Ecchymosis and meat quality following testosterone and cortisol exposure in prepubertally castrated fallow deer (*Dama dama*)

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Introduction

Ecchymosis or 'blood splash' is a muscle blemish, with severe instances resulting in meat condemnation, that is frequently detected in the loin and hind limb muscles of many commercial animal species. The amount of blood present in muscle blood vessels, at slaughter, is implicated in the severity of visible haemorrhage while the incidence of this phenomenon is apparently greater in head only electrically stunned animals (Kirton *et al.* 1981). Histological analysis has revealed that blood vessels rupture, around the time of slaughter, as a result of the strain of increased blood volume and muscular contractions (Shaw *et al.* 1971; Leet *et al.* 1977). Blood vessel dynamics are regulated by the β-adrenergic receptor (βAR) which is present on the surface of cells and in particular abundance on those lining the vascular system. Our focus has been on the steroid transcription factors, testosterone and cortisol, which modulate the expression and activity of the βAR (Mak *et al.* 1995; and Collins *et al.* 1988), thus altering vasodynamics. We have selected the fallow deer, which display marked responses to testosterone and cortisol, in order to elucidate the events resulting in ecchymosis.

Study 1. Elevated testosterone exposure

Materials and Methods

Two year old prepubertally castrated fallow deer (n=12) with mean ± SEM body weight of 46.5 ± 0.6 kg were maintained at pasture and habituated to frequent handling and blood sampling. Blood plasma was obtained at 0900hrs in order to determine, by radioimmunoassay (RIA), baseline concentrations of cortisol (Jones *et al.* 1989) and testosterone (Brown *et al.* 1989). In late February, treatment deer (n=6) received Primoteston® (500mg, testosterone enanthate in 2mL of castor oil, i.m.), and control deer (n=6) received castor oil (2mL, i.m.). The deer were then transported, during period of elevated testosterone, to the abattoir and remained in lairage overnight. Each animal was head only electrically stunned (Jarvis electrical stunner; 70V), followed by a transverse neck cut within 10sec of stunning. Blood plasma samples were collected and stored at -20°C prior to further RIA analyses. Heart, lung, liver, diaphragm, intercostal muscles, tender loin, *M. semimembranosus* and *M. semitendinosus* were inspected for the presence of ecchymosis, in the chiller, 2hr after slaughter. Loin and round (boned out) were evaluated for ecchymosis 24hr post slaughter employing the AUS-MEAT grading chart (1996). Loin muscle samples were obtained between the 6th and 13th thoracic vertebrae. A pH meter (TPS LC80-A) was used to measure pH and colour was quantified with a Minolta Chroma meter (CR-300) set on the L*a*b* colour system (L*= lightness, a*= red/greenness, b*= yellow/blueness). Both pH and colour measurements were performed 24hr after slaughter. Triplicate measurements of pH and 5 colour measurements per freshly cut loin surface were recorded. Mean loin peak shearforce (kg), on samples aged for 8 days at 4°C, was obtained from 2cm × 2cm cubes with a Warner-Bratzler attachment on a texture analyser (Stable Micro Systems TAXT2). Percentage moisture and fat were obtained from 12g samples of loin. Moisture was determined using an air oven (AOAC) and fat was extracted with a petroleum spirit in a continuous soxhlet extraction system (Büchi 810 soxhlet system). Vacuum packed microbial analysis of loins stored at 4°C was determined by conducting a standard plate count in accordance to Australian Standards (AS 1766.2.1) over 4 weeks. *Pseudomonas spp.* and *Lactobacillus spp.* were also quantified over the 4 week period using selective media and conditions outlined in the Oxoid manual (1990). A single factor ANOVA was utilised in statistical comparisons.

Results

Circulating testosterone concentrations rose from undetectable levels to 20.93 ± 3.08 ng/mL in the treatment group within 24hr (simulating the levels observed during the normal time of sexual activity). Ecchymosis of commercial importance (Grade 2 to 4) was not observed in either group. Microbiological evaluations revealed no treatment effect where all counts for monitored micro-organisms, over the 4 weeks, were within acceptable organoleptic limits. Mean plasma cortisol levels at the time of slaughter were 42.83 ± 4.01 ng/mL and 40.12 ± 7.60 ng/mL for the control and testosterone treated groups respectively and were not significantly different. Table 1 indicates no significant treatment changes in 24hr pH, percentage fat, peak shearforce and colour b*. However, colour L*, a* and percentage moisture were significantly altered following testosterone exposure ($P<0.05$).

Study 2. Elevated cortisol exposure

Materials and Methods

Female fallow deer (n=18) not habituated to intensive handling were electrically stunned and slaughtered by the same method described in Study 1. Blood was collected and plasma assessed by RIA for cortisol. Evaluation of ecchymosis was conducted 2hr after slaughter. Sites on the carcass examined for ecchymosis were the diaphragm, intercostal muscles, tender loin, *M. semimembranosus* and *M. semitendinosus*. In addition, muscle pH (2hr and 24hr) and external muscle colour (L* and a*) of the *M. semimembranosus* and *M. deltoideus* were also measured using the same equipment described in Study 1. A single factor ANOVA was applied to the data for statistical analysis.

Results

All the female fallow deer displayed ecchymosis with the exception of one animal. Mean ± SEM cortisol levels were 138.47 ± 1.41 ng/mL for those animals with ecchymosis. The individual not displaying ecchymosis had a cortisol concentration of 71.23 ng/mL at slaughter. Further, two animals with severe ecchymosis (Grade 4) had circulating cortisol levels greater than 200 ng/mL. The *M. semimembranosus* of the 2 severely blood splashed individuals had a pH>6.2 (24hr); this is in contrast with the non ecchymotic animal (pH 5.89; 24hr). There was a high incidence of ecchymosis expressed on the intercostal and diaphragm muscles. With increasing blood splash severity, in diaphragm and intercostal muscles, other sites on the carcass were more obviously affected including the tender loin, *M. semitendinosus*, and *M. semimembranosus*. The mean pH and colour readings of *M. semimembranosus* and *M. deltoideus*, 2hr and 24hr after slaughter, are presented in Table 2 showing a reduction in pH and a corresponding shift in colour values over this time.

Table 1. Measured *L. dorsi* attributes (mean ± SEM) in control and testosterone treated castrate fallow deer.

Attribute	Control	Testosterone
pH	5.43 ± 0.01	5.46 ± 0.01
fat (%)	1.10 ± 0.39	0.97 ± 0.27
moisture (%)	74.98 ± 0.22	75.71 ± 0.20†
shearforce (kg)	2.12 ± 0.07	2.25 ± 0.15
colour	L* 29.11 ± 0.15 a* 18.65 ± 0.16 b* 0.78 ± 0.14	L* 30.66 ± 0.23† a* 18.16 ± 0.17† b* 0.98 ± 0.17

† Indicates significant difference between treatments ($P<0.05$)

Table 2. Colour and pH readings (mean ± SEM) in two muscles 2hr and 24hr after slaughter in female fallow deer.

Attribute	Muscle	
	M. semimembranosus	*M. deltoideus*
2h pH	6.05 ± 0.05	6.61 ± 0.14
24h pH	5.87 ± 0.10	5.68 ± 0.07
2h colour	L* 38.33 ± 0.34 a* 9.15 ± 0.24	L* 41.01 ± 0.57 a* 9.24 ± 0.24
24h colour	L* 37.23 ± 0.65 a* 11.00 ± 0.17	L* 39.77 ± 0.52 a* 10.21 ± 0.21

Discussion

In this communication we report that testosterone, at reproductive concentrations normally experienced by the fallow deer during the breeding season, did not significantly affect ecchymosis expression. The increased percentage moisture due to testosterone exposure (Table 1) was also demonstrated by Cranwell *et al.* (1996) who treated beef cows with androgen implants. The shift in pH and colour over 24hr (Study 2, Table 2) has been previously observed (Katsarov, 1978; and Swan and Hall, 1995) confirming the relationship between pH and colour. Lower a* value readings in the testosterone group (Table 1) may be due to the action of adrenaline on an androgen upregulated βAR system as Bekaert *et al.* (1987) reported lower a* values in pig *L.dorsi* treated with the βAR-agonist cimaterol. The testosterone treated animals and their matched control group were habituated, on a weekly basis, to the same handling and blood sampling procedures for 4 months prior to the experiment. We have previously reported (Grogan, Mulley and Jones, 1996) that cortisol concentrations fall from 82.0 ng/mL to 55.0 ng/mL and remain stable as a result of extended handling and blood sampling habituation. We have also observed, in unpublished experiments, a low incidence of ecchymosis (16%; n=24) in fallow deer with a mean cortisol concentration of 57.85 ng/mL. The fallow deer exposed to elevated cortisol, in this report, were not habituated to any daily handling or blood sampling regime. Based on these data and unpublished files it appears the graded severity of ecchymosis is proportional to the vascular levels of cortisol observed at slaughter. Consequently, high cortisol may have a significant predisposing affect on ecchymosis expression. Animals that are not habituated are less able to cope with stressors during transport or at the abattoir and respond by secreting high levels of cortisol. Increased activation of the βAR in an abattoir environment is likely and in this enhanced physiological state a greater volume of blood would be diverted to muscle blood vessels in the classical 'fight-flight' response. Therefore at slaughter, animals in this enhanced physiological state would be more likely to display ecchymosis in concert with high cortisol. Transport and lairage has been shown to increase cortisol levels in a number of species (Kallweit *et al.* 1981; Hanssen *et al.* 1984; Shaw and Tume, 1992). Current industry practice for transport and lairage time before slaughter is at least 12hr. In this time frame cortisol can upregulate the activity of βAR (Mak *et al.* 1995) and potentially predispose the vascular system to ecchymosis. The implications of training or habituating animals to a variety of stressors is likely to be a means of reducing the expression of ecchymosis. Steroid and adrenergic interaction may also be implicated in other meat quality problems such as dark cutting meat. The two individuals, from Study 2, with severe ecchymosis and over 200 ng/mL of cortisol, had a high ultimate pH. Testosterone and cortisol increase βAR activity hence in stressful conditions stored glycogen, which is converted to glucose following βAR activation (Garret and Grisham, 1995), would diminish leading to conditions ideal for dark cutting meat. The importance of transport and lairage time is further supported by Katsarov (1978) who investigated muscle pH and darkness in bull calves held for 2hr and 24hr prior to slaughter. Bulls held for 24h had significantly darker meat and a pH of 6.83 while animals held for only 2hr had a low pH (5.77) and correspondingly lighter meat. The foregoing implicates the interaction between husbandry practices, cortisol and the βAR as important meat quality regulators. As a consequence of our research it is apparent testosterone and cortisol have different modes of transcriptional regulation affecting fallow deer physiology. Testosterone, as administered here, did not induce ecchymosis but did instigate significant colour and moisture changes ($P<0.05$) along with transient secondary sex characteristic permutations including behaviour (sparring) and increased neck girth. Cortisol appears to alter blood vessel dynamics, potentially through the βAR, to create vascular conditions that favour ecchymosis formation. We are currently investigating molecular events to clarify the relationship between cortisol, the βAR, and related transcriptional interactions, which give rise to ecchymosis and alter meat quality attributes.

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