EFFECT OF ANTE-MORTEM STRESS ON POST-MORTEM CHANGES OF TITIN I (α-CONNECTIN) INTO TITIN II (β-CONNECTIN) AND NEBULIN IN THE LIGHT AND DARK MUSCLE OF TAIWAN COUNTRY CHICKEN

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Summary

Purified myofibrils were prepared from ante-mortem stress and control lots of Taiwan country chicken breast and thigh muscles at death and after storage at 4℃ for 0, 1, 2, 3, and 7 days post-mortem. Sodium dodecyl sulfate polycrylamide gel electrophoresis (3.2%) and densitometer were used to examine the effect of ante mortem stress and control storage of muscle on titin and nebulin. Results indicated that titin and nebulin were more rapidly degraded in the control and the ante-mortem stress light muscles than in the control and ante-mortem dark muscles of Taiwan country chicken. In contrast, nebulin was shown to be more resistance to degradation in the ante-mortem stress dark muscle than in the control light muscle.

(Key Words: Ante-Mortem Stress, Titin, Nebulin, Taiwan Country Chicken)

Introduction

Titin exists as flexible filament connecting a thick (myosin) filament with a Z-line in a sarcomere (Maruyama et al., 1985; Frust et al., 1988). It is an elastic protein of striatel muscle found by Maruyama et al. (1976), and later named titin by Wang et al. (1979). Horowits et al. (1986) have shown an important physiological role of titin filaments; they keep the thick filament centered within a sarcomere during forcegeneration.

Skeletal muscle loss elasticity and become plastic with time post-mortem (de Fremery and Bool, 1960). The loss of elasticity seems to be closely related to the tenderization of meat during post-mortem aging (Yu and Lee, 1986).

There are two types of titin, insoluble titin I (α-connectin) and soluble titin II (β-connectin), whose molecular masses are estimated to be 2,800 and 2,100 KDa, respectively (Maruyama et al., 1984). It was found that in post-mortem skeletal muscle, the amount of insoluble α-connectin and elasticity of muscle decreased with increasing of storage time, suggesting that the splitting of connectin is responsible for post-mortem tenderization of meat (Takahashi and Saito, 1979). The amount of α-connectin decreased with post-mortem time, followed by the appearance of β-connectin (Takahashi et al., 1992).

Animals are exposed to many forms of stress during transportation to market for slaughter. Due to the subtropical climate in Taiwan, improper handling before slaughter such as long transportation, fasting and other stresses, would cause determinate effect on the meat quality of the country chicken. Examples of the effect of stress on muscle quality are PSE (pale, soft, exudative) pork and DFD (dark, firm, and dry) beef (Lawrie, 1958; Sayre et al., 1963a, 1963b). In a previous paper, we studied the effect of stress before slaughter on the physiologicalcal, biochemical, and physical characteristics, superprecipitation of natural actomyosin by scanning electron microscope (SEM) and ATP of the mule duck (Lin et al., 1990; Lin 1991a,b). We found that DFD-like muscle characteristics were observed in the breast and thigh of ducks that were stressed. In the present study, we have investigated the effect of postmortem storage on titin and nebulin in light and dark muscles of ante-mortem stress and normal Taiwan country chicken muscles.

Materials and Methods

Sample source

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Thirty Taiwan country chicken [three-way cross (BL_2D) among lines, B, L_2, and D bred by the National Chung-Hsing Univ. Taiwan, feeding periods: 16 wks] were divided into 2 lots. One lot was fasting for 12 h and forced to perform exercise (15 min). The other lot served as the control.

Muscle samples were obtained from breast and thigh muscles. All birds were killed by cutting the jugular vein and carotid arteries, skinned without scalding, and eviscerated. The carcasses were placed in a plastic bag and stored at 4℃.

Measurement of pH value

The pH was measured after homogenization of 5 g of breast and thigh (0, 1, 2, 3, 7 days) muscle in 20 ml of distilled water by means of a electric pH meter with glass electrode (Micro-computer; model 6200, Jenco. Elect. LTD).

Preparation of myofibrils

Purified myofibrils were prepared according to a modified procedure of Wang et al., (1979). Minced muscle tissue (5 g) was homogenized in a Waring Blender and centrifuged at 1,500 × g in Pyrophosphate relaxing buffer (0.1 M KCl, 2 mM $MgCl_2$, 2 mM EGTA, 10 mM Tris maleate, 0.5 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 2 mM $Na_2P_2O_7$, pH: 6.8) and Triton X-100 buffer, followed by washing in 100 mM KCl. An equal volume of ice cold glycerol was added to the myofibrils suspension. This was stirred gently until an even suspension was obtained, then stored at −20℃.

SDS-polyacrylamide gel electrophoresis

SDS-PAGE was performed on 3.2% acrylamide slab gels according to the procedure of Laemmli (1970). The protein samples were heated for 30 min at 50℃ in the sample buffer. Then 50 μg of protein were loaded into each gel lane. Gels were stained overnight in 0.25% Coomassie brilliant blue R-250, 7% (vol/vol) acid acetic, and 40% vol/vol methanol, and then were destained in an excess of the solution, excluding Coomassie brilliant blue. Densitometric scan of the gels were performed with a GS 300 Densitometer (Hoefer Sci. Instruments, U. S. A.) and a Model D-2500 chromato-integrator.

Measurement of the ratio of titin Ⅱ

The amount of ratio of titin Ⅱ was obtained according to the procedure of Tatsumi et al., (1988). Densitometric scan of the slab gels (including titin Ⅰ and titin Ⅱ) were performed with the densitometer at 620 nm and chromato-integrator.

$$\text{The amount of ratio of titin Ⅱ (\%)} = \frac{\text{titin Ⅱ}}{\text{titin Ⅰ} + \text{titin Ⅱ}} \times 100$$

Measurement of the relative amount of nebulin

The relative amount of nebulin was prepared according to a procedure of Tatsumi et al. (1988). Densitometric scan of the slab gels (including *myosin heavy chain* (MHC) and *nebulin*) were performed with the densitometer at 620 nm and chromato-integrator.

$$\text{The relative amount of nebulin} = \frac{\text{nebulin}}{\text{MHC (myosin heavy chain)}}$$

Measurement of protein concentration

Protein concentration was determined by the biuret method of Gornall et al. (1949), which had been standardized by bovine serum albumin.

Statistical analysis

Data were analysed by Statistical Analysis System (SAS) and Duncan's new multiple range test (Duncan, 1955).

Results and Discussion

The thigh and breast muscle pH of control and ante-mortem stress lots at 0, 1, 2, 3, 7 days after slaughter is show in figure 1. The thigh and breast muscle pH of the control lot is significantly lower than that of the ante-mortem stress lot at all other time examined. On the other hand, the muscle pH of the thigh is significantly higher than that of the muscle pH of the breast.

Muscle pH change and ante-mortem stress are closely correlated because are related to energy metabolism, particularly to metabolism glycogen (Newton and Gill, 1978). Muscle glycogen stores may be reduced when animal are fasted or exercised, muscle glycogen is utilized for energy, particularly to thigh muscle. If adequate feed and rest are not provided, glycogen deficiency may exist when slaughter occurs. Muscle glycogen deficiency in muscle causes a slow rate and limited extent of glycolysis after death. The resultant high pH in such tissue minimized the color change that

otherwise occurs in the post-mortem period (Ashmore et al., 1978; Judge et al., 1989).

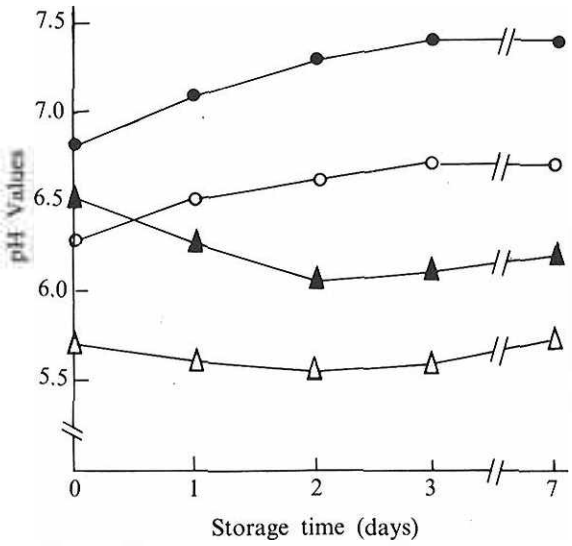
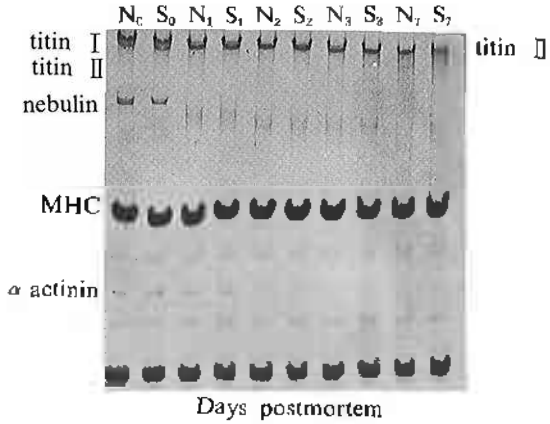


Figure 1. The effect of ante-mortem stress on pH values of Taiwan country chicken thigh and breast muscle.

SDS-PAGE of myofibrils of light muscle (breast) from ante-mortem stress and control lots were shown in figure 2. The electrophoretic pattern of the proteins in the control lot (0 day) (no ante-mortem stress) is typical of the patterns reported by Maruyama et al., (1976) and Wang et al. (1979). Titin migrated near the top of the gel as a closely spaced protein doublet, the components of which are referred to as titin Ⅰ and titin Ⅱ (Maruyama et al., 1976; Wang et al., 1979). Nebulin was clearly visible below titin. After 1 day of storage the amount of titin Ⅰ decreased, and titin Ⅱ increased with storage time in light chicken muscle. However, in the ante-mortem stress lot, titin Ⅰ and nebulin became less intense than in the control lots. After 2 days, the titin Ⅰ and nebulin bands of the control and ante-mortem stress lot were completely gone from the electrophoretogram. The titin Ⅱ band remained throughout the 7 days for both control and ante-mortem stress lots, but it became less intense with increasing storage time in control and ante-mortem stress lots.



N_0: normal	S_0: stress
N_1: stored 1 day	S_1: stored 1 days
N_2: stored 2 days	S_2: stored 2 days
N_3: stored 3 days	S_3: stored 3 days
N_7: stored 7 days	S_7: stored 7 days
MHC: myosin heavy chain	

Figure 2. Myofibrillar proteins on 3.2% SDS-PAGE electrophoretograms of control and ante-mortem stress muscles stored light country chicken (breast) muscle at 4℃ for 0, 1, 2, 3 and 7 days.

The amount of ratio (TⅡ/(TⅠ + TⅡ) × 100) of titin Ⅱ in myofibrils prepared from ante-mortem stress and control lots of light muscle was determined by densitometry. The results are shown in figure 3. The ratio of titin Ⅱ in ante-mortem stress and control lots increased with storage time. After 3 days for the ante-mortem stress lot, and 7 days for the control lot, the titin Ⅰ completely disappeared from the densitometry. This shows that the titin of myofibrils isolated from ante-mortem stress lot muscle seems to be more susceptible to postmortem degradation than that of the control lot.

Post-mortem changes in the relative amount of nebulin (nebulin/MHC) in myofibrils prepared from ante-mortem stress and control lots of light muscles were determined by densitometry. The results are shown in figure 4. At 0 day ($p < 0.05$) and 1 day ($p > 0.05$) of ante-mortem stress lot showed more less relative amount of nebulin

than those of the conrtrol lot. After 1 day for both lots, the relative amount of nebulin completely disappeared from the densitometry. It shows that the nebulin of the ante-mortem stress lot undergoes a more rapid post mortem protein degradation than that of the control lot.

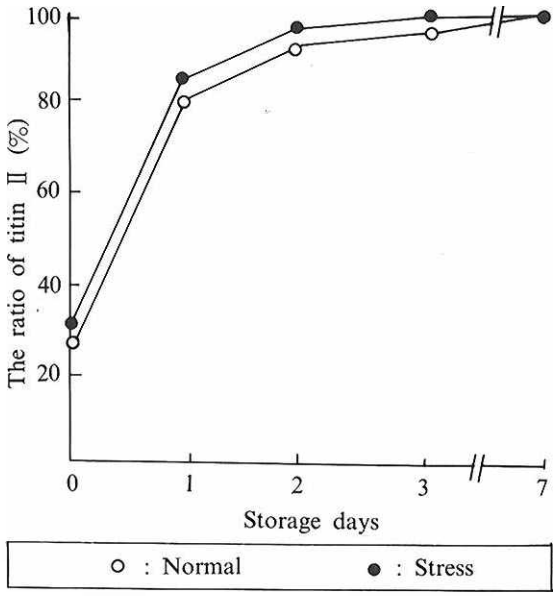


Figure 3. Post-mostem changes in the ratio {TII / (TI + TII) × 100} of titin II in myofibrils prepared from the ante mortem stress and control light muscles. The ratio was determined by densitometry of stained gels.

Myofibrils from dark chicken muscle (thigh) (figure 5) exhibited a pattern of degradation of gels different from those of light chicken muscle (figure 2). The degradation of titin I, titin II in the control lot was not evident during the storage period at 4℃, and the titin I and titin II bands remained sharp and distinct throughout the 7 days. But nebulin degraded more rapidly in the control lot than in the ante-mortem stress lot. Furthermore, 1,200 KDa subfragment of polypeptide bands (Takahashi et al., 1992) migrated between titin and nebulin after 1 day of storage in both control and ante-mortem stress lots. The amounts of the polypeptide increased with storage time (7 days) in the ante-mortem stress lot (thigh muscle).

The amount of ratio of titin II in myofibrils prepared from ante-mortem stress and control lots

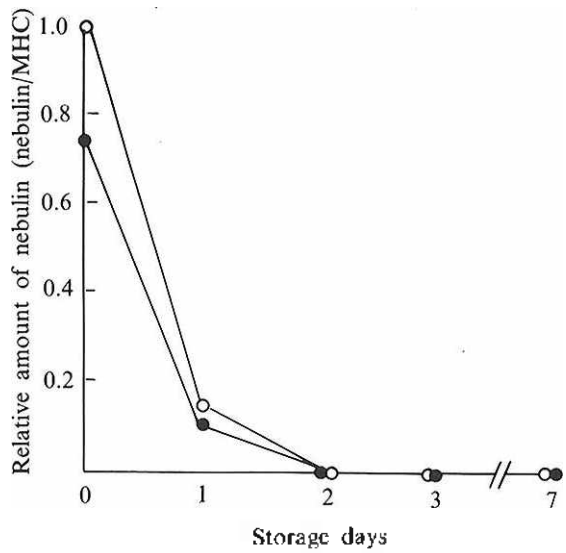
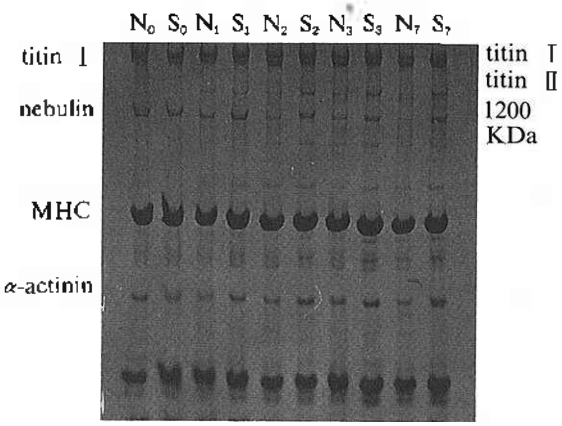


Figure 4. Post-mortem changes in the amount of nebulin in myofibrils prepared from the ante-mortem stress and control light muscles. The relative amount of nebulin to the amount of myosin heavy chain (MHC) was determined by densitometry of stained gels.
The symbols are as same as figure 3.

of dark muscle was determined by densitometry. The results are shown in figure 6. The ratio of titin II in ante-mortem stress and control lots increased with storage time. The amount of ratio of titin II in the ante-mortem stress lot was higher than that of control lot during storage for 7 days ($p < 0.05$). However, the titin I in both cases remained clearly visible from the densitometry after 7 days of storage. It shows that the titin I of the 30 ante-mortem stress lot and in light muscle seemed to be more susceptible to post-mortem degradation than titin I in the control lot and in dark muscle.

Post-mortem changes in the relative amount of nebulin (nebulin/MHC) in myofibrils prepared from ante-mortem stress and control lots of dark mucles were determined by densitometry. The results are shown in figure 7. The relative amount of nebulin of the control lot decreased with storage time. However, in the ante-mortem stress lot, it increased with storage time. This shows that the ante-mortem stress did not affect post-mortem protein degradation in nebulin. It was found that



N_0: normal	S_0: stress
N_1: stored 1 day	S_1: stored 1 days
N_2: stored 2 days	S_2: stored 2 days
N_3: stored 3 days	S_3: stored 3 days
N_7: stored 7 days	S_7: stored 7 days
MHC: myosin heavy chain	

Figure 5. Myofibrillar proteins on 3.2% SDS-PAGE electrophoretograms of control and ante-mortem stress muscles stored dark country chicken (thigh) muscle at 4℃ for 0, 1, 2, 3 and 7 days.

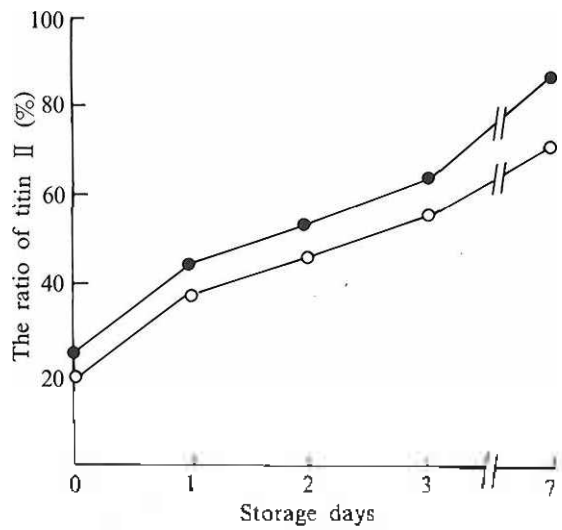


Figure 6. Post-mortem changes in the ratio {TII / (TI + TII) × 100} of titin II in myofibrils prepared from the ante-mortem stress and control dark muscles. The ratio was determined by densitometry of stained gels. The symbols are as same as figure 3.

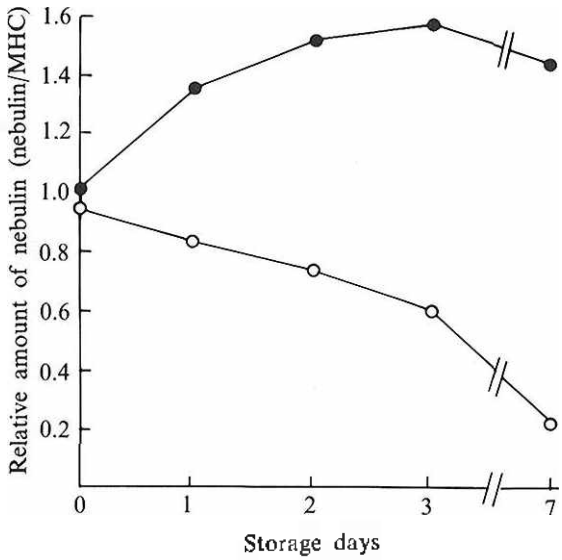


Figure 7. Post mortem changes in the amount of nebulin in myofibrils prepared from the ante-mortem stress and control dark. The relative amount of nebulin to the amount of myosin heavy chain (MHC) was determined by densitometry of stained gels. The symbols are as same as figure 3.

titin and nebulin in beef Longissimus were susceptible to post-mortem degradation, and that the extent of degradation was dependent on post-mortem storage time and temperature(Lusby et al., 1983). Moreover, Zeece et al., (1986) showed that calcium-activated factor (CAF) was responsible for the degradation of titin and nebulin. This experiment further demonstrated that the muscle type had an effect on protein degradation in post-mortem muscle. It was evident that degradation of titin and nebulin occurred more rapidly in light muscle (breast) than in dark muscle (thigh). Payhia and Parrish (1988) indicated that titin and nebulin were more rapidly degraded in light than in dark chicken muscle. It suggested that species and muscle type had an effect on protein degradation in post-mortem muscle. In addition, our present experiment showed that ante-mortem stress had an effect on protein degradation in post-mortem muscle. It was evident that degradation of titin I and nebulin occurred more rapidly in ante-mortem stress light muscle than in controls. Hay et al. (1973) used electron microscopy to examine myofibrils from poultry breast and leg

muscles. They found that after 48 h of post-mortem storage at 2℃, myofibrils prepared from leg muscle still had a typical, ordered appearance, but myofibrils from breast muscle were in random and exhibited highly degraded Z-lines and H-Z ones. In contrast to observation that protein degradation in light and dark Taiwan country chicken muscle in which titin of ante-mortem stress light muscle seemed to be more susceptible to degradation than that of control and ante mortem stress dark muscle, it was observed that nebulin from an ante-mortem stress lot showed no degradation in the dark muscle (figure 5)

In our previous studies (Lin et al., 1990), we found that the hardness of breast of duck decreased significantly as storage time increased, but that of thigh was not significantly different. Furthermore, the microstructure of both natural actomyosin gels of ante-mortem stress muscle revealed a finer network structure than that of the control muscle. In particular, the natural actomyosin gel of dark muscle from the ante-mortem stress lot had the finest network structure, and was more compact with increasing storage time. It may explain some of the differences observed between ante-mortem stress and control or between light and dark muscles cytoskeletal protein degradation during post-mortem storage.

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