104 (2025) 104996

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Full-Length Article

Growth patterns and heat tolerance analysis of dwarf chicken with frizzled feather

Guo Li [a,1], Tingting Xie [a,1], Zijing Zhu [a], Chengfeng Bin [a], Sadaqat Ali [a], Dongxue Guo [a], Xiaotong Wang [a], Lei Li [a], Xunhe Huang [c,d,2], Bing Zhang [b], Li Zhang [a,*]

[a] College of Coastal Agricultural Sciences, Guangdong Ocean University, Zhanjiang, 524088, Guangdong, China
[b] Guangxi Vocational University of Agriculture, Nanning, 530009, Guangxi, China
[c] Guangdong Provincial Key Laboratory of Conservation and Precision Utilization of Characteristic Agricultural Resources, Meizhou, 514015, Guangdong, China
[d] School of Life Science of Jiaying University, Meizhou, 514015, Guangdong, China

ARTICLE INFO

Keywords:
Chicken
Growth and development
Histological change
Heat resistance
Gene expression

ABSTRACT

Chickens are covered with feathers, lack sweat glands, and are sensitive to the thermal environment. Previously, our group bred a novel dwarf chicken strain with frizzled feather, named as dwarf chicken with frizzled feather (DFC). The cumulative growth of the chicken body weight and size were analyzed with 3 mathematical models. Subsequently, chickens were grouped to investigate the impact of heat stress (HS) on their slaughter performance, histomorphological development and gene mRNA change (*HSP70*, muscle development and appetite-related factors) using quantitative real-time PCR, tissue sections and Western Blot. In the HS group, chickens were placed at 34 ± 1°C for 8 hours (9:00 am - 17:00 pm) a day and lasted for 2 weeks, while in the control group, chickens were fed at 26 ± 1°C. Chicken tissue samples were collected at the age of 120 days to evaluate production performance, histological changes, and gene expression changes. Our results found that the Gompertz model was the best for fitting the body weight of DFC. The integrity of muscle, liver, spleen, and small intestine tissues was affected under HS conditions. Correspondingly, the length of the ileum was significantly decreased ($P < 0.05$), the thigh muscle development factor *MYOD1* expression was down-regulated ($P < 0.05$), while the expression of *MSTN* was up-regulated ($P < 0.001$). In addition, the jejunum VH / CD was reduced significantly ($P < 0.05$). The mRNA of appetite-promoting factors *AMPKα-1* and *AGRP* in the gut-brain axis were down-regulated ($P < 0.05$), while appetite-restrain factors *CCK, GHRL*, and *CART* were significantly up-regulated ($P < 0.05$ or $P < 0.01$). Moreover, the intestinal transport and absorption factors *ZO1, OCLN, PepT1, SGLT1*, and *CAT1* were up-regulated ($P < 0.05$ or $P < 0.01$), and *GLUT1* was down-regulated ($P < 0.05$). These results indicated that HS mainly impacted the appetite of chickens and did not significantly disrupt the nutrient absorption function of these chickens. The DFC appeared to be more tolerant to the hot environments for their frizzled feathers, small body size, and low basal metabolic rate.

Introduction

The chicken industry in tropical regions is encountering challenges due to global climate change. Yuexi frizzled feather chicken (**YFC**, Fig. S1A) and the yellow-feathered dwarf chicken (**YDC**, Fig. S1B) are two native breeds in South China. The frizzled feather gives the chicken body surface a better heat dissipation ability, while the dwarf chicken has short shins, small body weight, and low basal metabolic rate. The previous results of our laboratory showed that the frizzled feather of YFC was caused by the deletion of 15 bp in exon 2 of the keratin 75L4 (***KRT75L4***) gene (Dong et al., 2018). The dwarf trait of YDC was due to the deletion of 1.7 kb in the exon 9 and 3′UTR regions of the growth hormone receptor (***GHR***) gene. Based on this, YFC and YDC were used as parents to perform crossbreed and to combine the frizzled-feather and dwarf traits together into one novel strain (DFC), hoping to enhance their heat resistance. We used both F1 cross and backcross together to

* Corresponding author.
E-mail address: zhangl76@gdou.edu.cn (L. Zhang).
[1] Co-first author
[2] In Mountainous Areas

https://doi.org/10.1016/j.psj.2025.104996
Received 3 January 2025; Accepted 5 March 2025
Available online 6 March 2025

get the second generation of the pure strain ($Z^{dw}Z^{dw}33^{FF}$, $Z^{dw}W33^{FF}$). The breeding scheme of dwarf chicken with frizzled-feather (DFC) were listed in Fig. S2 and S3.

The growth and development of poultry typically follow nonlinear characteristics. The Logistic, Gompertz, and Von Bertalanffy models are commonly used to analyze the growth and development of poultry. The ideal growth curve model plays a crucial role in guiding the feeding management, nutritional requirements, and breeding practices of poultry at various stages (Kuhi et al., 2002; Sofaer et al., 2013). In this study, three growth curve models were used to analyze the body weight and size of DFC in order to get the optimal model.

Chickens are extremely sensitive to changes in environmental temperature. High-temperature environment can easily induce heat stress (**HS**) in chickens, impacting their feeding, growth, development, production performance and intestinal health. Heat shock proteins (**HSPs**) can be used as biomarkers in revealing physiological changes in HS (Bakthisaran et al., 2015). The expression level of HSPs in animals will increase, enhancing heat resistance and alleviating the damage caused by HS (Nawaz et al., 2021). The body temperature of chickens increased significantly after chronic HS treatment of 0-8 hours for 7 days, tissues including the pectoralis, thigh muscle, liver, and spleen suffered damage under the HS (Nawaz et al., 2023).

Many studies found that chicken feed intake decreased rapidly as the environmental temperature increasing (Kutlu and Forbes, 1993; Baziz et al., 1996). Feed consumption is correlated with chicken growth. Feed intake and its regulation process are very complicated. This process involves the integration of signals by the gut-brain axis and the hypothalamic nucleus (Wynne et al., 2005). At the same time, the hypothalamus and other brainstem regions interact to regulate feeding behavior. This interaction involves a variety of appetite regulators, such as *AMPKα-1, GHRL* and *AGRP*. Adenosine 5′-monophosphate-activated protein kinase (AMPK) plays a very important role in maintaining the body 's energy balance, and can regulate appetite through both central and peripheral pathways. Studies have found that changes in AMPK activity play an important role in regulating hypothalamic feeding, the decrease of AMPK activity in the hypothalamus inhibits feeding, and on the contrary promotes feeding (Carling et al., 2008). The regulation of appetite by hypothalamic AMPK is mainly through the action of downstream appetite-promoting factors and anorexia factors. In addition, *GHRL* (Ghrelin, *GHRL*) is a special appetite-promoting factor and has a strong feeding-promoting effect in many species (Tschöp et al., 2000). However, it has been reported that the regulation of *GHRL* on appetite in poultry seems to be opposite to that in mammals. Previous research has found that *GHRL* strongly inhibited the feed intake of chicks within 2 hours after intraventricular or peripheral injection of *GHRL* (Furuse et al., 2001; Buyse et al., 2009; Ocłoń and Pietras, 2011). The possible reason is that *GHRL* promotes the release of growth hormone, and the combined action of growth hormone, growth hormone releasing factor and its receptor inhibits the feeding of chickens (Small et al., 2001). Studies have found that *AGRP* (Agouti-related protein) is a natural antagonist of melanocorticosteroid and can compete with α-melanocyte stimulating hormone (α-MSH) to bind to melanocortin receptor 4 (MC4R), thereby inhibiting its anorexia (Chai et al., 2003). While some reports have indicated that central injection of *AGRP* can enhance appetite, and the duration of action is longer than NPY (neuropeptide Y) (Small et al., 2001). In addition, the intrinsic connection between the hypothalamus and the gastrointestinal tract ensures that neuropeptides regulate energy balance. in the hypothalamus, there are two interrelated appetite-regulating neurons: one is *NPY* and *AGRP*, and the other is proopiomelanocortin (*POMC*) and Cocaine-and amphetamine-regulated transcript (*CART*) (He et al., 2019). These neurons influence appetite by integrating peripheral signals and releasing signaling molecules. The intestinal tract is the main place where animals digest and absorb nutrients, playing crucial role in maintaining their basic life functions and overall health. The absorption and transport of nutrients are primarily facilitated by tight junction proteins and nutrient transporters. High environmental temperatures can impair intestinal barrier function by disrupting intestinal tight junctions, decreasing anti-inflammatory levels in the intestines, and elevating intestinal oxidative stress (Wang et al., 2022). In a word, changes in gut-brain axis hormones or gene expression will have negative impacts on chicken growth.

Selection of heat resistance is particularly important in tropical agriculture (Kumar et al., 2021). Previous researches indicate that reduced feather coverage improves heat tolerance (Cahaner et al., 1993; Yalcin et al., 1997; Yahav et al., 1998; Deeb and Cahaner, 1999). Featherless broilers do not suffer in hot conditions. They grow normally, yield significantly more breast meat and display better viability than feathered broilers (Somes and Johnson, 1982; Cahaner et al., 2003; Cahaner et al., 2008; Azoulay et al., 2011). The KRT75L4 frizzling mutation might reduce insulation, while the smaller body size are beneficial to a lower basal metabolic rate. Thus, the growth, heat resistance of DFC and their intestinal transport and absorption factors related gene expression are investigated in this study.

Materials and methods

Animals

The DFC and YFC chickens were raised using three-layer cages (single cage dimensions: length*width*height: 70*70*40 cm) at the Institute of Biotechnology, Guangdong Ocean University until 17 weeks of age. Chickens were provided free access to water and commercial basal diet. The animals and procedures involved in this experiment were examined and approved by the Animal Care and Use Committee of Guangdong Ocean University (SYXK-2021-0154).

Analysis patterns of chicken growth and development

The growth patterns of DFC and YFC were analyzed. From the 2nd to the 17th weeks, 30 female individuals were randomly selected from each breed to measure their body weight and body size, including keel length, tibia length, and tibia circumference. The growth models and parameters were shown in Table 1.

Heat stress experiment

When the DFC were raised to 105 days (15 weeks) old, 72 healthy female chickens were grouped for control and HS. Each group was designed 6 replicates with 6 chickens in each replicate. The temperature and relative humidity were maintained at 26 ± 1°C and 55 ± 5%, respectively for control group. While 34 ± 1°C and 65 ± 5% for HS group were for 8 hours per day (9:00 am - 17:00 pm) and were lasted for 14 consecutive days. And nighttime (17:00 pm - 09:00 am) heat stress group temperature returned to room temperature. The natural day and night temperature during the experiment period is 35 ± 3°C to 26 ± 1°C. The chicken rectal temperature was measured using a mercury thermometer on the 1st, 7th, and 14th day of the experimental period. The temperatures of back skin, feet and eyelid were measured with a Kefu infrared electronic temperature gun (KF-HW-002, Kefu Medical

Table 1
Three kinds of growth curve models and the parameters.

Model	Equation	Growth amount at inflection (IPGA)	Age at inflection (IPA)
Logistic	Y=A/(1+B*exp(-k*t))	A/2	(lnB)/k
Gompertz	Y=A*exp(-B*exp(-k*t))	A/e	(lnB)/k
Von Bertalanffy	Y=A*(1-B*exp(-k*t))**3	8A/27	(ln3B)/k

A: Maximum body weight; B: Parameter; k: Transient growth rate; t: Weekly age.

Equipment Flagship Store).

Tissue sample collection

After 14 days of HS treatment, animals were deprived of feed but provided with water for 12 h before analyzing. Experimental chickens were taken from each replicate to get the live body weight. Further the pectoralis muscle, thigh muscle, abdominal fat, liver, spleen, glandular stomach, gizzard, pancreas, duodenum, jejunum and ileum were collected to measure their weight or length. At the same time, two copies of each pectoral muscle, thigh muscle (iliotibial lateral muscle), liver, spleen, hypothalamus, pituitary, duodenum, jejunum, and ileum tissues were collected and stored in RNA preservation solution or 4% paraformaldehyde, respectively, for gene expression and tissue section analysis.

Tissue section analysis

The pectoral muscle, thigh muscle (iliotibial lateral muscle), liver, spleen, duodenum, jejunum, and ileum tissues of each replicate were collected and fixed in 4% paraformaldehyde to be observed histologically. The incidence of histopathology in various tissues was counted. Fixed tissue samples were paraffin-embedded and cut into 4 μm thick sections, which were then stained with hematoxylin and eosin (H&E). Tissue sections were examined with olympus optical microscope, and digital images were captured with Nikon Eclipse CI upright optical microscope connected to a Nikon DS-U3 camera (NIKON DS-U3, Japan).

RNA isolation and quantitative real-time PCR (qRT-PCR) analysis

According to the instructions of HiPure Universal RNA Kit (Magen Biotechnology Co., Ltd, Guangzhou, China), total RNA was obtained from pectoral muscle, thigh muscle (iliotibial lateral muscle), hypothalamus, and pituitary. The NanoDrop™ Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to assess its purity and integrity. RNA was reverse transcribed into cDNA using the PrimeScript RT Reagent Kit (TaKaRa, Kyoto, Japan). The reaction system and procedure were shown in Table S1. All cDNAs were diluted four fold and stored at -20°C.

The primers of growth, heat resistance, intestinal transport and

Table 2
Primers for qRT-PCR.

Genes	Accession NO.	Primer sequence (5′-3′)	Annealing temp (°C)	Product length (bp)
HSP70	NM_001006685.2	F: CAATGGCAAAGAGCTGAACA R: GGTGGGAATGGTGGTGTTAC	58	205
PPP1R7	NM_001277428.1	F: CGAATCGGAGGAGTCGGAAG R: GTGTCTCTGCTTCTCCTGGG	60	122
MYOD1	NM_204214.3	F: CGACGGCATGATGGAGTACA R: ATGCTTGAGAGGCAGTCGAG	60	144
MSTN	NM_001001461.2	F: CGGGTGAAGATGGATTGAACC R: TAGGGGACATCTTGGTGGGT	60	312
NPY	NM_205473.2	F: TCATCACCAGGCAGAGATATGG R: ATCCCATCACCACATCGAAGG	60	128
AGRP	NM_001398243.1	F: GGAACCGCAGGCATTGTC R: GTAGCAGAAGGCGTTGAAGAA	59	163
AMPKα-1	NM_001039603.2	F: ATCTGTCTCGCCCTCATCCT R: CCACTTCGCTCTTCTTACACCTT	60	125
CCK	NM_001001741.2	F: GGGAAGGAGGAAGCGATGTA R: CTCCCAGTGGAACCTTTCCG	59	284
GIP	NM_001080104.2	F: AGAGAGAAGAAAAGCGACAACG R: AGTCTTTGGCTTCGTGGCTG	60	112
PYY	NM_001361182.2	F: ATCCACCGAAGCCCGAAAG R: CGTCGATGTCAGACCACAGC	60	189
CART	XM_003643097.6	F: CGTCCCGAGAGAAGGAGCTGATC R: ACTGCTCTCCGGCGTCGCACAT	60	123
VIP	NM_001177309.2	F: CCAGAATTATTGATAGCTCCCAGG R: GGTGTCCTTCAGAGGTCCAA	59	268
GHRL	NM_001001131.2	F: GAAAACACATTTGAAGCACTGCC R: TGCCTCGGCGATGTAATCTT	58	194
ZO1	XM_040706827.2	F: AAGAGGAAGCTGTGGGTAACTC R: TGAAGAGTCACCGTGTGTTGT	60	200
OCLN	NM_205128.1	F: AGCCCTCAATACCAGGATGTG R: CGCTTGATGTGGAAGAGCTTG	60	125
CLDN2	NM_001277622.1	F: GATACGTGTAGCAGCAGCAG R: AGCTGGGATTTCTGAGCAGT	59	167
PepT1	NM_204365.2	F: CTTGGCAGATCCCTCAGTATTT R: GTTGGGCTTCAACCTCATTTG	58	300
GLUT1	NM_205209.2	F: AAGATGACAGCTCGCCTGA R: CCAATCATGCCTCCAACCGA	59	218
SGLT1	NM_001293240.2	F: AGGTTTGCACGAGCCAGATT R: TGTGGACACCAAATGCTTCA	60	175
CAT1	NM_001145490.2	F: TCTCCTTGGCTCCATGTTTC R: CAGGAGGGTCCCAATAGACA	58	205
ATP1A1	NM_205521.2	F: CTGCTATTGTTCGGCACCTG R: TGCAGTAGTCAAACCCCGAC	58	279
β-actin	NM_205518.2	F: CAGCCATCTTTCTTGGGTAT R: CTGTGATCTCCTTCTGCATCC	58	169

Abbreviations: *HSP70* = heat shock protein 70; *PPP1R7* = protein phosphatase 1 regulatory subunit 7; *MYOD1* = myogenic differentiation 1; *MSTN* = myostatin; *NPY* = neuropeptide Y; *AGRP* = agouti-related protein; *AMPKα-1* = protein kinase AMP-activated catalytic subunit alpha 1; *CCK* = cholecystokinin; *GIP* = gastric inhibitory polypeptide; *PYY* = peptide YY; *CART* = cocaine-and amphetamine-regulated transcript; *VIP* = vasoactive intestinal peptide; *GHRL* = ghrelin; *ZO1* = tight junction protein; *OCLN* = occluding; *CLDN2* = claudin 2; *PepT1* = peptide transporter 1; *GLUT1* = facilitative sugar transporters1; *SGLT1* = Na^+-dependent glucose transporters1; *CAT1* = cationic amino acid transporter 1; *ATP1A1* = ATPase Na^+/K^+ transporting subunit alpha 1; *β-actin* = beta actin.

absorption factors related genes were designed by Primer-BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and the primer sequences were shown in Table 2.

According to the instructions of ChamQ Universal SYBR qPCR Master Mix (Q711-02, Vazyme Biotech Co., Ltd, Nanjing, China), the CFX Connect Real-Time System (CFX Connect, BIO-RAD, Singapore) was used for the qRT-PCR reaction. The housekeeping gene *β-actin* was used as reference gene for qRT-PCR. The reaction mixture was detailed in Table S2. The reaction procedure was as follows: pre-denaturation at 95°C for 30 s; denaturation at 95°C for 5 s, annealing at for 30 s, repeated for 40 cycles. Three replicates were set up for the samples of each group. The target gene was quantified between the control group and the HS group with the $2^{-\Delta\Delta CT}$ method.

Western blot

The tissue protein were extracted referring to the literature (López et al., 2020). The blocking reagent was QuickBlock Blocking Buffer (Beyotime, Shanghai, China). The antibodies including β-actin, HSP70, CCK (1:1000 dilution, Abclonal, Wuhan, China) and HRP-labeled goat anti-rabbit IgG (1:500 dilution, Beyotime, Shanghai, China) were used for analysis, with at least 3 replicates in each group. The experiments were conducted with SDS-PAGE gel and blotting equipment, specifically the Mini-PROTEAN Tetra Cell Casting Module (BIO-RAD, Singapore), and Mini-PROTEAN Tetra Vertical Electrophoresis Cell for Mini Precast Gels, 4-gel, Mini Trans-Blot Module, PowerPac^{TM} HC Power Supply (BIO-RAD, Singapore). An automated chemiluminescence/fluorescence image analysis system (Tanon 4600, Shanghai, China) linked to the AllCap software was used to acquire the images. Gray scale analysis was performed with Image J software.

Statistical analysis

IBM SPSS Statistics 26.0 was used to perform a t-test on production data and gene expression results to evaluate the differences between groups. GraphPad Prism 9.0 software was used for creating graphs. * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$.

Results

The growth comparison between DFC and YFC

The results of the cumulative growth of body weight and body size of the DFC and YFC were presented in Table 3. It was evident that the growth rate of body weight, keel length, and tibia circumference of DFC was significantly slower than that of the YFC from the 2nd to the 17th week ($P < 0.05$). While the tibial length of DFC was significantly slower than that of YFC ($P < 0.001$) from the 4th to the 17th week.

The fitting models of the body weight and body size of the DFC were shown in Table 4. It can be seen that all the goodness of fit of the models was above 0.990, indicating that these 3 models can effectively simulate the body weight growth curve of DFC. Among them, the Gompertz model had the highest degree of fit for DFC, reaching 0.995. The predicted results were closer to the measured value, indicating that the Gompertz model was the optimal for DFC. In terms of body size, the results of a comprehensive comparison of R^2 values and the differences between predicted and measured values showed that the optimal model for keel length and tibia length of DFC was Logistic, while Bertalanffy was the best for tibia circumference.

Effect of heat stress on body temperature of DFC

Compared with the control group, the rectal temperature (**RT**) ($P < 0.05$ / $P < 0.01$), feet temperature (**FT**) ($P < 0.01$), and eyelid temperature (**ET**) ($P < 0.05$ / $P < 0.01$) of the DFC increased significantly at the 1st, 7th, and 14th days of HS. Additionally, the back skin temperature (**BST**) showed significant increase at the 1st day of HS ($P < 0.05$), with no significant differences existed at the 7th and 14th days ($P > 0.05$) (Table 5).

Effect of heat stress on production performance of DFC

From Table 6, it can be seen that the weight of slaughter body, pectoralis muscle, thigh muscle, spleen and Ileum showed decreasing trends, but the length of ileum significantly reduced ($P < 0.01$). There were no differences for other traits after 14 days of HS ($P > 0.05$).

Effects of heat stress on the histomorphology of muscle, liver and spleen, as well as the expression of HSP70 and muscle development factors in DFC

The effects of HS on the morphological development of muscle, liver, and spleen tissues in DFC were shown in Fig. 1. Compared with the control group (a1, b1, c1, and d1), HS (a2-a5, b2-b5) for 14 days resulted in mild fibrosis (a2, b2 and b3), expansion of interstitial tissues (a2, a3, b2 and b4), infiltration of fat tissues (a4, a5, b3 and b4), inflammatory cell infiltration (a3, a4, a5, b3 and b5) in pectoral and thigh muscles. For liver and spleen, HS (c2-c5, d2-d5) induced the red blood cell hyperplasia (c2, c3, c5, d2, d3 and d4), vacuole degeneration (c2, c3 and c4) and tissue haemorrhages with necrosis (c2 and c5). Both tissues showed inflammation with mononuclear cell infiltration (c2), mild inflammatory cells consumption (d3 and d5) and degeneration of red and white pulp (d4 and d5).

The amplification results of PCR products of *HSP70* and muscle development -related genes were shown in Fig. S4. After 14 days of HS,

Table 3
Body weight and body size accumulation growth of Yuexi frizzled feather chicken (YFC) and Dwarf chicken with frizzled feather (DFC).

Week	Body weight/g				Keel length/cm				Tibia length/cm				Tibia circumference/cm			
	YFC	DFC	SEM	*P*	YFC	DFC	SEM	*P*	YFC	DFC	SEM	*P*	YFC	DFC	SEM	*P*
2	152.43	137.63	3.43	0.03	3.89	3.69	0.05	0.03	3.53	3.54	0.04	0.91	2.42	2.32	0.03	0.046
4	385.12	284.97	9.36	0.00	5.74	5.10	0.07	0.00	5.24	4.31	0.07	0.00	2.99	2.84	0.03	0.007
6	649.46	478.79	15.89	0.00	6.94	5.94	0.09	0.00	6.44	4.95	0.11	0.00	3.40	3.05	0.03	0.00
7	798.80	521.30	21.85	0.00	7.28	6.10	0.10	0.00	6.76	5.32	0.11	0.00	3.53	3.06	0.04	0.00
8	990.53	708.49	25.38	0.00	8.13	7.14	0.10	0.00	7.53	5.92	0.12	0.00	3.78	3.42	0.04	0.00
9	1158.48	714.61	32.63	0.00	8.70	7.22	0.12	0.00	7.51	5.88	0.12	0.00	3.71	3.39	0.03	0.00
10	1360.12	860.75	36.96	0.00	9.20	8.18	0.10	0.00	8.17	6.08	0.15	0.00	4.09	3.39	0.05	0.00
11	1472.72	910.92	39.73	0.00	9.44	8.29	0.09	0.00	8.52	6.33	0.15	0.00	4.02	3.52	0.04	0.00
12	1643.17	1033.04	45.21	0.00	10.12	8.75	0.12	0.00	8.31	6.15	0.16	0.00	4.46	3.61	0.06	0.00
13	1703.94	1151.74	42.50	0.00	10.27	9.01	0.11	0.00	8.41	6.06	0.16	0.00	4.53	3.70	0.06	0.00
14	1804.19	1198.04	45.10	0.00	10.26	9.15	0.09	0.00	8.49	5.96	0.17	0.00	4.29	3.75	0.04	0.00
15	1988.97	1324.83	50.16	0.00	10.81	9.17	0.13	0.00	8.43	6.16	0.16	0.00	4.72	3.80	0.07	0.00
16	2101.54	1368.66	55.30	0.00	10.49	9.55	0.10	0.00	8.68	6.13	0.17	0.00	4.58	3.84	0.06	0.00
17	2139.29	1378.95	59.77	0.00	11.07	9.55	0.12	0.00	8.56	6.27	0.16	0.00	4.70	3.82	0.06	0.00

Abbreviations: YFC = Yuexi frizzled feather chicken; DFC = Dwarf chicken with frizzled feather.

Table 4
The results of three curves for different traits of dwarf chicken with frizzled feather (DFC).

Traits	Models	A	B	K	R^2	IPGA	IPA
Body weight	Logistic	1573.807	12.968	0.272	0.993	786.904	9.421
	Gompertz	1879.283	3.402	0.146	0.995	691.421	8.386
	Bertalanffy	2164.766	0.742	0.102	0.994	641.412	7.845
Keel length	Logistic	10.246	2.712	0.222	0.989	5.123	4.494
	Gompertz	10.802	1.467	0.154	0.988	3.974	2.488
	Bertalanffy	11.099	0.403	0.131	0.987	3.289	1.449
Tibia length	Logistic	6.270	1.657	0.343	0.963	3.135	1.472
	Gompertz	6.309	1.092	0.287	0.957	2.321	0.307
	Bertalanffy	6.326	0.319	0.269	0.955	1.482	-0.163
Tibia circumference	Logistic	3.984	0.986	0.192	0.977	1.992	-0.073
	Gompertz	4.041	0.732	0.158	0.978	1.487	-1.975
	Bertalanffy	4.065	0.221	0.147	0.979	1.204	-2.796

A: Maximum growth amount; B: Parameter; k: Transient growth rate; R^2 = coefficient of determination; IPA = age(week) at point of inflection; IPGA = growth amount (g/cm) at point of inflection.

Table 5
Effect of heat stress on body temperature of dwarf chicken with frizzled feather (DFC).

	Rectal temperature (°C)				Back skin temperature (°C)				Feet temperature (°C)				Eyelid temperature (°C)			
Time	CN	HS	SEM	*P*	CN	HS	SEM	*P*	CN	HS	SEM	*P*	CN	HS	SEM	*P*
1st D	40.4	42.2	0.29	0.00	41.3	42.2	0.24	0.04	39.6	42.0	0.41	0.00	39.5	40.4	0.19	0.01
7th D	40.2	40.6	0.08	0.02	40.7	41.3	0.27	0.28	37.3	40.0	0.48	0.001	37.8	39.4	0.31	0.003
14th D	39.9	41.2	0.21	0.00	41.5	41.7	0.17	0.53	38.3	40.6	0.38	0.00	38.7	40.0	0.30	0.02

Abbreviations: CN=Control groups; HS=Heat stress groups. 1st D, 7th D and 14th D mean that body temperature was detected at Day1, 7, and 14 after heat stress beginning. n = 6.

Table 6
Effect of heat stress on performance of dwarf chicken with frizzled feather (DFC).

Trait	CN	HS	SEM	*P*
Slaughter weight/g	1533.08	1516.77	67.72	0.91
Carcass weight/g	1375.70	1388.65	58.38	0.92
Pectoralis muscle weight/g	186.59	179.87	5.97	0.60
Thigh muscle weight/g	201.83	177.60	8.51	0.16
Abdominal fat weight/g	87.14	77.07	7.47	0.53
Liver weight/g	31.97	37.53	2.38	0.26
Spleen weight/g	3.61	2.03	0.69	0.27
Gizzard weight/g	18.16	15.75	1.46	0.44
Glandular stomach weight/g	3.49	4.27	0.25	0.12
Pancreas weight/g	2.96	2.75	0.19	0.60
Spleen length/cm	2.41	2.13	0.10	0.17
Spleen width/cm	1.84	1.70	0.10	0.53
Duodenum weight/g	6.74	7.38	0.34	0.37
Jejunum weight/g	8.69	10.00	0.64	0.33
Ileum weight/g	6.35	4.72	0.43	0.05
Duodenum length/cm	25.28	28.18	0.94	0.13
Jejunum length/cm	47.22	49.17	1.64	0.58
Ileum length/cm	44.00	31.08	2.52	0.003

Abbreviations: CN = Control groups; HS = Heat stress groups. n = 6.

there was no significant changes in the protein and mRNA expression levels of HSP70 compared with the control group ($P > 0.05$) (Fig. 2). Maybe their frizzled feather adaptation conferred lesser thermal load or they adapted quickly to chronic HS. In addition, HS significantly down-regulated the expression of *MYOD* (|fold change| ≥ 2, $P < 0.05$) and up-regulated the expression of *MSTN* in the thigh muscle (|fold change| ≥ 2, $P < 0.001$) (Fig. 2) which indicated that HS inhibited the development of thigh muscle.

Effects of heat stress on intestinal morphology, mRNA expression of gut-brain axis appetite factors and intestinal transport and absorption factor in DFC

The effect of HS on gene expression related with muscle and intestinal morphological development were shown in Fig. 2, Fig. 3 and Table 7. Compared with the control group, HSP70 was not significantly changed in liver and spleen ($P > 0.05$). Their frizzled feather adaptation maybe confer lesser thermal load. Hence, they adapted quickly to chronic HS. The ratio of jejunum villus height to crypt depth (VH / CD) was reduced after HS for 14 days ($P < 0.05$). Other traits were not statistically different ($P > 0.05$).

The results of PCR product detection of gut-brain axis appetite factors and intestinal transport absorption factors were shown in Fig. S5. Compared with the control group, the appetite-restrain factor showed an overall upward trend in the hypothalamus, pituitary and intestinal tissues, such as *CCK, GHRL* and *GIP*. While the appetite-promoting factor showed downward trends, including *AGRP, AMPKα-1* and *NPY* (Fig. 4). The protein expression *of CCK* in duodenum and jejunum exhibited increasing trends (Fig. 4).

The effects of HS on the expression of intestinal transport and absorption-related factors were shown in Fig. 5. The tight junction proteins showed upward trends while amino acid transporters showed downward trends in the duodenum, but there were no significant differences ($P > 0.05$). In jejunum and ileum, the tight junction proteins *ZO1* (|fold change| ≥ 1.5, $P < 0.01$) and *OCLN* (|fold change| ≥ 2, $P < 0.05$) showed upward trends. Also, the amino acid transporters *PepT1, SGLT1, CAT1* and *ATP1A1* exhibited upward trends, especially *PepT1* (|fold change| ≥ 2, $P < 0.05$), *SGLT1* (|fold change| ≥ 1.4, $P < 0.05$) and *CAT1* (|fold change| ≥ 1, $P < 0.01$) reached significant levels, while the *GLUT1* (|fold change| ≥ 1.4, $P < 0.05$) showed a downward trend.

Discussion

There is a subtropical and tropical monsoon climate in south China. The high temperature and humidity have a negative impact on the local chicken industry. Breeding of heat-resistant chicken strains is benefit for poultry production. Yuexi frizzled feather chicken is a local breed in south China. For their frizzled feather, they indicate good heat dissipation performance but with large body size. The YDC is a strain of dwarf chicken with small body size. The DFC strain combines the advantages of both YFC and YDC, with frizzled feathers across the entire body and the small body size. There are significant variations in the growth patterns among different breeds or strains of poultry, as well as variances in the

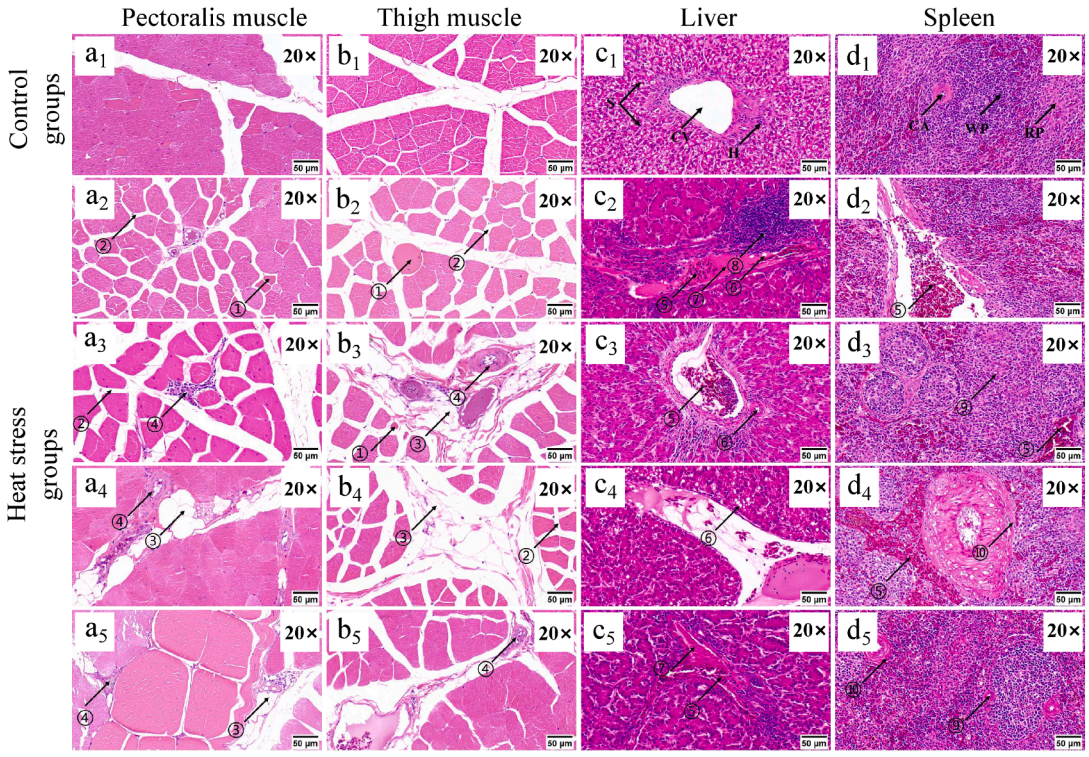

Fig. 1. Effect of heat stress on the pectoral muscle, thigh muscle, liver and spleen of dwarf chicken with frizzled feather. Magnification is 20 ×, and scale denotes 50 μm. a1, b1, c1 and d1 are the control groups. The control groups illustrate the central vein (CV), sinusoidal cells (S) and hepatocytes (H) in the c1. Whereas (RP) represents the red pulp, (WP) represents the white pulp, and (CA) represents the central artery in the d1. a2-a5, b2-b5, c2-c5 and d2-d5 are HS groups. For histological comparison in pectoral and thigh muscle, the mild fibrosis (①) occurred 3 times, expansion of interstitial tissues (②) occurred 4 times, infiltration of fat tissues (③) occurred 4 times and inflammatory cell infiltration (④) occurred 5 times in the HS group. In liver, red blood cell hyperplasia (⑤) occurred 3 times, vacuole degeneration (⑥) occurred 3 times, tissue haemorrhages with necrosis (⑦) occurred 2 times, tissue inflammation with mononuclear cell infiltration (⑧) occurred 1time in the HS group. In the spleen, red blood cell hyperplasia (⑤) occurred 3times, consumption of mild inflammatory cells (⑨) occurred 2times, degeneration of red and white pulp (⑩) occurred 2 times.

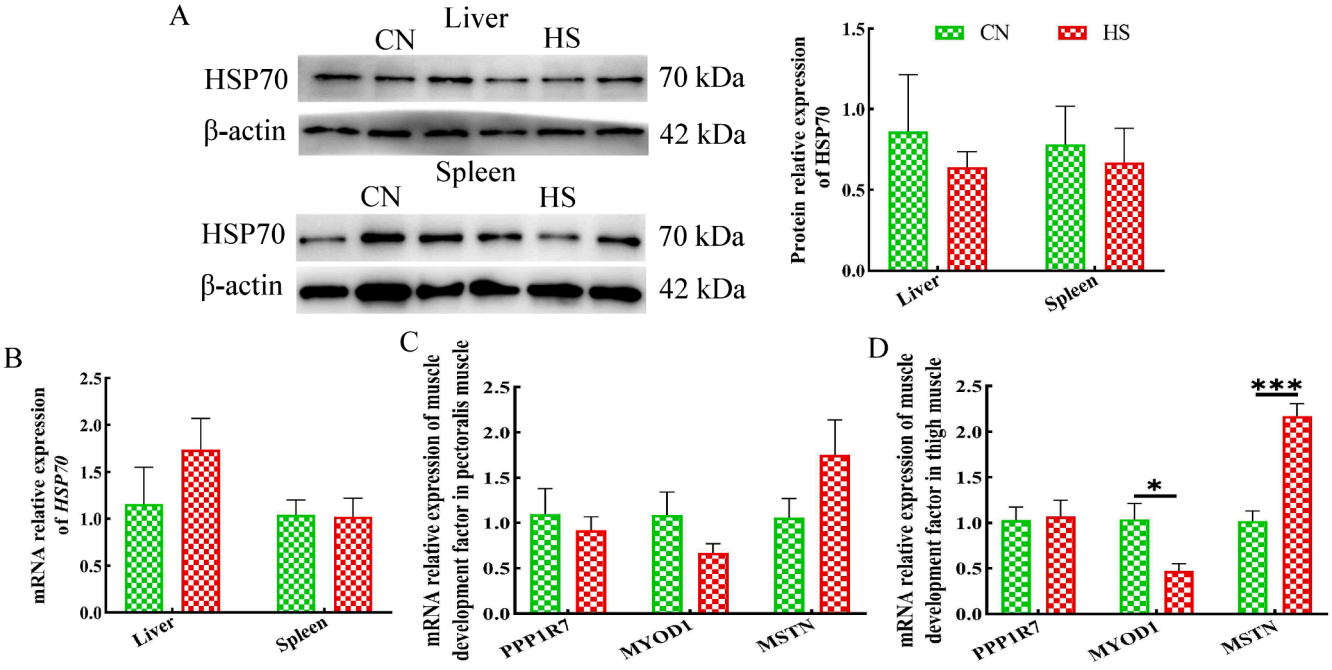

Fig. 2. Effect of heat stress on *HSP70* and muscle development related gene expression in dwarf chicken with frizzled feather. CN = Control, HS = Heat stress. HSP70 protein and mRNA expression level in the liver and spleen (A, B). The mRNA changes of muscle development factors in pectoral and thigh muscle (C, D). (mean ± SE; * $p < 0.05$; *** $p < 0.001$; n = 6).

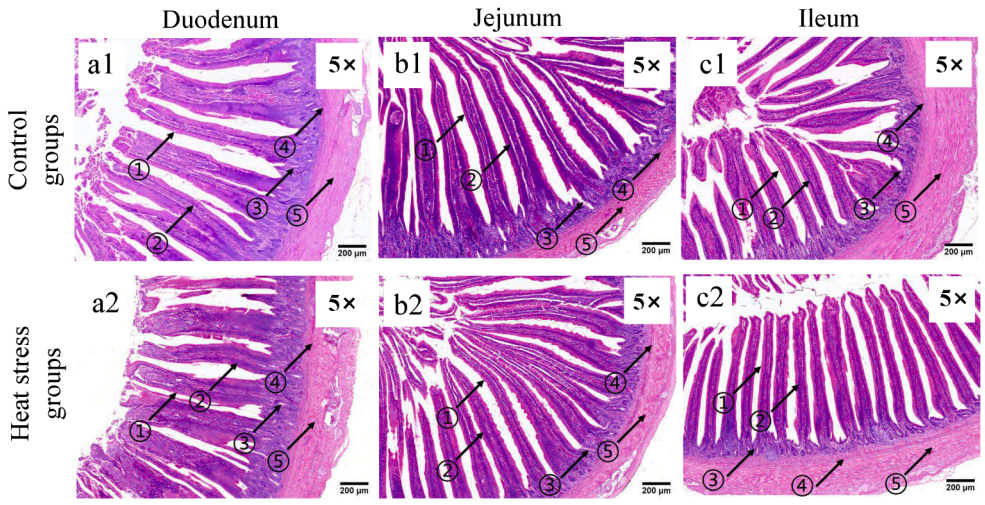

Fig. 3. Effect of heat stress on intestinal morphology of dwarf chicken with frizzled feather. Magnification is 5 ×, and scale denotes 200 μm. ① villus length, ② epithelial cell, ③ intestinal recess depth, ④ intestinal mucosa thickness, ⑤ intestine wall thickness.

Table 7
Effect of heat stress on intestinal morphology of dwarf chicken with frizzled feather (DFC).

Index	Duodenum				Jejunum				Ileum			
	CN	HS	SEM	*P*	CN	HS	SEM	*P*	CN	HS	SEM	*P*
Villus height (μm)	1105.97	920.24	55.33	0.09	1138.71	1054.55	40.15	0.34	960.23	924.76	18.76	0.43
Crypt depth (μm)	200.88	223.80	15.93	0.56	159.88	205.29	15.11	0.15	132.50	158.18	14.93	0.48
VH/CD	5.61	4.24	0.55	0.28	7.23	5.25	0.51	0.04	7.75	6.00	0.89	0.41
Intestinal mucosa thickness (μm)	25.12	31.86	2.87	0.31	28.12	33.61	1.54	0.07	41.01	43.19	2.99	0.77
Intestine wall thickness (μm)	227.12	186.66	20.10	0.40	164.77	166.30	10.51	0.95	265.24	221.99	40.02	0.67

CN = Control groups, HS = Heat stress groups.

optimal growth models. In this experiment, Logistic, Gompertz and Von Bertalanffy models were used to fit the growth curve of body weight and size of DFC. The best fitting model for body weight was Gompertz, with a fitting degree as high as 0.995. The results are consistent with that of Boonkum et al. (2021), Nguyen et al. (2021) and Mancinelli et al. (2023). In terms of body size, a comprehensive comparison of R^2 values and the differences between predicted and measured values indicated that the optimal model for keel length and tibia length of DFC was Logistic, while Bertalanffy is the best for tibia circumference.

Studies have shown that high temperatures have negative effects on the behavior, growth, and physiology of chickens, and can cause an increase in chicken mortality rate (Xie et al., 2015). The body temperature including comb, feet, eyelid, and rectal was also influenced (Nascimento et al., 2014; Adu-Asiamah et al., 2021). Our results indicate that, at the 1st, 7th, and 14th days of treatment, high-temperature significantly increased the body surface and rectal temperature of DFC. While, there was no significant difference in BST at the 7th and 14th days ($P > 0.05$). Previous studies have shown that HS can reduce live weight and pectoral muscle weight, while increase fat deposition (Shakeri et al., 2018; Liu et al., 2023). In addition to that, the current study found that the full intestinal length reduced, especially the ileum length of DFC. The shortening of intestinal length means that the intake of food and intestinal peristalsis are reduced, the time of food staying in the intestinal tract is shortened, which indicates that heat stress affects the intestinal development and finally affects chicken growth (Garriga et al., 2006).

The HS has been shown to damage chicken tissue in previous study (Adu-Asiamah et al., 2021). Therefore, we collected pectoral muscle, thigh muscle, liver, and spleen of DFC to evaluate the tissue damage caused by HS. The results of histological analysis revealed interstitial tissue expansion, fibrosis, and inflammatory cell infiltration in the muscle tissue. Red blood cell hyperplasia, inflammatory cell infiltration, and tissue necrosis with hemorrhage were observed in the liver and spleen. In the literature, similar results have been reported (Adu-Asiamah et al., 2021; Nawaz et al., 2023). In addition, we further explored the mRNA change of muscle development factors. In addition to its role in myoblast differentiation, *MYOD1* is required for rapid fiber formation (Kitamura et al., 2007; Cao et al., 2010). *MSTN* also negatively regulates *MYOD1* expression in muscle (Hennebry et al., 2009). This study found that HS notably decreased *MYOD1* expression but increased *MSTN* levels in thigh muscle. The results are consistent with previous research (Li et al., 2021).

The HSP70 is the most conserved and important class of proteins that regulate the cell cycle and maintain cell stability (Mayer and Bukau, 2005). Under adverse conditions such as HS, the synthesis of HSP70 will be significantly increased to enhance the body's ability to resist stress. In various tissues, HSP70 has been shown to be increased in high-temperature environments (Cedraz et al., 2017; Pawar et al., 2023). Both HSP70 and HSP90 of pectoral major muscle are highly expressed in Peloco and Caneluda within thermal stress, these breeds proved to be very resistant to high temperature (Cedraz et al., 2017). Another study has demonstrated that HSP70 in the liver of Kuchi varieties was significantly up-regulated after HS for several days. However, there are no significant differences in HSP70 expression between Ching 'wekwe and Morogoro medium varieties (Khondowe et al., 2021). We investigated the expression of HSP70 protein and mRNA in the liver and spleen of DFC. The results showed that no significant differences existed, which was consistent with previous studies. The expression level of HSP70 is related to the heat tolerance of local chickens (Cedraz et al., 2017; Kang and Shim, 2021). The mRNA of *HSP70* in the DFC liver

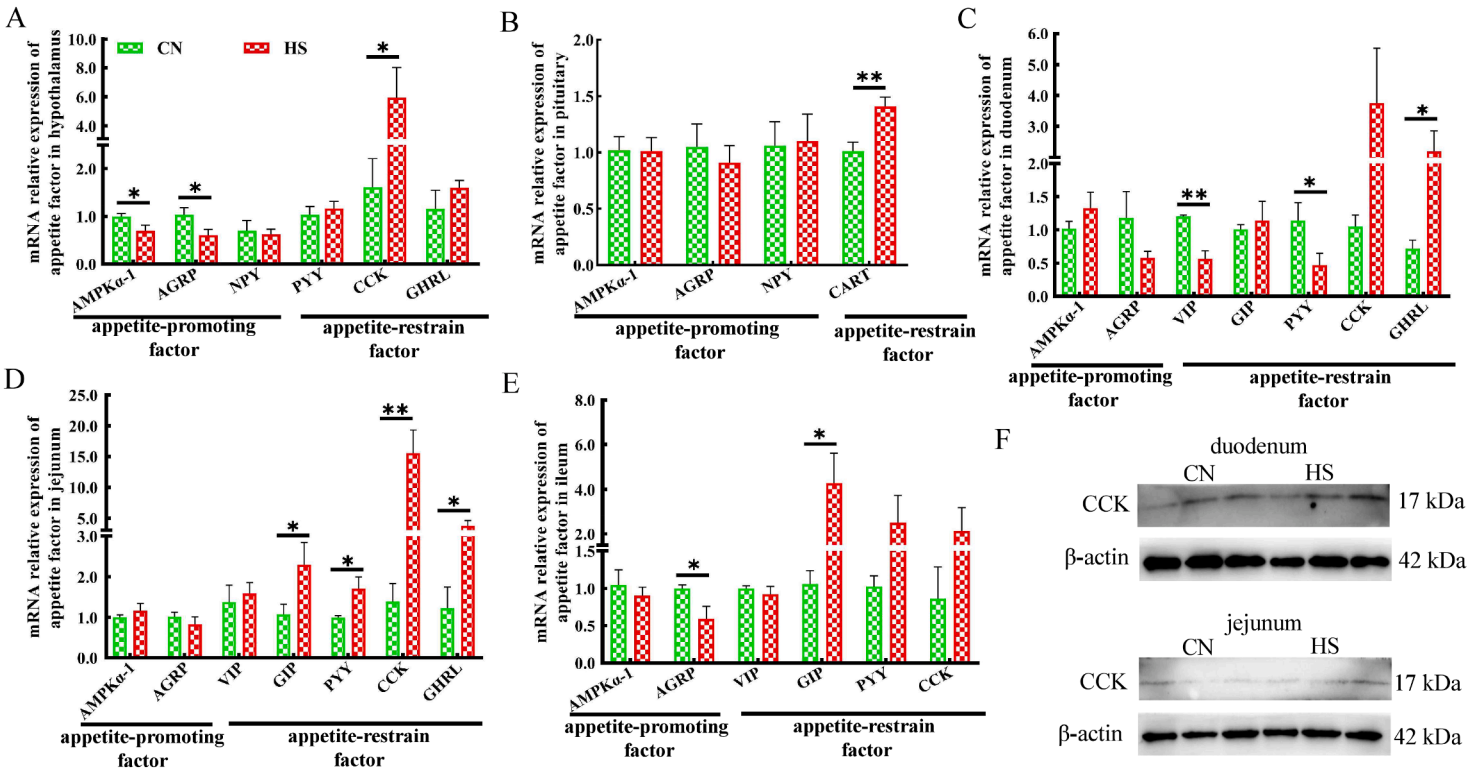

Fig. 4. Effect of heat stress on appetite related factors in dwarf chicken with frizzled feather. CN = Control, HS = Heat stress. Appetite factor mRNA expression in hypothalamus (A), pituitary (B), duodenum (C), jejunum (D) and ileum (E). (F) CCK protein expression level in duodenum and jejunum (mean ± SE; * $p < 0.05$; ** $p < 0.01$; n = 6).

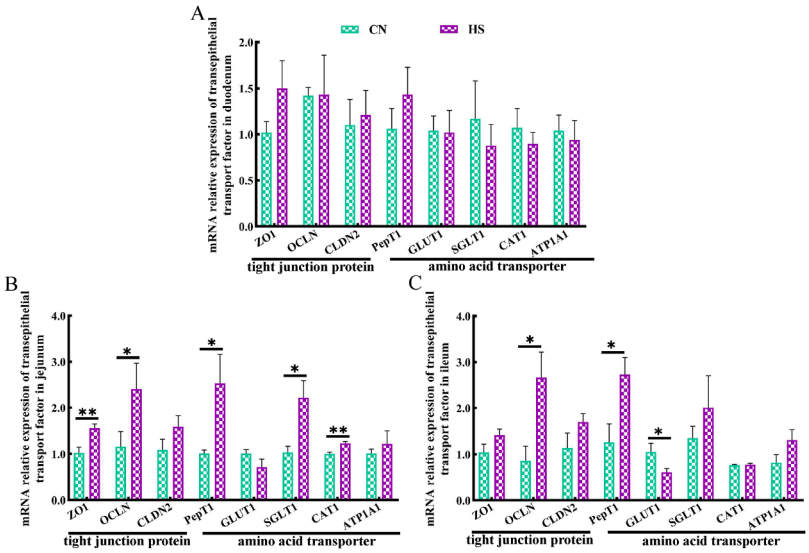

Fig. 5. Effect of heat stress on transport absorption factors in dwarf chicken with frizzled feather. CN = Control, HS = Heat stress. Transport absorption factor mRNA changes in duodenum(A), jejunum (B) and ileum (C) (mean ± SE; * $p < 0.05$; ** $p < 0.01$; n = 6).

exhibits an upward trend, though it does not reach the statistically significant, which may be due to the fact that DFC belong to hybrids of local heat-resistant chicken breeds, and it also may be that the DFC have a certain degree of acclimatization to the thermal environment after 14 days of chronic HS. Further exploration is needed to determine the specific mechanism for this adaptation. HS inhibits the feeding behavior of chickens, and the reduction of chicken feed intake has been considered typical signs of HS. The hypothalamus, as the central regulatory center for the body's homeostasis, plays a crucial role in regulating feeding behavior. The small intestine is the primary site for animal digestion and absorption. High-temperature environment can damage the health of the small intestine, disrupt its integrity, and subsequently impact the normal growth of animals. The study demonstrated that HS significantly decreased *AMPKα-1* and *AGRP* in the hypothalamus while increased *CCK* in the hypothalamus and *CART* in the pituitary, which was similar to previous researches (He et al., 2018a). Under heat stress conditions, the expression of appetite-promoting factors in the hypothalamus and pituitary feeding centers were decreased, while the appetite-restrain factors were up-regulated, indicating that the appetite of chickens was inhibited and ultimately affecting their development. The results of this study showed that HS significantly reduced the villus height to crypt depth ratio (VH / CD) in the jejunum of DFC, which was consistent with those of Peng et al. (2022) and He et al. (2018b). The VH / CD decreases under HS maybe detrimental for chickens. Also, HS significantly down-regulated the expression of *VIP* and *PYY* and up-regulated the *GHRL* in the duodenum. While HS significantly up-regulated the expression of *GIP, PYY, CCK*, and *GHRL* in the jejunum. Simultaneously, *AGRP* was down-regulated while *GIP* was up-regulated in the ileum, which was consistent with the findings of He et al. (2018b) and Lei et al. (2013). Therefore, HS may inhibit the intestinal appetite promoting factor and activate the appetite inhibition, thus the appetite of chickens was inhibited.

Animals' intestines play a vital role in supporting their overall health and fundamental life functions, including digestion and absorption of nutrients. The absorption and transport of nutrients are primarily facilitated by tight junction proteins and nutrient transporters. Several studies have shown that HS can reduce the absorption efficiency of nutrients in the small intestine (Habashy et al., 2017a; 2017b). Cheng et al. (2019) found, after 42 days of HS, the expression of *OCLN* in the jejunum and *ZO1/OCLN* in the ileum were significantly down-regulated. The similer results were obtained by others (Peng et al., 2022; Wang et al., 2022). In addition, the results of Orhan et al. (2019) showed that after 12 weeks of HS (34 ± 2°C), the ileal nutrient transporters *PepT1, GLUT1, SGLT1*, and *CAT1* were down-regulated. This study showed that after 14 days of HS (34 ± 1°C), a significant increase in tight junction proteins and amino acid transporters was observed in jejunum and ileum, including *ZO1, OCLN, PepT1, SGLT1* and *CAT1*. The results differed from those of the previous studies, which could be attributed to variations in HS duration and strains. Furthermore, feed intake, feed conversion ratio (FCR), metabolic rate and water intake are critical variable under heat stress. Future research need to pay more attentions on these traits although gene expression can give some clues to some extent.

This study explored the growth patterns of DFC and investigated factor changes in brain-gut axis of DFC under hot environment. The appetite-promoting factors and appetite-restrain factors exhibited the opposite trends. While the transport and absorption factors were up-regulated, indicating that environmental factors affected the regulation of the brain-gut axis on appetite, thereby affecting its development. The *KRT75L4* frizzling mutation might reduce insulation, while the smaller body size are beneficial to a lower basal metabolic rate. But, how genetic traits such as frizzled feathers and dwarfism might impart better heat tolerance, the underlying mechanism needs further research.

Conclusion

The best fitting model for body weight is Gompertz for DFC. The HS can cause tissue damage in DFC. Also, HS mainly affects the appetite of DFC but does not disrupt the nutrient absorption function. Further, the DFC exhibited heat resistance to some extent for the HSP*70* shows no differences between the control and the HS groups, which may be due to their frizzled feathers and small body size.

Author contributions

GL: Conceptualization, investigation, methodology, data curation, writing-original draft. **TX:** Formal analysis, investigation, methodology, resources, writing - review & editing. **ZZ:** Methodology, data curation. **CB:** Methodology, investigation. **SA:** Formal analysis, visualization. **DG:** Investigation, validation. **XW:** Formal analysis, software. **LL:** Software, visualization. **XH:** Investigation, resources. **BZ:** Investigation, resources. **LZ:** Project administration, funding acquisition, methodology, resources, supervision, writing - review & editing.

Ethics approval

The animals and procedures covered in this experiment were examined and approved by the Animal Care and Use Committee of Guangdong Ocean University (SYXK-2021-0154). All of the experiments were conducted was in completely in line with the policies of animal welfare in China.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors did not use any Al and Al-assisted technologies

Data and model availability statemen

None of the data were deposited in an official repository. The datasets generated and analyzed during the current study are available from the corresponding author upon request.

Financial support statement

This work was supported by the Natural Science Foundation of China [grant number 32472893], the Special Project of Seed Industry Revitalization of the Agriculture and Rural Department Guangdong Province [grant number 2024-XPY-00-008], the Open Fund Project of Guangdong Provincial Key Laboratory of Conservation and Precision Utilization of Characteristic Agricultural Resources in Mountainous Areas [grant number 2023JYKF06], program for scientific research start-up funds of Guangdong Ocean University [grant number 060302052303], and Science and technology projects of Guangxi Agricultural and Rural Department [grant number Z2022120].

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgments

Authors would like to thank the whole staff of “Animal Husbandry Research Institute of Guangxi, China” for their assistance during this experiment.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.104996.

References

Adu-Asiamah, P., Zhang, Y., Amoah, K., Leng, Q.Y., Zheng, J.H., Yang, H., Zhang, W.L., Zhang, L., 2021. Evaluation of physiological and molecular responses to acute heat stress in two chicken breeds. Animal 15 (2), 100106.

Azoulay, Y., Druyan, S., Yadgary, L., Hadad, Y., Cahaner, A., 2011. The viability and performance under hot conditions of featherless broilers versus fully feathered broilers. Poult. Sci. 90 (1), 19–29.

Bakthisaran, R., Tangirala, R., Rao, C.M., 2015. Small heat shock proteins: role in cellular functions and pathology. Biochim. Biophys. Acta 1854 (4), 291–319.

Baziz, H.A., Geraert, P.A., Padilha, J.C.F., Guillaumin, S., 1996. Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. Poult. Sci. 75 (4), 505–513.

Boonkum, W., Duangjinda, M., Kananit, S., Chankitisakul, V., Kenchaiwong, W., 2021. Genetic effect and growth curve parameter estimation under heat stress in slow-growing Thai native chickens. Vet. Sci. 8 (12), 297.

Buyse, J., Janssen, S., Geelissen, S., Swennen, Q., Kaiya, H., Darras, V.M., Dridi, S., 2009. Ghrelin modulates fatty acid synthase and related transcription factor mRNA levels in a tissue-specific manner in neonatal broiler chicks. Peptides 30 (7), 1342–1347.

Cahaner, A., Ajuh, J.A., Siegmund-Schultze, M., Azoulay, Y., Druyan, S., Zárate, A.V., 2008. Effects of the genetically reduced feather coverage in naked neck and featherless broilers on their performance under hot conditions. Poult. Sci. 87 (12), 2517–2527.

Cahaner, A., Deeb, N., Gutman, M., 1993. Effects of the plumage-reducing naked neck (Na) gene on the performance of fast-growing broilers at normal and high ambient temperatures1. Poult. Sci. 72 (5), 767–775.

Cahaner, A., Druyan, S., Deeb, N., 2003. Improving broiler meat production, especially in hot climates, by genes that reduce or eliminate feather coverage. Br. Poult. Sci. 44 (SUPPL. 1), S22–S23.

Cao, Y., Yao, Z., Sarkar, D., Lawrence, M., Sanchez, G.J., Parker, M.H., MacQuarrie, K.L., Davison, J., Morgan, M.T., Ruzzo, W.L., Gentleman, R.C., Tapscott, S.J., 2010. Genome-wide MyoD binding in skeletal muscle cells: a potential for broad cellular reprogramming. Dev. Cell 18 (4), 662–674.

Carling, D., Sanders, M.J., Woods, A., 2008. The regulation of AMP-activated protein kinase by upstream kinases. Int. J. Obes. 32 (4), S55–S59.

Cedraz, H., Gromboni, J.G.G., Garcia, A.A.P.J., Farias Filho, R.V., Souza, T.M., Oliveira, E.R.d., Oliveira, E.B.d., Nascimento, C.S.d., Meneghetti, C., Wenceslau, A. A., 2017. Heat stress induces expression of HSP genes in genetically divergent chickens. PLoS One 12 (10), e0186083.

Chai, B.-X., Neubig, R.R., Millhauser, G.L., Thompson, D.A., Jackson, P.J., Barsh, G.S., Dickinson, C.J., Li, J.-Y., Lai, Y.-M., Gantz, I., 2003. Inverse agonist activity of agouti and agouti-related protein. Peptides 24 (4), 603–609.

Cheng, Y.F., Chen, Y.P., Chen, R., Su, Y., Zhang, R.Q., He, Q.F., Wang, K., Wen, C., Zhou, Y.M., 2019. Dietary mannan oligosaccharide ameliorates cyclic heat stress-induced damages on intestinal oxidative status and barrier integrity of broilers. Poult. Sci. 98 (10), 4767–4776.

Deeb, N., Cahaner, A., 1999. The effects of naked neck genotypes, ambient temperature, and feeding status and their interactions on body temperature and performance of broilers. Poult. Sci. 78 (10), 1341–1346.

Dong, J., He, C., Wang, Z., Li, Y., Li, S., Tao, L., Chen, J., Li, D., Yang, F., Li, N., Zhang, Q., Zhang, L., Wang, G., Akinyemi, F., Meng, H., Du, B., 2018. A novel deletion in KRT75L4 mediates the frizzle trait in a Chinese indigenous chicken. Genet. Sel. Evol. 50 (1), 68.

Furuse, M., Tachibana, T., Ohgushi, A., Ando, R., Yoshimatsu, T., Denbow, D.M., 2001. Intracerebroventricular injection of ghrelin and growth hormone releasing factor inhibits food intake in neonatal chicks. Neurosci. Lett. 301 (2), 123–126.

Garriga, C., Hunter, R.R., Amat, C., Planas, J.M., Mitchell, M.A., Moretó, M., 2006. Heat stress increases apical glucose transport in the chicken jejunum. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290 (1), R195–R201.

Habashy, W.S., Milfort, M.C., Adomako, K., Attia, Y.A., Rekaya, R., Aggrey, S.E., 2017a. Effect of heat stress on amino acid digestibility and transporters in meat-type chickens. Poult. Sci. 96 (7), 2312–2319.

Habashy, W.S., Milfort, M.C., Fuller, A.L., Attia, Y.A., Rekaya, R., Aggrey, S.E., 2017b. Effect of heat stress on protein utilization and nutrient transporters in meat-type chickens. Int. J. Biometeorol. 61 (12), 2111–2118.

He, X., Lu, Z., Ma, B., Zhang, L., Li, J., Jiang, Y., Zhou, G., Gao, F., 2018a. Chronic heat stress damages small intestinal epithelium cells associated with the adenosine 5′-monophosphate-activated protein kinase pathway in broilers. J. Agric. Food Chem. 66 (28), 7301–7309.

He, X., Lu, Z., Ma, B., Zhang, L., Li, J., Jiang, Y., Zhou, G., Gao, F., 2018b. Effects of chronic heat exposure on growth performance, intestinal epithelial histology, appetite-related hormones and genes expression in broilers. J. Sci. Food Agric. 98 (12), 4471–4478.

He, X., Lu, Z., Ma, B., Zhang, L., Li, J., Jiang, Y., Zhou, G., Gao, F., 2019. Chronic heat stress alters hypothalamus integrity, the serum indexes and attenuates expressions of hypothalamic appetite genes in broilers. J. Therm. Biol. 81, 110–117.

Hennebry, A., Berry, C., Siriett, V., O'Callaghan, P., Chau, L., Watson, T., Sharma, M., Kambadur, R., 2009. Myostatin regulates fiber-type composition of skeletal muscle by regulating MEF2 and MyoD gene expression. Am. J. Physiol. Cell Physiol. 296 (3), C525–C534.

Kang, D., Shim, K., 2021. Early heat exposure effect on the heat shock proteins in broilers under acute heat stress. Poult. Sci. 100 (3), 100964.

Khondowe, P., Mutayoba, B., Muhairwa, A., Phiri, E., 2021. Effects of heat stress and a low energy diet on blood parameters and liver hsp70 and iNOS gene expressions in local chickens. Vet. Anim. Sci. 14, 100221.

Kitamura, T., Kitamura, Y.I., Funahashi, Y., Shawber, C.J., Castrillon, D.H., Kollipara, R., DePinho, R.A., Kitajewski, J., Accili, D., 2007. A Foxo/Notch pathway controls myogenic differentiation and fiber type specification. J. Clin. Invest. 117 (9), 2477–2485.

Kuhi, H.D., Kebreab, E., López, S., Sciences, F., 2002. A derivation and evaluation of the von Bertalanffy equation for describing growth in broilers over time. J. Sci. Food Agric. 11 (1), 109–125.

Kumar, M., Ratwan, P., Dahiya, S.P., Nehra, A.K., 2021. Climate change and heat stress: impact on production, reproduction and growth performance of poultry and its mitigation using genetic strategies. J. Therm. Biol. 97, 102867.

Kutlu, H.R., Forbes, J.M., 1993. Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. Livest. Prod. Sci. 36 (4), 335–350.

Lei, L., Hepeng, L., Xianlei, L., Hongchao, J., Hai, L., Sheikhahmadi, A., Yufeng, W., Zhigang, S., 2013. Effects of acute heat stress on gene expression of brain–gut neuropeptides in broiler chickens (Gallus gallus domesticus)1. J. Anim. Sci. 91 (11), 5194–5201.

Li, X., Zhang, M., Feng, J., Zhou, Y., 2021. Myostatin and related factors are involved in skeletal muscle protein breakdown in growing broilers exposed to constant heat stress. Animals 11 (5), 1467.

Liu, Y., Liu, Z., Xing, T., Li, J., Zhang, L., Zhao, L., Gao, F., 2023. Effect of chronic heat stress on the carbonylation of glycolytic enzymes in breast muscle and its correlation with the growth performance of broilers. Poult. Sci. 102 (12), 103103.

López, S.M., Balog-Alvarez, C., Canessa, E.H., Hathout, Y., Brown, K.J., Vitha, S., Bettis, A.K., Boehler, J., Kornegay, J.N., Nghiem, P.P., 2020. Creation and

characterization of an immortalized canine myoblast cell line: Myok9. Mamm. Genome 31 (3), 95–109.
Mancinelli, A.C., Menchetti, L., Birolo, M., Bittante, G., Chiattelli, D., Castellini, C., 2023. Crossbreeding to improve local chicken breeds: predicting growth performance of the crosses using the Gompertz model and estimated heterosis. Poult. Sci. 102 (8), 102783.
Mayer, M.P., Bukau, B., 2005. Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol. Life Sci. 62 (6), 670.
Nascimento, S.T., da Silva, I.J.O., Maia, A.S.C., de Castro, A.C., Vieira, F.M.C., 2014. Mean surface temperature prediction models for broiler chickens—a study of sensible heat flow. Int. J. Biometeorol. 58 (2), 195–201.
Nawaz, A.H., Amoah, K., Leng, Q.Y., Zheng, J.H., Zhang, W.L., Zhang, L., 2021. Poultry response to heat stress: its physiological, metabolic, and genetic implications on meat production and quality including strategies to improve broiler production in a warming world. Front. Vet. Sci. 8, 699081.
Nawaz, A.H., Lin, S., Wang, F., Zheng, J., Sun, J., Zhang, W., Jiao, Z., Zhu, Z., An, L., Zhang, L., 2023. Investigating the heat tolerance and production performance in local chicken breed having normal and dwarf size. Animal 17 (3), 100707.
Nguyen Hoang, T., Do, H.T.T., Bui, D.H., Pham, D.K., Hoang, T.A., Do, D.N., 2021. Evaluation of non-linear growth curve models in the Vietnamese indigenous Mia chicken. Anim. Sci. J. 92 (1), e13483.
Ocłoń, E., Pietras, M., 2011. Peripheral ghrelin inhibits feed intake through hypothalamo-pituitary-adrenal axis-dependent mechanism in chicken. J. Anim. Feed Sci. 20 (1), 118–130.
Orhan, C., Tuzcu, M., Deeh, P.B.D., Sahin, N., Komorowski, J.R., Sahin, K., 2019. Organic chromium form alleviates the detrimental effects of heat stress on nutrient digestibility and nutrient transporters in laying hens. Biol. Trace. Elem. Res. 189, 529–537.
Pawar, S.S., Kurade, N.P., Bhendarkar, M.P., Bhosale, S.V., Nirmale, A.V., Kochewad, S. A., 2023. Modulation of heat shock protein 70 (HSP70) gene expression ex vivo in response to heat stress in chicken. Anim. Biotechnol. 34 (9), 5168–5172.
Peng, X.Y., Xing, T., Li, J.L., Zhang, L., Jiang, Y., Gao, F., 2022. Guanidinoacetic acid supplementation improves intestinal morphology, mucosal barrier function of broilers subjected to chronic heat stress. J. Anim. Sci. 101, skac355.
Shakeri, M., Cottrell, J.J., Wilkinson, S., Ringuet, M., Furness, J.B., Dunshea, F.R., 2018. Betaine and antioxidants improve growth performance, breast muscle development and ameliorate thermoregulatory responses to cyclic heat exposure in broiler chickens. Animals 8 (10), 162.
Small, C.J., Kim, M.S., Stanley, S.A., Mitchell, J.R.D., Murphy, K., Morgan, D.G.A., Ghatei, M.A., Bloom, S.R., 2001. Effects of chronic central nervous system administration of Agouti-related protein in pair-fed animals. Diabetes 50 (2), 248–254.
Sofaer, H.R., Chapman, P.L., Sillett, T.S., Ghalambor, C.K., 2013. Advantages of nonlinear mixed models for fitting avian growth curves. J. Avian. Biol. 44 (5), 469–478.
Somes, R.G., Johnson, S., 1982. The effect of the scaleless gene, sc, on growth performance and carcass composition of Broilers1,2. Poult. Sci. 61 (3), 414–423.
Tschöp, M., Smiley, D.L., Heiman, M.L., 2000. Ghrelin induces adiposity in rodents. Nature 407 (6806), 908–913.
Wang, Y., Sun, W., Wu, E., Wang, K., Chen, X., Cui, Y., Zhang, G., Lv, F., Wang, Y., Peng, X., Si, H., 2022. Polysaccharides from Abrus cantoniensis hance modulate intestinal microflora and improve intestinal mucosal barrier and liver oxidative damage induced by heat stress. Front. Vet. Sci. 9, 868433.
Wang, Z., Shao, D., Wu, S., Song, Z., Shi, S., 2022. Heat stress-induced intestinal barrier damage and dimethylglycine alleviates via improving the metabolism function of microbiota gut brain axis. Ecotoxicol. Environ. Saf. 244, 114053.
Wynne, K., Stanley, S., McGowan, B., Bloom, S., 2005. Appetite control. J. Endocrinol. 184 (2), 291–318.
Xie, J., Tang, L., Lu, L., Zhang, L., Lin, X., Liu, H.C., Odle, J., Luo, X., 2015. Effects of acute and chronic heat stress on plasma metabolites, hormones and oxidant status in restrictedly fed broiler breeders. Poult. Sci. 94 (7), 1635–1644.
Yahav, S., Luger, D., Cahaner, A., Dotan, M., Rusal, M., Hurwitz, S., 1998. Thermoregulation in naked neck chickens subjected to different ambient temperatures. Br. Poult. Sci. 39 (1), 133–138.
Yalcin, S., Testik, A., Ozkan, S., Settar, P., Celen, F., Cahaner, A., 1997. Performance of naked neck and normal broilers in hot, warm, and temperate climates. Poult. Sci. 76 (7), 930–937.