

RELATIONSHIP BETWEEN LACTATE AND GLYCOGEN CONTENTS AND pH VALUES IN *POST MORTEM* LONGISSIMUS MUSCLE OF THE PIG

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INTRODUCTION

The rate and extent of *post mortem* glycogenolysis and glycolysis in muscle are of prime importance for pork quality. The former depends on the speed of both lactate build-up and ATP hydrolysis, while the latter is mainly determined by the content of glycogen in muscle at time of slaughter (BENDALL, 1973). BENDALL (1973) thoroughly discussed the relationships between i/ ultimate pH (pH_U , 24 h *post mortem*) and initial glycogen content and ii/ pH_U and final lactate content. However, to our knowledge, less is known about the relationship between rate of lactate production and pH changes in *post mortem* muscle.

The present experiment was performed in order to study the relationships between i/ *Longissimus* muscle glycolytic potential (GP, an indicator of glycogen level at time of slaughter) determined on *post mortem* samples and pH_U and ii/ lactate content and pH value at 45 min *post mortem* (pH_1).

MATERIAL AND METHODS

The study involved 59 Large White, 60 Landrace and 51 Pietrain pigs of the three "sex types", i.e. boars, castrates and gilts. Pigs were reared under commercial conditions in 7 series of unequal size, but in each of these series the three breeds were represented. The live weight at slaughter was c.a. 105 kg.

Approximately 40 to 45 min *post mortem*, pH was measured in *Longissimus* muscle (LD) at the level of the last rib and in the middle of the *semimembranosus* (SM) muscle, owing to a portable pH-meter equipped with a combined xerolyte electrode ("Ingold", France). A small sample of m. LD was taken at the level of the last rib and immediately frozen in liquid nitrogen. Ultimate pH (pH_U) was measured 24 h *post mortem* using the same equipment as for pH_1 .

Free-dried samples were used for simultaneous determination of glycogen, glucose and glucose-6-phosphate (DALRYMPLE and DALRYMPLE, 1973), and lactate (BERGMEYER, 1974).

In order to estimate glycogen content in muscle at time of slaughter, GP was calculated according to the formula proposed by MONIN and GUEBLEZ (1985):

$$GP = 2 ([\text{glycogen}] + [\text{glucose}] + [\text{glucose-6-phosphate}]) + [\text{lactate}], \text{ expressed as } \mu\text{mol lactate} \cdot (\text{g fresh muscle})^{-1}, \text{ assuming a moisture content of 75 per cent.}$$

This calculation allows one to take into account the degradation of glycogen which occurs during the first 45 min *post mortem*.

Variance analysis was performed in order to test the effects of slaughter series, breed, sex and those of the corresponding first order interactions, i.e. "slaughter series x breed", "slaughter series x sex" and "breed x sex".

The relationships between GP and pH_U and between lactate and pH_1 were estimated with different models (linear, curvilinear and segmented), using various procedures of the SAS system. The segmented model gave the best estimation for both relationships.

Table 1: Effects of slaughter series, breed and sex on pH values in LD and SM muscles, and on lactate and glycolytic potential (GP) in LD muscle.

	LD		SM		Lactate(1)	GP(2)
	pH ₁	pH _u	pH ₁	pH _u		
Slaughter series						
1 (n= 7)	6.24	5.48	6.77	5.57	33.7 ^b	156 ^a
2 (n= 28)	6.14	5.58	6.30	5.64	38.5 ^a	147 ^a
3 (n= 30)	6.39	5.58	6.48	5.67	35.0 ^{a,b}	160 ^a
4 (n= 17)	6.33	5.55	6.50	5.68	34.0 ^b	160 ^a
5 (n= 49)	6.13	5.51	6.30	5.64	41.0 ^a	157 ^a
6 (n= 25)	6.23	5.62	6.23	5.68	40.9 ^a	156 ^a
7 (n= 14)	6.16	5.28	6.31	5.71	40.3 ^a	161 ^a
MSE	0.13	0.02	0.12	0.04	69.3	498 ^a
Breed(2)						
LW (n= 59)	6.36	5.56	6.49	5.65	34.6 ^b	158 ^a
P (n= 51)	5.85	5.56	6.05	5.66	46.8 ^a	146 ^b
LRF (n= 60)	6.40	5.54	6.50	5.66	35.2 ^b	163 ^a
MSE	0.07	0.02	0.09	0.04	45.6	455
Sex						
Gilts (n= 58)	6.22	5.57	6.33	5.67	38.1 ^a	156 ^{a,b}
Boars (n= 59)	6.22	5.53	6.42	5.62	38.9 ^a	163 ^a
Castrates (n= 53)	6.22	5.56	6.33	5.69	38.4 ^a	150 ^b
MSE	0.13	0.02	0.13	0.04	75.8	480
Significance(3)(4)						
Slaughter series	**	*	***	NS	***	NS
Breed	***	NS	***	NS	***	***
Sex	NS	NS	NS	NS	NS	**

(1), glycolytic potential (GP) and lactate are expressed as μmol lactate/g fresh tissue.
(2), LW, Large White; P, Pietrain; LRF, French Landrace.
(3), results from variance analysis are reported as: ***, P<0.001; **, P<0.01; *, P<0.05; NS, P> 0.05.
(4), the first order interactions (series x breed, series x sex, breed x sex) were not significant.

RESULTS AND DISCUSSION

Results from variance analysis show that most of the traits under study were significantly affected by slaughter series (Tab. 1). In general an effect of slaughter date on meat quality traits such as pH is to be expected (see for instance, MONIN and SELLIER, 1985 and FERNANDEZ et al, 1991) since stress conditions pre-slaughter vary from a day to another. The values of pH₁ in both LD and SM muscles were significantly affected by breed (Tab. 1). This result is not surprising since stress-susceptible animals were likely to be present in this population and especially within Pietrain pigs, as indicated by the lower pH₁ values in this breed. Subsequently, lactate content 45 min *post mortem* was significantly higher in LD muscle of Pietrain pigs than in the two other breeds, thus indicating a faster glycolysis. GP was higher in boar muscles compared to gilts and castrates (Table 1). This finding is contradictory to most studies on this subject. Generally boars exhibit higher pH_u than gilts and castrates probably due to lower muscle glycogen store at slaughter since boars exhibit a more aggressive behaviour during the pre-slaughter period (see review by FERNANDEZ and TORNBERG, 1991). With regard to the examination of the relationships between metabolite contents and pH values, data were not corrected for the above discussed effects. The use of raw data was preferred to that of corrected ones in order to preserve a large variability in the traits under study. This choice was supported by the lack of effect of first order interactions for all items (Tab. 1). The best prediction of the relationship between glycolytic potential and ultimate pH was obtained using a segmented quadratic model with plateau (r = -0.81, P < 0.001) (Figure 1). pH_u decreases following a curvilinear regression when GP increases until the convergence point (GP= 173 μmol/g). Above this threshold, pH remains at a constant value (5.50) regardless of GP. WARRIS et al (1989) studied the relationship between pH_u and glycogen store in *Adductor femoris* (AD) muscle. They also found a non-linear relationship. From the observation of the corresponding plot, it seems that a plateau in m. AD pH_u (≈ 5.7) was reached for glycogen content above 61 μmol/g. Similarly, MONIN (1988) reported a non-linear relationship between GP and pH_u in pig m. *Semimembranosus*.

Figure 1: Relationship between glycolytic potential and ultimate pH (the model used was a segmented quadratic model with plateau)

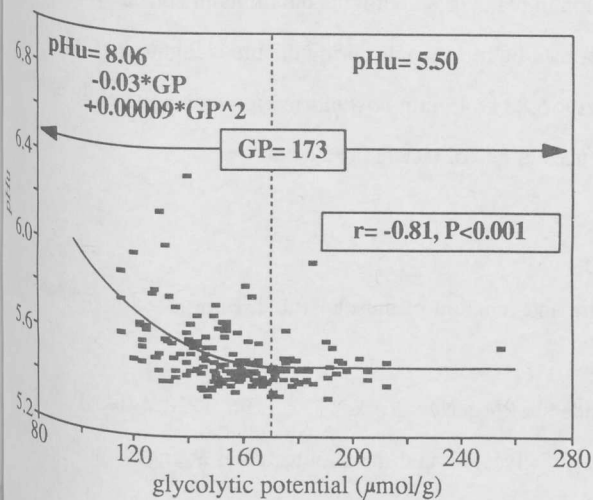
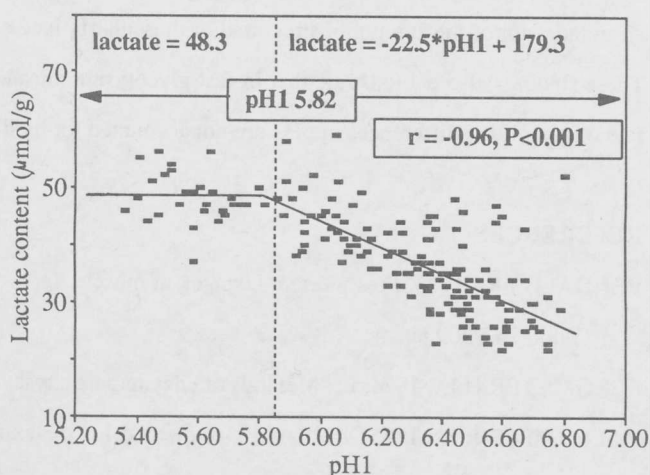


Figure 2: Relationship between lactate and pH₁ (the model used was a segmented linear model with plateau)



determinism of pH_u is not fully understood. *Post mortem* pH fall may stop even in the presence of residual glycogen (SAYRE et al, 1971). According to SCOPES (1971), under conditions where glycogen is not the limiting factor, glycolysis stops when adenosine triphosphate (ATP) is no more available. Indeed, ATP is a cofactor for glycogenolytic and glycolytic enzymes. In *post mortem* muscle, ATP is gradually desaminated by AMP-desaminase (SCOPES, 1971). According to SAHLIN (1978) glycolytic enzymes are inactivated when pH reaches low values (< 5.4). The sensitivity of glycolytic enzymes to pH may also explain the stop of *post mortem* pH fall. However, the model used in this study gives a plateau corresponding to $pH_u = 5.50$, a value at which glycolytic enzymes should be still active.

The relationship between lactate content at 45 min *post mortem* and pH_1 was tested using several models. The segmented linear model with plateau gave the best prediction of the relationship ($r = -0.96$, $P < 0.001$) (Figure 2). This model gives an estimated constant value of 48.3 $\mu\text{mol/g}$ for pH_1 varying between 5.3 and 5.8. Above the convergence point ($pH_1 = 5.82$), lactate decreases linearly when pH_1 increases. Similar relationship has been previously reported within the latter pH range (FERNANDEZ et al, 1992). This results suggests that below $pH_1 = 5.82$, glycolysis has reached its maximum speed since no variation in lactate content is recorded within this pH range. The apparent plateau in the speed of glycolysis, pH still varies between muscles. This finding indicates that the rate of *post mortem* pH fall may not be explained solely by the rate of glycolysis.

It has often been stated that the lactate produced during *post mortem* glycolysis is the direct cause of the pH fall. When looking more in detail at the biochemical reactions taking place in *post mortem* muscle, it may be seen that glycolysis *per se* leads to an alkaline change since a net consumption of one proton occurs for one glycosyl unit broken down to lactate (BENDALL, 1973). However, 3 ATP are produced during that phase and their subsequent hydrolysis by the ATPase systems leads to the release of 3 protons in the cell. Thus, 2 protons are released for one glycosyl unit metabolised to lactate. From this consideration, it comes out that the rate of *post mortem* pH fall is mainly determined by the rate of ATP hydrolysis, as shown by SCOPES (1971) using cell-free glycolysing systems. However, ATP breakdown cannot occur at a faster rate than its synthesis through the glycolytic pathway. Thus, an uncoupling between ATP hydrolysis and glycolysis cannot explain the plateau in lactate values observed below $pH_1 = 5.82$.

It might be supposed that, within the pH region corresponding to a maximum rate of lactate production ($pH_1 < 5.82$), the observed differences in pH_1 are due to individual variability in muscle buffering capacity.

CONCLUSIONS

These results confirm that ultimate pH cannot be predicted in a linear manner from values of muscle glycogen content at slaughter. Large variations in glycogen content may not be associated with any variation in pH_{u} . This highlights the limits in considering ultimate pH as an indicator of the amount of stress and/or muscular fatigue experienced by the animals during the pre-slaughter period. These findings also demonstrate that in fast glycolysing muscles ($\text{pH} < 5.82$ at 45 min *post mortem*), which apparently exhibit maximum rate of glycolysis, differences in pH_1 are not accounted for by differences in rate of lactate production.

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