EFFECTS OF PITHING ON pH AND ATP-RELATED COMPOUNDS OF BEEF MUSCLES

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Keywords: beef, slaughter, pithing, pH, ATP

OBJECTIVES

Pithing procedures have been used in some abattoirs to reduce the hazard to slaughtermen. When a cattle is stunned by pole-axe or captive bolt pistol, a wire rode is thrust into the cranial cavity through the hole made in the skull, destroying the brain and spinal cord and causing vigorous movements and muscle spasms. There are some reports on the effects of different methods of stunning or slaughter on post-mortem changes of muscle, but studies on the pithing are few In the present report, the effects of pithing of fattening steers and calves on pH, ATP and its breakdown products were studied.

EXPERIMENT AL METHODS

Analysis of ATP and its breakdown products

ATP-related compounds (ATP, ADP, AMP, IMP, Inosine(Ino), Hypoxanthine(Hyp), Xanthine(Xan)) in beef were analyzed by HPLC equipped with microparticle reverse-phase Shimpack CLC-ODS(M) (4.6 mm× 15 cm) column from Shimadzu Corporation was used.

Fattening steers

A total of ten fattening steers of 25 months old (355.5 ± 16.0 kg carcass weight) were used. Five of them were pithed and another five were not pithed at slaughter. Concentrations of A TP-related compounds and pH at 2.0 hours after slaughter were measured on *Psoas major* muscles.

Calves

Nine calves (12.6 ± 1.7 months old) were divided into three groups; anesthetized (A-group), pithed calves (P-group) and non-pithed calves (N-group) at slaughter *Biceps femoris* (BF), *Longissimus dorsi* (LD) and *Psoas major*(PM) muscles were excised from the carcass of each group at one hour after stunning, and muscles were incubated at 37 ℃. Changes of pH and A TP-related compounds during incubation were measured.

Comparison between fattening steers and calves

A total of eight calves (13 months old) were used. Four were pithed (CP-group) and the remainder were not pithed (CN-group) at slaughter. For the fattening cattle, eight steers (27 months old) were used. Half of them were pithed (FP-group) and the other half (FN-group) were not pithed. At one hour after stunning, pH was measured on PM and LD muscles in each group. Another three calves (C-group) and three fattening cattle (F-group) without pithing were determining the time from stunning to cease the movements or spasm as a means of judging death.

RESULTS AND CONCLUSIONS

Analytical conditions for ATP-related compounds

The analytical method for ATP-related compounds by HPLC was successfully developed. The best separation (Figure 1-(a)) was obtained with the following conditions; the first eluant was 0.1M KH_2PO_4 (buffer-1) and the second was 0.1M KH_2PO_4 containing 10% (V/V) methanol (buffer-2). The pH of this buffer was adjusted to 4.0 with H_3PO_4 before HPLC analysis. For the first 5-min, 100% of the first buffer was run, followed for the next 25-min by linear gradient from 0 to 50% of buffer-2, and then buffer-2 was brought up to 100% in 10 min. During the last 10-min, the gradient was held and returned to buffer-1 in one min. The initial conditions were restored in about 15-min. The flow rate was 1.0 mL/min, and the column temperature was 24-28 ℃. In this procedure, however, the purification of IMP and ATP was unsatisfactory when ageing has progressed (Table 1-(a)). In this case, 0.02M KH_2PO_4 adjusted to pH 4.8 with KOH was used for IMP and ATP (Figure 1-(b), Table 1-(b)).

Difference between pithed and non pithed fattening steers

Figure 3 shows the difference of PM muscle between pithed and non pithed group. Creatine phosphate (CP) was not detected in any PM muscles from pithed steers, and there was a lower pH and a more rapid change from ATP to IMP on pithed steers. However, significant differences were not observed between the groups for the mean values of pH and any compound, because of the large variance of pH value, ATP, IMP, and Ino levels on non-pithed group.

Difference between anesthetized, pithed and not pithed calves

For the PM muscle, the mean values of pH from P and N-groups were significantly lower ($P<0.01$) than that of A-group at 1, 3 and 4 hour post-mortem, and the values were always lower in the order P-group < N-group < A-group until reaching ultimate pH (PM muscle in figure 4). No significant differences were observed between the groups on the pH of LD and BF muscles (Figure 4). When the rate of ATP degradation was described in terms of the Ka value (Ka=(IMP+Ino+Hyp+Xan)/(ATP+ADP+AMP+IMP+Ino+Hyp+Xan)), significant differences ($P<0.01$) between N-group and P-group were observed for the PM muscle at one hour post-mortem and the differences were also observed for the LD muscle at two hours post-mortem but were not significant ($P>0.05$). No differences were observed for the BF muscle until 3 hours post-mortem (Figure 5).

Comparison of fattening steers and Calves

The level of pH was in the order of FP<FN<CP<CN (Figure 6). Significant difference were observed between CN and FP ($p<0.01$), and

CP and FP ($p<0.05$). Significant differences were also observed between fattening steers and calves ($p<0.01$) and between pithed and non pithed cattle ($p<0.05$). Differences of time from stunning to death on non pithed cattle were quite large between fattening steers and calves (Figure 7). The pH differences on PM muscle at 1.0 h post-mortem might be explained by the differece in death struggle which are caused by different times from stunning to death and by whether the pithing procedure was used or not. Hence, by 1.0 after slaughter, pH values (5.6 ± 0.08) close to ultimate were observed in all PM muscles.

Our explanation for the first turnover in PM muscle is that the end site of PM muscle is adherent to *M.iliacus* and connects with the *trochanter minor* of the femur, the movement of hind legs at slaughter possibly causes the depletion of CP and ATP and the rapid glycolysis, so that a lower initial pH was observed in PM muscles. These movements were possibly enhanced by pithing.

The live weight of fattening steers in Japan is about 600kg and pithing is common procedure in abattoirs, but the kidney fat is not excised. This situation badly affects the PM muscle, which becomes similar to a PSE condition.

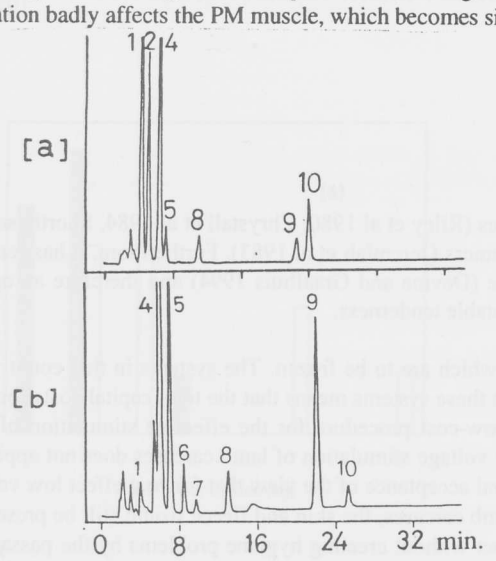

Figure 1. Chromatograms of ATP-related compounds in meat extract.
(a) at 4 hr after slaughter analyzed by the eluant of 0.1 M KH_2PO_4 (pH 4.0).
(b) at 6 days after slaughter analyzed by the eluant of 0.02 M KH_2PO_4 (pH4.8).
1; ATP 2; ADP 4; IMP 5; Hypoxanthine 6; Xanthine 8; AMP 9; Inosine 10;NAD

Table 1. Absorbance ratios (254 nm/280 nm) of ATP-compounds and those in meat extract at 4 hr, 6 days and 10 days after saughter.

(a) In case of 0.1M KH_2PO_4 (pH 4.0) eluant.

Compound	Standard	4 hr	6 days	10 days
ATP	*5.86*	*5.76*	*6.55*	*7.66*
ADP	6.10	6.01	5.98	6.20
IMP	*7.85*	*7.05*	*6.45*	*5.54*
Hyp	16.88	16.51	14.40	16.00
Xan	1.64	1.70	1.53	1.59
AMP	6.17	6.31	5.92	6.20
Ino	7.73	7.10	7.78	7.83

(b) In case of 0.02M KH_2PO_4 (pH 4.8) eluant.

Compound	Standard	4 hr	6 days	10 days
ATP	*6.48*	*6.30*	*6.15*	Not detected
IMP	*7.88*	*7.98*	*7.90*	*7.70*

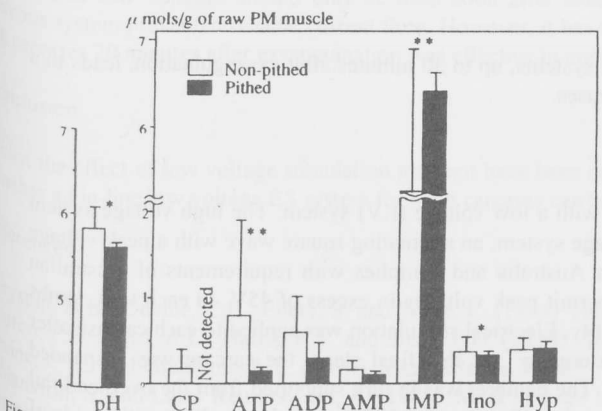

Figure 3. Significant differnces were observed on the variance (* $p<0.05$, ** $p<0.01$)

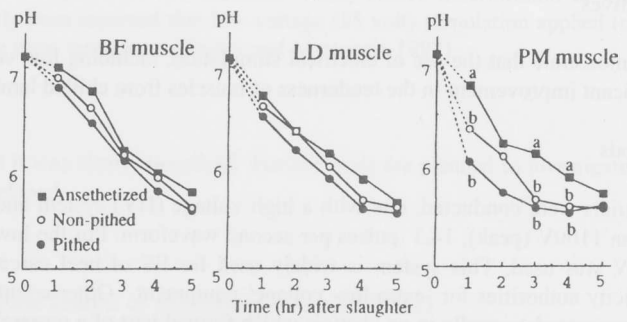

Figure 4. Changes of pH with time after slaughter at 37℃ for Pithed(●), Non-pithed (○) and Anesthetized(■) groups of biceps femoris (BF), longissimus doris (LD) and psoas major (PM) muscle.
a, b : Means at same time with different letter differ significantly ($p<0.05$).

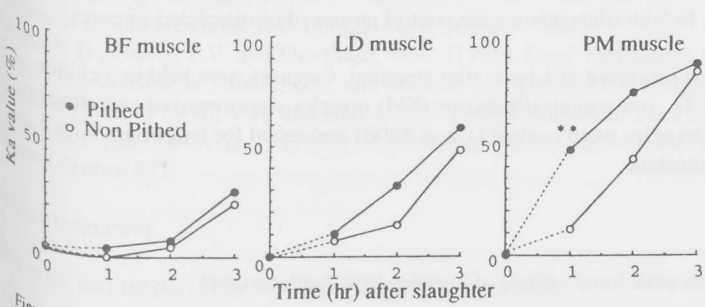

Figure 5. Changes of Ka valuer at 37℃ for *biceps femoris* (BF), *longissimus dorsi* (LD) and *psoas major* (PM) muscles. The data at 0 hr after slaughter were obtained from anesthetizd calves.
Ka value = (IMP+Ino+Hyp+Xan)/(ATP+ADP+AMP+IMP+Ino+Hyp+Xan)
**; Significant difference was observed between Pithed and Non-pithed group.

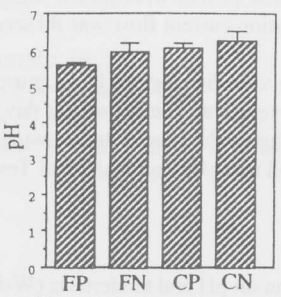

Figure 6. pH changes between the groups at 1-h post-mortem.

FP: Pithed fattening steers. FN:Non-pithed fattening steers.
CP: Pithed calves. CN: Non-pithed calves.

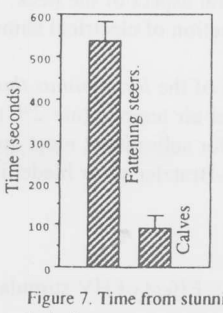

Figure 7. Time from stunning to death.