EFFECT OF ANTEMORTEM STRESSES ON CHANGES OF PHYSIOLOGICAL BIOCHEMICAL AND PHYSICAL CHARACTERISTICS OF DUCK MUSCLE

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INTRODUCTION

It has been recognized that any stressors can cause the metabolites of muscle changed and result in different meat quality. The nature of the effects depends on such factors as the duration and severity of the stress. The effects of the changes on meat have been reported restrictly on pork, beef, lamb, but not poultry, particularly, duck meat. In Taiwan, we have found ducks occurring DFD-like duck muscle when they suffered transporting, fassting, forcing exercise and any other stressors. Therefore, this experiment was to study the changes of physiological, biochemical and physical characteristics of the duck muscle when they were antemortem stressed.

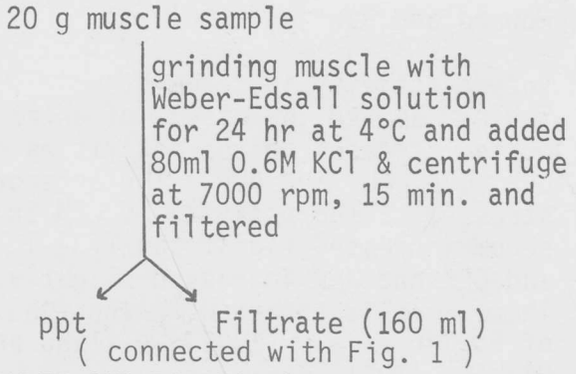
MATERIALS AND METHODS

Sixty market mule ducks (body weight: 3.0±0.2 Kg) at 75±5 days of age were randomly assigned to three treatments ---the control (without stress), 8 hr and 24 hr fasting plus enforced exercise for 10 min. preslaughter. The birds were sacrificed by a conventional method but were not defeathered, and excised the breast and thigh muscle within 10 min. postmortem. The muscle samples were wrapped in PVC film and stored at 4°C for 24 hours. During the storage time the samples were taken out to determine some characteristics.

pH values of blood and muscle were measured with H18424 Microprocessor pH-meter portable Instrument HANNA and LTD-SP 35 digital pH/MV meter, SUNTEX Instrument Co., separately. Lactic acid in serum and muscle from the breast and thigh at different storage times were determined according to the method described by Barker and Summerson (1941). The muscle---M. gastrocneminus was used to measure the pattern of extensibility changes during the development of rigor mortis with a rigorometer which was designed by our lab. Enzyme activity determination: Lactate Dehydrogenase (LDH), creatine phosphokinase (CPK), alkaline phosphatase & acid phosphatase (ALP & ACP) were determined using the methods described by Caband and Wroblewski (1958), Tanzer and Gilvary (1959), Bessey-Lowry-Brock (1964), respectively.

Myofibrillar proteins of duck breast muscle excised from the carcass stored for 1, 8 and 24 hours were extracted according to the procedures as shown in Fig. 1 (Brisky and Fukazawa, 1971).

ATP ase activity was determined by the method of Martin and Daty (1949). Dodecyl sulfate-polyacylamide slab gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (1970). The color of duck muscle of breast and thigh was measured by the colorimetry (Tokyo Denshoku Co., Model TC-III).



RESULTS AND DISCUSSION

Development of rigor mortis of duck muscle

The rigor pattern of muscle completed within 30 min. postmortem from the stressed ducks, but the completion of rigor was taken at least 1.5 hours postmortem for the control. It could be noted that the rigor process occurred in the stressed duck muscle earlier than the muscle of the control. The pH of the stressed duck was at the range between 6.9 and 7.1, while the control was between 6.0 and 6.3.

Lactate and pH of blood and muscle postmortem

The pH and lactate content of serum increased slightly with progressive fasting time, but this result was not significantly different (Table 1). This seemed to be affected by acid-base balance or self-buffering action in blood, thus, the pH value did not chaNGE Remarkably. The changes of pH and lactate content of duck breast and thigh muscles obtained from different antemortem stresses during postmortem storage at 4°C were shown in Fig. 2. The result showed that antemortem fasting and stressing caused pH increased and lactate content decreased in the breast and thigh muscles. In other words, the muscle with high pH, the lactate content decreased. The result also found that the lactate content in thigh muscle was less than in the breast muscle, especially, the muscle from the duck of 24 hour fasting and 10 min. forced exercise had lowest in lactate content and highest in pH value. These results agreed with the findings of the works of Newton & Gill (1980-81), Liu (1985), Bate-Smith & Bendall (1949), and Howard and Rawrie (1956).

Enzyme activity in serum

Table 2 showed the effect of antermortem stresses on the activities of LDH, CPK, ALP and ACP. The antemortem stresses caused activity of LDH in serum increased significantly ($p<0.05$), and CPK and ALP increased slightly. These results agreed with the findings of Fowler et al. (1962), Altland and Highman (1961).

Changes in myofibrillar protein extractability and electrobphoretic behavior of duck muscle

Table 3 showed the changes in the myofibrillar protein extractability and electrobphoretic behavior of the duck muscle during storage at 4°C caused by antemortem stresses. The results were noted that the extractability of myofibrillar protein was affected limitedly by antemortem stress, and there was no significantly difference among the treatments. The extractability of the myofibrillar protein increased with storage time.

The electophoretogram changes of the myofibrillar protein of duck muscle during storage were shown in Fig. 3. The difference between breast and thigh muscles was found the position of one component between the bands of actin and tropomyosin had different molecular weight with 40Kdalton and 37 K dalton, respectively. There were two components degradated gradually as storage time increased, most seriously occurred in the control muscle. And it was also found that the myofibrillar protein in the breast muscle was more seriously degradated than in the thigh muscle. The concentration of the component of 30K dalton in the gel increased with storage time, and the concentration in the breast was significantly higher than in the thigh. The band of 30K dalton which appeared as the result from the troponin-T degradation. This result agreed with the works of Koohmaraie et al.(1984), Olson et al. (1977) and Penny et al.(1974).

ATPase activity of myofibrillar protein

Table 4 and 5 showed the effect of antemortem stress on ATPase activity during storage postmortem. ATPase activity of the myofibrillar protein of the breast muscle in the antemortem stressed duck was higher than in the control one hour postmortem, and there was no significant difference among the treatments. The ATPase activity of the myofibrillar protein both in the control and the antemortem stressed ducks was higher in the samples 24 hours postmortem than in the samples one hour postmortem but the activity of ATPase in the myofibrillar protein of the thigh muscle was not affected by the stress and storage time. However, APTase activity in the breast muscle increased with the storage time which was shown in Table 4.

Color change in the duck muscle

Tables 6 and 7 showed that the breast of the stressed duck resulted in significantly lower L- and b- values, but slightly affected a-value of the duck muscle. Consequently, DFD-like muscle color was observed in the

breast and thigh muscles of the ducks which were stressed.

CONCLUSION

Fasting time and stresses could enhance or encourage rigor process occurring earlier in the duck muscle. Consequently, DFD-like duck muscle resulted from the breast and thigh antemortem stressed. The darkness of the duck muscle was intensified with the fasting time. The muscle obtained from the duck without stress and only fasting for 2-4 hours could produce a preferable bright red color. The components of 40Kdalton in the breast muscle and 37 K dalton component in the thigh muscle were also found in the duck. These results of this experiment would recommend that the ducks should not be stressed and fasted too long time before they were slaughtered.

ACKNOWLEDGEMENT

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Filtrate (160 ml)

↓ dilute to 0.1 M (864 ml)

300 ml (0.1 M)

↓ centrifuging at 7,000 rpm, 20 min.

Sediment

↓ adjusted to 0.6 M & stirred and centrifuged at 7,000 rpm, 15 min.

Supernanant (0.6 M) 50 ml

↓ adjusted to 0.2 M

150 ml (0.2 M)

↓ centrifuging at 7,000 rpm, 15 min.

Sediment (adjusted to 0.6 M)

↓ adjusted to 0.2 M

150 ml (0.2 M)

↓ centrifuging at 7,000 rpm, 15 min.

Sediment

↓ washing with 100 ml 0.2 M KCl and centrifuging at 7,000 rpm, 15 min.

Sediment

↓ adjusted to 0.6 M & centrifuging at 10,000 rpm, 15 min.

Supernanant

Fig. 1. The flow chart of myofibrillar protein extraction.

Table 2 Effect of ante-mortem stress on the activity of lactate dehydrogenase (LDH), creatine phosphokinase (CPK), acid phosphatase (ACP), alkaline phosphatase (ALP) activity in serum.

屠前緊迫 Antemortem stress	血清 serum			
	LDH[1]	CPK[2]	ACP[3]	ALP[3]
對照組 Control	1128±203 [a]	226±100 [a]	0.61±0.28 [a]	10.8±3.2 [a]
絕食8小時追趕10分鐘 Fasted-8hr-forced-exercise-10'	1439±244 [b]	319±104 [a]	0.80±0.24 [a]	13.7±4.5 [a]
絕食24小時追趕10分鐘 Fasted-24hr-forced-exercise-10'	1546±182 [b]	302±144 [a]	0.57±0.18 [a]	13.3±3.5 [a]

#. 平均值±標準偏差 means ± S.D.

a,b: 表同行中不同字母者數字間有顯著差異($p<0.05$)

#. means ± S.D.

a,b: Means of the same column without the same superscript are significantly different ($P<0.05$).

1. B-B (Berger-Broida) Unit/ml
2. umole/min/l
3. 1 BLB Unit=umole/hr/l

Table 3. The effect of ante-mortem stress and storage time on extractibility of myofibrillar protein of breast.

屠前緊迫 ante-mortem stress	抽出率 (extractibility) (%) 貯存時間 (小時) storage time (hour)		
	1小時 1 hr	8小時 8 hr	24小時 24 hr
對照組 (control)	26.6 ± 12.1 [a]	28.6 ± 11.0 [ab]	36.0 ± 14.1 [b]
絕食8小時追趕10分鐘 fasting-8hr-enforced exercise-10 min	28.8 ± 8.5 [a]	33.7 ± 9.5 [ab]	36.2 ± 13.7 [b]
絕食24小時追趕10分鐘 fasting-24hr-enforced exercise-10 min	27.8 ± 9.0 [a]	30.6 ± 8.6 [ab]	39.2 ± 8.2 [b]

#: means ± S.D.

a,b: Means of the same row without the same superscript are significantly different ($P<0.05$).

*: show the percentage of total protein content.

Table 4. Effect of ante-mortem stress and storage time on the ATPase activity of myofibrillar protein (breast).

屠前緊迫 ante-mortem stress	活性 (activity) (μ mole/mg/min) 貯存時間 (小時) storage time (hour)		時間差異 time difference
	1小時 1 hr	24小時 24 hr	
對照組 (control)	0.098 [a]	0.139 [a]	*
絕食8小時追趕10分鐘 fasting-8hr-enforced exercise-10 min	0.131 [b]	0.151 [a]	*
絕食24小時追趕10分鐘 fasting-24hr-enforced exercise-10 min	0.121 [b]	0.155 [a]	*

a,b,c: Means of the same column without the same superscript are significantly different ($P<0.05$).

* : Means between treatments of storage time are significantly different ($p<0.05$).

Table 5. The effect of ante-mortem stress and storage time on the ATPase activity of myofibrillar protein (thigh).

屠前緊迫 ante-mortem stress	活性 (activity) (μ mole/mg/min) 貯存時間 (小時) storage time (hour)		時間差異 time difference
	1小時 1 hr	24小時 24 hr	
對照組 (control)	0.117 [a]	0.113 [a]	NS
絕食8小時追趕10分鐘 fasting-8hr-enforced exercise-10 min	0.102 [a]	0.116 [a]	NS
絕食24小時追趕10分鐘 fasting-24hr-enforced exercise-10 min	0.098 [a]	0.120 [a]	NS

a,b,c: Means of the same column without the same superscript are significantly different ($P<0.05$).

NS: Means between treatments of storage time are not significantly different ($p>0.05$).

Table 6. Effect of ante-mortem stress on pH of blood and lactate content of serum.

屠前緊迫 ante-mortem stress	血液 pH 值 blood pH	血清中乳酸 lactate in serum
對照組 (control)	7.71 ± 0.06	9.29 ± 2.27
絕食8小時追趕10分鐘 fasting-8hr-enforced exercise-10 min	7.75 ± 0.08	9.92 ± 3.51
絕食24小時追趕10分鐘 fasting-24hr-enforced exercise-10 min	7.75 ± 0.09	9.59 ± 2.01

A. umole/ml serum

B. 平均值±標準偏差 means ± S.D.

Table 6. Effect of ante-morten stresse on the color and pH of breast muscle after 24 hours post-morten at 4℃.

屠前緊迫 ante-morten stress	L 值 L value	a 值 a value	b 值 b value	pH 值 pH value
對照組 (control)	31.84 [a]	13.43 [a]	6.12 [a]	5.78 [a]
絶食8小時追趕10分鐘 fasting-8hr-enforced exercise-10 min	29.40 [b]	12.14 [ab]	4.79 [b]	5.98 [b]
絶食24小時追趕10分鐘 fasting-24hr-enforced exercise-10 min	27.18 [c]	11.27 [b]	3.80 [c]	6.25 [c]

a,b,c:Means of the same column without the same superscript are significantly different (P<0.05).

Table 7. Effect of ante-morten stresse on the color and pH of thigh muscle after 24 hours post-morten at 4℃.

屠前緊迫 ante-morten stress	L 值 L value	a 值 a value	b 值 b value	pH 值 pH value
對照組 (control)	31.32 [a]	16.16 [a]	6.07 [a]	6.06 [a]
絶食8小時追趕10分鐘 fasting-8hr-enforced exercise-10 min	24.91 [b]	13.33 [a]	4.07 [b]	7.01 [b]
絶食24小時追趕10分鐘 fasting-24hr-enforced exercise-10 min	24.00 [b]	14.35 [a]	3.78 [b]	7.14 [b]

a,b,c:Means of the same column without the same superscript are significantly different (P<0.05).

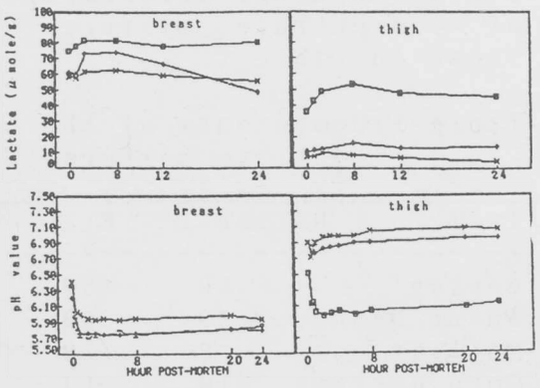

Fig 2. The changes of pH and lactate content of duck breast and thigh muscle obtained from different ante-morten stress during post-morten storage at 4℃.

□:對照組 ●:絶食8小時追趕10分鐘
×:絶食24小時追趕10分鐘
□:control ●:fasting-8hr-enforced exercise-10 min.
×:fasting-24hr-enforced exercise-10 min.

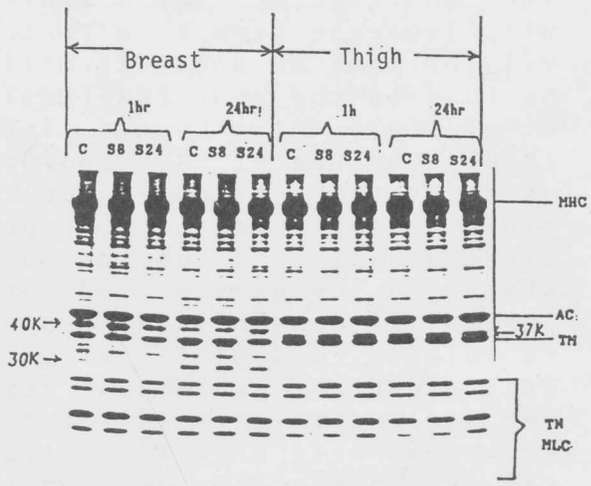

Fig.3. SDS-polyacrysamide gel electrophoresis of natural actomyosin.
C:對照組 C:control
S8:絶食8小時追趕10分鐘 S8:fasting-8hr-enforced exercise-10 min
S24:絶食24小時追趕10分鐘 S24:fasting-24hr-enforced exercise-10 min
MHC:myosin heavy chain;AC:actin;TN:tropomyosin;
MLC:myosin light chain;TN:troponin