Influence of free-range days on growth performance, carcass traits, meat quality, lymphoid organ indices, and blood biochemistry of Wannan Yellow chickens

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ABSTRACT This study investigated the effects of free-range days on growth performance, carcass traits, meat quality, lymphoid organ indices, and blood biochemical parameters of Wannan Yellow chickens. A total of 1,000 one-day-old male Wannan Yellow chickens were reared to 56 D. At 56 D of age, 960 birds with similar body weight (BW) were randomly allocated to free-range treatment at 56, 70, 84, and 98 D of age (assigned to free-range treatment for 42, 28, 14, and 0 D, respectively); 6 replicates with 40 chickens per treatment. In the free-range system, chickens were reared in indoor floor pens with an outdoor free-range paddock measuring 4 × 7 m (28 m^2, 1.5 birds/m^2). Results showed that BW of birds decreased significantly in the first 2 wk after birds were assigned to free-range treatment compared with those in the conventional treatment ($P < 0.05$). Average daily gain (ADG) and average daily feed intake (ADFI) decreased significantly ($P < 0.05$) for chickens assigned to free-range treatment from 56 to 70 D of age, while feed conversion ratio increased significantly ($P < 0.05$). Breast yield increased linearly with increasing free-range days ($P < 0.05$), whereas leg and foot yields decreased linearly ($P < 0.05$). Drip loss and L* value of thigh muscle decreased linearly with increasing free-range days ($P < 0.05$), while shear force improved linearly ($P < 0.05$). Additionally, the absolute thymus weight and thymus to BW ratio showed significant increasing and then decreasing quadratic responses to increasing free-range days ($P < 0.05$). Furthermore, serum glucose, total protein, cholesterol, and triglyceride content declined linearly, while high-density lipoprotein cholesterol (HDL-C) content increased linearly with increasing free-range days ($P < 0.05$). In conclusion, increasing free-range days had positive effects on breast yield, shear force, thymus weight, and HDL-C content, but negatively affected leg yield, foot yield, drip loss, L* value of thigh muscle, glucose, total protein, cholesterol, and triglyceride levels of Wannan Yellow chickens.

Key words: free-range days, meat quality, lymphoid organ index, blood biochemical parameters, Wannan Yellow chicken

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INTRODUCTION

Free-range rearing affects chickens' health, welfare, and production efficiency, and poultry products derived from free-range systems have become increasingly popular (Chen et al., 2013a; Tong et al., 2014; Wang et al., 2015). Previous studies indicated that free-range rearing can positively affect both farm animal welfare and meat quality (Yamak et al., 2016). Moreover, many consumers prefer to purchase free-range products (McFadden and Huffman, 2017) and are willing to pay a premium for them (Zhang et al., 2018a). Free-range products have good market potential globally but high premiums rely on positive consumer impressions of superior meat quality and higher welfare standards (Tong et al., 2014). In addition, since the 1990s, some governments and markets in North America, Australia, and Europe have led initiatives to promote animal welfare, with a focus on the abolition of confinement systems. The commodification and market segmentation of higher welfare standards has increased the demand for cage-free and free-range products in these countries (Scrinis et al., 2017).

Products from free-range systems are generally healthier and have higher welfare standards from a consumer's standpoint than products from conventional confined systems (Rehman et al., 2017; Scrinis et al., 2017). It is well documented that birds from free-range systems have higher antibody titers for Newcastle

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disease virus and infectious bronchitis virus (Rehman et al., 2017). Moreover, previous studies have indicated that free-range systems are an important part of organic farming as they conform to the natural habits of animals (Ferrante et al., 2009; Pettersson et al., 2016). A previous study showed that free-range rearing systems affected the meat quality, composition, and taste of local chicken products (Yamak et al., 2016). However, the effects of free-range systems remain controversial as there are many variables to be considered, including free-range days (Stadig et al., 2016), artificial structuring (Zeltner and Hirt, 2003), and pasture intake (Ponte et al., 2008). The number of free-range days plays a key role in free-range rearing systems. It has been reported that free-range days can directly and indirectly influence the productivity and quality of local chickens in terms of their growth performance, carcass yield, meat quality, and immune performance (Tong et al., 2014; Stadig et al., 2016).

Wannan Yellow chicken is one of the most important and popular local breeds in eastern China, especially in Anhui Province, and it was recorded as an indigenous poultry genetic resource in 2003. In recent years, this indigenous chicken has gained much attention due to its broad adaptability, strong disease resistance, special phenotypic features (yellow plumage, beak, and shanks), and conserved gene pools (Bao et al., 2009). The average egg production for 72 wk is 150 to 160. In addition, it is a slow-growing and medium-size local chicken breed that is known for its superior meat quality, special flavor, and high nutritional value (Chen et al., 2013b). Therefore, it is a well-known meat and egg dual-purpose chicken breed in China and is widely raised. Slow-growing birds are better suited to free-range systems (Chen et al., 2013a) as fast-growing birds have a lower degree of adaptation and prefer to stay indoors to reduce energy expenditure (Branciari et al., 2009). Moreover, it has been reported that consumers prefer local organic products to other organic products (Thøgersen et al., 2017). Hence, Wannan Yellow chicken, a typical slow-growing local chicken breed, was selected for the present study.

To date, there are still relatively few studies on the effects of free-range days on Wannan Yellow chickens. The objective of this study, therefore, was to examine the influence of free-range days on growth performance, carcass traits, meat quality, lymphoid organ indices, and blood biochemical parameters of Wannan Yellow chickens. This study will provide relevant information on free-range systems for farmers and breeders of Wannan Yellow chickens and other similar local chicken breeds in China.

MATERIALS AND METHODS

Ethics Statement

The experiment was carried out according to the Guidelines for Experimental Animals from the Ministry of Science and Technology (Beijing, China; revised in 2004). All procedures and protocols involving birds were reviewed and approved by the Animal Care and Use Committee of Anhui Agricultural University (approval number: SYXK 2016-007).

Birds, Housing, and Diets

This experiment was conducted at Qingyang Pingyun Poultry Conservation and Breeding Co., Ltd., Chizhou, China (30°68′ North, 117°93′ East; 24 m above sea level) from September to October. A total of 1,000 one-day-old male Wannan Yellow chickens with complete pedigree information obtained from Qingyang Pingyun Poultry Conservation and Breeding Co. Ltd., Anhui, China, were chosen as the experimental population. All chickens were sexed, wing-banded, and weighed individually on the day of hatching. All chickens were reared in indoor floor pens with an area of 4 × 7 m (6.5 birds/m^2). The indoor floor pens were covered with wood shavings with a density of 280 kg/m^3, and the height of wood shavings in every indoor floor poultry house was 8.5 cm. An appropriate vaccination schedule was administered to all chickens according to the company's management procedures: Marek's disease vaccine was provided on day 1, Newcastle disease vaccine on days 7 and 28, and infectious bursal disease on days 14 and 24.

At 56 D of age, 960 birds with similar BW (477 ± 25 g) were selected and randomly assigned to free-range treatment at 56, 70, 84, and 98 D of age (i.e., assigned to a free-range treatment for 42, 28, 14, and 0 D, respectively). Each treatment comprised 6 replicates, and each replicate contained 40 birds (240 birds per treatment). The remaining 40 chickens were not used in these experiments owing to the missing pedigree or phenotype information. In the free-range treatment, birds were reared in an indoor floor poultry house (6.5 birds/m^2) connected to an outside free-range paddock. The indoor floor house was covered with litter with a height of 8.5 cm. The outside free-range paddock measured 4 × 7 m (1.5 birds/m^2), and was fenced with 2 m high barbed wire with a plastic net on the top to exclude predators. The outside paddock also provided an activity area without any pasture or vegetation intake to eliminate interference with the normal feed intake of chickens. Each replicate was separated with the help of fish net. Furthermore, there were some perches used for birds to rest on. Feeders and plastic water tanks (2 indoors and 1 outdoor) provided ad libitum feed and water for experimental birds in each replicate. The birds in the free-range treatment had free daytime access to the outside free-range paddock (from 06:00 to 18:00 h) but were confined to the indoor house at night. During the experimental period, the indoor and outdoor environmental conditions were similar (average temperature: 20.0 to 25.0°C; relative humidity: 65.0 to 70.0%; indoor lighting period: 22 to 23 h).

Table 1. Ingredients and nutrient levels of the basal diet for experimental chickens.

Items	Content
Ingredients(%)	
Corn	65.19
Soybean meal	26.30
Wheat bran	3.90
Limestone	1.20
Dicalcium phosphate	1.30
Choline chloride	0.12
Salt	0.30
Lysine	0.46
Methionine	0.23
Premix[1]	1.00
Total	100.0
Calculated nutrient levels (%)	
Metabolizable energy ($MJ\cdot kg^{-1}$)	12.96
Crude protein	19.00
Crude fiber	4.15
Calcium	0.95
Available phosphorus	0.42
Lysine	0.85
Methionine	0.36

[1]Premix provided per kg of diet: vitamin A, 9,500 IU; vitamin D3, 2,200 IU; vitamin E, 20 IU; vitamin K_3, 2.65 mg; vitamin B_1, 2.8 mg; vitamin B_2, 7.0 mg; vitamin B_6, 1.50 mg; vitamin B_{12}, 0.025 mg; nicotinic acid, 27.0 mg; folic acid, 1.20 mg; biotin, 0.20 mg; $NaHCO_3$ 1050 mg; Fe, 80 mg; Cu, 8.0 mg; Mn, 60 mg; Zn, 50 mg; I, 0.35 mg, Se, 0.15 mg.

All chickens were provided with the same diet. The ingredients and nutrient levels of the basal diet are listed in Table 1 and were formulated according to the nutrient requirements of chickens of China (criterion code: NY/T 33-2004). Mortality was recorded when it occurred. Considering the mortality and incomplete phenotype data, within each treatment, there were 6 replicates with 37 chickens each for further analysis at the end of the trial.

Growth Performance

The birds and feed for each replicate were weighed weekly to determine BW and feed intake. At the end of the experiment, the ADG, ADFI, and FCR were calculated after a 12 h overnight fast on a per replicate basis.

Sample Collection

On day 98, after fasting for 12 h, 8 chickens were randomly selected from each replicate, weighed individually, and manually exsanguinated. The feathers, head, and feet were immediately removed and weighed. Carcasses were weighed and then eviscerated and weighed to calculate slaughter yield and eviscerated carcass yield. The wings, lung, abdominal fat, breast and thigh muscles, liver, spleen, thymus, and bursa were removed and weighed. The percentage of head, foot, wing, lung, breast muscle, thigh muscle, and abdominal fat were calculated as a percentage of eviscerated carcass weight. In addition, the percentages of liver, spleen, thymus, and bursa were calculated as a percentage of final live weight of the bird after fasting. Muscle samples were collected from the same place in the right side of the thigh muscle for meat quality detection.

Physiochemical Features

Meat color of the thigh muscle was evaluated using a colorimeter (CR-300; Minolta Camera, Osaka, Japan), which was calibrated against white and black tiles according to the manufacturer's manual before analysis. For each sample, 3 different points were detected around the thigh muscle. Each point reading was repeated 3 times and the final value for each sample was the average of these readings. Meat color was shown as lightness (L*), redness (a*), and yellowness (b*) in accordance with the International Commission on Illumination (CIE) color systems.

For drip loss, the thigh muscle was stripped of adjacent fascia and then cut into strips (3.5 × 2.0 × 1.5 cm) parallel to the longitudinal orientation of the muscle fibers. Samples were subsequently weighed and then hung in a sealed plastic bag in a refrigerator at 4°C for 24 h. The muscle sample was weighed again when taken out of the bag. Drip loss was defined as the weight lost over 24 h and was presented as a percentage of the initial muscle weight.

Muscle shear force was determined on the same day using a digital texture analyzer (C-LM4; Northeast Agricultural University Ltd., Harbin, China). Muscle samples were cooked in plastic bags in a water bath at 80°C to an internal temperature of 70°C, and then removed and chilled to room temperature. The samples were cut into strips with an average size of 1.0 × 0.5 × 2.5 cm. Each strip was cut 3 times vertical to the muscle fiber direction to acquire an average value for shear force.

The pH values of the thigh muscle were measured at 45 min postmortem at 1 cm depth using a portable pH meter (Testo 205; Testo Instruments International Trading Co. Ltd., Shanghai, China). Before analysis, the meter was adjusted using buffers with pH values of 4.01, 7.00, and 9.01 at room temperature. The average pH value was defined from 3 measured values of the same area, and the procedures were the same for all of the samples.

Blood Biochemical Parameters

Blood samples (3.5 mL per bird) were collected from the brachial vein and centrifuged at 4,450 × g for 15 min at 4°C to separate the serum, which was immediately frozen at −20°C until further analysis. The concentrations of triiodothyronine (T3), thyroxine (T4), aspartate aminotransferase (**AST**), uric acid, glucose, total protein, albumin, globulin, cholesterol, triacylglycerol, high-density lipoprotein cholesterol (**HDL-C**), and low-density lipoprotein cholesterol (**LDL-C**) were determined using a double beam UV-visible light spectrophotometer (Biomate 5; Thermo Electron Corporation,

Table 2. Effects of free-range days on body weight, daily weight gain, daily feed intake, feed conversion ratio, and mortality.[1]

Items[2]	Free-range days 0	14	28	42	SEM[3]	*P*-value	Linear	Quadratic
BW (g)								
70 D	665.87[a]	665.87[a]	665.87[a]	629.48[b]	10.244	0.024	–	–
84 D	840.46[a]	840.46[a]	796.93[b]	785.03[c]	9.644	0.035	0.036	0.552
98 D	1073.83	1055.74	1047.63	1077.70	11.304	0.118	0.201	0.588
56 to 70 D								
ADG (g/D)	13.59[a]	13.59[a]	13.59[a]	10.89[b]	0.238	0.011	–	–
ADFI (g/D)	53.98[a]	53.98[a]	53.98[a]	52.13[b]	0.295	0.024	–	–
FCR (g/g)	3.97[a]	3.97[a]	3.97[a]	4.79[b]	0.068	0.023	–	–
71 to 84 D								
ADG (g/D)	13.23	13.23	12.70	13.31	0.293	0.253	0.120	0.083
ADFI (g/D)	55.23	55.23	53.11	55.09	0.484	0.312	0.302	0.123
FCR (g/g)	4.17	4.17	4.18	4.14	0.196	0.213	0.535	0.195
85 to 98 D								
ADG (g/D)	14.01[a]	14.51[b]	15.29[c]	15.87[c]	0.110	0.011	0.010	0.001
ADFI (g/D)	62.29[a]	62.30[a]	63.99[b]	65.89[c]	0.648	0.021	0.005	0.003
FCR (g/g)	4.45[a]	4.29[b]	4.19[c]	4.15[c]	0.026	0.030	0.011	0.584
56 to 98 D								
ADG (g/D)	12.53	12.58	12.13	12.01	0.330	0.6351	0.4828	0.565
ADFI (g/D)	58.17	57.50	55.70	56.45	0.450	0.4536	0.4726	0.4862
FCR (g/g)	4.64	4.53	4.59	4.70	0.090	0.5648	0.2835	0.4791
Mortality(%)	1.10	1.78	2.21	1.12	0.010	0.6870	0.3860	0.5652

[1]Data are means of 6 replicates of 37 birds each.
[2]ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.
[3]Pooled standard error of the mean.
[a–d]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Rochester, NY) and commercial kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China).

Statistical Analysis

Performance data for individuals were subjected to repeated measures analysis, and the average values for replicates were considered as the experimental units for further analysis. All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The homogeneity of variance and normality of the data were verified by quantile-quantile plots, formal statistical tests, and histograms as part of the UNIVARIATE procedure of SAS 9.4. All data were analyzed as a complete randomized design and subjected to a one-way ANOVA using the GLM procedure in SAS 9.4. The significance of differences among treatments was tested using Duncan's multiple-range test. Orthogonal polynomial contrasts were used to determine linear and quadratic responses to each treatment. Data were considered to be statistically significant at $P < 0.05$.

RESULTS

Growth Performance

Growth performance of Wannan Yellow chickens in each treatment is shown in Table 2. Body weight of birds at 70 and 84 D of age significantly decreased after they were assigned to free-range treatment compared with those from the conventional treatment ($P < 0.05$). The ADG and ADFI significantly decreased from 56 to 70 D of age after birds were assigned to free-range systems ($P < 0.05$), whereas the FCR significantly increased ($P < 0.05$). The ADG and ADFI increased linearly with increasing free-range days from 85 to 98 D of age after birds were assigned to free-range systems ($P < 0.05$), while FCR significantly decreased ($P < 0.05$). However, there was no significant influence of free-range days on the final BW at 98 D of age ($P > 0.05$), and there was also no significant influence on ADG, ADFI, FCR, and mortality from 56 to 98 D of age ($P > 0.05$).

Carcass Traits

Carcass yield data for the chickens are presented in Table 3. Breast yield increased linearly with increasing free-range days ($P < 0.05$), whereas the leg, foot, and abdominal fat yields decreased linearly with increasing free-range days ($P < 0.05$). However, no differences were noted in the slaughter, eviscerated carcass, thigh, wing, head, or lung yields among the treatments ($P = 0.068$, $P = 0.100$, $P = 0.297$, $P = 0.211$, $P = 0.251$, and $P = 0.496$, respectively).

Meat Quality

The effect of free-range days on meat quality of thigh muscles is shown in Table 4. The shear force increased linearly with increasing free-range days ($P < 0.05$). Furthermore, drip loss and L* value decreased with

Table 3. Effects of free-range days on carcass traits of Wannan Yellow chickens.[1]

Items (%)	Free-range days 0	14	28	42	SEM[2]	Significance	Linear	Quadratic
Slaughter yield	92.80	93.02	92.09	92.15	0.002	0.068	0.069	0.801
Eviscerated carcass yield	70.89	72.12	70.84	69.46	0.013	0.100	0.202	0.512
Breast yield	14.30[a]	14.60[a]	15.77[b]	16.34[c]	0.022	0.025	0.034	0.468
Thigh yield	15.52	16.39	16.26	16.06	0.013	0.297	0.372	0.108
Wing yield	12.64	11.98	11.73	11.90	0.011	0.211	0.110	0.181
Leg yield	29.62[a]	29.78[a]	27.48[b]	27.44[b]	0.130	0.017	0.013	0.866
Abdominal fat yield	1.38[a]	1.21[a]	0.89[b]	0.90[b]	0.001	0.019	0.025	0.173
Head yield	5.69	5.29	5.61	5.51	0.008	0.251	0.795	0.373
Foot yield	7.38[a]	6.86[a,b]	6.26[b]	6.05[c]	0.022	0.003	0.031	0.455
Lung yield	1.07	1.01	1.01	1.03	0.021	0.496	0.463	0.185

[1]Data are means of 6 replicates of 8 chickens each.
[2]Pooled standard error of the mean.
[a–c]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Table 4. Effects of free-range days on meat quality of Wannan Yellow chickens.

Items	Free-range days 0	14	28	42	SEM[2]	Significance	Linear	Quadratic
Drip loss (%)	1.82[a]	1.65[b]	1.38[c]	1.24[d]	0.001	0.021	0.011	0.832
Shear force (kg)	2.08[a]	2.24[a]	2.54[b]	2.76[c]	0.059	0.034	0.012	0.872
Meat color[1]								
L*	50.64[a]	50.22[a]	48.55[b]	47.80[b]	0.422	0.015	0.038	0.046
a*	8.34	8.15	8.19	8.24	0.332	0.306	0.082	0.578
b*	15.46	14.93	15.33	16.56	0.420	0.578	0.334	0.310
pH	6.95	6.65	6.64	6.43	0.038	0.321	0.235	0.541

[1]L* = lightness; a* = redness; b* = yellowness.
[2]Pooled standard error of the mean.
[a–c]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

Table 5. Effects of free-range days on lymphoid organ indices of Wannan Yellow chickens.[1]

Items	Free-range days 0	14	28	42	SEM[2]	Significance	Linear	Quadratic
Liver weight (g)	25.99	24.52	23.86	25.51	0.179	0.403	0.628	0.113
(liver weight/BW) × 100	2.45	2.41	2.52	2.67	0.047	0.048	0.081	0.115
Spleen weight (g)	2.41	2.24	2.13	2.58	0.109	0.364	0.215	0.440
(spleen weight/BW) × 100	0.22	0.21	0.22	0.26	0.011	0.376	0.414	0.873
Thymus weight (g)	4.06[a]	4.81[a]	5.51[b]	5.43[b]	0.205	0.013	0.653	0.017
(Thymus weight/BW) × 100	0.33[a]	0.40[a]	0.52[b]	0.51[b]	0.019	0.034	0.901	0.021
Bursa weight (g)	1.66	1.73	1.92	1.93	0.106	0.115	0.161	0.217
(Bursa weight/BW) × 100	0.15	0.16	0.18	0.19	0.001	0.129	0.091	0.221

[1]Data are means of 6 replicates of 8 chickens each.
[2]Pooled standard error of the mean.
[a–c]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

increasing free-range days ($P < 0.05$). However, the a* and b* values and pH were not affected by free-range days ($P = 0.306$, $P = 0.578$, and $P = 0.321$, respectively).

Lymphoid Organ Indices

The effect of free-range days on lymphoid organ indices is shown in Table 5. The thymus weight and thymus weight to BW ratio showed an increasing and then decreasing quadratic response to increasing free-range days ($P < 0.05$). However, there was no significant effect of free-range days on liver weight, liver weight to BW ratio, spleen weight, spleen weight to BW ratio, bursa weight, or bursa weight to BW ratio ($P > 0.05$).

Blood Biochemical Parameters

The influence of free-range days on blood biochemical parameters is shown in Table 6. The serum glucose and total protein decreased linearly with increasing free-range days ($P < 0.05$). The serum cholesterol and

Table 6. Effects of free-range days on blood biochemical indices of Wannan Yellow chickens.[1]

Items[2]	Free-range days 0	14	28	42	SEM[3]	Significance	Linear	Quadratic
T3 (nmol/L)	1.16	1.21	1.12	1.19	0.079	0.197	0.303	0.105
T4 (nmol/L)	23.39	24.56	23.10	22.76	0.407	0.528	0.589	0.308
AST (U/L)	207.86	208.02	207.74	206.12	4.596	0.076	0.019	0.385
Uric acid (μmol/L)	220.97	226.79	225.33	235.62	7.808	0.334	0.246	0.887
Glucose (mmol/L)	14.57[a]	13.02[b]	12.19[c]	12.08[c]	0.275	0.002	0.005	0.005
Total protein (g/L)	47.47[a]	43.39[b]	41.94[c]	39.03[d]	1.006	0.011	0.002	0.156
Albumin (g/L)	14.50	13.58	12.84	13.91	0.286	0.215	0.331	0.084
Globulin (g/L)	25.46	26.89	26.10	25.81	0.673	0.114	0.087	0.113
Cholesterol (mmol/L)	3.43[a]	3.17[a]	3.15[a]	2.83[b]	0.076	0.038	0.034	0.035
Triglyceride (mmol/L)	1.06[a]	0.69[b]	0.39[c]	0.37[c]	0.001	0.035	0.036	0.132
HDL-C (mmol/L)	2.01[a]	2.23[a]	2.29[a]	2.65[b]	0.042	0.023	0.028	0.016
LDL-C (mmol/L)	1.71	1.70	1.72	1.83	0.030	0.105	0.296	0.076

[1]Data are means of 6 replicates of 8 chickens each.
[2]T3: triiodothyronine; T4: thyroxine; AST: aspartate aminotransferase; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol.
[3]Pooled standard error of the mean.
[a–d]Means within a row lacking a common superscript differ significantly ($P < 0.05$).

triglyceride levels decreased linearly with increasing free-range days ($P < 0.05$), whereas the serum HDL-C content increased linearly ($P < 0.05$). However, no significant differences were observed in serum T3, T4, AST, uric acid, albumin, globulin, and LDL-C contents among treatments ($P > 0.05$).

DISCUSSION

Growth Performance

Growth performance indicators are often used to assess poultry production (Zhang et al., 2018b). Previous studies have indicated that poultry growth performance indicators are affected by rearing systems (Wang et al., 2009; Liu et al., 2011; Wang et al., 2015). In the present study, the ADG and ADFI significantly decreased, whereas FCR increased in the first 2 wk after birds were assigned to free-range systems. These results were probably due to the birds adjusting to the new environmental conditions. The growth performance of free-range local chickens can be influenced by many factors, including ambient temperature, size of exercise area, and stocking density (Wang et al., 2009; Yamak et al., 2016).

In terms of ambient temperature fluctuations, birds in each treatment were confined within the indoor house at night and provided with new litter to mitigate the effects of temperature fluctuations. However, the temperature of the free-range area was more variable than that of the indoor house during the daytime, due to changes in sunlight, rain, wind, and other natural factors. The outdoor paddock of free-range systems provided an exercise area for birds and decreased the stocking density of birds. In addition, the exercise areas promote higher energy expenditure of birds (Chen et al., 2013a). A previous study indicated that a lower stocking density decreased the physiological stress of chickens (Najafi et al., 2015). In short, the feed intake and body growth of free-range chickens are affected by ambient temperature fluctuations, energy expenditure, and physiological stress, which ultimately affect the ADFI, ADG, and FCR of chickens when birds are moved into free-range systems.

Throughout the experimental period, growth performance from 56 to 98 D of age was unaffected by increasing free-range days. This finding is consistent with a previous study using local chickens (Tong et al., 2015). However, other studies on broiler chickens found that ADG and ADFI were affected by free-range days (Castellini et al., 2002; Wang et al., 2009). This difference might be attributed to the different experimental breeds. Commercial broiler breeds differ from local chicken breeds in terms of both their growth and developmental patterns (Verdiglione and Cassandro, 2013).

Carcass Traits

Carcass yield is an important trait for poultry production and may affect both consumers' purchase intention and poultry production profits (Tong et al., 2015; Zhang et al., 2018b). In the current study, leg and foot yields decreased linearly with increasing free-range days, which may be ascribed to the fact that birds assigned to more free-range days had more physical exercise than those in the conventional treatment. More physical exercise may lead to higher energy expenditure, and accordingly may decrease leg and foot yields in chickens. A previous study, using an indigenous chicken breed, reported that 4 activity-related organ yields (leg, thigh, thigh bone, and foot yields) decreased with increasing free-range days (Tong et al., 2014).

In the present study, breast yield increased linearly with increasing free-range days, which might be due to increased motor activity of birds with increasing free-range days, which would further promote breast muscle mass. This result is supported by previous studies

(Sales, 2014; Tong et al., 2015). However, several studies have indicated that free-range systems do not affect activity-related organ yields (Fanatico et al., 2005; Wang et al., 2009). Differences might have arisen because these studies did not compare birds from treatments with different numbers of free-range days, but compared birds from a free-range system with individuals from other rearing systems.

Abdominal fat yield decreased linearly with increasing free-range days in the present study, which can be attributed to greater energy expenditure by the birds. Increased motor activity usually increases birds' metabolic rate and energy and fat expenditure, and thus reduces abdominal fat deposition in birds (Li et al., 2016). In agreement with our findings, previous studies reported that birds from free-range treatments showed lower abdominal fat yields (Wang et al., 2009; Jiang et al., 2011).

Meat Quality

In the modern poultry industry, meat quality is crucial for poultry products (Chen et al., 2013a). Consumers tend to buy free-range or organic products because they are characterized as having better meat quality and flavor (Sales, 2014; McFadden and Huffman, 2017). In addition, meat quality is a complex trait which is influenced by genetic strain (Castellini et al., 2008), physical activity (Tong et al., 2014), and pasture intake (Ponte et al., 2008). In the current study, meat quality was evaluated by drip loss, shear force, meat color, and pH of the thigh muscle.

Drip loss is a common indicator of meat water-holding capacity (Huff-Lonergan and Lonergan, 2005). A higher drip loss means greater loss of soluble nutrients and flavor substances (Liu et al., 2011). In this study, the drip loss decreased with increasing free-range days. Exercise stress is regarded as one of the most important factors affecting the drip loss of meat (Young et al., 2009). The lower drip loss with more free-range days might be ascribed to the increased exercise stress on thigh muscles.

Shear force is an important parameter of tenderness, which is the so-called eating quality (Piorkowska et al., 2016). In the present study, shear force increased linearly with increasing free-range days, indicating that the tenderness of thigh muscles decreased with increasing free-range days. This finding might be due to the increased motor activity with increasing free-range days; more exercise would contribute to development of the skeletal muscle, and thus influence the shear force of the thigh muscle.

Meat color is another important indicator of meat quality, especially in boneless products, and is also a critical parameter for consumers (Chen et al., 2013a). In the current study, the L* value of the thigh muscle decreased with increasing free-range days. The L* value indicates the degree of paleness, which is related to poor meat quality. This result is in accordance with that of a previous study (Castellini et al., 2002). Moreover, it was demonstrated that the meat from indoor birds had higher L* values than that from free-range birds (Fanatico et al., 2007).

Muscle pH is a good indicator of the stability and preservation of meat, and a high muscle pH leads to inferior shelf-life stability, especially since it is associated with microbial growth. In addition, the ultimate pH value is determined by the glycogen content of the muscle (Nissen and Young, 2006). In the present study, there was no significant influence of free-range days on the muscle pH, which was consistent with the results of previous studies that reported that the muscle pH of local chicken breeds was nearly unaffected by free-range treatment (Wang et al., 2009; Tong et al., 2014).

Lymphoid Organ Indices

Lymphoid organ weights and organ to BW ratios are commonly used to roughly estimate the immune response of poultry (Pope, 1991). A previous study indicated that the developmental status of the lymphoid organ and the associated organ to BW ratios played key roles in the immune responses of chickens (Gore and Qureshi, 1997). In the present study, the thymus was the only lymphoid organ that showed a significant increase with increasing free-range days. This finding was confirmed by that of a previous study showing that free-range days affected thymus weight and thymus to BW ratio (Tong et al., 2014). This finding is likely due to increasing free-range days promoting bird activity and animal welfare, which leads to lower susceptibility to infection (Alpigiani et al., 2017). Exercise can also promote immune function (Souza et al., 2017). Furthermore, a previous study suggested that reducing welfare problems could not only improve broiler welfare, but also decrease the risk of Campylobacter contamination (Alpigiani et al., 2017). The thymus is a primary lymphoid organ of the immune system, and loss of the thymus at an early age can lead to severe immunodeficiency and result in high susceptibility to infection (Khoso et al., 2015).

Blood Biochemical Parameters

Blood biochemical parameters are commonly used indicators to infer the physiological and metabolic status of poultry (Zhang et al., 2018b), and provide basic knowledge for studies in immunology and comparative avian pathology (Rehman et al., 2017). In the present study, serum glucose and total protein decreased linearly with increasing free-range days. Glucose is important for maintenance of energy levels and biochemical metabolism (Humphrey et al., 2004), and serum total protein reflects the quality of dietary protein (Akbarian et al., 2015). Our results might be attributed to the increase in free-range days promoting motor activity. Exercise stimulates insulin secretion, which accelerates glucose metabolism (Rehman et al., 2017). Similarly, more exercise could improve adrenal function and promote protein synthesis, which lead to nitrogen loss (Lumeij and Westerhof, 1987).

In the present study, the albumin, globulin, and AST contents were unaffected by increasing free-range days. Albumin and globulin, which are abundant proteins in blood serum, are good indicators of inflammation. Moreover, serum AST activity reflects the health of the liver, and high levels indicate a higher risk of hepatocellular damage (Han et al., 2008). Therefore, increasing free-range days did not appear to increase the risk of inflammation in local chickens.

Cholesterol, triglyceride, and HDL-C are strongly associated with fat deposition (Zhang et al., 2015). Cholesterol plays an essential role in membrane structure and as a precursor for the synthesis of metabolic substances, including steroid hormones, bile acids, and vitamin D. LDL-C plays an important role in the transportation of total cholesterol (**TC**) from the liver to other tissues, while HDL-C plays a crucial role in the transportation of TC from other tissues to the liver for excretion (Xiao et al., 2017). Triglyceride is an ester derived from glycerol and fatty acids, and it is difficult for triacylglycerol-rich lipoproteins, such as chylomicrons and large VLDL, to cross the endothelial barrier and enter the arterial intima (Brighenti, 2007). In the present study, the levels of cholesterol and triglyceride decreased with increasing free-range days, while the level of HDL-C increased. These results indicate that free-range days have a negative effect on fat deposition, which is consistent with the measurements of abdominal fat in the present study (linear decrease with increasing free-range days). Therefore, increasing free-range days reduce fat deposition in Wannan Yellow chickens.

In summary, BW of birds decreased significantly in the first 2 wk after birds were assigned to free-range treatments. Furthermore, increasing free-range days advantageously affected breast yield, shear force, thymus weight, thymus weight to BW ratio, and HDL-C content, but led to decreased leg yield, foot yield, drip loss, L* value of thigh muscle, glucose, total protein, cholesterol, and triglyceride levels of Wannan Yellow chickens. The findings of this study have important implications for the future breeding and farming of Wannan Yellow chickens.

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AUTHORS' CONTRIBUTIONS

ZG and SJ conceived and designed the experiments; LY, HZ, YX, XC, and PL performed the experiments and collected the samples; SJ, YL, and HZ analyzed the data; SJ and YL wrote the manuscript; SJ and ZG participated in the revision of the manuscript. All authors read and approved the final version of the manuscript.

COMPETING INTERESTS

The authors have declared that no conflict of interest exists.

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