Rural Development Administration
National Institute of Animal Science

Comparison of Stress Markers for Measuring Heat Stress of Meat-Type Ducks: A Field Study

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Animal Welfare research team

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Contents

Introduction

- Heat stress is a major concern in the poultry industry due to its effect on productivity and animal welfare.
- Developing strategies to reduce heat stress is essential, requiring research on its measurement and the development of appropriate indicators.
- Blood corticosterone is a classical indicator of heat stress. But, it is a sensitive to handling time and is an invasive method that can elevate stress levels.
- With rising concerns for animal welfare, many non-invasive markers (e.g., corticosterone in saliva, feces and feathers) are being developed (Sejian et al., 2021).
- Due to the different environmental conditions between field and laboratory, the selection of appropriate indicators may vary accordingly.
- **The objective of this study is**
    - to perform a comparative analysis of stress markers (corticosterone, Hsp70, H/L ratio) in meat-type ducks to identify a suitable method under field conditions.

Materials & Methods

Experimental Design

- Experiment was conducted from August until October in 2023.
- The treatments are divided into **summer** (28~36 ℃) and **fall** (16~27 ℃).
- **Animals**
  - A total of **50 ducks** (20 ducks in the summer, 30 ducks in the fall) were taken for sampling.
  - Ducks were chosen by random selection at each sampling date.
  - Ducks were rearing in experimental site from day 13 until day 42, 43 (summer, fall).

Materials & Methods

Experimental Site

Figure 1. Photograph of experimental site

Table 1. Growth Performance of Meat-Type Ducks by Seasons

Parameter	summer	fall
Final body weight (kg)	3.25 (3.25*)	3.65 (3.6*)
Feed intake (kg)	6.33	6.73
Water intake (L)	16.73	14.63
FCR	1.95 (2.10*)	1.85 (1.91*)
Breeding days	43 (46.8*)	42 (42.5*)
Livability (%)	95.1 (95.2*)	100.2 (97*)
Mortality rate (%)	7.3	4.4

* Average Growth Performance of meat-type ducks in south korea (2015-2017)

- **Facility information**
  - House type: Open housing with naturally ventilating system
  - Total number of ducks: 19,000
  - Stocking density: 3.6 birds / m^2
  - House size: 65m (length), 10m (width), 4.5m (height)
  - Location: Jincheon, Chungbuk

Materials & Methods

Analysis Parameters

- **THI (Temperature Humidity Index)**

$$THI = (1.8 \times T_{db} + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T_{db} - 26.8)$$

- **Growth performance** (Body weight, Feed intake, Water intake, FCR, Mortality)
- **Stress Markers**

- Corticosterone in blood, pulp, feather
- Hsp70 in blood, pulp, feather
- H/L ratio

Table 2. Temperature-Humidity sensor (HST20) specification

parameter	
Temperature range	-30 ℃ ~ 80 ℃ (± 0.5 ℃)
Humidity range	0 ~ 100 % (± 3 %)
Operating temperature range	-40 ℃ ~ 80 ℃
Operating humidity range	5 ~ 90 %

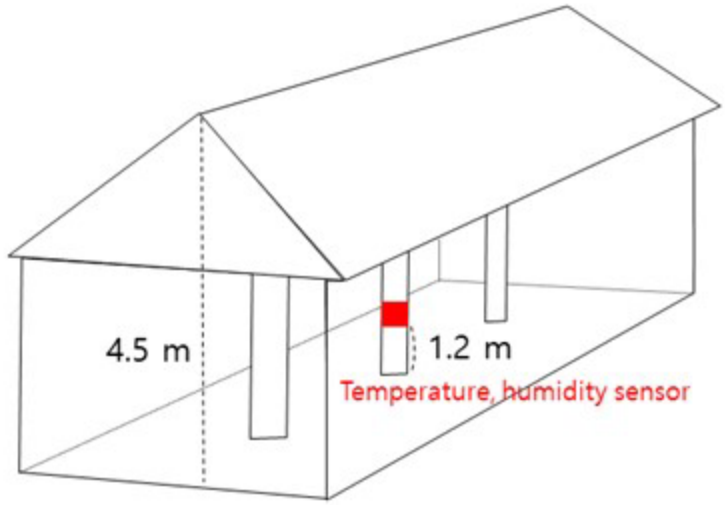
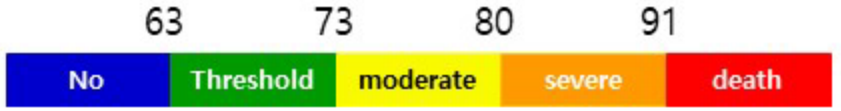


Figure 2. Location of Temperature and Humidity sensor in duck house

Materials & Methods

THI change



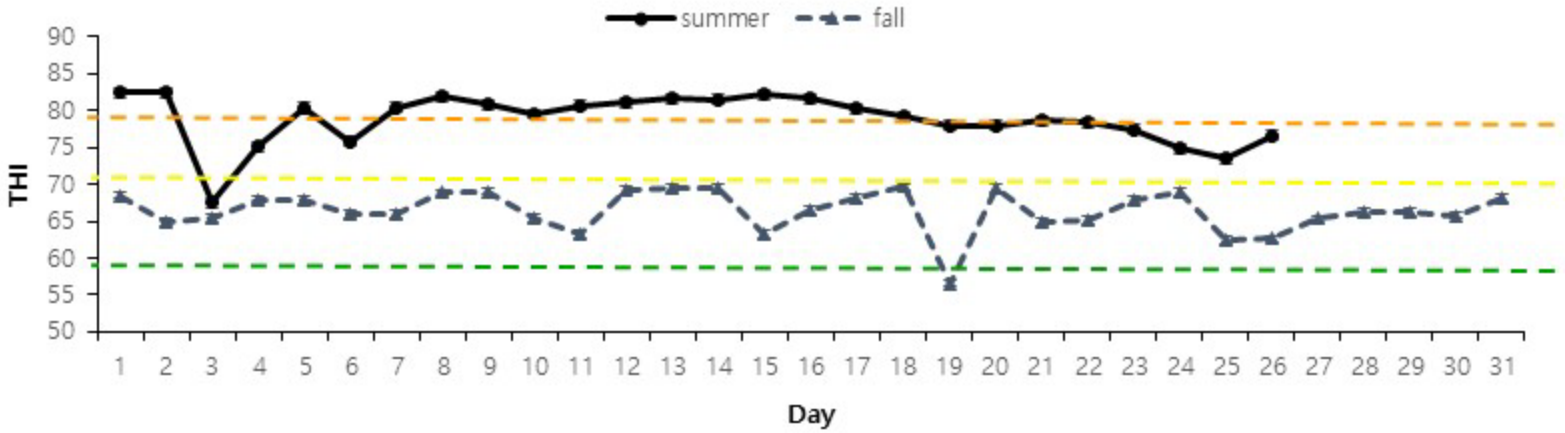


Figure 3. Daily THI change

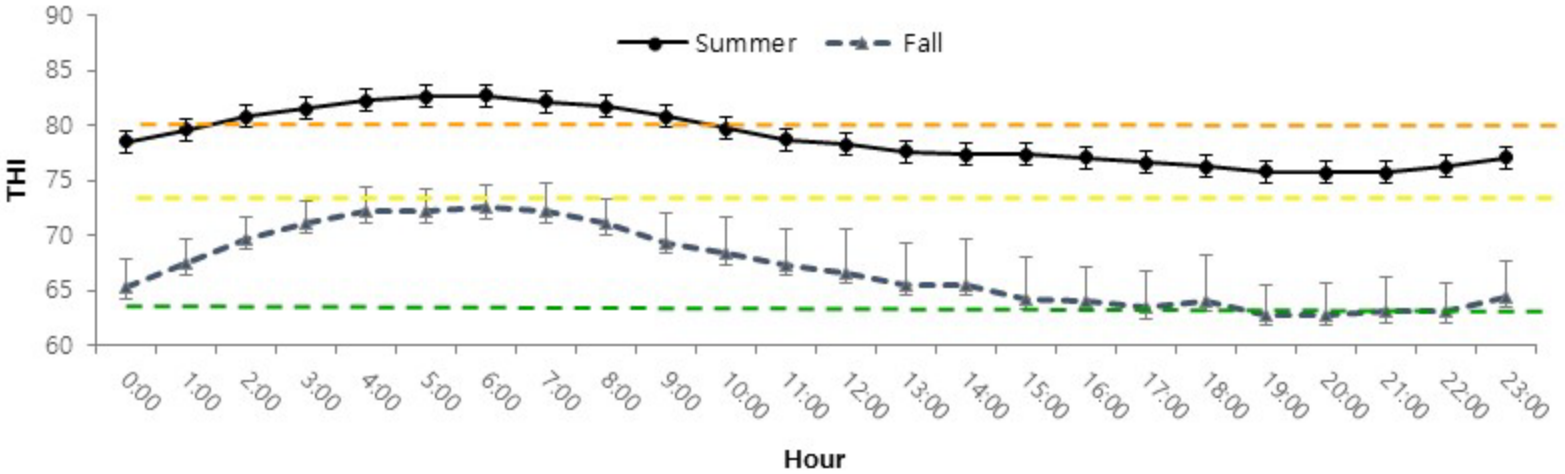


Figure 4. Hourly THI change

- Summer period 78.57 ± 1.02 (67.51 ~ 82.4) was mostly measured in degree of moderate, and fall period 66.43 ± 1.24 (56.6 ~ 69.65) was mostly measured in degree of threshold.
- In both seasons, the highest THI was recorded in the pre-dawn (3 ~ 7 o'clock), and the lowest in the evening (16 ~ 21 o'clock).
- In summer, the lowest and highest daily THI were recorded as 75.74 and 82.78. It ranged from degree moderate to severe.
- In fall, the lowest and highest daily THI were recorded as 62.8 and 72.53. It ranged from degree no stress to threshold.
- This data also shows that summer was heat stress environment in this experiment.

Materials & Methods

Sampling & Analysis

Figure 5. Blood collection by wing vein

Figure 6. feather collection by wing (a), scapula (b)

- Blood samples

  - 4.5ml of blood was taken from the wing vein.

- Feather samples

  - 1~2g of feather collection by scapula

  - 5 growing feathers (for Pulp) was taken from wing.

Materials & Methods

Sampling & Analysis

Figure 7. Analysis part of feather (a), feather cutting (b)

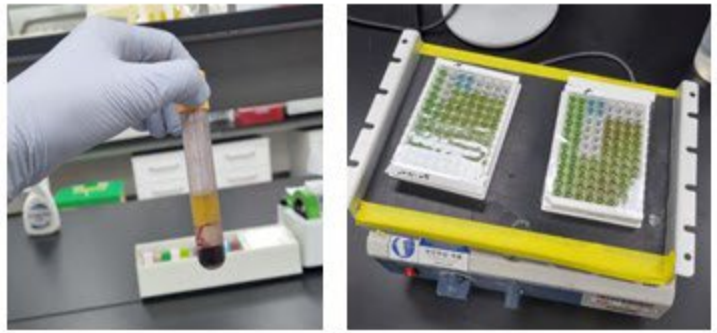


Figure 8. Centrifugation of blood (a), ELISA analysis (b)

- Pulp was collected from the bottom 10mm of growing feather.
- The measurement of Corticosterone, HSP70 were performed with ELISA kits.
  - Corticosterone ELISA kit (Enzo Life Sciences, cat no. ADI-900-097)
  - Chicken heat shock protein 70 ELISA kit (Cusabio, cat no. CSB-E11196Ch)
- H/L ratio was measured by Wright's staining.

Materials & Methods

Statistical analysis

- SAS analysis system (SAS Enterprise Guide 7.1)
- Shapiro-Wilk test was used to assess normality.
- Independent t-test, Wilcoxon test were used to determine significant differences.
- H/L ratio was assessed using Wilcoxon test.
- All differences were considered significant at $p < 0.05$.

Results

Weight and Stress Markers

Table 3. Effect of seasons on weight and stress markers of meat-type ducks

Parameters	Source	Summer	Fall	*p*-value
weight (kg)		2.96 ± 0.23	3.68 ± 0.27	< 0.0001
Hsp70	blood (ng/ml)	1.36 ± 0.44	1.56 ± 0.63	0.3171
	pulp (ng/mg)	0.14 ± 0.12	0.22 ± 0.17	0.2000
	feather (ng/mg)	0.46 ± 0.16	0.20 ± 0.13	<.0001
corticosterone	blood (pg/ml)	946.53 ± 632.00	1634.07 ± 967.96	0.0137
	pulp (pg/mg)	6.01 ± 2.74	5.40 ± 3.40	0.5223
	feather (pg/mg)	6.73 ± 2.47	6.60 ± 2.37	0.8541
H/L ratio		1.31± 0.69	1.06 ± 0.86	0.0155

- The body weight of summer group is significantly lower than that of fall group, likely due to heat stress.
- The markers did not show significant differences may either not reflect the heat stress or be less sensitive indicators.
- Feather Hsp70 and H/L ratio levels were significantly higher in the summer group than in the fall group.
- However, Blood corticosterone level in summer is significantly higher than fall.
- Corticosterone was significantly correlated with handling time, but HSP and H/L ratio were not affected by handling time (O'Dell, 2014).

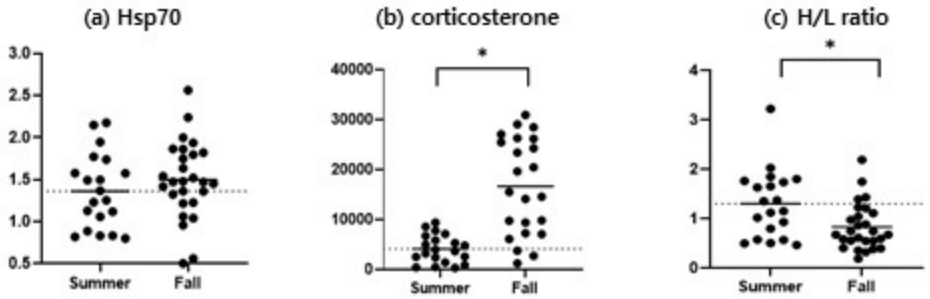


Figure 9. Distribution of stress markers in blood.

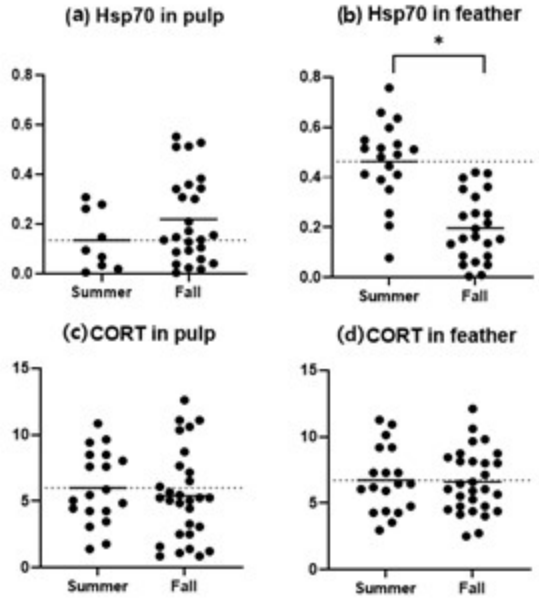


Figure 10. Distribution of stress markers in pulp and feather.

Results

Correlation analysis of Weight and Stress Markers

Table 4. Correlation of stress markers and weight

Variable	R^2	*p*-value	N
weight vs Hsp70(b)[a]	-0.0591	0.6835	50
weight vs Hsp70(f)[a]	-0.6481	<0.0001	42
weight vs Hsp70(p)[a]	0.1909	0.2720	35
weight vs CORT(b)[a]	0.3222	0.0272	47
weight vs CORT(f)[a]	0.0234	0.8747	48
weight vs CORT(p)[a]	-0.1076	0.4667	48
weight vs H/L[b]	-0.2858	0.0515	47

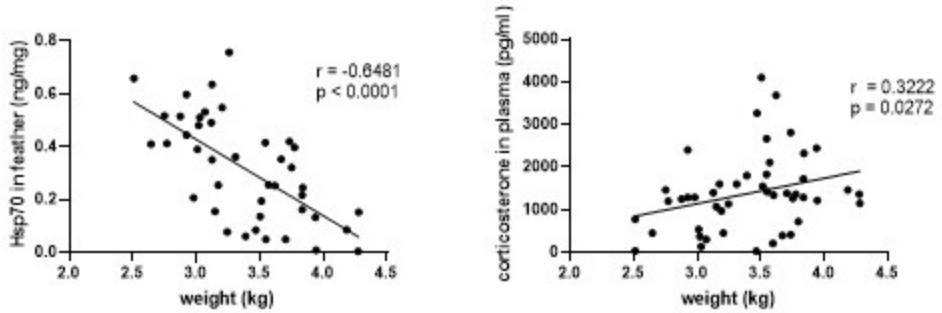


Figure 11. Scatter plots of stress markers and weight.

- Feather Hsp70 were negative correlated with body weight, while blood corticosterone showed a positive correlation.
- Blood corticosterone may be more strongly influenced by factors other than heat stress.
- It is believed to be the feather Hsp70 is appropriate indicator of heat stress.

Conclusion

- Comparing summer and fall, there was a difference in thermal environment (THI).
- It leads to significant difference in growth performance and stress markers.
- As a result of comparing stress indicators, feather Hsp70, blood corticosterone, H/L ratio are a significant difference by seasons.
- Among other stress markers used, **feather Hsp70 was assumed to be the most significant indicator of heat stress** owing to the thermal environment of the meat-type duck.
- Considering **animal welfare** and **reliability**, feather is an alternative method to blood.
- The data can be used as basic for developing heat stress marker and complementary research.

Thank you for your attention

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