Thigh Muscle Response of Broilers to Cold Stress in Comparison to Breast Muscle

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Abstract— **The objective of this study was to assess the effect of acute cold exposure on broiler physiology, breast and thigh muscle metabolites, and meat quality. One hundred sixty male birds at ages of 5 and 6 wk were exposed to temperatures of -9 to -15°C, and +20ºC (control) in a simulated transport chamber for 3 h followed by 0 or 2 h of lairage prior to slaughter. Core body temperature was monitored every minute using i-Button data loggers, and live shrink and blood glucose were assessed. Total glucose and lactate concentrations at 30 h post-mortem, as well as ultimate pH (pH_u), color, and water holding attributes were evaluated on *Pectoralis* major muscle of breast and *Iliotibialis* muscle of thigh. A significant ($P < 0.05$) drop in body temperature and blood glucose and a substantial increase in live shrink were observed at temperatures below 0°C. Following cold exposure, thigh muscle was almost depleted of glycogen compared to a small but significant reduction in breast muscle glycogen when exposure temperature was below -8ºC. Similarly much greater effects of simulated cold transport were observed on thigh pH_u, and quality attributes compared to breast. In addition, 84% incidence of the dark, firm, dry (DFD) quality defect was observed for thigh ($pH_u > 6.4$, $L^* < 44$) compared to 42% incidence of DFD for breast ($pH_u > 6.1$, $L^* < 46$) when transportation temperature was below 0°C. Results of this study showed that thigh muscle was affected more severely than breast muscle by exposure of broilers to cold temperatures prior to slaughter.**

Keywords— **Broilers, cold stress, breast and thigh**

I. INTRODUCTION

The effect of cold winter transportation has only been reported on breast meat [1, 2]. The majority of studies conducted on the effect of pre-slaughter stress in broilers [3, 4, 5] and turkeys [6, 7] have also focused on breast meat quality, perhaps due to its higher economic value compared to thigh meat. Only a few studies have investigated the effect of pre-slaughter transport [8] on thigh meat quality. The aim of the current study was to determine the effect of cold-stress during simulated transport on thigh meat quality and incidence of DFD meat and compare it with breast meat.

II. MATERIALS & METHODS

A total of 160 male broilers at 5 and 6 wk of age were exposed to one of the assigned exposure temperatures of -15, -12, -9, and +20ºC for a duration of 3 h in an indoor test chamber. Birds exposed to +20°C are referred to as "control" birds and the remainder as "cold stressed."

Birds were taken off feed 7 h prior to start of the trial. They were wing banded, weighed, and orally dosed with small temperature logging devices (iButton Thermochron, DS1922L, Maxim Integrated Products, CA) into the proventriculus to measure core body temperature (CBT) 2 h prior to start of the chamber exposure. The temperature and humidity near each bird was also monitored (Hygrocon iButton DS19223). Birds were individually housed in a 2 partitioned drawers. Due to variation in microclimate temperature, birds were grouped based on the temperature in their immediate surroundings into 4 groups: control (21.7±0.7), 0 to -8 (-5.37±2.1), -8 to -11 (-9.5±0.9) and colder than -11ºC (-12.2±1.0) during simulated transport.

Birds were randomly assigned to 0 or 2 h of lairage upon removal from the chamber. Blood was taken from the brachial vein using a heparinised syringe. Birds were processed as described by Dadgar et al. [2]. Breast meat quality was evaluated by measuring color, ultimate pH (pH_u; 30 h post-mortem), water binding capacity (WBC) and processing cook yield (PCY) as previously described [1, 2]. Color, pH_u, and thaw and cook losses of thigh was assessed. Color of thigh was measured on the external surface of the *iliotibialis*

muscle. Thaw loss was determined on the entire thigh. Cook loss was assessed on *iliotibialis* muscle of thigh, by cooking the samples for 12 min in an 80ºC water bath to internal temperature of 76-78ºC.

Concentrations of total glucose and lactate were assessed at 30 h post-mortem on breast and thigh muscles using the methods described by Dadgar et al. [2]. Glycolytic potential (GP) was calculated according to Monin and Sellier [9] as follows: GP= 2 (total glucose) + lactate.

Samples were classified as normal ($pH_u < 6.1$, $L^* > 46$) and DFD breast ($pH_u > 6.1$, $L^* < 46$) [10] or normal ($pH_u < 6.4$, $L^* > 44$), and DFD thigh ($pH_u > 6.4$, $L^* < 44$). Thigh meat classification was performed based on pH_u and color within the population in our laboratory, as no classification was available for this muscle in the literature.

A completely randomized design with a 4 x 2 x 2 factorial arrangement was employed, with 10 birds per treatment combination (n = 160). The model included main effects of microclimate temperature (below -11, -11 to -8, -8 to 0, and control), age (5 and 6 wk), and lairage (0 or 2 h) and the interactions between them as the main sources of variation. Data were subjected to analysis of variance (ANOVA) using the General Linear Models procedure of SAS. Differences among means were evaluated using the Duncan's multiple comparison test option of SAS.

III. RESULTS & DISCUSSION

A. Effect of Cold Stress on Physiology Parameters

In the current study, the average CBT of male broilers dropped significantly as the immediate temperature surrounding them decreased from 0 to -14ºC compared to the control birds which maintained a normal CBT throughout the 3 h simulated transportation and lairage (40.5ºC). At all of the cold temperatures the average CBT steadily fell during exposure and then rose again during lairage, though most did not return to pre-test levels by slaughter time.

Significant ($P < 0.05$) effect of age was observed for CBT, with 5 wk birds showing lower average CBT compared to the 6 wk birds when exposed to temperatures below -8ºC (30.1 vs. 36.3ºC, when exposed to -8 to -11ºC; 26.0 vs. 31.2 ºC when exposed to below -11ºC for 5 wk compared to 6 wk old birds, respectively).

Live shrink was observed to be higher for all cold treatment temperatures (~3.3%) tested compared to the control group (1.7%), as found previously [2]. A higher live shrink was observed for 5 wk birds (3.6%) compared to 6 wk birds (2.3%). Blood glucose, measured only on the 6 wk birds, showed a significant decline as the exposure temperature decreased from 0 to -14ºC. The highest blood glucose values were observed in the control birds (12.3±0.5) and the lowest were observed in birds exposed to temperatures colder than -11ºC (6.9±2.0). This is similar to results of an earlier study by the same authors [2].

B. Effect of Cold Stress on Breast and Thigh Muscle Metabolites and Meat Quality

Exposure temperature showed a significant relationship to levels of thigh muscle metabolites, with thigh muscle lactate and GP at 30 h post-mortem being inversely related to microclimate temperature (**Table 1**). It should be noted that these differences in thigh muscle metabolites based on microclimate temperature were more pronounced than in breast meat (**Table 2**). Lactate concentration and GP of thigh meat from birds exposed to control temperatures were over twice higher than those exposed to temperatures of 0 to -8ºC and over three times higher for birds exposed to temperatures below -8ºC (**Table 1**).

Significant effect of age and lairage was observed on thigh muscle metabolites, with 6 wk birds showing higher GP for thigh meat compared to 5 wk birds. In addition, the 2 h lairage caused significant reduction in thigh muscle metabolites (**Table 1**).

In the present study, breast muscle metabolites were affected by interaction effects of microclimate temperature with age of the birds and lairage prior to slaughter. Five wk birds exposed to temperatures below 0ºC had lower breast meat GP_{30h} (87.0±19.8 µmol/g) compared to the control 5 wk birds (105.1±17.2 µmol/g). But, no difference was observed in 6 wk birds breast muscle GP (83.8±14.5µmol/g) based on the temperature grouping. The 2 h lairage prior to slaughter resulted in lower breast muscle GP (75.3±16.0 µmol/g) compared to those slaughtered immediately (98.5±19.8 µmol/g) after removal from temperatures below -11ºC.

The difference in thigh meat pH_u was more noticeable (0.7- 0.9 units) compared to breast meat (0.1- 0.2 units) based on cold temperature exposure prior to slaughter (**Tables 1 & 2**). Based on the literature [8, 11], it is likely that thigh muscle (slow-twitch fiber) is more prone to glycogen depletion compared to breast muscle (fast-twitch fiber) due to differences in fiber type, initial glycogen reserve and type of stress (cold stress and transport) prior to slaughter, leading to a larger variation in GP, which consequently resulted in higher variation in pH_u and ultimate meat quality.

Color and processing properties of breast and thigh muscles were significantly affected by microclimate temperature (**Tables 1 & 2**). Both thigh and breast meat of birds exposed to temperatures below 0°C were darker, redder and less yellow compared to the control group, but no significant difference was observed between the cold temperature groupings. However, the difference in color parameters were of much bigger magnitude for thigh meat of birds exposed to cold temperatures vs. the controls, compared to breast meat (10 units difference in L*, compared to 2-3 units difference, respectively). Some effects of cold stress on water holding ability of both thigh and breast meat were observed, with cold exposure prior to slaughter causing an increase in water holding ability of both muscles.

C. Effect of Cold Stress on Incidence of DFD in Breast and Thigh Meat

Birds exposed for 3 h to temperatures below 0°C showed an overall 42% incidence of DFD breast meat compared to 85% incidence of DFD for thigh meat. Birds exposed to +20ºC did not show any DFD thigh, but 20% DFD breast meat. The high DFD incidence may be related to the individual housing of birds during treatment and their inability to huddle together in order to maintain their CBT at acceptable levels.

IV. CONCLUSIONS

Exposure to cold temperatures (< 0ºC) during transportation influenced bird physiological parameters, shown with the significant drop in CBT and blood glucose and a significant increase in live shrink of birds, which was greater for 5 wk birds compared to the 6 wk birds.

Exposure of birds to temperatures below 0°C during simulated transport resulted in significant effects on muscle metabolites, pH and color of both breast and thigh, with the greatest effect on the thigh meat. The difference in GP based on microclimate temperature was much less for breast compared to thigh meat. The pH_u of thigh meat from the cold stressed birds was 0.8 units higher than the controls compared to a difference of only 0.2 units in breast pH_u between the control and cold-stressed birds. This difference between breast and thigh meat in their response to cold stress is likely due to the fiber type differences between the two muscles and differences in muscle function during simulated cold transport. The incidence of DFD was higher in thigh meat of the cold-stressed birds compared to the breast meat.

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Table 1. Effect of microclimate temperature, lairage and age on thigh muscle metabolites and meat quality parameters

Variables[1]	Groupings	n	Lactate 30 h (µmol/g)	GP 30 h (µmol/g)	pH_u	Thigh L*	Thigh a*	Thigh b*	Thaw loss (%)	Cook loss (%)
Temperature	20<T≤24	40	63.0±7.2^{a}	71.7±8.1^{a}	6.18±0.1^{c}	50.5±1.8^{a}	4.4±0.9^{b}	5.2±1.3^{a}	1.18±0.4^{a}	13.9±2.6^{a}
	-8<T≤0	71	24.0±13.0^{b}	31.3±13.1^{b}	6.91±0.2^{b}	41.6±3.0^{b}	6.5±1.6^{a}	1.3±1.8^{b}	0.53±0.2^{b}	4.8±1.8^{b}
	-11<T≤-8	31	13.4±8.0^{c}	20.1±8.7^{c}	6.95±0.1^{b}	40.5±2.0^{b}	7.0±1.7^{a}	1.0±1.3^{b}	0.43±0.1^{b}	4.0±1.2^{bc}
	-14<T≤-11	18	12.4±5.7^{c}	18.9±6.0^{c}	7.07±0.1^{a}	41.1±2.2^{b}	6.4±1.7^{a}	1.1±1.3^{b}	0.44±0.2^{b}	3.7±0.8^{c}
P-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Age (wk)	5	80	27.69^{b}	34.94^{b}	6.78	43.37	6.26^{a}	1.78^{b}	0.75^{a}	6.76
	6	80	33.31^{a}	40.78^{a}	6.72	43.76	5.89^{b}	2.65^{a}	0.58^{b}	6.81
P-value			0.0399	0.0277	NS	NS	0.019	0.0009	0.0001	NS
Lairage (h)	0	80	27.69^{b}	40.76^{a}	6.80^{a}	43.51	5.93	2.22	0.67	6.37
	2	80	33.32^{a}	34.96^{b}	6.71^{b}	43.62	6.21	2.21	0.66	7.21
P-value			0.009	0.001	0.012	NS	NS	NS	NS	0.047

$^{a-c}$ Means±SD with different letters are significantly different at $P < 0.05$
[1]L* (lightness); a* (redness); b* (yellowness); GP (glycolytic potential)

Table 2. Effect of microclimate temperature, lairage and age on breast muscle metabolites and meat quality parameters

Variables[1]	Groupings	n	Lactate 30 h (µmol/g)	GP 30 h (µmol/g)	pH_u	Breast L*	Breast a*	Breast b*	WBC (%)	PCY (%)
Temp.	20<T≤24	40	88.5±15.2^{a}	96.1±17.5^{a}	6.10±0.1^{c}	47.4±1.8^{a}	3.2±0.7^{b}	4.8±1.2^{a}	33.6±9.2^{c}	94.6±8.7^{c}
	-8<T≤0	71	80.9±16.7^{ab}	88.5±17.7^{ab}	6.20±0.2^{b}	46.0±2.5^{b}	3.8±1.0^{a}	4.1±2.4^{ab}	44.3±11.3^{b}	107.1±15.7^{b}
	-11<T≤-8	31	75.3±15.4^{b}	82.9±16.7^{b}	6.31±0.2^{a}	45.4±3.7^{bc}	4.0±1.4^{a}	3.5±1.9^{b}	50.4±17.7^{a}	120.1±25.4^{a}
	-14<T≤-11	18	76.5±18.4^{b}	84.3±20.6^{b}	6.31±0.2^{a}	44.8±2.5^{c}	4.0±1.0^{a}	3.2±3.2^{b}	46.3±15.1^{ab}	113.9±23.1^{ab}
P-value			0.0003	0.0003	<0.0001	<0.0001	0.0002	0.0006	<0.0001	<0.0001
Age (wk)	5	80	82.40	91.38	6.24	46.17	3.79	3.63	46.63	113.83
	6	80	80.09	86.29	6.18	46.02	3.63	4.49	39.41	100.62
P-value			NS	0.028	NS	NS	0.084	0.031	0.003	0.0002
Lairage (h)	0	80	82.91	90.75	6.19	47.10	3.39	4.42	38.67	102.45
	2	80	79.58	86.91	6.23	45.09	4.04	3.70	47.37	112.0
P-value			0.030	0.020	0.013	<0.0001	<0.0001	0.009	<0.0001	<0.0001

$^{a-c}$ Means±SD with different letters are significantly different at $P < 0.05$
[1] pH_u (ultimate pH measured at 30 h post-mortem); GP (glycolytic potential) ; L* (lightness); a* (redness); b* (yellowness); WBC (water binding capacity); PCY (processing cook yield).