

THE STRESS SYNDROME AND MEAT QUALITY

POST MORTEM GLYCOLYSIS IN THE SEMITENDINOSUS, PSOAS AND LONGISSIMUS DORSI MUSCLES OF CAPTIVE BOLT SLAUGHTERED AND ANAESTHETIZED BOER GOATS (CAPRA HIRCUS)

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The rates of post mortem glycolysis in some muscles of captive bolt slaughtered and halothane-anaesthetized goats were measured. Unlike the pig it is shown that the captive bolt slaughter technique does not produce a very rapid post mortem glycolysis. Initial pH values in the semitendinosus, psaos and longissimus dorsi muscles of the slaughtered animals were in the region of 6,8, while the pH₁ values were about 6,6. The longissimus dorsi was affected less by captive bolt slaughter than either the psaos or the semitendinosus. Data on levels of ATP, phosphocreatine and glucose-6-phosphate in these muscles are also presented, and are related to the fibre types of the muscles as determined by succinic dehydrogenase histochemistry.

POST MORTEM GLYCOLYSE IN DEN MUSKELN SEMITENDINOSUS, PSOAS UND LONGISSIMUS DORSI VON MIT SCHLAGBOLZEN-PISTOLE UND NARKOSE GETÖTETEN BOERBOK ZIEGE (CAPRA HIRCUS)

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Die Gewindigkeit der post mortem Glycolyse wurde in einigen Muskeln von Ziegen gemessen welche mit Schlagbolzen-pistole oder Halothane-narkose getötet waren. In Gegensatz zum Schwein verursachte die Tötung mit Schlagbolzen-pistole keine stark erhöhte post mortem Glycolyse. Die pH Werte in den semitendinosus, psaos und longissimus dorsi Muskeln wurde weniger von der Tötung mit Schlagbolzen-pistole beeinflusst als sowohl die M.psaos wie auch die M.semitendinosus. Die Werte der Mengen an ATP, Phosphocreatin und Glucose-6-phosphat werden diskutiert, und ihre Beziehung zu dem auf Grund der Succinat-Dehydrogenase histochemisch identifizierten Fasertyps der Muskel.

LA GLYCOLYSE POST MORTEM DES MUSCLES SEMITENDINOSUS, PSOAS ET LONGISSIMUS DORSI DES CHÈVRES BOER (CAPRA HIRCUS) ABATTUES (ET ANESTHÉSIÉES) AU MOYEN DU PISTOLET D'ABATTOIR (CAPTIVE BOLT)

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Les taux de glycolyse post mortem de certains muscles de chèvres abattues et anesthésiées au halothane ont été mesurés. Contrairement à ce qui se produit chez le porc il est montré que la technique d'abattage au pistolet d'abattoir: "captive bolt" ne produit pas très rapidement une glycolyse post-mortem. Les valeurs initiales des muscles semitendinosus, psaos et longissimus dorsi des animaux abattus se situèrent dans la région de 6,8 tandis que les valeurs pH₁ furent d'environ 6,6. Le longissimus dorsi fut moins affecté par l'abattage au pistolet "captive-bolt", que le psaos ou le semitendinosus. Des données dans les niveaux de l'ATP, phosphocreatine et glucose-6-phosphate de ces muscles sont également présentées, et sont mises en relation avec les types de fibre des muscles par détermination succinic-dehydrogenase histochimique.

POST MORTEM GLYCOLYSIS В МУСКЛАХ SEMITENDINOSUS, PSOAS И LONGISSIMUS DORSI КОЗ, [CAPRA HIRCUS] УБИТЫХ СПОСОБОМ „CAPTIVE-BOLT“ ПОД НАРКОЗОМ И БЕЗ НАРКОЗА

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Скорость post-mortem glycolysis в некоторых мышцах коз, убитых способом „captive-bolt“ под наркозом halothane, без наркоза, была измерена. Было установлено, что в отличие от свиньи, метод убоя не приводит к очень быстрой post-mortem glycolysis. Начальные значения pH в мышцах semitendinosus, psaos и longissimus dorsi в убитых животных были около 6,8, тогда как значения pH₁ были около 6,6. Воздействие на мышцу longissimus dorsi при таком способе убоя было слабее, чем на psaos или на semitendinosus. Данные об уровнях ATP, phosphocreatine и glucose-6-phosphate в этих мышцах также представлены и связаны с типами мышечных волокон с помощью гистохимического метода succinic-dehydrogenase.

THE STRESS SYNDROME AND MEAT QUALITY

POSTMORTEM GLYCOLYSIS IN THE SEMITENDINOSUS, PSOAS AND LONGISSIMUS

DORSI MUSCLES OF CAPTIVE-BOLT SLAUGHTERED AND ANAESTHETISED BOER

GOATS (*CAPRA HIRGUS*)

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It is well known that the rate of postmortem glycolysis in the skeletal musculature of the pig determines the quality of the pork as assessed by colour, texture and juiciness (1, 2). The postmortem glycolytic rate of porcine muscles is affected by many factors, notably, the method of stunning, genetic composition, environmental stressors and physiological status. The incidence of the pale, soft, exudative muscle condition (PSE) is greatest in the so-called stress susceptible breeds of pig, the Pietrain, Poland China and Landrace breeds. The most affected muscles are the longissimus dorsi and the semimembranosus, and to a lesser extent the rectus femoris. The longissimus dorsi is affected to different degrees along its length, the most susceptible regions being the tenth thoracic and third lumbar vertebral regions (3). The method of stunning can produce sufficiently rapid rates of postmortem glycolysis to cause the PSE condition in the musculature of stress susceptible and stress resistant pigs alike (4, 5). It appears that the physical stimulation which pigs encounter before slaughter is accentuated in the stress susceptible breeds by failure of the mechanisms which regulate the resting rate of glycolysis in muscle (6). Of the three methods of stunning, carbon dioxide, electrical and captive-bolt, the latter seems to be the most severe in terms of its effects on postmortem muscle metabolism (4, 5). Apparently the impact of the bolt produces neural discharges which pass down the spinal column in the intact motor tracts and activate the voluntary muscles (2).

To our knowledge there is little comparative information on the effects of the stunning method on the rate of postmortem muscle metabolism of bovines, sheep, or goats. In the present study the effect of the captive-bolt stunning method on the rate of postmortem glycolysis in the skeletal muscles of the Boer goat (*Capra hircus*) is examined. The Boer goat, raised in many parts of Southern Africa, has not been selected for high total muscularity or growth rate unlike the various

breeds of pig already referred to. Furthermore the fibre types of the muscles are predominantly red while those of the pig are mainly white. It was of interest to determine the rate of postmortem glycolysis in the muscles of goats stunned with the captive-bolt and to compare the results with those of pigs stunned by the same method.

Materials and Methods

Animals: Adult Boer goats, reared on the Institute farm, were used. The animals were in the liveweight range 35 to 50 kg and were fed on a stock diet ad libitum.

Slaughter and anaesthesia: A polythene bag, having two apertures for inspiration and expiration of gases, was placed over the animal's head. Anaesthesia was induced with 5% halothane and an oxygen flow rate of 2 litres per minute, delivered from a closed circuit anaesthetic apparatus by an endotracheal tube inserted in the inspiration aperture of the polythene bag. The concentration of halothane was controlled with a 'Fluotac' vaporiser in the closed circuit. After a suitable plane of anaesthesia was obtained, the upper region of the trachea was exposed and partially transected and a tracheal tube was inserted. Anaesthesia was maintained with 1.5 to 2.5% halothane and an oxygen flow rate of 0.5 litres per minute. After twenty minutes had elapsed, the muscles were excised. Goats were stunned with the standard captive-bolt pistol, exsanguinated immediately and the muscles removed from five to ten minutes after stunning.

Biochemical measurements: Three muscles were used in the present investigation, namely, the *m. semitendinosus*, *longissimus dorsi* and *psaos*. The *semitendinosus* and *psaos* were excised as intact as possible while the *longissimus dorsi* was sampled between the second and fourth lumbar vertebrae. In the case of the stunned animals the time of excision of the muscles was standardised to five to ten minutes after stunning. After removal the muscles were placed at 37°C in an incubator having a moist nitrogen atmosphere. One gram samples were taken from each muscle at one hour intervals for pH determination and for analysis of phosphocreatine, ATP and glucose-6-phosphate. For analysis of the organic phosphate compounds, the muscle samples were frozen in a dry-ice/acetone mixture and stored at -40°C.

Muscle pH was measured on homogenates prepared in five volumes of 5 mM iodoacetate, pH 7.0 at 25°C. A Radiometer pH meter with glass electrode and scale expansion accessory was used to make the measurements.

Phosphocreatine, ATP and glucose-6-phosphate were determined by the coupled enzymatic method of Lampracht and Stein (7). The frozen muscle samples were extracted with 9.25 volumes of ice-cold 5% perchloric acid by homogenisation with a 'Ultra-Turrax' homogeniser. The extracts were filtered at 0°C with Whatman No. 1 paper supported on a 'Millipore' microanalysis filter holder. The filtrate was neutralised with saturated potassium carbonate and the precipitated perchlorate allowed to settle out for fifteen minutes at 0°C. 0.05 ml. aliquots of the filtrate were assayed in duplicate using a Beckman 'Acta' III spectrophotometer at 340 nm. Concentrations of the phosphate compounds are expressed as μ moles per gram of tissue, wet weight.

Fresh muscle samples were taken from the three muscles already named for histochemical demonstration of succinic dehydrogenase using the nitro-blue tetrazolium method of Malaty and Bourne (8). Fibres were classified into three groups according to the intensity of staining i.e. red, intermediate and white (9).

Results and Discussion

Biopsy specimens were taken from five anaesthetised goats, and the initial pH values and levels of phosphocreatine, ATP and glucose-6-phosphate determined. Postmortem changes in these parameters were followed for several hours under anaerobic conditions at 37°C. Ultimate values (after 24 hours) were also obtained for each parameter. Similar measurements were made on the muscles of the captive-bolt stunned animals, the first ones being made eight minutes (mean value) after stunning. The results obtained from the anaesthetised goats are used as reference values, and departure from these indicates the magnitude of the effect of the captive-bolt stunning.

The initial pH values of the *semitendinosus*, *longissimus dorsi* and *psaos* muscles from the anaesthetised goats were 7.08 ± 0.05 (S.E.M.), 7.22 ± 0.03 and 7.13 ± 0.06 , respectively. In contrast, the corresponding values for the five stunned goats were 6.64 ± 0.05 , 6.84 ± 0.05 and 6.59 ± 0.03 . The effect of stunning on the initial pH is significantly greater in the case of the *semitendinosus* and *psaos* muscles than in the *longissimus dorsi* ($P < 0.05$). Generally the initial pH values of the three muscles are higher than the initial values for pig *longissimus dorsi* after stunning, by 0.3 to 0.5 pH units (10). The initial values for the muscles of the live animals are 0.1 to 0.2 pH units higher than those obtained for anaesthetised pigs (10), but are the same as initial value of 7.2 reported for the *longissimus dorsi* of anaesthetised pigs which were also curarised (11). Fig. 1 shows the rate of postmortem glycolysis, measured as decrease in muscle pH, in the three muscles from the anaesthetised and stunned goats during the three hours immediately postmortem and after twenty four hours. The rates of pH decrease in all three muscles from the anaesthetised animals are greatest in the first hour, being about 0.25 units, thereafter levelling off to about 0.15 units, similar to the rates of pH decrease in the muscles of the stunned goats. It is shown that the glycolytic rates after the first hour are the same in the muscles from the anaesthetised and stunned animals. In all instances the ultimate pH values fall in the range 5.45 to 5.65. It is clear from Fig. 1 that stunning produced the greatest burst of glycolysis in the *psaos* muscle, and least in the *longissimus dorsi*, the values being 0.54 and 0.38 pH units, respectively. pH decreases of 0.7 units in five minutes have been reported for the *longissimus dorsi* of captive-bolt stunned, stress resistant Landrace and Large White pigs (10) while the subsequent rate of pH decrease was 0.3 units per hour compared with 0.2 units per hour in the same muscle from anaesthetised pigs. Thus the effect of captive-bolt stunning differs considerably between the goat and pig. The goat responded with a burst of glycolysis about half that reported for the pig but the rate continued for several hours after the initial burst at the same value as for the muscles from the anaesthetised animals. It is worth noting that the glycolytic rate observed here in the muscles from the anaesthetised goats is similar to reported rates for pigs anaesthetised with halothane (10).

The results for phosphocreatine content of the muscles from the two groups of experimental animals are shown in Table 1. Only the values at zero time and one hour postmortem are shown here. The phosphocreatine levels of the three muscles from the anaesthetised animals at zero time are just slightly lower than the values reported for porcine *longissimus dorsi* (10). Seventeen μ moles of phosphocreatine per gram were broken down in eight minutes in the *psaos* muscle of the stunned goats similar to the amount hydrolysed in the pig for the same degree of pH decrease. As in the case of pH, the least hydrolysis of phosphocreatine occurred in the *longissimus dorsi*. In pig muscle about 50% of the resting levels of phosphocreatine were hydrolysed when the pH reached 6.8, while it was almost completely depleted at pH 6.6 (10). In the present study only 10% of the initial

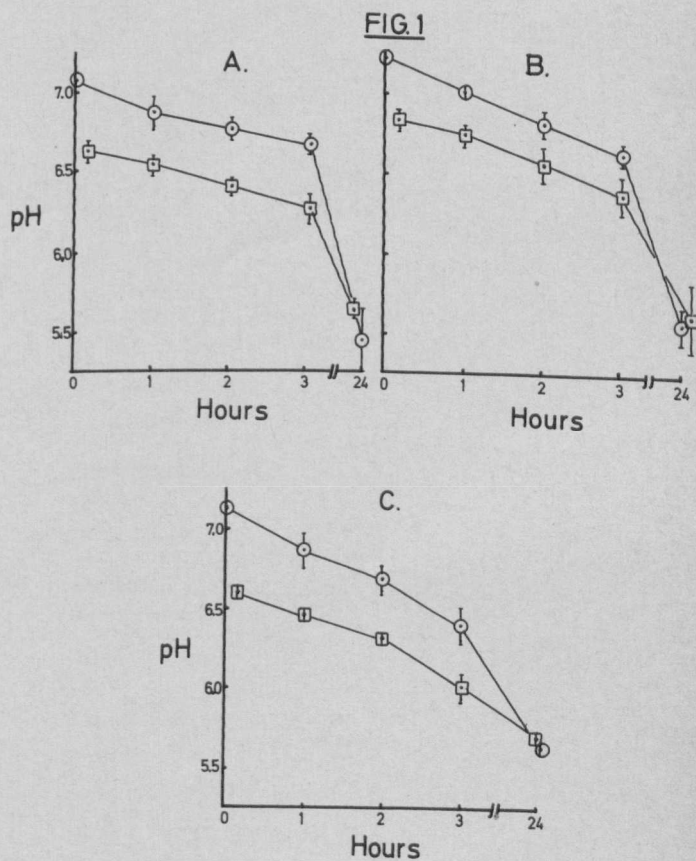


Fig. 1 Rates of postmortem glycolysis in the *semitendinosus* (A), *longissimus dorsi* (B) and *psaos* (C) of anaesthetised and stunned goats. Circles indicate anaesthetised and squares indicate stunned animals. The diameter of a circle or side of a square corresponds to 0.075 pH units, and represent $2 \times$ S.E.M. where bars are absent.

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TABLE 1

Phosphocreatine content of three muscles of anaesthetised and stunned Boer goats

Muscle	Anaesthetised		Stunned	
	0 Hours	1 Hour	0 Hour	1 Hour
Semitendinosus	*16.7±2.1	8.7±2.0	3.3±0.4	3.0±0.3
Longissimus dorsi	20.1±1.9	9.6±1.2	7.4±1.8	4.0±0.5
Psoas	19.3±2.1	6.8±1.4	2.6±0.1	3.1±0.5

*Phosphocreatine content is expressed as μ moles per gram of tissue, wet weight, mean values \pm S.E.M., 5 animals per group.

phosphocreatine remained at pH 5.6. Thus, in the goat as well as in the pig, the extent of phosphocreatine hydrolysis is the most sensitive indicator of the physical stimulation elicited by the stunning procedure. The ATP values for the three muscles at zero time in both anaesthetised and stunned animals are shown in Table 2. As already noted for the pH and phosphocreatine, the amount of ATP hydrolysed during the initial burst of glycolysis was greatest in the psoas of the stunned goats while it was intermediate in the semitendinosus. Surprisingly, the initial ATP content of the longissimus dorsi was greatest in the stunned goats.

TABLE 2

ATP and glucose-6-phosphate content of muscles of anaesthetised and stunned goats

Muscle	Anaesthetised		Stunned	
	*ATP	*G-6-P ₄	ATP	G-6-P ₄
Semitendinosus	9.2±0.4	1.7±0.7	7.3±0.6	8.0±0.6
Longissimus dorsi	7.4±0.7	0.5±0.1	8.4±0.7	4.5±1.0
Psoas	9.6±0.4	1.1±0.3	6.3±0.3	4.8±0.9

*Values are given as μ moles per gram of tissue, wet weight, means \pm S.E.M., 5 animals per group.

The resting levels of glucose-6-phosphate (G-6-P₄) in the anaesthetised goats, shown in Table 2, show the same trend as the pH and phosphocreatine values. There is no apparent correlation between glucose-6-phosphate levels and initial pH in the muscles of the stunned animals. It may be that the lack of correlation at the initial time point is due to non-steady state fluxes of hexose monophosphates through the glycolytic cycle soon after the stunning trauma.

Histochemical staining of the psoas and semitendinosus muscles for succinic dehydrogenase activity showed that the former was composed of 54% red, 14% intermediate and 32% white fibres, while the latter consisted of 45% red, 21% intermediate and 34% white fibres. Enzyme measurements on the longissimus dorsi have yet to be made. It was noted that the semitendinosus of the goat was not visually divided into distinct red and white areas as occurs in the pig and other species.

It is clear that the muscles of the goat used in the present study are composed predominantly aerobic fibres i.e. red and intermediate fibres, and it may be that the apparent resistance of the goat muscles to stunning with the captive-bolt compared with the corresponding pig muscles is due to the dominant aerobic fibre type of the muscles of the former. A further difference between the goat and pig was observed in the present study, namely, the slowing of the glycolytic rate to the resting rate after the initial burst of glycolysis in the stunned animals. At present it is not known what is the extent of neurological differences in the motor units of the pig and goat, since differences at this level could also determine the responsiveness of the muscular system to the trauma of the stunning method. It is also possible that glycolytic control mechanisms are more labile in the predominantly white muscles of the pig compared with the mechanisms which operate in red fibres of animals such as the goat.

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