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The effects of photoperiod on physical and chemical properties of broiler muscle

Carl Andre Dejoie

To the Graduate Council:

I am submitting herewith a thesis written by Carl Andre Dejoie entitled "The effects of photoperiod on physical and chemical properties of broiler muscle." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Herschel V. Shirley, Major Professor

We have read this thesis and recommend its acceptance:

Kelly Robbins, Marvin J. Riemann

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

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M. James Riemann

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Date May 22, 1986

THE EFFECTS OF PHOTOPERIOD ON PHYSICAL AND CHEMICAL PROPERTIES OF BROILER MUSCLE

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Carl Andre Dejoie

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ABSTRACT

A series of experiments were conducted on broiler males to study the effects of photoperiod, fasting, time post-mortem and exercise on the time required for the development of rigor mortis, tenderness of breast and thigh muscle, muscle glycogen levels, and histopathological characteristics of muscle.

Shear values of breast muscle of birds reared on photoperiods of 24L:0D and 16L:8D were not significantly different when tested at either 2 or 20 hrs. post mortem. Excising the muscle sample from the bone immediately following slaughter resulted in higher shear values than those of muscle allowed to remain on the bone prior to cooking. This effect was not as great with respect to the thigh muscle as it was for the breast muscle and especially so for birds reared on 24L:0D.

The light treatment of 24L:0D resulted in a very rapid onset of rigor mortis approximately one-half the time required by birds reared on 16L:8D. Exercising for 1 min. prior to slaughter reduced rigor time significantly in the 24L:0D group but not in the 16L:0D birds. Fasting for 24 hours prior to slaughter significantly reduced rigor time in both light treatment groups. The combination of the 24L:0D photoperiod, exercise and fasting were additive in their effects and resulted in extremely rapid onset of rigor mortis. These birds evidenced rigor on an average of 3.6 min. post slaughter.

No significant effect of the two photoperiods on muscle glycogen levels could be established. Muscle glycogen was found to decrease

rapidly following slaughter of the birds in both treatments. The glycogen level of muscle excised from anesthetized but living birds averaged 3-4 x of that from slaughtered birds.

The myosin ATPase reaction revealed no information regarding the effects of photoperiod. Evidence of vacuolar degeneration or necrosis was present in sections of breast muscle from the 24L:0D birds but not in the 16L:8D birds. This difference was thought to be possibly related to the anaerobic glycolytic metabolism of white muscle as opposed to the oxidative aerobic metabolism of the red thigh muscle.

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CHAPTER I

INTRODUCTION

Tenderness of broilers is of concern to today's broiler industry. In spite of their young age at time of slaughter, approximately 7 weeks, some birds show a degree of toughness. Several factors have been shown to influence the tenderness of broiler meat. Strain, sex, age, ration and season are the ones more often recognized. However, the toughness experienced by processors in some groups of broilers is not explainable by the above factors.

It is also known that there is a close relationship between the onset of rigor mortis following slaughter, its time of resolution and the final tenderness of the meat. Muscle in rigor is tougher than pre-rigor or post-rigor muscle. In unpublished studies, Shirley (1986) has observed that birds reared under continuous light develop rigor very rapidly when compared to birds receiving light-dark cycles similar to natural light patterns. Most broiler producers, in order to accelerate growth rate, expose their birds to continuous light throughout the growing period. Thus there is the possibility that the rapidity of rigor mortis, and possibly the degree of muscle contraction, as influenced by these light treatments, has an effect on the tenderness of the broiler meat.

The objectives of this study were to investigate the effect of light on the onset of rigor mortis, its influence on muscle metabolism as measured by muscle glycogen content, and its effect on muscle tenderness.

CHAPTER II

REVIEW OF LITERATURE

Rigor Mortis and Tenderness

Rigor mortis is a physical change which occurs in all muscles following death. It is characterized by stiffness and lost of extensibility of the musculature and by shortening of the muscle fibers. This post-mortem change in the physical characteristics of the muscle lasts over a variable period of time according to the physical and physiological state of the animal before its death. Generally, it is completed in 2 to 4 hours after slaughter but, in processing plants, under commercial conditions, it may develop within 10 minutes after the birds are slaughtered (Shrimpton, 1960). These physical changes are the results of chemical reactions occurring in the muscle after the death of the animal. Koonz et al. (1954) showed that there is considerable variation among the tenderness of the principal muscles of the poultry carcass as well as among individual birds. Dodge and Stadelman (1959) stated "since in most cases the groups of birds tested were raised under identical management conditions and treated the same during slaughter, it would seem that some presently uncontrolled factor, possibly physiological, causes the variation." Nevertheless, the obligatory condition for the onset of rigor is the depletion of energy reserve in the muscle following reactions such as glycolysis and loss of adenosine triphosphate (ATP), which in the resting, recently excised muscle acts as a plasticiser

to maintain it in a flexible and easily extensible state (Erdos, 1943; Bate-Smith and Bendall, 1947; Bendall, 1951; Lawrie, 1953). It has been reported that the onset of rigor occurs when the ATP level decreases to one-third of its initial concentration (Bate-Smith, 1948; de Fremery and Pool, 1960; Khan, 1975). All of this depends originally on the glycogen level in the muscle: ATP being generated via glycolysis, the depletion of glycogen causes the cessation of ATP production and ATP is irreversibly broken down by muscle contraction (Bate-Smith, 1948). When the ATP concentration decreases to a low level, permanent crossbridges are formed between actin and myosin which lock the muscle in contraction and rigor develops (Forrest et al., 1975). Although the loss of ATP is established as the main cause of rigor, the influence of other factors is less well understood.

Photoperiod Effects

No reports were found in the literature on the effect of light on the onset of rigor mortis and tenderness of the chicken meat. Nevertheless, it is known that poultry are very sensitive to the effect of light. Several experiments have shown that the length of the photoperiod has an effect on birds. Light affects growth rate, pigmentation, the activity pattern of the birds, etc. Chickens subjected to continuous light (24L:0D) develop various abnormalities such as: glaucoma, crooked toes, leg and back abnormalities, twisted wing feathers, abdominal hernias and a high incidence of the sudden

death syndrome (Buckland et al., 1976; Buckland et al., 1973; Shirley, 1986). These symptoms suggest a general physiological disturbance. Light has been shown to affect thyroid and adrenal function. On the other hand, continuous darkness brings about a reduction of thyroid functions. Constant or continuous light stimulates the hypothalamic-pituitary-thyroid axis in rabbits (Dale et al., 1972). Although many observations indicate the thyrotrophic effect of light, the question of how this came about remains unanswered. However, it seems that the thyroid activity may have an effect on glycolysis and indirectly on rigor mortis and tenderness. The effect of certain hormones such as cortisone and epinephrine on phosphorylase activity in skeletal muscle has been reported. Cortisone increases glycogen storage in rabbit muscle and inhibits the activity of the enzyme phosphorylase which catalyses reversibly the conversion of glucose phosphate to glycogen (Kerppola, 1952). Epinephrine increases or maintains this enzyme activity in the isolated rat diaphragm (Sutherland, 1951). In an experiment conducted by Osei (1981) it was shown that broilers grown on 24 hours light had significantly higher plasma corticoid levels than those grown on intermittent light. This is evidence that intermittent lighting is less stressful than continuous lighting. Another way by which the light duration may affect tenderness is related to activity of the birds. It has been observed that chickens raised under patterns of alternating light and dark, eg. 16 hrs. of light per day (16L:8D) are much more active than those raised under 24 hrs. of light per day (Shirley, 1986).

Of the known effects of the photoperiod in birds, the thyroid gland seems to play a major role. Relationships in such major functions as growth rate, reproduction, molting and migration have been established. The thyroid affects the metabolic rate of all cells and a wide variety of interactions between the thyroid hormone and other hormones have been reported. Several experiments have shown that the photoperiod has a significant effect on the basic metabolism of the chicken. Squidd (1971) demonstrated that the level of amino and nucleic acids in the muscle of growing chicks are related to light-dark cycles. He showed that the level of glucose, glycogen and free fatty acids increased significantly in the liver and other tissues during the first half of the diurnal period (Squidd, 1974).

Twiest and Smith (1970) and Smith (1972) reported a circadian pattern in blood glucose levels with a maximum occurring during the light period even in starved birds.

Newcomer (1974) found that thyroid function fluctuates sinusoidally in a 24 hour pattern associated with the light dark cycle.

Long photoperiods stimulate pituitary thyrotrophin producing cells (Tixer-Vidal et al., 1967) although they seem to inhibit the thyroid function as measured by ^{131}I uptake (Tixer-Vidal and Assenmacker, 1966). Metabolic activity and heat production are higher during the light phase and thiouracil suppression of thyroid activity reduces the amplitude of the durinal variation (Washburn et al., 1962).

Effects of Some Biological and Physical Factors on Tenderness of Chicken Meat

Some researchers suggest that many factors which are not directly associated with the development of rigor mortis in chickens may have an effect on the tenderness of their meat. Among these factors are:

Strain and breed. Shrimpton and Miller (1960) compared a fast-growing strain of White Rocks with a lighter, slower-growing strain of Brown Leghorns and found that the male Leghorns were tougher than the male White Rocks. In contrast, Goodwin (1964) found that neither sex nor strain had any significant effect on meat tenderness when compared in six strains of Broad Breasted Bronze Turkeys. Paul et al. (1959) found no difference in tenderness of chicken of two different strains. Goodwin et al. (1969) also found no differences in tenderness associated with strains.

Age. As it is in other species, age is a factor in poultry tenderness. The size of the muscle fiber varies with age and they become tougher as the bird ages. In the case of broilers, as they are slaughtered at a very young age--less than seven weeks--the effect of age should be of little importance. Seven weeks seems to be the best age to slaughter broilers from the stand point of tenderness (Evans et al., 1976).

Sex. Shrimpton and Miller (1960), Gilpin et al. (1960) and Carlson et al. (1962) reported that sex has little or no effect on

tenderness. On the other hand, Goodwin et al. (1969) found that male chickens are slightly more tender than are females.

Ration. So long as the diet is well balanced and optimum for growth, it appears to have no effect on tenderness. Marsden et al. (1957), Harkin et al. (1958), and Goertz et al. (1961) showed that different types of diet have little or no influence on tenderness of chicken broilers.

Season. Several reports have suggested that the season of the year may have an influence on broiler tenderness. Simpson and Goodwin (1975) showed that chickens produced in the fall have lower shear values in comparison with those produced in the other seasons of the year. Broilers produced during the summer months seem to be tougher than those grown during other seasons of the year (Hargus and Lee, 1973).

White vs dark muscle. In the chicken, the white muscle, such as the breast muscle is generally found to be tougher than the dark muscle as found in the leg or thigh. This observation is reinforced by the fact that most consumer complaints about chicken toughness are associated with the breast. When compared to breast muscle, thigh muscle showed a lower shear value (6 to 7 kg/g vs 9 to 11 kg/g) (Goodwin et al., 1969). Koonz et al. (1954) found that breast muscle is tougher than thigh muscle even after the aging process. Cunningham and Lee (1975) froze birds and cooked them either from the frozen

state or after thawing and found that, in both cases, the breast muscles were less tender than those of the thigh. Marion (1967) suggested that with aging the tenderness of thigh muscle tended to increase more than that of breast muscle. Van den Berg et al. (1964), de Frimery and Streeter (1969) stated that thigh muscle tenderizes more slowly than breast muscle. Hanson et al. (1942) reported that tenderness of breast muscle did not change in New York dressed broilers after three hours post-mortem at 1.6°C but increases in thigh tenderness continued up to 42 hours post-mortem. These observations were confirmed by Canadian workers. Van den Berg et al. (1964) found that breast muscle tenderness increased during the first 1-2 days post-mortem when kept in ice. Leg muscle was found to undergo a second phase of tenderization 2-5 days later.

The Influence of Muscle Glycogen on the Tenderness of Broiler Meat

Several workers have established the relationship between the glycogen reserve in the muscle and its tenderness. Rose and Peterson (1951) found that the glycogen reserve in the muscle was lowered by starvation and seriously depleted by exercise. The combination of these two factors made the loss of glycogen much greater than either separately. When sugar was fed, the glycogen reserve took about six hours to reach its initial level. Silvette and Britton (1934) analyzed the breast and thigh muscle from 20 Brown Leghorn cockerels and pullets and found that the breast muscle contained three times as much glycogen as the thigh muscle. The average glycogen

values were 0.89 and 0.24%, respectively. Mellor et al. (1958) suggest that the glycogen level of the tissues at the time of death may be one of the basic factors influencing tenderness. They found that the higher the glycogen content at death the more tender is the meat. The amount of glycogen in the muscle seems to vary with the individual bird.

Chickens killed with an overdose of anesthesia so as to prevent struggling at slaughter showed great variation in the glycogen content (42 to 62 umol/g muscle) (Grey et al., 1974). Khan and Nakamura (1970) confirmed the above observation by finding that accelerated glycolysis immediately before slaughter, which would lower the initial glycogen content, resulted in less tender meat. They concluded that minimizing pre-slaughter glycolysis in well fed and well rested birds would increase tenderness. Prevention of glycogen depletion by administering epinephrine 12 or more hours before slaughter contributed to tenderness of the meat (de Fremery, 1966 and Khan, 1975). When post-mortem glycolysis was minimized by epinephrine injections, sodium iodoacetate, or rapid cooking, the meat was tender without aging. They state that since these treatments accelerate rigor mortis, it is the acceleration of post-mortem glycogen depletion, not the acceleration of rigor mortis, which induces toughness.

CHAPTER III

MATERIALS AND METHODS

A series of experiments were conducted to study the effect of the photoperiod, fasting, and exercise on physical and chemical properties of muscle.

Rearing Phase

Eighty males of a commercial broiler strain (Hubbard x White Mountain) was brooded in 4 light-proof rooms approximately 4 ft. x 5 ft. Two rooms were subjected to continuous light (24L:0D) and 2 rooms received 16 hrs. of light per day (16L:8D). The light treatments were initiated at 1 day of age. Light was provided by 1 25W incandescent bulb in each room with an intensity of 14.5 lux. Feed and water was provided ad libitum. A ration containing 23% protein and 2835 Kcal/kg. was fed to 3 weeks of age followed by a 21% protein--2884 Kcal ration to 7 weeks of age.

Experiment 1--Muscle Tenderness

Ten male chickens from each light treatment, at approximately 7 weeks of age, were used to determine the effects of light on muscle tenderness. The birds were on feed ad libitum up to the time of slaughter and care was taken to avoid excitement and struggling during catching and slaughtering. The birds were killed by severing the jugular veins, bled, scalded in 60°C water for 1 minute and picked on a free floating cyclic picker for 1 minute.

The first set of samples were taken on the breast (Pectoralis major) and thigh muscle (Biceps femoris) on the left side of each bird immediately following picking. The samples were approximately 1 cm x 1 cm x 8 cm. They were placed in plastic bags and these placed in ice slush for approximately 2 hours. The samples were then cooked in boiling water for 30 minutes and then submitted to the shear test using a Dynamometer scale with the Warner-Bratzler shear attachment. The measurements were taken at four different points on each muscle sample. The average value of these measurements was considered as the force applied to shear the meat. A second set of samples was taken 20 hours later from the same birds which had been kept in ice. The entire breast of each bird was removed, and then cooked in boiling water for 30 minutes. A strip of muscle of the size previously stated was taken and the shear test was performed according to the procedures described earlier.

Experiment 2--Rapidity of Rigor Mortis

A series of trials were conducted to compare the time required following death for the onset of rigor mortis. The birds were killed by cervical dislocation and placed on their backs at room temperature (approximately 21°C) and at intervals of approximately 1 minute were checked for rigor by slightly lifting the legs. Rigor was considered to have occurred when a definite degree of stiffness was detected.

In Trial 1, rigor time of broiler males reared from 1 day of age to 5 weeks of age under photoperiods of 24L:0D and 16L:8D

were determined when the birds were subjected to four conditions prior to slaughter: (1) full fed with no forced exercise; (2) full fed plus 1 minute forced exercise; (3) 24 hours of fasting with no exercise; and (4) fasting plus exercise.

Trial 2 consisted of 6-week-old broiler males reared under 24L:0D and 16L:8D light treatments subjected to 0 hours of fasting and to no forced exercise.

Trial 3 was similar to Trial 2 with the exception that the birds were 7 weeks of age at the time the rigor test was conducted.

Experiment 3--Muscle Glycogen

Trial 1. At 5 weeks of age, 5 males from each of the 24L:0D and 16L:8D reared groups were anesthetized to the surgical level by wing vein injection of nembutal. A strip of the pectoralis major muscle approximately 1 x 1 x 5 cm was removed from each bird and a 1 gram sample was quickly weighed and then placed in a dry ice-alcohol preparation. The approximate time elapsing between the removal of the sample from the bird and the frozen state was 1 minute.

Trial 2. Each bird was killed by cervical dislocation and a strip of Pectoralis major muscle was removed approximately 3 minutes later. The muscle was placed in an individual plastic bag to prevent loss of moisture and kept at 21°C. A 1 gm. sample was removed at 3, 120, and 240 minutes post slaughter and frozen as in trial 1. Muscle samples were taken from 5 males from each light treatment group.

Trial 3. Six week old male broilers were slaughtered by severing the jugular veins. At 3, 15, 30, 60 and 120 minutes post slaughter, 1 gm. samples of the Pectoralis major muscle was removed and frozen in a dry ice-alcohol preparation. A total of 16 birds, 8 from the 24L:0D and 8 from the 16L:8D treatment groups were used in this study.

All birds in the 3 trials were on feed prior to slaughter and an effort was made to avoid excessive activity during handling that would tend to deplete the muscle glycogen. The muscle samples that were frozen in dry ice were later stored in a refrigerator at -40°C until glycogen determinations were made. Glycogen determination was made by the phenol sulfluric acid method of Dubois et al. (1956).

Histopathological Study

Samples of the Pectoralis major and the Biceps femoris muscles were taken from 3 7-week-old male broilers from each of the 24L:0D and 16L:8D treatment groups. The sample blocks were frozen in iospentane cooled in liquid nitrogen and stained by the myosin ATPase reaction with hematoxylin and eosin. The remnants of each block were kept in the refrigerator overnight and fixed in 10% buffer neutral formol the following day.

Statistical Analysis

The statistical significance of the data obtained in these studies was evaluated by a paired "t" test when only two mean values were compared. The Student-Newman-Keuls test was used as mean

separation method when more than two means were compared. Orthogonal contrasts were used to compare the means when several sets of data were considered in an experiment. In experiment 2, trial 1, the analysis of variance was performed to determine the significance of the main effects and their interaction.

CHAPTER IV

RESULTS AND DISCUSSION

Muscle Tenderness

The average values for the shear force are given in Table I for the breast muscle and in Table II for the thigh muscle. There were no significant differences ($P>.05$) in shear values of cooked meat of both the breast and the thigh muscle when compared in regard to the light treatments. However, there were significant differences ($P<.05$) in the shear values of 2 hours post slaughter samples and 20 hours post slaughter samples of breast meat. This agrees with the finding of Koonz et al. (1954) who reported that cut muscle was always tougher than uncut muscle as in the case of the 20 hours post slaughter samples in which the muscle was cooked on the bone. This difference can also be attributed in part to the effects of aging as the two sets of samples were about 18 hours different in age at the time they were sheared. In the case of the thigh muscle, shear values of the excised vs. intact samples from birds receiving the 24L:0D treatment were not significantly different. Leaving the muscle on the bone did improve tenderness in the 16L:8D birds. As the tenderness of the thigh muscles increases less rapidly than that of the breast, probably because of the higher connective tissue content (Lowe et al., 1946), this might be an explanation why the shear value of the 2 hours post slaughter sample and 20 hours post slaughter of the thigh did not show a significant difference in the 24 hours

TABLE I. The Effect of Photoperiod and Time Post Slaughter on Shear Values[1] of Cooked Breast Muscle From Seven Week Old Broiler Males

Bird No.	2 hrs. post slaughter[2]		20 hrs. post slaughter[3]	
	24L:0D	16L:8D	24L:0D	16L:8D
	----------(lbs. of force)----------			
1	7.90[3]	11.87	3.50	4.25
2	7.50	13.56	2.69	4.12
3	9.94	7.94	3.87	4.25
4	5.69	11.62	4.69	5.44
5	7.62	7.25	4.12	8.44
6	7.81	11.12	9.44	3.96
7	9.19	8.44	3.44	4.25
8	14.0	5.37	6.87	4.56
9	8.75	8.56	5.62	2.56
10	10.94	12.50	4.87	4.69
Mean	8.93[a]	9.83[a]	4.99[b]	4.75[b]
S.E.M.	.72	.83	.56	.35

[1]Average of shear values at 4 points on each muscle strip.

[2]Muscle samples were removed from the bone at the time of slaughter and prior to cooking at 2 hrs. post slaughter.

[3]Muscle samples were removed from the bone after cooking at 20 hrs. post slaughter.

[a,b]Means with different superscripts are significantly different ($P<.01$).

TABLE II. The Effects of Photoperiod and Time Post Slaughter on Shear Values[1] of Cooked Thigh Muscle From Seven Week Old Broiler Males

Bird No.	2 hrs. post slaughter[2]		20 hrs. post slaughter[3]	
	24L:0D	16L:8D	24L:0D	16L:8D
	(lbs. of force)			
1	4.75[3]	9.19	2.56	7.37
2	7.62	7.06	4.12	5.81
3	7.50	10.19	5.25	7.24
4	7.81	9.62	6.12	7.19
5	2.69	9.37	7.31	8.19
6	4.44	11.93	3.87	6.00
7	12.87	8.62	7.50	5.87
8	11.69	8.18	9.19	5.25
9	7.44	8.56	7.06	5.12
10	8.87	9.00	7.50	6.62
Mean	7.56[a]	9.17[a]	6.04[ab]	6.46[b]
S.E.M.	.99	.40	.65	.32

[1]Average of shear values at 4 points on each muscle strip.

[2]Muscle samples were removed from the bone at the time of slaughter and prior to cooking at 2 hrs. post slaughter.

[3]Muscle samples were removed from the bone after cooking at 20 hrs. post slaughter.

[a,b]Means with different superscripts are significantly different ($P<.01$).

light treatment. Also, as the mean shear values of the 24L:0D groups tended to be lower than those of the 16L:8D groups a greater difference due to leaving the muscle in site would have been required for significance (Table III).

Rigor Mortis

The time required following death for the onset of rigor mortis is shown in Table IV and V for trials one, two and three. The birds exposed to the 16L:8D treatment took a significantly longer time for the onset of rigor mortis. Those exposed to continuous light entered the rigor state very rapidly. When full-fed and non-exercised, the 16L:8D reared birds took an average of 22.7 minutes for the onset of rigor mortis while those exposed to continuous light required an average of 12 minutes. These differences are statistically significant ($P<.05$). It is generally agreed that the onset of rigor occurs when the ATP levels fall to about one-third of its initial value and that ATP is regenerated by glycolysis. The rapidity of the ATP depletion in the post mortem muscle depends on the glycogen level in the muscle at slaughter time. Therefore, the birds exposed to the 16L:8D treatment seem to have a more efficient ATP regeneration capability. The 24L:0D birds seem to have a defective ATP system which became more pronounced with age, in fact the 7 week old birds reared on 24L:0D required one-half the time taken by the 6 week old birds for the onset of rigor, 6.2 minutes vs 12 minutes. In trial one, the main effects of feed, exercise, photoperiod, and

TABLE III. Orthogonal Contrast for Shear Values of Muscle From Seven Week Old Broiler Males

Contrast[1]	DF	SS	F value	Pr>F
1 vs 2	1	3.95	0.89	0.3475
3 vs 4	1	12.86	2.91	0.9234
5 vs 6	1	0.33	0.08	0.7837
7 vs 8	1	0.87	0.20	0.6579
1 vs 5	1	80.92	18.32	0.0001**
2 vs 6	1	71.25	16.13	0.0001**
3 vs 7	1	11.55	2.61	0.1103
4 vs 8	1	36.61	8.29	0.0053**

[1]1 = 24L:0D excised breast muscle; 2 = 16L:8D excised breast muscle; 3 = 24L:0D excised thigh muscle; 4 = 16L:8D excised thigh muscle; 5 = 24L:0D *in situ* breast muscle; 6 = 16L:8D *in situ* breast muscle; 7 = 24L:0D *in situ* thigh muscle; and 8 = 16L:8D *in situ* thigh muscle.

**Significantly different at P<.001.

TABLE IV. The Effects of Photoperiod, Fasting and Exercise on the Time of Onset of Rigor Mortis of Broiler Males

Trial No.	Age of Birds (wks.)	Treatments: Feed and Exercise State	Photoperiod: 24L:0D	Photoperiod: 16L:8D
			------- Minutes -------	
1[a]	5	Full fed, no exercise	12.2±3.0	22.2±1.2
		Full fed, exercised	6.8±1.6	20.6±2.8
		Fasted, no exercise	6.6±0.5	16.0±2.8
		Fasted, exercised	3.6±0.2	6.0±0.7
2[b]	6	Full Fed, no exercise	12.0±1.4	22.7±1.4
3[b]	7	Full Fed, no exercise	6.2±1.7	23.0±4.1

[a]The main effects of feed, exercise, photoperiod, and their three way interaction were significant ($P<.05$).

[b]Photoperiod effect was significant ($P<.05$).

TABLE V. Orthogonal Contrast for the Effects of Feed, Fasting, Exercise on Time for Onset of Rigor Mortis of Five, Six and Seven Week Old Broiler Males

Contrast[1]	DF	SS	F Value	Pr>F
1 vs 2	1	250.000	16.39	0.0003**
3 vs 4	1	476.100	31.22	0.0001**
5 vs 6	1	220.900	14.49	0.0006**
7 vs 8	1	14.400	0.94	0.3385

[1]1 = 16L:8D photoperiod, full fed and no exercise; 2 = 24L:0D photoperiod, full fed and no exercise; 3 = 16L:8D photoperiod, full fed and exercised; 4 = 24L:0D photoperiod, full fed and exercised; 5 = 16L:8D photoperiod, fasted and no exercise; 6 = 24L:0D photoperiod, fasted and no exercise; 7 = 16L:8D photoperiod, fasted and exercised; and 8 = 24L:0D photoperiod, fasted and exercised.

**Significantly different at P<.001.

their three way interaction were significant ($P<.05$). After 1 minute of exercise, the 24L:0D birds entered rigor in approximately one-half the time required for the non-exercised group. Exercise reduced rigor time in the 16L:8D birds by very little. This observation indicates that the birds exposed to continuous light are more sensitive to the effects of exercise, in fact they are less active than those reared under 16L:8D. For the 24L:0D birds, 24 hours of fasting had almost the same effect on rigor time as 1 minute of exercise. Fasting for 24 hours also significantly reduced rigor time of the 16L:8D group. In both photoperiod groups, the combination of fasting and exercise had an additive effect in reducing the time of onset of rigor. The time required for the onset of rigor thus being approximately one-fourth that of full-fed and non-exercised birds.

Histopathological Findings

Lesions were detected in the formalin fixed H & E stained sections of muscle and consisted of scattered muscle fibers undergoing either vacuolar degeneration or necrosis. Longitudinal sections frequently revealed that this was segmental.

The major significant finding was that both types of muscle (*Pectoralis major* and *Biceps femoris*) from the 24L:0D birds were affected while only the thigh muscle of the 16L:8D reared birds evidenced these lesions.

These results suggest that the thigh muscles are under greater stress compared with the breast muscle. This is a common finding in

metabolic diseases of muscle. It also implies the possibility that dark muscles which are type 1 muscles and are oxidative aerobic in metabolism may be more susceptible than the white, type 2 glycolytic or anaerobic-glycolytic muscle fibers.

These types of degenerative changes in muscle fibers can be caused by a variety of factors such as toxins, deficiencies and errors of metabolism. In this study, it is clearly evident that continuous light (24L:0D) results in a greater stress on breast muscle which is glycolytic in metabolism.

Muscle Glycogen

The results of the experiments regarding muscle glycogen in response to light and other treatments are shown in Table VI. The post-mortem glycogen level in the breast muscle taken from the live bird (Trial 1) is approximately the same in both groups of birds, 6290 $\pm$ 424 µg/gm. for the birds exposed to 24L:0D versus 6580 $\pm$ 840 µg/gm. for the 16L:8D photoperiod. The statistical analysis of these data did not show any significant difference ($P>.05$). In Trial 2 in which the birds were slaughtered by cervical dislocation there is also no significant difference ($P>.05$) between the two groups of birds in regard to the effect of light. Similarly, in Trial 3, in which the birds were killed by severing the jugular, the differences between light treatments were not statistically significant. There was clear cut evidence of decreasing muscle glycogen with respect to time in both Trials 2 and 3. At 3 minutes post-slaughter the

TABLE VI. The Effects of Photoperiod, Method of Slaughter and Time Post Mortem on Breast Muscle Glycogen of Broiler Males

Trial No.	State of Bird	Minutes Post Mortem	Photoperiod 24L:0D	Photoperiod 16L:8D
			--- Glycogen--μg/gm ------	
1	Anesthetized[1]	0	6290±424[a]	6580±848[a]
2	Slaughtered by cervical dislocation	3	1832±526[a]	1362±406[a]
		120	646±191[b]	378±89[b]
		240	322±109[b]	270±92[b]
3	Slaughtered by severing jugular	3	804±97[ab]	1383±275[a]
		15	818±187[ab]	1139±203[ab]
		30	796±144[ab]	972±214[ab]
		60	506± 81[b]	863±183[ab]
		120	405±84[b]	426±45[b]

[a,b]Means within each trial with different superscripts are significantly different (P<.05).

[1]The mean values of the anesthetized birds of both photoperiod treatments were significantly different from all other means in table (P<.01).

glycogen level in the breast muscle was higher than at 120 minutes and 240 minutes. After 240 minutes following death the glycogen level was about one-fifty of the initial value. In Trial 3, the 16L:8D treated birds seemed to have a higher glycogen level at 3 minutes post slaughter compared with the 24L:0D birds but the statistical analysis of the data did not confirm that difference ($P>.05$). There was great variation within the treatment groups, thus large differences in mean values were required for statistical significance. Great care was exercised to reduce experimental error, however, it was apparent that excessive error still occurred. It was also evident that there was much variation among the birds in the initial muscle glycogen.

CHAPTER V

SUMMARY AND CONCLUSIONS

A series of experiments were conducted to study the effects of photoperiod, fasting, time post-mortem, and exercise on the time required for the development of rigor mortis, tenderness of breast and thigh muscles, muscle glycogen levels, and histopathological characteristics of muscle.

Shear values for the breast muscle of birds receiving 24L:0D or 16L:8D were not significantly different at either 2 or 20 hours post mortem. However, breast muscle that was excised from the bone immediately following slaughter was significantly tougher than that which remained on the bone prior to cooking. The advantage of leaving the thigh muscle in situ before cooking was not as great as it was for the breast muscle. This was particularly so for the birds that had received the 24L:0D treatment.

The light treatment of 24L:0D resulted in a very rapid onset of rigor mortis, approximately one-half the time required for the 16L:8D reared birds. Exercising for 1 minute prior to slaughter reduced rigor time in the 24L:0D birds but not in the 16L:8D birds. Fasting for 24 hours prior to slaughter significantly reduced rigor time in both photoperiod groups. When exercise and fasting were combined, rigor was extremely rapid. This was to be expected as these factors result in a depletion of muscle glycogen. When combined with the 24L:0D photoperiod, exercise and fasting resulted in an

average rigor time of 3.6 minutes. It is apparent that continuous light has a similar effect as exercise and fasting on time of rigor. Continuous light apparently interferes with the glycolytic pathway by which muscle energy is derived.

The photoperiod did not result in a significant difference in muscle glycogen as might be expected from its effects on rigor time. Much variation was present in the data probably reflecting both a lack of precision and unexplainable individual differences. The glycogen level of muscle from live but anesthetized birds was found to be 3-4 x that of slaughtered birds. Muscle glycogen was also found to diminish rapidly with time following slaughter.

The myosin ATPase reaction did not provide any useful information for comparing the effects of 24L:0D vs. 16L:8D treatments. Evidence of vacuolar degeneration or necrosis was present in sections of breast muscle from the 24L:0D birds but not from the 16L:8D treated birds. Thigh muscle was comparable in both groups. The implication is that white muscle, because of its anaerobic glycolytic metabolism, is affected to a greater extent in the continuous light treated birds.

Considering the known relationships of rigor mortis to tenderness, it is difficult to reconcile the lack of differences in tenderness and in muscle glycogen levels of birds receiving the two photoperiods. Especially is this so in the light of the great differences in rigor time caused by these photoperiods.

Further studies are called for to elucidate the nature of the metabolic effects of continuous light in growing broilers. This should include the role of the thyroid and adrenal hormones on ATP regeneration. The results of the muscle studies should also be related to the broader and economically important light stress syndrome, with its many diseases, of commercial broilers.

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VITA

Carl Andre Dejoie was born on February 6, 1957 in Cap Haitian, Haiti. He completed his secondary instruction at "Centre d'Etude Secondaires," Port-au-Prince in 1977. The same year, he was admitted as a student in agriculture at the Universite D'etat D'Haiti, Faculte d Agronomie et de Medecine Veterinaire, and received his Bachelor of Science in agriculture in 1981.

He was employed for three years at the "Haitian American Meat Provision Company" as a farm manager. In 1982, he was admitted as a teaching assistant in his former agriculture school. Two years later, he was granted a scholarship to persue post graduate studies in the U.S.A.

In September 1984 he was admitted to The University of Tennessee, Knoxville as a graduate student in the Animal Science Department. He obtained the M.S. degree in Animal Science in June 1986.