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POSTMORTEM GLYCOLYTIC METABOLISM IN THE SKELETAL MUSCLES OF ANAESTHETISED AND STUNNED BOER GOATS, *CAPRA HIRCUS*

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OPSOMMING: BEPALING VAN NADOODSE GLIKOLITIESE METABOLISME IN DIE SKELETSPIERE VAN BOERBOKKE, *CAPRA HIRCUS*, WAT DEUR NARKOSE OF DIE PENPISTOOL BEDWELM IS

Die spoed van nadoodse glikolise is by 37°C bepaal in 'n aantal spiere van boerbokke wat met halotaan of die penpistool bedwelm is. Die spiere wat by die ondersoek betrokke was, is die *M. semitendinosus, M. psoas major* en die thorakale en lumbale gedeeltes van die *M. longissimus dorsi*. Daar is gevind dat penpistool-bedwelming nie 'n baie vinnige nadoodse glikolise in die skeletspier veroorsaak nie. Die pH-aanvangswaarde van die *M. semi-tendinosus, M. psoas major* en *M. longissimus dorsi* was 6,8 in vergelyking met die aanvangswaarde van 7,1 by diere wat onder narkose was. Die pH_1 waardes in die penpistool-groep was 6,6 en die pH was nog steeds bokant 6,0 vier ure nadoods. Die *M. longissimus dorsi* was minder beïnvloed deur die penpistool-bedwelming as beide die *M. psoas major* en *M. semitendinosus*. Gegewens oor die vlak van ATP, fosfokreatien en glukose-6-fosfaat in dieselfde spiere word ook aangegee en is verwant aan die spierveseltipe soos histochemies bepaal deur barnsteensuur-dehidrogenase. Die gegewens is in teenstelling met gepubliseerde gegewens van varkspiere wat aantoon dat penpistool-bedwelming 'n nadelige uitwerking op vleis-kwaliteit het.

SUMMARY:

The rates of *postmortem* glycolysis in several muscles of halothane anaesthetised and captive-bolt stunned Boer goats were measured at 37°C. The muscles examined were the *M. semitendinosus*, the *M. Psoas major* and the *M. longissimus dorsi* at the thoracic and lumbar regions. It is shown that captive-bolt stunning does not produce a very rapid postmortem glycolysis in the skeletal musculature. Initial pH values in the *M. semitendinosus, M. psoas major* and *M. longissimus dorsi* of the stunned animals were in the region of 6,8 compared with an initial value of 7,1 in the anaesthetised animals. pH_1 values in the muscles of the stunned goats were about 6,6 and the pH was still above 6,0 at four hours *postmortem*. The *M. longissimus dorsi* was less affected by stunning than either the *M. psoas major* or the *M. semitendinosus*. Data on the levels of ATP, phosphocreatine and glucose-6-phosphate in the same muscles are also presented, and are related to the fibre type of the muscles as determined by succinic dehydrogenase histochemistry. The results are contrasted with previously published data which showed that captive-bolt stunning has a detrimental effect on the meat quality of the muscles of the pig.

It is well known that the rate of *postmortem* glycolytic metabolism in the skeletal musculature determines the quality of pork as assessed by colour, texture and juiciness (Briskey, 1964; McLoughlin, 1971). 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. It is seldom seen in the Large White Breed, and then only in a mild form (McLoughlin, 1971, 1974). The muscles which are affected most are the *M. longissimus dorsi*, the *M. semimembranosus*, and to a lesser extent the *M. rectus femoris*. The *M. longissimus dorsi* is affected to different extents along its length, the most susceptible regions being at the tenth thoracic and the third lumbar vertebrae (Lawrie & Gatherum, 1961). 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 (McLoughlin, 1965; van der Walt, 1971). 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 levels of glycolysis in skeletal muscle (Lister, Scopes & Bendall, 1969). Of the three methods of stunning, carbon dioxide, electrical and captive-bolt, the latter appears to be the most severe in terms of its effect on the rate of *postmortem* muscle metabolism (McLoughlin, 1965; van der Walt, 1971). Apparently, the impact of the captive-bolt pistol produces neural discharges which pass down the spinal column in the intact motor tracts and activate the voluntary muscle (McLoughlin, 1971).

To our knowledge there is little comparative information on the effects of the stunning method on the rates of *postmortem* muscle metabolism in 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 is examined. This goat has not been intensively bred for high total muscularity, food conversion or growth rate unlike the various breeds of pig already referred to. Furthermore the muscle fibre types of the intensively selected pigs 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.

Procedure

Animals: Adult Boer goats (*Capra hircus*), reared on the Institute farm, were used. The animals had body masses in

the range 35 to 50 kg and were fed on a stock diet *ad libitum*. They were housed in a spacious pen and were subjected to a minimum of pre-slaughter stress.

Anaesthesia and slaughter: Individual goats were brought into the operating theatre where a polythene bag, having apertures for inspiration and expiration of gases, was placed over the head of the animal. Anaesthesia was induced with 6% 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 "Fluotec" 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 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 small biopsies of the muscles were taken for biochemical analysis. The muscles were then excised and placed in an incubator having a moist nitrogen atmosphere at 37°C. The goats were then sacrificed with an overdose of anaesthetic. Goats were also stunned with the captive-bolt pistol. The animals were exsanguinated immediately and the muscles removed five to ten minutes after stunning and placed in the incubator. Five goats were anaesthetised and five were stunned.

Biochemical measurements: Three muscles were used in the present investigation, namely, the *M. semitendinosus, M. psoas major* and the *M. longissimus dorsi*. The *M. semitendinosus* and *M. psoas major* were excised as intact as possible while the *M. longissimus dorsi* was sampled at the tenth thoracic and third lumbar regions. In the case of the stunned animals, the time of excision of the muscles was standardised to five to ten minutes after stunning. 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 at –75°C and stored at –40°C.

Muscle pH was measured on homogenates prepared in five volumes of 5mM sodium 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 in the frozen muscle samples were determined by the coupled enzymatic method of Lamprecht & Stein, (1965) using a Beckman "Acta" III spectrophotometer. Concentrations of these compounds are expressed as μ moles per gram of tissue, wet mass.

Enzyme histochemistry: Fresh muscle samples were taken from the muscles already named for histochemical demonstration of succinic dehydrogenase using the nitro-blue tetrazolium method of Malaty & Bourne (1953). Fibres were classified into three groups according to the intensity of staining i.e. red, intermediate and white (Gauthier, 1971).

Statistical analysis: Significances of the differences between the pH values obtained by the different treatments were determined with the Mann-Whitney test for non-parametric, unpaired measurements (Snedecor & Cochran, 1972). Other parameters were analysed with the "Student" t-test.

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. Similar measurements were made on the muscles of five captive-bolt stunned goats, the first ones being made eight minutes (mean value) after stunning. The results obtained from the anaesthetised animals are used as reference values, and departure from these indicates the magnitude of the effect of captive-bolt stunning.

The initial pH values of the *M. semitendinosus, M. psoas major* and the lumbar and thoracic regions of the *M. longissimus dorsi* from the anaesthetised goats were 7,08 ± 0,05 (S.E.M.), 7,13 ± 0,06, 7,22 ± 0,03 and 7,26% ± 0,02 respectively. In contrast, the corresponding values for the stunned goats were 6,64 ± 0,05, 6,59 ± 0,03, 6,84 ± 0,05 and 6,82 ± 0,13. The effect of stunning on the initial muscle pH is significantly greater in the case of the *M. semitendinosus* and *M. psoas major* than in the *M. longissimus dorsi* ($P < 0,05$). Generally, the initial pH values of the three muscles of the stunned goats are higher than the initial values for pig *M. longissimus dorsi* after stunning (Tarrant, McLoughlin & Harrington, 1972) by about 0,3 to 0,5 pH units. They are roughly the same as the initial pH values reported for pigs which were exsanguinated without prior stunning (McLoughlin & Heffron, 1974). The initial pH values of the muscles of the anaesthetised animals are 0,1 to 0,2 pH units higher than those obtained for anaesthetised pigs (Heffron & McLoughlin, 1971; Tarrant, McLoughlin & Harrington, 1972) but are the same as the initial values of 7,2 reported for the *M. longissimus dorsi* of pigs which were anaesthetised and curarised (Bendall, 1966). Figs. 1 and 2 show the rates of *postmortem* glycolysis, measured as the decrease in muscle pH, in the *M. semitendinosus* and *M. psoas major* of anaesthetised and stunned goats during the five hours immediately *postmortem* and after 24 hours. Figs. 3 and 4 show the corresponding results for the lumbar and thoracic regions of the *M. longissimus dorsi*. The rates of pH decrease in all three muscles from the anaesthetised animals are greatest in the first hour, being about 0,25 units per hour, thereafter levelling off to about 0,15 pH units per hour, similar to the rate of pH decrease in the muscles of the stunned goats. In all instances the ultimate pH values fell in the range 5,45 to 5,65 indicating that the animals experienced little, if any, pre-slaughter stress. It is clear from comparison of Figs. 1 to 4 that stunning produced the greatest burst of glycolysis in the *M. psoas major*, and least in the *M. 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 *M. longissimus dorsi* of captive-bolt stunned, stress-resistant Landrace and Large White pigs (Tarrant, McLoughlin & Harrington, 1972),

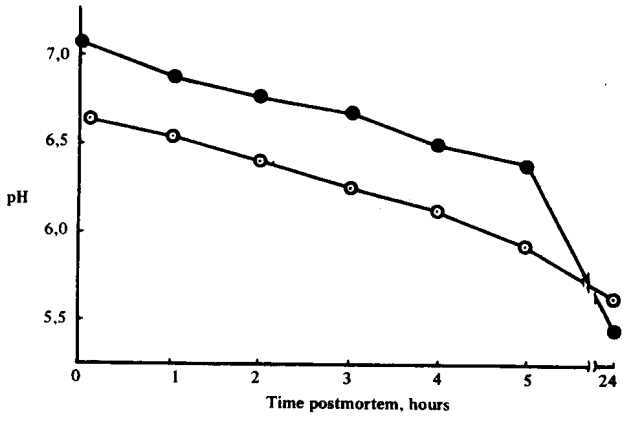

Fig. 1 *Rates of* postmortem *glycolysis in the* M. semitendinosus *of anaesthetised (●) and stunned (⊙) goats at 37°C*

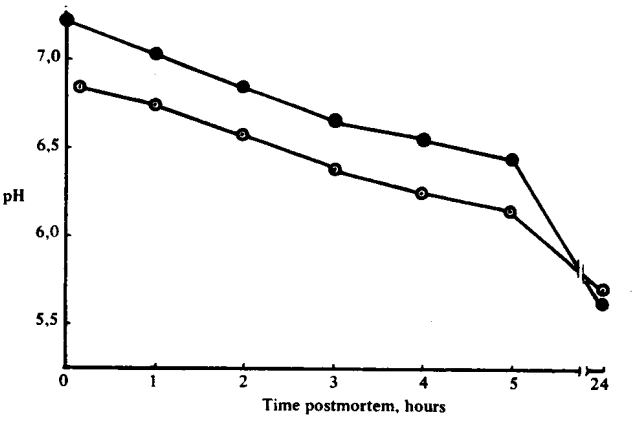

Fig. 3 *Rates of* postmortem *glycolysis in the lumbar region of the* M. longissimus dorsi *of anaesthetised (●) and stunned (⊙) goats at 37°C*

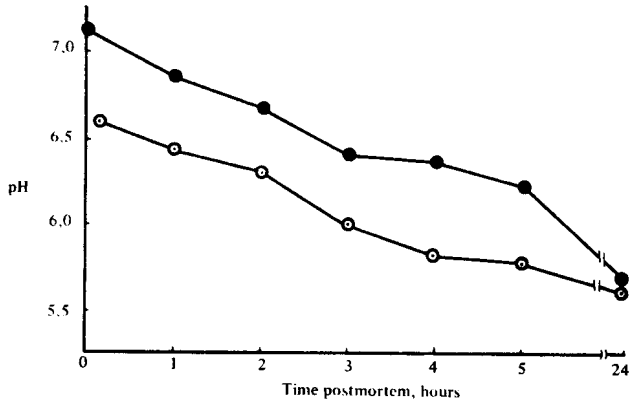

Fig. 2 *Rates of* postmortem *glycolysis in the* M. psoas major *of anaesthetised (●) and stunned (⊙) goats at 37°C*

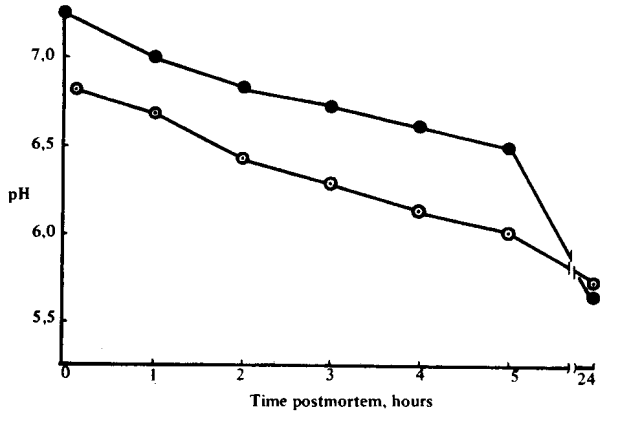

Fig. 4 *Rates of* postmortem *glycolysis in the thoracic region of the* M. longissimus dorsi *of anaesthetised (●) and stunned (⊙) goats at 37°C*

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 the 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. In the pig, stunning causes the glycolytic rate to remain at an elevated level during the complete time course of *postmortem* glycolysis. The observation points to a more stable glycolytic control mechanism in the goat muscles. It is worth noting that the glycolytic rate observed here in the muscles of the anaesthetised goats is similar to reported rates of muscles of anaesthetised pigs (Tarrant, McLoughlin & Harrington, 1972). It is also noted that similar rates of glycolysis occur in both regions of the *M. longissimus dorsi* examined. Unlike the goat muscle the *M. longissimus dorsi* of the pig exhibits very rapid rates of *postmortem* glycolysis and is very prone to development of the PSE condition.

The phosphocreatine levels 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. The phosphocreatine levels of the three muscles of the anaesthetised goats at zero time are just slightly lower than the values reported for porcine *M. longissmus dorsi* by Tarrant, McLoughlin & Harrington (1972). This is probably due to the greater red fibre content of the goat muscles compared with the pig. Seventeen micromoles of phosphocreatine per gram were broken down in eight minutes in the *M. psoas major* of the stunned goats, similar to the amount hydrolysed in pig muscle for the same extent of pH decrease. As in the case of pH, the least hydrolysis of phosphocreatine occurred in the *M. longissimus dorsi.* 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 levels in the three muscles at zero time in both anaesthetised and stunned animals are presented in Table 2. As already noted for pH and phosphocreatine, the amount of ATP hydrolysed during the initial burst of glyco-

Table 1

Phosphocreatine content of three muscles of anaesthetised and stunned goats

Muscle	Anaesthetised		Stunned	
	0 Hour	+1 Hour	0 Hour	+1 Hour
M. semitendinosus	*16,7 ± 2,1	8,7 ± 2,0	3,3 ± 0,4	3,0 ± 0,3
M. psoas major	19,3 ± 2,1	6,8 ± 1,4	2,6 ± 0,1	3,1 ± 0,5
M. longissimus dorsi:				
Thoracic region	18,1 ± 3,7	9,0 ± 2,7	8,1 ± 1,8	4,3 ± 1,3
Lumbar region	20,1 ± 1,9	9,6 ± 1,2	7,4 ± 1,8	4,0 ± 0,5

+ One hour *postmortem*. * Phosphocreatine content expressed as μmoles per gram of tissue, wet weight, mean values ± S.E.M., 5 animals per group.

Table 2

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

Muscle	Anaesthetised		Stunned	
	ATP	G-6-PO_4	ATP	G-6-PO_4
M. semitendinosus	9,2 ±0,4	1,7 ± 0,7	7,3 ± 0,6	8,0 ± 0,6
M. psoas major	9,6 ± 0,4	1,1 ± 0,3	6,3 ± 0,3	4,8 ± 0,9
M. longissimus dorsi:				
Thoracic region	7,9 ± 0,7	0,8 ± 0,4	7,3 ± 0,5	4,2 ± 1,1
Lumbar region	7,4 ± 0,7	0,5 ± 0,1	8,4 ± 0,7	4,5 ± 1,0

Zero time values of ATP and glucose-6-phosphate (G-6-PO_4) are expressed as μmoles per gram of tissue, wet weight, mean values ± S.E.M., 5 animals per group.

lysis was greatest in the *M. psoas major* of the stunned goats while it was intermediate in the *M. semitendinosus*. It is shown that there is no significant difference between the ATP levels of either region of the *M. longissimus dorsi* of the stunned and anaesthetised animals ($P = 0,05$). Clearly this is due to the higher level of phosphocreatine in the *M. longissimus dorsi* of the stunned goats at zero time (Table 1), this level being sufficient to maintain the ATP concentration at its resting value. The initial levels of glucose-6-phosphate (G-6-PO_4) in the anaesthetised goats, shown in Table 2, exhibit the same trend as the pH and phosphocreatine values. Although the G-6-PO_4 levels are significantly elevated ($P < 0,05$), in all three muscles from the stunned animals compared with the anaesthetised ones, there is no apparent relation between the G-6-PO_4 levels and initial pH in the muscles of the stunned animals. This may be due to the non-steady state fluxes of hexose monophosphates through the glycolytic cycle soon after the stunning trauma. Such high levels of G-6-PO_4 nevertheless prove that stunning causes considerable glycogenolysos in goat muscles as well as in pig muscles.

Histochemical staining of the *M. psoas major* and *M. semitendinosus* for succinic dehydrogenase activity showed that the former was composed of 54% red, 21% intermediate and 32% white fibres, while the latter consisted of 45% red, 21% intermediate and 34% white fibres. Enzyme measurements on the *M. longissimus dorsi* have yet to be made. Thus the muscles of the goat are composed of 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 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 the neurological differences in the motor units of the pig and goat, and differences at this level could also determine the responsiveness of the muscular system to the trauma of the stunning method. Clearly further and more detailed biochemical investigations into the glycolytic control mechanisms operating in the muscles of the goat are essential to an understanding of the fundamental basis of meat quality.

Conclusions

The muscles of the Boer goat showed high pH_1 values when stunned with the captive-bolt pistol. There was no tendency to produce an undesirable muscle quality as is well known to occur in the musculature of pigs stunned in the same manner. The comparatively low rates of *postmortem* glycolysis in the muscles of the stunned goats are probably due to the presence of high proportions of aerobic muscle fibres.

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