SESSION 1. MUSCLE BIOLOGY AND BIOCHEMISTRY

REVIEW: MUSCLE BIOLOGY AND BIOCHEMISTRY

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INTRODUCTION

Advances are taking place at several levels of investigation into the biology and biochemistry of muscle in meat animals, improving our knowledge and control of meat quality. At the level of the cell nucleus, it may soon be possible to separate the positive and negative effects of genes of major economic importance, such as the halothane gene (Archibald, 1987). At the level of the cytoskeleton and myofibril, progress is being made in our understanding of the mechanism of degradation of the important structural proteins of the myofibre during the conditioning of meat. At the level of the cell membrane there is renewed interest in the functioning of the β adrenergic receptors, the sites of action of a new generation of growth promoters, the repartitioning agents. The amount of information available on the quantitative effects of these agents is in contrast to the paucity of information about their effects on meat quality. Finally, at the level of the intact organism, progress has been made in understanding the link between specific animal behaviours, muscle metabolism and meat quality. Some of these developments at the interface between muscle biology and meat quality are the subject of this review.

The Nature of PSS

Research into the PSS (porcine stress syndrome) continues and is of great practical relevance to the pork industry. This is so, not only because of the continuing presence of the halothane gene and therefore PSE in many pig breeds, but also because there is a good economic arguement for retaining the halothane gene. By producing a heterozygous slaughter generation from a halothane positive sire line and a halothane negative dam line it may be possible to gain significant advantages in carcass composition without the deterioration in meat quality and mortality rate that are found in halothane reactors (Webb et al, 1987).

The possibility that a basic lesion may give rise to the multiple symptoms of the PSS syndrome is suggested by analogy with the closely-related MH (malignant hyperthermia) syndrome in pigs. In MH, experimental results suggest that there may be a fundamental defect in the regulation of free Ca^{2+} in the muscle cell (Heffron, 1987). In normal resting muscle, the free Ca^{2+} concentration in the sarcoplasm is very low compared with that in the SR (sarcoplasmic reticulum), mitochondria or extracellular fluid. Consequently, a defect in the Ca^{2+} transporting systems of the respective membranes could result in an uncontrolled rise in free intracellular Ca^{2+}. This would enhance muscle glycolysis by activating actomyosin ATPase and phosphorylase kinase, precipitating MH symptoms in vivo and PSE post mortem.

There is much evidence, although none of it conclusive, that the calcium-transporting mechanisms of the muscle cell are defective in MH pigs and, by implication, in PSS pigs. The SR is the primary system which releases and sequesters Ca^{2+} during excitation-contraction coupling and relaxation. There is disagreement as to whether uptake of Ca^{2+} by the SR is unaltered or somewhat decreased in MH. However, all of the reports agree that the calcium threshold for calcium-induced calcium release (CaIR) is significantly reduced in SR from MH compared with normal pigs. While the CaIR mechanism is abnormally sensitive in MH pig muscle, this observation was made at Ca^{2+} concentrations above the physiological range. Also, the significance of the CaIR mechanism of the SR in the normal excitation-contraction coupling cycle must be established before assigning it a role as a primary event in MH. Rather than indicating the site of the fundamental defect in MH, this finding may be indicative of a more widespread membrane lesion (Heffron, 1987).

The Ca^{2+} pump in the plasma membrane also controls intracellular Ca^{2+}, but its relative importance is not yet known because of difficulties in obtaining pure sarcolemmal membranes. However, there is evidence for multiple defects in the excitation-contraction coupling cycle in MH porcine muscle, including defective t-tubule control of calcium release from SR and a decreased rate of plasma membrane repolarisation. Such evidence points to an abnormality in the plasma membrane of MH muscle.

Concerning the mitochondria, Cheah and Cheah (1978) observed that, under anaerobic conditions, the efflux of Ca^{2+} was greater in mitochondria from MH muscle than in controls. Subsequently they postulated that this effect was mediated by phospholipase A_2. This enzyme is located in the mitochondrial membrane and is activated by Ca^{2+} and by anoxia. MH pigs have enhanced phospholipase A_2 activity in the presence of Ca^{2+}. In resting muscle the enzyme is latent, since oxygen is readily available and the Ca^{2+} concentration in the sarcoplasm is low (about 10^{-7} M). Under anaerobic conditions the mitochondria release Ca^{2+} thereby activating phospholipase A_2 which in turn liberates unsaturated fatty acids. These uncouple the mitochondria, stimulating the efflux of Ca^{2+} from the mitochondria. The free fatty acids may also influence the SR to release Ca^{2+} through the mechanism of fatty acid-induced Ca^{2+} release or CaIR or by both mechanisms (Cheah et al, 1986).

The arguement against mitochondria being the site of the primary defect in MH is that there is adequate evidence that tissue oxygenation is maintained during the onset and early phase of porcine MH. The significance of anaerobically-induced Ca^{2+} efflux in MH mitochondria may be in determining the reversibility of the established syndrome (Heffron, 1987).

In summary, the primary defect in the MH or PSS syndromes has not yet been identified, although there is considerable evidence that the basic abnormality lies in the control of free Ca^{2+} in the muscle cell. This may be a consequence of a specific defect in muscle cell membranes or part of a more generalised membrane defect. There is evidence that the catecholamines may play a permissive role in triggering PSS (Lister, 1987). The sympathetic nervous system is more responsive, and the muscle, heart and fat tissues contain more β adrenergic receptors, in PSS compared with stress-resistant pigs. Thus, adrenaline and nor-adrenaline may be key factors in precipitating the stress syndrome, by potentiating the strength and duration of muscle contractions and by stimulating glycogenolysis, respectively.

On-line Detection of PSE

The pig industry is moving rapidly towards automatic carcass grading, using probes such as the Fat-O-Meater and the Hennessy Grading Probe. Because these probes use optical systems to identify the interface between fat and lean tissue, they can be used to simultaneously record internal muscle reflectance or back-scatter of incident light. Probe readings increase during the development of rigor mortis, as a consequence of the denaturation of muscle proteins, and are high in PSE meat. Reliable detection of PSE at grading would allow the processor to include meat quality in payment for pigs, to select carcasses with different quality for appropriate end uses and would facilitate management action to reduce the incidence of PSE.

A serious obstacle to achieving these aims is the inability of probe measurements made on the killing line to identify all PSE carcasses.

PSE develops at different rates in different carcasses; severe PSE can be detected at about 45 min post mortem, but a substantial proportion of PSE gets past a quality checkpoint on the killing line (Lundstrom et al, 1987; Van der Wal et al, 1987). These late developers escape detection unless the carcasses are re-examined later.

The key question is: How soon after slaughter can a reasonable estimate of drip be made using optical probe measurements? In Irish Landrace and Large White pigs, the correlation between drip and optic probe value was not significant at 35 min after slaughter ($r = 0.19$), but was significant at 1 h ($r = 0.55$) and increased further by 4 h after slaughter ($r = 0.66$). In contrast, probe pH values were well correlated with drip as early at 45 min post mortem ($r = -.65$) but the correlation declined thereafter (Somers et al, 1985; Tarrant and Long, 1986).

The time lag between pH fall and the resulting denaturation of muscle proteins is responsible for the critical delay in the development of muscle opacity, the property measured by the present generation of optic probes. This delay is of no consequence, except where measurements are made on the hot carcass. In that case, a better prediction of drip loss and PSE would be provided by pH rather than opacity values. This could be done by immobilizing a reagent phase (chromophore) on or around the face of the optical fibre. Chromophores such as fluorescein, phenol red and bromothymol blue are used as pH indicators. Interaction with hydrogen ions leads to a change in the optical properties of the immobilized chromophore which may be detected through the optic fibre. There is a logarithmic relationship, based on the Beer-Lambert law, between the detected signal and pH (M O'Dowd pers comm). In the future, such a development may increase the potential of optical probes for detecting PSE at grading.

It is inevitable that some potentially 'drippy' meat will escape detection on the killing line, due to the existance of the so-called 'Hampshire effect' (Monin and Sellier, 1987; Sellier, 1987). In this case, higher drip loss and lower processing yields are a consequence of an abnormally low ultimate pH rather than a rapid rate of pH fall. Whereas rapid pH fall appears to be inherited as a recessive or partly recessive trait associated with the halothane locus, the low ultimate pH charact erising the Hampshire breed appears to be inherited as a dominant or partially dominant trait. The condition manifests itself in vivo as an abnormally high content of glycogen in the musculature.

DFD and Glycogen Metabolism before Slaughter

Dark, firm, dry meat is a potential problem in all meat species, but has been most closely examined in pork and particularly in beef muscle, where the valuable trade in fresh chilled beef has given rise to stringent quality requirements for colour and shelf-life (Poulanne et al, 1981). DFD is a consequence of antemortem breakdown of muscle glycogen, giving rise to abnormally high ultimate pH values in meat.

Glycogen metabolism in beef muscle is similar to that in monogastric species, despite differences in the nature of the dietary supply of energy (McVeigh and Tarrant, 1982). Glycogen breakdown in muscle is under dual control by the sympathetic and somatomotor nervous systems which exercise their control by increasing the contractions of the key intracellular messengers, cyclic AMP and Ca^{2+} respectively. Cyclic AMP activates protein kinase and Ca^{2+} activates phosphorylase kinase, two enzymes that regulate sequential steps in the so-called glycogenolytic cascade. One or both of these mechanisms may be activated in any particular glycogen-wasting situation (McVeigh and Tarrant, 1983).

In beef, physical exercise before slaughter is by far the most common cause of dark-cutting. The strenuous activity accompanying social regrouping of young bulls greatly accelerates glycogen breakdown, which is activated by the rise in free Ca^{2+} concentration in the sarcoplasm that triggers muscle contraction. Although the sympathetic-adrenal medullary system may also be stimulated by the challenge of social regrouping, this was shown not an important factor in activating glycogen breakdown in beef muscle, because an effective blockade of the β adrenoceptor sites on the muscle cell membranes failed to protect the muscle glycogen store during social regrouping (McVeigh and Tarrant, 1983).

As expected, it is the working muscles that are depleted of glycogen and become dark-cutting. These are principally M. longissimus dorsi (LD) and M semitendinosus, with other major hindlimb muscles affected to a lesser degree (Tarrant and Sherington, 1980; Kenny and Tarrant, 1984). In these working muscles, it is the fast twitch fibres that lose glycogen most rapidly, suggesting that they are recruited in preference to the slow twitch fibres, which undergo a more gradual loss of glycogen (Lacourt and Tarrant, 1985). In contrast, the reverse situation prevails when the adrenergic mechanism is activated. After adrenaline injection, the glycogen store in the slow fibres is rapidly and completely exhausted, whereas the fast fibres are only partially depleted.

Observation of the behaviour of beef cattle before slaughter clarified the relationship between physical activity and dark-cutting. A high correlation ($r = -0.85$) was found between muscle glycogen concentration and mounting activity in beef heifers

$$g = 77.16 - 0.56\ n$$

where g = glycogen concentration of the LD (in micromoles of glucose per gram of wet tissue) and n = number of mounts performed during the preslaughter period. Thus, each time an animal mounted, it lost 0.56 µmoles of muscle glycogen (Kenny and Tarrant, 1984). This relationship may vary somewhat because of differences between individuals in the oxidative capacity of skeletal muscle. The important point is that it shows the need to control physical activity in cattle. An animal mounting ten times per hour may not be conspicuous in a busy slaughter-yard, yet over a 10 h period it will lose 56 µmoles of muscle glycogen, well over half of the typical resting store. If we consider an ultimate pH value below 6.0 to be a prerequisite for quality in fresh meat, then the minimum concentration of glycogen necessary to achieve this value in the LD is 40 µmol glucose/g wet tissue. The glycogen requirement to achieve other pH_u values may be estimated from the relationship,

$$pH_u = 7.1 - 0.028g \quad \text{(McVeigh, 1980)}$$

Straightforward procedures for controlling animal behaviour are quite successful. An overhead electrified wire grid was used to modify the behaviour of young bulls mixed in pens at an abattoir (Kenny and Tarrant, 1987). The overhead grid was effective in significantly lowering mounting activity. This resulted in higher muscle glycogen content, lower meat ultimate pH values, and reduced carcass bruising. In two trials, the incidence of dark-cutting was reduced from 64% to zero and from 35% to zero by use of the modified pens. Interestingly, on the basis of circulating cortisol, the electric grid did not constitute an additional source of stress for the cattle.

Beta agonists and meat quality

The use of implants as growth promoters is no longer permitted in the European Community and, as a consequence, alternative acceptable methods of reducing fatness are being sought. Discovery of the powerful 'repartitioning' effect that β agonists have on carcass composition has aroused considerable interest. When used as feed additives, agents such as clenbuterol and cimaterol improved production efficiency and lean content, and reduced fat content in ruminants, pigs and poultry (Ingle and Dalrymple, 1986).

These compounds possess similar structural and functional features to the natural catecholamines. The β_1 agonists increase heart rate and mobilize fat from adipose tissue, β_2 agonists stimulate glycogenolysis and vasodilation in skeletal muscle. The useful property of the repartitioning agents is that, instead of increasing heat production, they divert energy from fat into the synthesis of muscle protein by an as yet unknown mechanism. In view of their efficacy at improving many quantitative traits (Allen et al, 1986; Hanrahan et al, 1986; Ingle and Dalrymple, 1986) there is interest in the effect of β agonists on meat quality.

One possible side-effect of treatment with β_2 agonists might be an increase in DFD meat, due to adrenergic activation of muscle glycogenolysis. Indeed, such an effect was observed in lamb fed cimaterol until two days before slaughter (Allen et al, 1985). Loin muscle pH_u values were significantly elevated after medium and high rates of dosage (2.29 and 11.42 mg of cimaterol/kg of feed) causing an increase in the incidence of DFD meat in the lamb carcasses. In further work where cimaterol was withdrawn 7 days before slaughter the muscle pH_u values were normal. Although muscle glycogen content was lower in the treated lambs, there was sufficient for normal meat quality. In subsequent trials on steers, a 7-day withdrawal period was observed and uniformly normal ultimate pH values were obtained (Allen et al, 1986). However, any treatment that lowers muscle glycogen content at preslaughter will inevitably, under commercial conditions, push a larger proportion of animals into the DFD category.

In the Friesian steers, cimaterol caused hypertrophy of the hindquarters, the area of the LD increased by 40% and lean meat yield increased by 9% (Allen et al, 1986). The fat content of the LD decreased and meat quality was adversely affected. The drip loss from slices of LD doubled. In addition, the shear force value of the cooked steaks doubled, and a taste panel rated the steaks lower than those from control animals for all quality characteristics (Allen et al, 1987).

How cimaterol increases shear force and drip loss in treated beef has not been established. Possible mechanisms for the increase in toughness are:-

1. an increase in the quantity and/or degree of cross-linking of collagen in muscle, and
2. a decrease in the activity of the endogenous proteases responsible for meat tenderization during conditioning.

In this respect it is of interest that Reeds et al (1986) showed that clenbuterol increased in vivo muscle protein deposition in the rat by having an inhibitory effect on protein degradation.

Concerning the increase in drip, the appearance of beef from cimaterol-treated cattle did not suggest a PSE condition. Post mortem pH fall in beef is accelerated by antemortem adrenaline treatment, but a direct acceleration of pH fall by cimaterol was unlikely due to the 7 day withdrawal period. It is possible that changes in muscle water-holding capacity may be associated with hypertrophy of the fast twitch fibres. These increased in cross-sectional area by 50% in cimaterol-fed lambs (Kim et al, 1986). There was no effect on the cross-sectional area of slow twitch fibres, or on muscle fibre type composition.

There may be an analogy between the effects of orally administered β agonists and the naturally-occurring increased responsiveness to catecholamines observed in lean breeds of pigs. Pietrains are thought to develop their leanness through enhanced responsiveness to noradrenaline, but the higher responsiveness of the sympathetic nervous system in such breeds may contribute to the porcine stress syndrome and PSE meat (Lister, 1987). Oral administration may provide a controlled mechanism for the supply of β agonists, without the hazard of a naturally hyperactive sympathetic nervous system. The increased drip in β agonist treated cattle may be associated with chronic changes in tissue composition rather than with acute metabolic disturbances, such as those seen at slaughter in stress-prone animals.

Calpains and Cathepsins in Meat Conditioning

Tenderness is an important quality characteristic in most meat products and is considered to be the primary attribute of eating quality in red meats. Interest in the natural tenderising enzymes of meat is stimulated by consumer resistance to food additives, including tenderizers, particularly in traditional foods such as fresh meat. The main components of texture in meat are the fibrous proteins of connective tissue and the myofibril, located outside and within the muscle cell respectively. The increase in tenderness during conditioning may be ascribed to changes occurring in these proteins. Examination of changes in collagen structure during conditioning of meat has not revealed transformations comparable in extent to those in the myofibrillar structure including the recently described cytoskeletal framework. Thus, the central mechanism in the conditioning process may be the limited hydrolysis of myofibrillar proteins by endogenous muscle enzymes (Goll et al, 1983).

Two proteolytic systems may be involved, the calpains and the cathepsins. The former is activated by Ca^{2+} at neutral pH (Busch et al, 1972) and has been isolated from skeletal muscle in two forms, one of which is optimally active at 1 mM Ca^{2+} and the other at about 0.1 mM Ca^{2+} (Dayton et al, 1981; Penny et al, 1985). Some changes that occur in the myofibril during meat conditioning can be reproduced in vitro by calpain. The second system comprises certain lysosomal enzymes including cathepsins B, D and probably also H and L. These enzymes are active under acidic conditions and also induce changes similar to those found during conditioning (Schwartz and Bird, 1977; Zeece et al, 1986; Ouali et al, 1987; and others).

It is necessary to establish what proteolytic system is responsible for meat tenderisation and how soon after slaughter the process of enzymic tenderisation commences. It may then be possible to optimise the conditioning process by devising carcass handling techniques that stimulate the activity of the relevant proteases by altering the intracellular environment. Important variables in this respect are pH, temperature, free Ca^{2+} concentration and the ultrastructural configuration of the substrate proteins (Locker, 1982, 1985; Marsh, 1985; Dutson and Pearson, 1985).

One approach to establishing the relative importance of the neutral versus acidic proteolytic systems is to

examine protein breakdown in muscles with widely different post mortem pH histories. The premise is that, by identifying the conditions that favour post mortem proteolysis, we may shed light on which enzyme system is responsible. Using this approach with beef carcasses, we induced exceptionally low (pH below 6 within an hour of slaughter) or high (ultimate pH above 6.4) post mortem pH values, using ES (electrical stimulation) or antemortem adrenaline, respectively. The LD muscles were then conditioned at high (37°C) or low (4°C) storage temperature. Myofibrils from the two treatments and from conventionally slaughtered controls were compared using SDS-PAGE, with emphasis on detecting changes during the early post mortem period (Troy and Tarrant, 1987; Troy et al, 1987).

The results showed that muscle pH has a major effect on the nature and rate of degradation of the myofibrillar proteins. In the molecular weight range below myosin heavy chain (210 kDa) the changes were, in general, greater and occurred at an earlier time post mortem in ES than in control meat, and the least amount of change was observed in the high pH meat. Within 4 h of slaughter new polypeptide bands were detected in myofibrils from ES meat. These bands (at 154, 145, 90, 33 and 30 kDa) are likely proteolytic degradation products. In control meat these bands were detected between 6 and 8 h after slaughter, and their appearance was further delayed in high pH meat. The band at 33 kDa appeared in control and ES muscle but was absent in the high pH muscle. Degradation products which appeared in the M- and C-protein region, and which may originate from myosin heavy chain, were much less evident and appeared at a later stage post mortem in high pH meat compared with control and ES meat.

In contrast to the above, cleavage of high molecular weight proteins (> 210 kDa) was favoured in meat of high pH. Nebulin and the band directly beneath it were depleted by 8 h post mortem in high pH meat and not until later in the control and ES meat. Furthermore, troponin-T was degraded to a much greater extent in high pH meat than in control or ES meat.

The above changes occurred in muscle stored at 37°C. In muscle held at 4°C the earliest degradation products of the myofibrils were not detected until 2 days post mortem in ES and high pH meat and 3 days post mortem in control meat. Again, ES muscle showed the most pronounced changes although certain discrete changes occurred preferentially in high pH muscle. These included partial loss of α-actinin and troponin T, and complete loss of filamin.

These results indicate that breakdown of the myofibrillar proteins commenced during the very early post mortem period. Separate degradative pathways were favoured by high or low post mortem pH respectively. However, the relationship between cleavage of specific proteins and changes in meat texture is unknown. The fact that the mechanism of proteolytic attack is substantially different under conditions that favour either calpain or cathepsin activity poses a question as to where the best advantage lies with respect to meat tenderising. To answer this question we need to know more about the effect of cleavage of specific myofibrillar proteins on meat texture.

CONCLUSIONS

Progress is being made in understanding the biological and biochemical basis of meat quality, ultimately for the benefit of both the meat industry and the consumer. Fundamental questions remain to be answered, for example in the diverse fields of PSS, the mechanisms of muscle hypertrophy and the biochemistry of conditioning. New questions are continually being generated as a consequence of advances in breeding, growth manipulation and slaughter technology that alter the properties of skeletal muscle.

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TABLE 1: Proximate composition of semitendinosus muscle (Values expressed as % of fresh tissue)

	WATER(Fat-Free)%		PROTEIN(Fat-Free)%		LIPID %		WATER/PROTEIN	
	7 m.	10 m.	7 m.	10 m.	7 m.	10 m.	7 m.	10 m.
C.S.	77.6±0.5^{a}	78.0±0.8^{a}	21.4±0.5^{a}	21.1±0.6^{a}	4.2±1.5^{ab}	8.3±3.1^{a}	3.6±0.1^{a}	3.7±0.1^{a}
B.La.	78.4±0.7^{a}	78.0±0.1^{a}	20.5±0.7^{a}	20.9±0.1^{a}	3.6±1.3^{ab}	2.2±1.2^{b}	3.8±0.2^{a}	3.7±0.02^{a}
I.La.	78.1±0.8^{a}	77.5±0.4^{a}	20.8±0.7^{a}	21.4±0.4^{a}	2.3±0.5^{b}	3.7±2.3^{ab}	3.8±0.2^{a}	3.6±0.1^{a}
D.	77.6±0.5^{a}	77.5±0.4^{a}	21.4±0.5^{a}	21.4±0.4^{a}	6.3±2.4^{a}	5.8±1.2^{ab}	3.6±0.1^{a}	3.6±0.1^{a}
P.	77.9±1.2^{a}	77.9±0.2^{a}	21.1±1.2^{a}	21.0±0.2^{a}	2.7±1.4^{b}	2.6±0.6^{b}	3.7±0.3^{a}	3.7±0.04^{a}
L.W.	77.9±1.1^{a}	77.4±0.7^{a}	21.1±1.1^{a}	21.5±0.7^{a}	2.9±1.5^{ab}	2.2±0.5^{b}	3.7±0.2^{a}	3.6±0.2^{a}
MEAN	77.9±0.3^{a}	77.7±0.3^{a}	21.0±0.3^{a}	21.2±0.2^{a}			3.7±0.1^{a}	3.7±0.1^{a}

TABLE 2: pH of the 7 months group

	LONGISSIMUS DORSI		SEMIMEMBRANOSUS		BICEPS FEM.	SEMITENDIN.
	45'	48h	45'	48h	48h	48h
C.S.	6.81±0.21^{a}	5.68±0.09^{a}	6.46±0.09^{a}	5.68±0.07^{b}	5.78±0.04^{bc}	5.99±0.09^{ab}
B.La.	5.93±0.08^{c}	5.57±0.06^{ab}	6.12±0.15^{b}	5.94±0.17^{a}	5.78±0.15^{bc}	6.15±0.17^{a}
I.La.	6.43±0.18^{b}	5.57±0.02^{ab}	6.46±0.04^{a}	5.70±0.06^{b}	6.16±0.16^{a}	5.73±0.16^{bc}
D.	6.50±0.03^{ab}	5.56±0.02^{b}	6.37±0.04^{a}	5.64±0.06^{b}	6.02±0.12^{ab}	5.65±0.09^{bc}
P.	5.74±0.02^{c}	5.40±0.04^{c}	5.89±0.12^{c}	5.59±0.14^{b}	5.61±0.15^{c}	5.92±0.21^{ab}
L.W.	6.44±0.20^{b}	5.52±0.03^{b}	6.30±0.11^{a}	5.62±0.02^{b}	5.93±0.19^{abc}	5.57±0.14^{c}

TABLE 3: pH of the 10 months group

	LONGISSIMUS DORSI		SEMIMEMBRANOSUS		BICEPS FEM.	SEMITENDIN.
	45'	48h	45'	48h	48h	48h
C.S.	6.53±0.12^{a}	6.03±0.21^{a}	6.45±0.13^{a}	6.19±0.25^{a}	6.24±0.24^{a}	6.20±0.17^{ab}
B.La.	5.62±0.04^{c}	5.72±0.06^{bc}	5.91±0.20^{b}	5.58±0.15^{b}	5.80±0.08^{b}	6.02±0.13^{ab}
I.La.	6.15±0.43^{abc}	5.92±0.09^{ab}	6.41±0.19^{a}	5.87±0.16^{ab}	5.87±0.12^{ab}	6.40±0.21^{a}
D.	6.36±0.41^{abc}	5.72±0.04^{bc}	6.28±0.08^{ab}	5.79±0.05^{ab}	5.79±0.11^{b}	5.98±0.18^{ab}
P.	5.75±0.16^{bc}	5.68±0.03^{c}	6.00±0.40^{ab}	5.50±0.07^{b}	5.77±0.04^{b}	5.87±0.12^{b}
L.W.	6.43±0.20^{ab}	5.66±0.08^{c}	6.36±0.11^{ab}	5.73±0.09^{b}	5.58±0.05^{b}	6.00±0.23^{ab}

TABLE 4: Glycolytic parameters (Values expressed as g % of freeze-dried muscle)

	LONGISSIMUS DORSI					SEMIMEMBRANOSUS				
	Glycogen	Glucose	Lactic Ac.	Glycolytic Potential	Lact.Ac.% Glyc.Pot.	Glycogen	Glucose	Lactic Ac.	Glycolyt. Potent.	Lact.Ac.% Glyc.Pot.
C.S.	1.2±0.5^{b}	0.2±0.1^{c}	1.9±0.7^{a}	4.6±0.8^{b}	41.30	0.7±0.4^{c}	0.2±0.1^{b}	2.6±0.7^{ab}	4.6±0.7^{cd}	56.52
B.La.	0.9±0.2^{b}	0.7±0.1^{ab}	3.7±0.5^{a}	7.0±1.2^{ab}	52.86	1.0±0.2^{bc}	0.7±0.2^{ab}	4.0±0.5^{a}	7.4±0.8^{abc}	54.05
I.La.	1.2±0.9^{b}	0.5±0.1^{bc}	2.7±1.0^{a}	6.2±1.4^{b}	43.55	1.0±0.4^{c}	0.4±0.1^{ab}	2.1±0.8^{b}	4.9±1.5^{d}	42.86
D.	1.6±0.5^{ab}	0.3±0.2^{bc}	2.7±1.3^{a}	6.6±1.1^{ab}	40.91	1.2±0.4^{bc}	0.5±0.1^{ab}	2.8±0.2^{ab}	6.3±1.0^{bcd}	44.44
P.	1.6±0.4^{ab}	0.9±0.1^{a}	4.0±0.2^{a}	8.9±1.3^{a}	44.94	2.0±0.5^{ab}	0.8±0.4^{a}	3.2±1.5^{ab}	8.8±1.7^{ab}	36.36
L.W.	2.7±0.6^{a}	0.4±0.2^{bc}	2.2±0.7^{a}	8.3±1.1^{a}	26.51	2.3±0.6^{a}	0.5±0.1^{ab}	3.3±1.2^{ab}	8.9±1.4^{a}	37.08

TABLE 5: Protein solubility and non protein nitrogen in ST muscle (Values expressed as % of total proteins)

	SOLUBLE PROTEINS			NON PROTEIN NITROGEN		
	7 m.	10 m.	7 m.+ 10 m.	7 m.	10 m.	7 m.+ 10 m.
C.S.	83.4±6.4[a]	86.2±8.8[a]	84.6±7.2[a]	1.64±0.10[a]	1.69±0.05[a]	1.67±0.08[a]
B.La.	71.7±18.7[a]	72.7±10.8[a]	72.1±14.8[a]	1.66±0.01[a]	1.67±0.14[a]	1.67±0.08[a]
I.La.	82.2±4.9[a]	88.2±8.1[a]	84.9±6.9[a]	1.66±0.07[a]	1.61±0.16[a]	1.64±0.11[a]
D.	76.6±9.8[a]	72.7±13.5[a]	74.9±11.0[a]	1.61±0.06[a]	1.77±0.04[a]	1.68±0.10[a]
P.	65.1±18.4[a]	66.8±19.8[a]	65.6±17.6[a]	1.64±0.11[a]	1.81±0.13[a]	1.71±0.14[a]
L.W.	71.7±13.0[a]	77.4±12.3[a]	74.2±12.2[a]	1.75±0.06[a]	1.76±0.09[a]	1.75±0.07[a]

TABLE 6: Correlation coef. and prob. levels between ST 48h pH and protein solubility and non protein nitrogen

	Sol. Prot.	Non Prot. Nitrogen
pH	0.86	-0.76
	0.0001	0.0001

TABLE 7: Correlation coefficients and probability levels between muscles pH and "L" and "b" values

	LONGISSIMUS DORSI		SEMIMEMBRANOSUS		BICEPS FEM.	SEMITENDIN.
	45'pH	48h pH	45'pH	48h pH	48h pH	48h pH
L	-0.62	-0.56	-0.72	-0.54	-0.47	-0.66
	0.0001	0.0001	0.0001	0.0002	0.0002	0.0001
b	-0.50	-0.42	-0.76	-0.68	-0.74	-0.47
	0.0001	0.002	0.0001	0.0001	0.0001	0.0005

TABLE 8: Correlation coefficients and probability levels between "L","b" and protein solubility and non protein nitrogen.

	Sol.Prot.	Non Prot. Nitrogen
L	-0.80	0.65
	0.0001	0.0009
b	-0.65	0.55
	0.0001	0.006

TABLE 9: Correlations coefficients and probability levels between "L","a" and "b" values of thigh muscles.

	SM/B.F.	SM/ST	B.F./ST
L	0.79	0.60	0.47
	0.0001	0.0001	0.004
a	0.43	0.06	0.18
	0.007	0.73	0.30
b	0.74	0.48	0.40
	0.0001	0.003	0.01