitamin E on extracellular matrix.

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