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Serum Cortisol Concentration in Different Sex-Types and Slaughter Weights, and its Relationship with Meat Quality and Intramuscular Fatty Acid Profile

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Abstract: A group of 84 crossbred lambs comprising 21 lambs for each of 4 sex-types (entire ram, vasectomized ram, castrate and ewe) were subdivided within each sex-type into 7 slaughter weights (32, 36, 40, 44, 48, 52, 56 kg). There were 3 lambs per slaughter weight. Lambs were born in the latter part of spring and were out on pasture with their dams, but were housed by September and fed a concentrate diet and hay. Lambs were slaughtered in a nearly abattoir and blood samples were collected for cortisol determination. Meat quality and fatty acid profile were assessed using the 6^{th}-12^{th} rib section of the *Longissimus dorsi* muscle. The mean serum cortisol concentrations ranged from 103.3-117.7 nMol L^{-1} and differences due to sex - type were not significant (P>0.05). However, serum cortisol concentration was positively correlated with slaughter weight (r = 0.34, P<0.01) and age (r = 0.43, P<0.001). Whereas serum cortisol level was negatively correlated with initial pH and positively correlated with intramuscular fat in castrates (P<0.05), the same correlations in other sex-types were not significant (P>0.05). Cortisol level was negatively correlated with cooking loss in all sex-types (P < 0.01) and also significantly related to fatty acid profile (P<0.05).

Key words: Cortisol, sheep, meat quality, fatty acid

Introduction

The relative importance of stress during animal production and in the immediate pre-slaughter period as a major determinant of ultimate meat quality has long been recognized (Briskey, 1964; Trenkle and Topel, 1978; Lawrie, 1991). Pre-slaughter stress changes the rate and extent of postmortem glycolysis and acidification (Chrystall *et al.*, 1982; Lawrie, 1991). Various parameters have been used as indices of the magnitude of stress in different livestock species. These include changes in plasma concentrations of adrenocorticotrophic hormone (ACTH), cortisol, growth hormone, glucose and free fatty acids (Marple *et al.*, 1972), changes in plasma concentrations of thyroxine and cortisol (Moss and Robb, 1978), agonistic behaviour, such as threats, head blows, bites, butting, fighting and pursuit (Dantzer and Mormele, 1983), homosexual activity (Mohan Raj *et al.*, 1991), restlessness, anxiety and escape attempts (Johnson and Vanjonak, 1976) and reduction in productivity (Colditz and Kellaway, 1972). However, plasma concentrations of cortisol, creatine phosphokinase (CPK) and glycogen metabolites appear to be the most widely accepted and more generally used measures of stress sensitivity and the magnitude of stress associated with most systems of animal husbandry and pre-slaughter animal handling.

Cortisol is synthesized in the adrenal cortex, and its production is stimulated by physical stress, emotional or psychological stress and hypoglycaemia (Emslie-Smith *et al.*, 1988). Reid and Mills (1962) observed higher cortisol and glucose levels in grazing sheep than in housed sheep after both groups were transported. Experience and age are known to moderate behavioural responses to stressors in lambs (Reid and Mills, 1962; Morberg *et al.*, 1980). Transporting sheep to the abattoir from markets compared with transportation from farms (Jarvis *et al.*, 1996) and on rough versus smooth roads (Ruiz-de-la-Torre *et al.*, 2001) resulted to increased plasma cortisol levels. Increase in the complexity of transportation treatment in bulls resulted in corresponding increase in cortisol level (Kenny and Tarrant, 1987). Similarly, plasma cortisol, glucose and creatine kinase activity of Friesian steers increased with increase in stocking density during transportation to abattoir (Tarrant *et al.*, 1998). The authors concluded that animal welfare and carcass quality were adversely affected under high stocking density, relative to low or medium densities. Although cortisol levels were not affected, high stocking rate resulted in higher CPK levels in pigs (Barton Grade and Christensen, 1998; Lee *et al.*, 2000). It has been shown that differences in handling pigs in same abattoir (Moss and Robb, 1978) and handling cattle in the same way but in different abattoirs (Tume and Shaw, 1992) resulted in differences in plasma cortisol levels. Recently, it has been reported

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that blood cortisol level in pigs was higher in winter than in summer (Gispert *et al.*, 2000). Male animals tended to be more excitable, aggressive and stress sensitive than females. This was observed in pigs (Moss and Robb, 1978), cattle (Tennessen *et al.*, 1985), laboratory animals (Gray, 1971), but not in sheep (Horton *et al.*, 1991). Apparently, sheep tolerate stressful conditions better than pigs (Warriss *et al.*, 1990).

Compared with untreated counterparts, steers treated with cortisol acetate grew significantly less, and contained more fat and less protein (Carrol *et al.*, 1963). Trenkle and Topel (1978) showed that plasma corticoid concentration in the bovine was positively correlated with lipid content in the carcass, and fat content of the *Longissimus* muscle. Lundstrom *et al.*, (1983) reported that plasma cortisol level was lower in a line of pigs selected for leanness compared with an opposite line selected for fatness. Studies on effects of cortisol concentration on fatty acid distribution are scanty or non-existent. This study reports on the effects of sex-type (entire ram, vasectomized ram, castrate and ewe) and live weight on serum cortisol level, and the relationships between serum cortisol concentration and meat quality and fatty acid distribution.

Materials and Methods

Experimental animals comprised 84 cross-bred lambs (Greyface dams x Dutch Texel sires) and included 21 ewes and 63 rams. All animals were born in March or April at the Sheep Unit of the Agricultural Research Institute of Northern Ireland, Hillsborough (ARIH). The 63 rams were allotted to one of three sex-types, hence 21 rams remained entire, 21 rams were emasculated by traditional surgical castration and the remaining 21 rams were epididymectomised as described by McCaughey and Martin (1980). Lambs were 3 days old when castration and epididymectomy were performed. Lambs together with their dams were out on pasture until September when they were housed and fed a concentrate diet and hay. The concentrate contained 150 g kg^{-1} crude protein and 10.6 MJ Kg^{-1} metabolizable energy and each lamb received 500 g of the diet per day. Hay was offered *ad libitum*. The 21 animals in each sex-type were divided into 7 slaughter weights (32, 36, 40, 44, 48, 52 and 56 kg live weight). Age at slaughter ranged from 180 to 390 days. When animals attained their allotted slaughter weights, they were transported in a truck, and on smooth road to a nearby commercial abattoir. Duration of transport was less than 1 h. After a brief lairage rest animals were slaughtered following conventional practice.

Blood collection and cortisol determination: Blood samples were collected at the abattoir at the point of sticking, into 25 ml plastic bottles (Sterillin). Blood samples were transported to the laboratory and the serum was recovered by centrifugation, at 1000 rpm for 15 min. Serum samples were stored at -20°C. Serum cortisol was determined by the radioimmunoassay (RIA) technique. A reagent kit supplied by Immunodiagnostic Sytems Ltd (UK) was used. Each kit contained a set of seven standard cortisol solutions, radioactive cortisol solution (^{125}1- Cortisol) and polypropylene tubes coated with anti-cortisol IgG lined to the inner surface. The analytical procedure described on the method sheet supplied by Immunodiagnostic Systems Ltd was followed. Briefly, samples were allowed to thaw under room temperature conditions, and an aliquot of 25 µl of each standard or sample was pipetted into the antibody-coated tubes using the Tecan Liquid Dispenser. Next, 1 mL of radioactive cortisol was added into all the tubes, including two additional tubes set aside for total counts. All tubes were thoroughly mixed, incubated at 37°C for 45 min decanted and subsequently counted for 1 min using the GAMMatic II gamma counter (Kontron Analytical, Munchenstein, Switzerland). All analyses were performed in duplicate. Per cent bound cortisol was derived by dividing mean counts for each sample (or standard solution) by the mean of total counts and then multiplied by 100. Percent bound values for the standards were regressed on their respective cortisol concentrations and the regression curve was used to predict the cortisol concentration of the samples.

Meat quality determination: Initial pH (pHi) was measured at the abattoir, 45 min post slaughter by inserting the spear-head electrode of the pH meter (RE 357 Tx Microprocessor pH meter, EDT Instruments) into the 9th rib region of the *Longissimus dorsi* (LD) muscle. After a 24 h chill at the abattoir, the right shoulder joint from each carcass was removed and taken to the laboratory, and held at 2°C until sampled for analysis. Ultimate pH (pHu) was measured in the laboratory, after 48 h post-slaughter using the same probe method. From the shoulder joints, the 6th - 10th rib sections were removed and used for the determination of cooking loss and shear forcer. The 10th - 12th rib sections were used for sarcomere length and intramuscular lipid assessment.

Cooking loss, shear force and sarcomere length determination: Muscle samples were placed individually in self sealing polythene bags and cooked at 75°C for 35 min. Thereafter, samples were cooled to room temperature in a bucket containing ice. Cooking loss was calculated by dividing the weight lost during cooking by the fresh sample weight, and multiplying the result by 100. Six cores were drilled from each sample along the fibre long axis and transversely sheared using a Warner - Bratzler device mounted on the Instron Universal Testing Machine (model 6021). Samples for sarcomere length determination were fixed in buffered 5 % gluteraldehyde solution (Koolmees *et al.*, 1986), and measured by laser diffraction (Cross *et al.*, 1981).

Intramuscular lipid and fatty acid determination: Muscle samples were freeze-dried and milled. The lipid present in 4 g sample (weighed to the nearest 1 mg) was extracted using chloroform : methanol solution (Folch *et al.*, 1957). Fatty acids were methylated according to method 6 of B. S. 684 (1980) and thereafter measured using the Varian Star 3400 Gas Chromatograph, equipped with a capillary silicon based column, CP-SIL 88 (Chrompack, The Netherlands).

Statistical analyses: Serum cortisol concentration was fitted against sex-type and slaughter weight, and thereafter sex-type and slaughter age together with their respective interactions, using the linear model of Genstat 5 (1990). Quadratic ($y = c + ax + bx^2$) and log-log ($\log y = c + b \log x$) regressions were also carried out to test for possible improvements in correlation. Similarly each meat quality parameter and fatty acid was fitted against cortisol concentration and sex-type, and interactions using the linear model. The coefficient of correlation was calculated. The significance of each term was tested using the accumulated analysis of variance test.

Results

The mean cortisol values for the 4 sex-types were castrate, 103.3, entire ram, 107.4; vasectomized ram, 108.1 and ewe, 117.7 nmol L^{-1}. Differences were not statistically significant ($P>0.05$). The log-log model yielded the highest degree of correlation between serum cortisol concentration and slaughter weight or age. Serum cortisol concentration was positively correlated with slaughter weight ($r = 0.34$, $P<0.01$) and slaughter age ($r = 0.43$, $P<0.001$).

Linear correlations between serum cortisol concentration and meat quality and intramuscular fatty acid profile are presented in Table 1. Correlations were significant for a number of parameters, but generally low. Significant ($P<0.05$) cortisol by sex interaction effects on initial pH (pHi) and intramuscular lipid were detected. Whilst increasing serum cortisol concentration was significantly ($p<0.05$) associated with decreasing pHi levels and increasing intramuscular lipid content in castrates, effect on other sex-types were very small and not significant. In all sex-types serum cortisol concentration was negatively correlated with cooking loss ($P<0.01$), myristic acid, $C_{14:0}$ ($P<0.05$) and linolenic acid $C_{18:3}$ ($P<0.001$), and positively with oleic acid, $C_{18:1}$ ($P<0.05$).

Table 1: Linear correlation of different meat quality characteristics against serum cortisol concentration (n = 84)

Parameters	Correlation coefficient (r)
pHi	-0.12[1]
pHu	0.016
Sarcomere length	-0.10
Cooking loss	-0.35**
Shear force	-0.09
Intramuscular lipid	0.11[1]
$C_{10:0}$ (capric acid)	-0.14
$C_{12:0}$ (lauric acid)	-0.24
$C_{14:0}$ (myristic acid)	-0.34*
$C_{14:1\ cis}$ (myristoleic acid)	-0.11
$C_{15:0}$ (pentadecylic acid)	-0.20
$C_{16:0}$ (palmitic acid)	-0.03
$C_{16:1\ trans}$ (palmitelaidic acid)	-0.16
$C_{16:1\ cis}$ (palmitoleic acid)	-0.15
$C_{17:0}$ (margaric acid)	-0.02
$C_{18:0}$ (stearic acid)	0.04
$C_{18:1\ cis}$ (oleic acid)	0.29*
$C_{18:2\ trans}$ (linoelaidic acid)	-0.25
$C_{18:2\ cis}$ (linoleic acid)	0.11
$C_{18:3}$ (linolenic acid)	-0.41***
Totals:	
Saturated fatty acids	-0.11
Monounsaturated fatty acids	0.14
Polyunsaturated fatty acids	-0.07

[1]Significant cortisol by sex interaction effect (P<0.05); * = P<0.05, ** = P<0.01; *** = P<0.001.

Discussion

Our results show that serum cortisol concentration was similar across the sex-types of entire rams, vasectomized rams, castrates (wethers) and ewes ($P>0.05$). This is consistent with the report of Horton *et al.* (1991) on sheep, but contradicts other reports on cattle (Gettys *et al.*, 1988; Lee *et al.*, 1990). This in itself does not entirely suggest that stress sensitivity does not differ across the sexes in sheep. Since animals were not subjected to any stressful treatment beyond that associated with normal handling operation, it may be that none of the sex-types was stressed sufficiently to show any differences. Another probable reason is that cortisol secretion in sheep is not very sensitive to minor stress levels. Increased blood glucose concentration in sheep after transportation (Warriss *et al.*, 1990) and changes in triiodothyronine uptake ratio and serum thyroxine level in pigs after overnight lairage (Moss and Robb, 1978) have been observed, without any concurrent change in cortisol level. In contrast, significant differences in plasma cortisol concentration have been observed in sheep subjected to apparently more stressful conditions. Transporting sheep on a rough road versus transporting sheep on a smooth road (Ruiz-de-la-Torre *et al.*, 2001) and subjecting wether lambs to treadmill exercise for 10 min at either 5.6, 7.2 or 8.8 km h^{-1} on a 9^{o} incline, followed by a 10 min walk at 4.0 km h^{-1} on the horizontal plane (Apple *et al.*, 1994) resulted to significantly increased plasma cortisol levels. Generally, cortisol levels reported in this study are in line with other literature values on sheep (Horton *et al.*, 1991;

Shaw and Tume, 1992). Although caution must be used in comparing these values since preliminary studies in our laboratory indicate that different radioimmunoassay methodology and test kit may result in different cortisol values (Moss, pers. comm.). The result from any one methodology should in general be considered relative and not absolute unless suitable standards for each farm animal species have been used as quality control. Cortisol level increased with slaughter weight and age, and the correlation with age ($r = 0.43$, $P < 0.001$) was stronger than the correlation with weight ($r = 0.34$, $P<0.01$). A similar study in cattle reported correlation coefficient values of 0.53 ($P<0.01$) for age and 0.32 ($P>0.05$) for weight (Trenkle and Topel, 1978). This demonstrates that if all other factors remain constant, cortisol level increases as the animal increases in age. The observation that cortisol concentration was not related to shear force is consistent with Purchas (1973), but contradicts Chrystall *et al.* (1982).

Serum cortisol level was negatively correlated with initial pH and positively with intramuscular lipid in castrates alone, and not in any other sex-type. Since the serum cortisol level was the same in all the sex-types, the likely explanation would be that the sensitivity of sheep to cortisol is affected by sex hormones metabolism. However, reports on cattle suggest that cortisol and sex hormone levels are negatively correlated. Significantly lower cortisol levels in steers implanted with trenbolone acetate (androgenic) and estradiol (oestrogenic) than in other steers that were not implanted has been reported.(Lee *et al.*, 1990). The authors also observed that castration was followed by a decrease in insulin like growth factor 1 (IGF-1) and an increase in cortisol secretion in cattle. Jones *et al.* (1991) reported that bulls implanted with trenbolone acetate and zeranol (oestrogenic) had lower cortisol levels and delayed puberty. Our results show that cooking loss was negatively correlated ($P<0.01$) with cortisol concentration in all sex-types. Our earlier report (Okeudo and Moss, 2005) and that of Bailey (1985) showed that cooking loss was positively related to collagen content, particularly thermally stable collagen content. The reports of Gerrard *et al.* (1987) suggest that testosterone has a stimulating effect on collagen synthesis. Since cortisol is known to stimulate increased proteolysis (Emslie-Smith *et al.*, 1988), it is likely that testosterone specially protects the collagen fibres from degradation. This may be the likely explanation why cooking loss was negatively correlated with serum cortisol concentration. Although serum cortisol level had no general effect on intramuscular lipid content, it was negatively correlated with linolenic acid, $C_{18:3}$ ($P<0.001$), myristic acid $C_{14:0}$ ($P<0.05$) and positively correlated with oleic acid $C_{18:1\ cis}$ ($P<0.05$) suggesting that the effect of cortisol on fatty acid distribution is not through its general effect on fat deposition.

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