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Original Research Article

A comparative study to determine the effects of breed and feed restriction on glucose metabolism of chickens

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ABSTRACT

The glucose metabolism of poultry draws wide attention as they have nearly twice the fasting blood glucose than that of mammals. To define the relationship between glucose metabolism and breed of chicken, the outcomes from different growth rate chickens showed that Arbor Acres (AA) broilers, a well-known fast-growing breed, had a lower fasting blood glucose concentration and glucose clearance rate when compared to Silky chickens, a Chinese traditional medicinal chicken with black skin and a slow growth rate. Moreover, AA broilers had a relatively slow rise in blood glucose in response to oral glucose solution than the Silky chickens on 21 and 42 d ($P < 0.05$), which is probably attributed to downregulated expression of pancreatic insulin (*INS*), and upregulated transcription of phosphoenolpyruvate carboxy kinase 1 (*PCK1*) and glucose transporter 2 (*GLUT2*) in the liver of AA broilers ($P < 0.05$). In response to feeding restriction from 7 to 21 d, both the fasting blood glucose and the response speed of AA broilers to oral glucose were increased on d 21 ($P < 0.05$), and the serum glucose concentrations after 3 weeks compensatory growth were improved by early feed restriction in AA broilers. Feed restriction could also upregulate the mRNA level of pancreatic *INS* on d 21 and 42, as well as decrease the expressions of *PCK1*, glucose-6-phosphatase catalytic (*G6PC*), and *GLUT2* in the liver on d 21 ($P < 0.05$) when compared to the free feeding group. These results revealed that Silky chickens have a stronger capability to regulate glucose homeostasis than AA broilers, and feed restriction could improve the fasting blood glucose and the response to oral glucose of AA broilers.

1. Introduction

As an important process for maintaining cell energy metabolism and life activities, glucose metabolism is directly related to the feed conversion rate and therefore economic benefits in the production of livestock and poultry. Glucose metabolism disorders might lead to metabolic disturbance, a major risk factor for insulin resistance and type 2 diabetes (Després and Lemieux, 2006; Wilmot and Idris, 2014), which are characterized by impaired fasting glucose and glucose tolerance (Tsuchida et al., 2020). Of note, poultry are noted to have nearly twice the fasting blood glucose than mammals (Braun and Sweazea, 2008). It is probably associated with insulin resistance (Akiba et al., 1999), i.e. the bird is less sensitive to insulin or the known diabetogenic streptozotocin than mammals (Modak et al., 2007; Sinaiko and Caprio, 2012). Accordingly, chickens could provide an alternative model to analyze specific aspects of diabetes and insulin resistance for domestic animals and humans (Datar and Bhonde, 2011).

Data from our recent research indicated that the glucose metabolism of chickens depends on their breed, as evidenced by faster blood glucose recovery under exogenous insulin stimulation in Silky chickens, a Chinese traditional medicinal chicken with black skin and a slow growth rate, compared with Arbor Acres (AA)

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broilers, which is one well-known fast-growing breed (Ji et al., 2020). Sung et al. also deemed that the difference in growth rate may be related to a difference in glucose metabolism mechanism (Sung et al., 2020). This suggests that the relationship between glucose metabolism and breed of chicken needs to be further defined. In addition, dietary restriction is widely used in both human and animal production to control body weight (BW) and avoid many obesity-related diseases to improve metabolic adaptability (Murphy et al., 2012), glucose balance, lipid distribution, and cardiovascular health (Walford et al., 1992; Heilbronn et al., 2006). As far as chickens are considered, they have undergone intensive genetic selection for approximately 60 generations for rapid growth (Cônsolo et al., 2020). One unwanted side effect of this genetic gain is the increase in the incidence of metabolic disorders (Sumners et al., 2014). Early feed restriction could possibly promote balanced organ development (Hansen et al., 1995), improve feed conversion efficiency, enhance the disease resistance of broilers (Urdaneta-Rincon and Leeson, 2002; Orso et al., 2019), and, promote the glucose metabolism of modern chickens.

As mentioned above, we hypothesized that different breeds of chickens have different glucose metabolism mechanisms, and restricted feeding might be involved in regulating the glucose metabolism process in chickens. Specifically, the objectives of this work were: 1) to understand the effects of breed on the glucose tolerance of chickens at different ages; and 2) to study the relationship between restricted feeding and glucose metabolism in AA broilers.

2. Materials and methods

2.1. Animal ethics statement

All the procedures in this experiment were approved by the Henan Agricultural University Laboratory Animal Welfare and Animal Experimental Ethical Inspection Committee (SKLAB-B-2010-003-01) and conducted following the Guidelines for Experimental Animals.

2.2. Animals and management

One-day-old AA broilers ($n = 200$) and Silky chickens ($n = 100$) were obtained from a commercial hatchery. After sex determination, birds were labeled and reared in individual cages (1.9 m × 0.5 m × 0.5 m) situated in a temperature- and humidity-controlled room. The temperature was 32 °C for 1 to 7 d and then gradually reduced to 22 °C by 20 d based on normal management practices. The light schedule was 23 L:1 D and 18 L:6 D during d 1 to 7 and beyond, respectively. Birds were fed the same diet, which was formulated to meet or exceed the nutrient requirements of both AA broilers and Silky chickens according to our previous research as shown in Table S1 (Wang et al., 2020).

Experiment 1: comparison between silky and AA broilers regarding glucose metabolism.

Glucose tolerance tests (GTT) were performed at d 21 and 42. In detail, after fasting for 16 h, the male chickens were given glucose solution at 2 g/kg BW orally, and then the blood glucose concentration was measured at 0, 5, 15, 30, 60, 120, 240, and 300 min via the wing vein using a blood glucose meter (ACCU-CHEK Performa, Roche, Germany). The area under the blood glucose curve (AUC) and the blood glucose clearance rates from 15 to 120 min were calculated according to a previous method (Gilbert et al., 2011; Purkayastha et al., 2011).

Experiment 2: glucose metabolism response to restricted feeding in AA broilers. One-day-old mixed-sex AA broilers were fed to 7 d ad libitum, then those birds with similar BW were randomly divided into a restricted feeding group (RG) and a free feeding group (FF). In the restricted feeding stage (d 7 to 21), the feeding time of the RG group was 08:00 to 13:00, while the birds of the FF group had access to feed at libitum. From d 22 to 49, all groups had free access to feed and water (Fig. S1). After fasting for 16 h, GTT were performed on the broilers at d 21 and 42. At d 21, 42 and 49, 12 chickens from each group were euthanized by cervical dislocation after being fasted overnight for further sampling.

2.3. Data collection and sampling

All chickens were weighed individually on d 7, 21 and 42, respectively, and the average daily gain (ADG) of broilers was calculated. After 16 h fasting, pancreas and liver tissues of each chicken were collected at d 21 and 42, respectively (male, $n = 8$). Pancreatic and liver samples were obtained, immediately snap-frozen in liquid nitrogen, and stored at −80 °C for determination of glucose regulatory gene expression. At 49 d, blood samples were harvested from each male bird at basal status for the analysis of serum biochemical parameters. After euthanasia, the heart, liver, spleen, pancreas, thymus, breast muscle, leg muscle, and abdominal fat pad were excised and weighed immediately. The relative organ weights (% BW) were calculated. The pancreases of the chickens were fixed in 10% neutral buffered formalin for histological analysis sectioning.

2.4. Histological analysis of pancreas

Harvested pancreas samples were fixed, embedded, and sliced. For microarchitecture, the sections were stained using hematoxylin and eosin, the micrographs of sections were taken through a microscope (Olympus CKX53, Japan), and an image analyzer (Image Pro-Plus, Rockville, MD). The islet area and islet number were measured based on a previous report (Sumners et al., 2014). Total cross-sectional area was quantified using the automated measurement feature by employing the intensity threshold method using image analysis software (Image J v1.8.0). The manual draw feature was used to trace the islet areas and all areas were quantified using the formula: % pancreatic islet area (Pancreas islet proportion) = islet area/pancreas area × 100%.

2.5. Serum biochemical parameters

Serum metabolites including glucose (GLU, A154-1-1), urea (UREA, C013-2-1), uric acid (URIC, C012-2-1), cholesterol (CHOL, A111-1-1), high density lipids (HDL, A112-1-1), low density lipids (LDL, A113-2-1), triglycerides (TG, A110-1-1), alanine aminotransferase (ALT, C009-2-1), aspartate aminotransferase (AST, C010-2-1), glutamyl transpeptidase (GGT, C017-2-1), and creatine kinase (CK, A032-1-1) were determined by an automatic biochemistry analyzer (Hitachi 7600, China). The assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.6. Gene expression assays

Total RNA was extracted from the liver and pancreas using Trizol reagent (TransGen Biotech Co. Ltd, Beijing, China), and then reverse transcribed into cDNA using a reverse transcription kit (Vazyme Biotech Co. Ltd, Nanjing, China). The obtained cDNA was used to determine the mRNA expression of genes involved in glucose regulation using a BioRad CFX96 system (BioRad, America) with SYBR qPCR Master Mix (Vazyme Biotech Co. Ltd, Nanjing, China). In detail, target cDNA was amplified by 35 cycles (1 cycle: 95 °C for

10 s, 60 °C for 30 s) and a standard curve was generated to estimated reaction efficiency and gene expression. Primers were designed using online Primer 3 and are shown in Table S2, which were further determined once the amplification efficiency ranged from 80% to 120%. Each gene was performed in triplicate and *β-actin* was used as the reference gene to normalize the expression level of the targeted gene using the $2^{-\triangle\triangle CT}$ method (Livak and Schmittgen, 2001).

2.7. Statistical analysis

The statistical analysis was performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA) statistical software. The data obtained were analyzed by the Shapiro–Wilk and Levene's tests to assess normal distribution and homogeneity of variances, in which all data were normally distributed except leg muscle rate. Therefore, the leg muscle rate was analyzed by the Mann–Whitney U test, and the rest of data were compared using the two-tailed unpaired Student's *t*-test. In addition, blood glucose at different time points in the GTT was analyzed using one-way analysis of variance (ANOVA) and differences among treatments were detected by Tukey's post hoc comparison. Statistical significance was assigned at $P < 0.05$. All data were presented as means ± standard deviation (SD).

3. Results

3.1. Effect of breed on performance and the relative organ weights

As shown in Table 1, the BW and ADG of AA broilers at different stages was higher than that of Silky chickens ($P < 0.01$). At 49 d, the liver index, thymus index, and abdominal fat ratio were higher ($P < 0.05$) in Silky chickens compared with AA broilers (Table 2). Of note, the proportion of pancreas in Silky chickens was significantly higher ($P < 0.01$) than that in AA broilers.

3.2. Impact of breed on serum biochemistry

The effects of breed of chicken on serum biochemical indicators are presented in Table 3. The level of serum glucose in Silky chickens was higher than that in AA broilers at 49 d ($P < 0.01$), whereas the contents of CHOL, HDL, CK, TG and LDL in Silky chickens were lower than those in AA broilers ($P < 0.05$).

3.3. Differences between silky chickens and AA broilers regarding glucose metabolism

The fasting blood glucose of Silky chickens at 21 d was higher ($P = 0.015$) than that of AA broilers (Fig. 1A), but there was no difference in fasting blood glucose level between the 2 breeds at 42 d ($P > 0.05$; Fig. 1B). At 21 d, after oral glucose challenge, the blood glucose of AA broilers did not peak until 30 min, with differences between breeds at 15, 30, 240 and 300 min ($P < 0.05$). After normalizing the initial blood glucose, the mean blood glucose of Silky chickens was higher ($P < 0.01$) than that of the AA broilers at 15 min (206.67 vs.123.00, Fig. 1C) and 30 min (196.75 vs.132.25, Fig. 1C). At 42 d, the Silky chickens responded more quickly to the oral glucose, having a peak in mean blood glucose at 15 min, but the AA broilers did not peak until 60 min, with a difference between breeds at 5, 15 and 60 min ($P < 0.05$). After normalizing the initial blood glucose, the blood glucose of Silky chickens was higher than that of AA broilers at 15 min ($P < 0.05$), while it was lower at 60, 120 and 300 min ($P < 0.05$) after oral glucose (Fig. 1D). Even though AUC did not differ between two breeds at 21 and 42 d (Fig. 1E and F), the Silky chickens were more efficient in clearing circulating glucose from the bloodstream and their glucose clearance rate was higher in the GTT at 21 and 42 d ($P < 0.01$; Fig. 1G and H).

Table 1
Effects of breed on growth performance of chickens.

Item	Silky chicken	AA broiler	*P*-value
Body weight, g			
1 d	34.72 ± 0.57	43.24 ± 1.00	<0.001
7 d	67.83 ± 0.86	128.47 ± 3.02	<0.001
21 d	228.07 ± 7.64	849.69 ± 23.75	<0.001
42 d	601.35 ± 22.43	2,428.89 ± 75.80	<0.001
Average daily gain, g/d			
1–7 d	5.52 ± 0.17	14.20 ± 0.47	<0.001
8–21 d	11.45 ± 0.51	51.52 ± 1.64	<0.001
22–42 d	17.78 ± 0.79	75.20 ± 3.27	<0.001
1–42 d	13.82 ± 0.55	58.19 ± 1.84	<0.001

Data represent means with standard deviation (male, $n = 10$).

Table 2
Effects of breed on carcass performance of chickens at 49 d (%).

Item	Silky chicken	AA broiler	*P*-value
Heart index	0.36 ± 0.01	0.31 ± 0.03	0.110
Liver index	2.30 ± 0.06	1.77 ± 0.08	0.001
Pancreas index	0.22 ± 0.01	0.15 ± 0.01	0.001
Thymus index	0.51 ± 0.08	0.17 ± 0.03	0.011
Half net carcass rate	88.45 ± 0.59	86.97 ± 0.48	0.110
Whole net carcass rate	71.05 ± 0.66	75.74 ± 0.43	0.001
Abdominal fat rate	1.82 ± 0.29	0.97 ± 0.13	0.034
Breast muscle rate	4.78 ± 0.12	8.75 ± 0.45	0.002
Leg muscle rate	6.65 ± 0.10	7.86 ± 0.49	0.018

Values are expressed as a percentage of BW just before slaughter. Data are presented as means with standard deviation (male, $n = 8$).

3.4. Alteration in islet numbers of silky chickens and AA broilers

As illustrated in Fig. 2A, the number of islets in Silky chickens was higher compared with AA broilers. Quantitatively, the proportion of islets in Silky chickens was higher than in AA broilers ($P = 0.012$; Fig. 2B).

3.5. Glucose regulatory gene expression in silky chickens and AA broilers

In the pancreas, the mRNA expression of insulin (*INS*) and glucagon (*GCG*) in Silky chickens was higher (both $P < 0.05$) than in

Table 3
Effects of breed on serum parameters of chickens at 49 d (mmol/L).

Item	Silky chicken	AA broiler	*P*-value
UREA	0.65 ± 0.02	0.60 ± 0.11	0.688
URIC, μmol/L	329.00 ± 53.63	224.50 ± 54.24	0.219
GLU	14.56 ± 0.45	7.71 ± 0.38	<0.001
CHOL	2.37 ± 0.145	3.33 ± 0.12	0.002
TG	0.25 ± 0.02	0.41 ± 0.05	0.011
HDL	1.96 ± 0.10	2.43 ± 0.06	0.005
LDL	0.39 ± 0.05	0.94 ± 0.18	0.007
ALT, U/L	1.00 ± 0.13	1.95 ± 0.46	0.130
AST, U/L	261.88 ± 19.34	496.30 ± 83.26	0.063
AST to ALT ratio	283.19 ± 34.88	275.38 ± 37.09	0.886
GGT, U/L	22.83 ± 1.42	19.25 ± 1.49	0.132
CK, U/L	8,512.67 ± 1,440.63	16,459.25 ± 1,107.06	0.004

UREA = urea; URIC = uric acid; GLU = glucose; CHOL = cholesterol; TG = triglycerides; HDL = high density lipids; LDL = low density lipids; ALT = alanine aminotransferase; AST = aspartate aminotransferase; AST to ALT ratio = aspartate aminotransferase to alanine aminotransferase ratio; GGT = glutamyl transpeptidase; CK = creatine kinase.
Data are presented as means with standard deviation (male, $n = 8$).

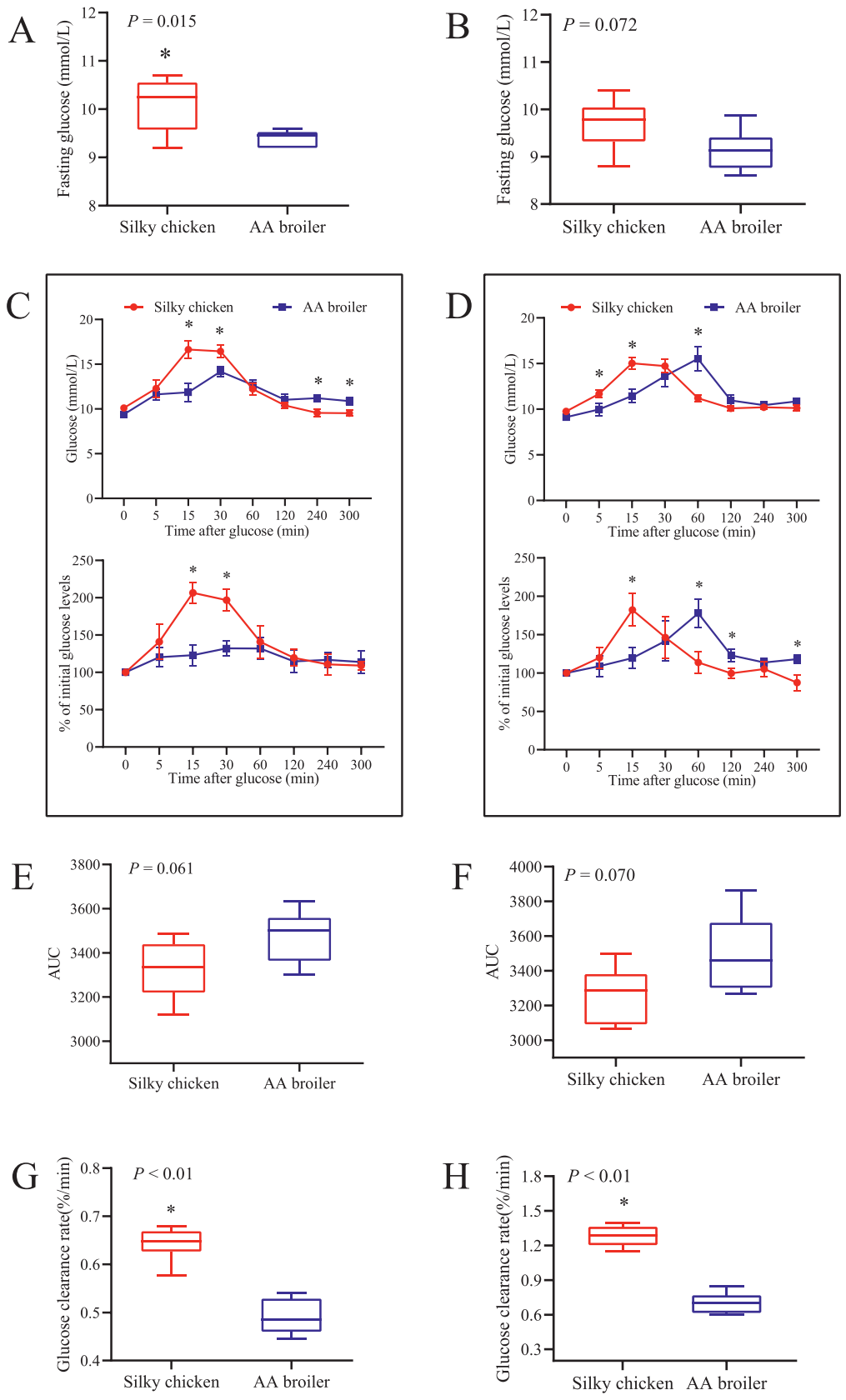

Fig. 1. Differences in glucose metabolism between Silky chickens and AA broilers. (A) Comparison of fasting blood glucose at 21 d. (B) Differences in fasting blood glucose at 42 d. (C and D) Glucose tolerance test (GTT) results and blood glucose change relative to initial value after glucose treatment at 21 and 42 d. Silky chickens and AA broilers were fasted for 16 h. Chickens were given oral glucose (2 g/kg body weight; 20% wt/vol H_2O). (E and F) The area under the curve of blood glucose (AUC) at 21 and 42 d. (G and H) Glucose clearance rate (%/min) at 21 and 42 d. Data are presented as means with standard deviation (male, $n = 6$, $^*P < 0.05$).

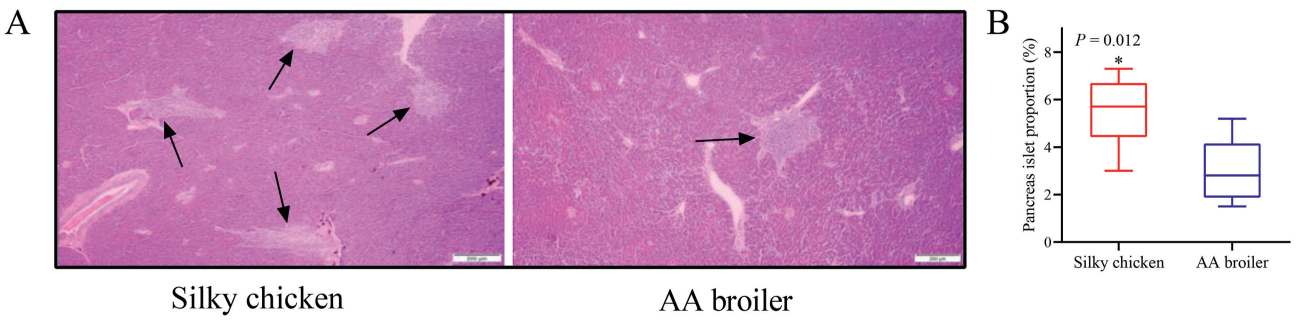

Fig. 2. Effect of breed on pancreatic physiology at 49 d. (A) Representative pancreas section of Silky chickens and AA broilers (magnification 100 × , scale bar = 200 μm). (B) Relative pancreas islet (percentage pancreas weight, male, $n = 6$). Arrows indicate pancreas islet. Mean values with an asterisk are significantly different from AA broilers (*$P < 0.05$).

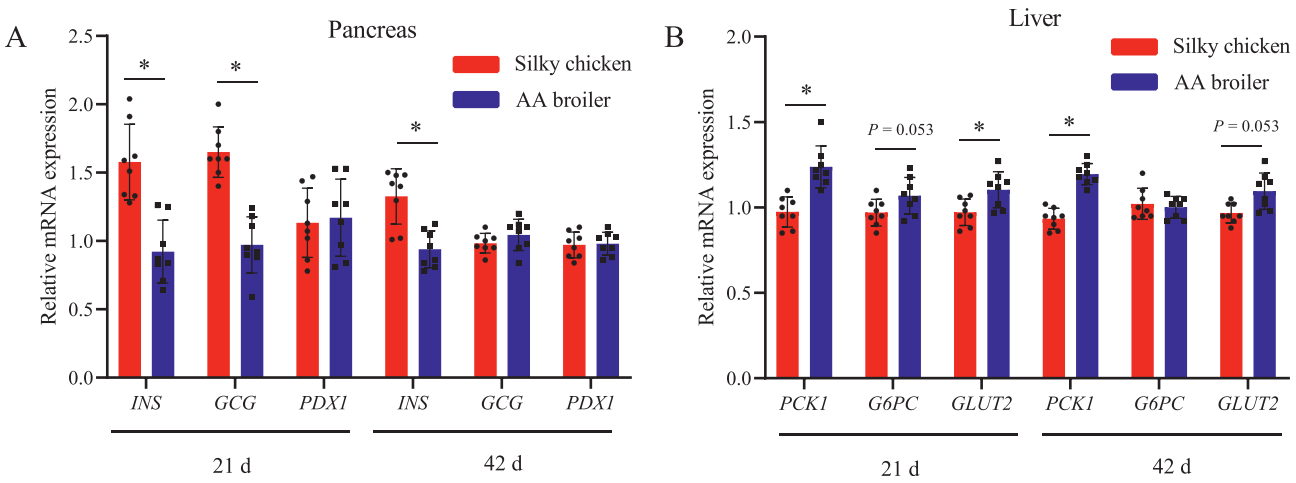

Fig. 3. Expression of glucose-regulated genes in the pancreas and liver between Silky chickens and AA broilers. (A) Transcription level of insulin (*INS*), glucagon (*GCG*), pancreatic and duodenal homeobox 1 (*PDX1*) in the pancreas at 21 and 42 d. (B) The mRNA expression of hepatic glucose regulatory genes at 21 and 42 d, including phosphoenolpyruvate carboxy kinase 1 (*PCK1*), glucose-6-phosphatase catalytic (*G6PC*), glucose transporter 2 (*GLUT2*). Mean values with an asterisk are significantly different from AA broilers (male, $n = 8$, *$P < 0.05$).

AA broilers at 21 d (Fig. 3A). At 42 d, the breeds failed to affect the expression of duodenal homeobox 1 (*PDX1*) and *GCG* mRNA, whereas the mRNA level of *INS* was notably increased ($P < 0.05$) in Silky chickens (Fig. 3A). Regarding the mRNA expression of hepatic glucose regulatory genes, the mRNA levels of phosphoenolpyruvate carboxykinase 1 (*PCK1*), glucose-6-phosphatase catalytic (*G6PC*), and glucose transporter 2 (*GLUT2*) were decreased in the liver of Silky chickens when compared with AA broilers at 21 d (Fig. 3B). Similarly, the transcription levels of *PCK1* and *GLUT2* but not *G6PC* were downregulated in Silky chickens relative to AA broilers at 42 d (Fig. 3B).

3.6. Performance and the relative organ weights in response to restricted feeding in AA broilers

The effect of feed restriction on BW and ADG are presented in Table 4. After feed restriction from d 7 to 21, the BW at d 21 and ADG over that period of the FF group were higher than the RG group ($P < 0.01$). However, in the compensatory growth period from 22 to 42 d, the ADG of the RG group was higher than that of the FF group ($P < 0.05$; Table 4). At 49 d, the thymus index of the FF group was lower than that of the RG group ($P < 0.05$; Table 5).

3.7. Serum biochemistry response to restricted feeding in AA broilers

The serum glucose content and AST to ALT ratio of the RG group were higher than that of the FF group ($P < 0.05$; Table 6). No differences were found for the rest of the serum biochemical parameters between the RG and FF group at 49 d (Table 6).

3.8. Effect of restricted feeding on glucose metabolism in AA broilers

Restricted feeding increased ($P = 0.02$) fasting blood glucose of chickens in 21 d (Fig. 4A), while after 3 weeks' compensatory growth it was only slightly higher at 42 d ($P = 0.133$; Fig. 4B). At 21 d, the blood glucose of the RG group peaked at 15 min after oral glucose, whereas that of the FF group peaked at 30 min. The blood glucose of the RG group was higher than that of the FF group at

Table 4
Effects of restricted feeding on growth performance of AA broilers (g).

Item	FF	RG	*P*-value
Body weight			
1 d	42.29 ± 0.62	41.72 ± 0.47	0.103
7 d	129.35 ± 2.52	137.12 ± 2.65	0.058
21 d	832.00 ± 18.58	488.73 ± 9.05	<0.001
42 d	2337.14 ± 70.64	2258.33 ± 45.87	0.547
Average daily gain			
1–7 d	14.34 ± 0.40	15.90 ± 0.47	0.107
8–21 d	50.19 ± 1.27	25.11 ± 0.53	<0.001
22–42 d	71.67 ± 3.20	84.27 ± 2.31	0.011
1–42 d	55.89 ± 1.72	54.06 ± 1.11	0.607

The free feeding group (FF) was fed ad libitum and the restricted feeding group (RG) was fed from 08:00 to 13:00 for a restricted feeding period of 7–21 d. Data are presented as means with standard deviation (male, $n = 10$).

Table 5
Effects of restricted feeding on carcass performance of AA broilers at 49 d (%).

Item	FF	RG	*P*-value
Heart index	0.30 ± 0.02	0.32 ± 0.01	0.587
Liver index	1.82 ± 0.08	1.92 ± 0.07	0.603
Thymus index	0.17 ± 0.01	0.28 ± 0.03	0.026
Pancreas index	0.15 ± 0.01	0.17 ± 0.01	0.234
Half net carcass rate	87.71 ± 0.71	86.99 ± 0.42	0.795
Whole net carcass rate	76.98 ± 0.78	76.54 ± 0.83	0.936
Abdominal fat rate	1.16 ± 0.12	1.17 ± 0.15	0.982
Breast muscle rate	9.82 ± 0.46	9.50 ± 0.37	0.804
Leg muscle rate	7.55 ± 0.30	7.29 ± 0.25	0.758

Values are expressed as a percentage of BW just before slaughter. The free feeding group (FF) was fed ad libitum, the restricted feeding group (RG) was fed from 08:00 to 13:00 for a restricted feeding period of 7–21 d. Data are presented as means with standard deviation (male, $n = 8$).

15 min after oral glucose was given ($P < 0.05$; Fig. 4C). After the initial blood glucose was normalized, the blood glucose of the FF group was higher than that of the RG group at 60 min ($P < 0.05$) after oral glucose at 21 d (Fig. 4C). After 3 weeks' growth compensation, the blood glucose of the RG group reached the peak at 30 min after oral glucose at 42 d, while that of the FF group reached the peak at 60 min. The blood glucose of the FF group was higher than that of the RG group at 60 min after oral glucose ($P < 0.05$; Fig. 4D). After normalizing the initial blood glucose, the blood glucose of the FF group was higher ($P < 0.05$) than that of the RG group at 60 min after oral glucose at 42 d (Fig. 4D). However, no differences were found for AUC ($P > 0.05$; Fig. 4E and F) and the glucose clearance ($P > 0.05$; Fig. 4G and H) between the FF and RG groups after GTT at d 21 and 42.

3.9. Glucose regulatory gene expression response to restricted feeding in AA broilers

Compared with the FF group, the expression of glucose regulatory genes was increased in the pancreas of RG chickens at 21 d, especially *INS* and *GCG* ($P < 0.01$; Fig. 5A). At 42 d, early feed restriction notably increased the mRNA level of *INS* after compensatory growth ($P < 0.01$), whereas it failed to affect the mRNA levels of *PDX1* and *GCG* (Fig. 5A). In liver, restricted feeding reduced the expression of *PCK1*, *G6PC* and *GLUT2* at 21 d ($P < 0.05$; Fig. 5B), but after 3 weeks of compensatory growth, these trends were eliminated ($P > 0.05$; Fig. 5B).

Table 6
Effects of restricted feeding on serum parameters of AA broilers at 49 d.

Item	FF	RG	*P*-value
UREA, mmol/L	0.65 ± 0.06	0.69 ± 0.04	0.594
URIC, μmol/L	208.33 ± 38.03	166.38 ± 24.60	0.382
GLU, mmol/L	8.40 ± 0.34	11.60 ± 0.47	<0.001
CHOL, mmol/L	3.73 ± 0.24	4.27 ± 0.22	0.122
TG, mmol/L	0.44 ± 0.04	0.37 ± 0.02	0.155
HDL, mmol/L	2.69 ± 0.12	3.01 ± 0.13	0.086
LDL, mmol/L	0.99 ± 0.01	1.08 ± 0.10	0.610
ALT, U/L	2.06 ± 0.26	2.13 ± 0.18	0.833
AST, U/L	587.11 ± 63.17	486.43 ± 49.76	0.238
AST to ALT ratio	298.78 ± 21.32	231.17 ± 16.79	0.027
GGT, U/L	24.78 ± 2.28	27.88 ± 2.72	0.393
CK, U/L	21,022.44 ± 4014.50	19,920.88 ± 2076.60	0.818

UREA = urea; URIC = uric acid; GLU = glucose; CHOL = cholesterol; TG = triglycerides; HDL = high density lipids; LDL = low density lipids; ALT = alanine aminotransferase; AST = aspartate aminotransferase; AST to ALT ratio = aspartate aminotransferase to alanine aminotransferase ratio; GGT = glutamyl transpeptidase; CK = creatine kinase.
The free feeding group (FF) was fed ad libitum and the restricted feeding group (RG) was fed from 08:00 to 13:00 for a restricted feeding period of 7–21 d. Data are presented as means with standard deviation (male, $n = 8$).

4. Discussion

In the present study, in line with the breed standard (Rodrigues and Choct, 2019), AA broilers exhibited higher BW than Silky chickens, which was related to the genetic characteristics of high feed intake and fast growth rate of AA broilers (Te Pas et al., 2020). Accordingly, feed restriction decreased the feed intake and thus decreased the BW and ADG of AA broilers at 21 d, followed by compensatory growth from d 22 to 42 when removing feed restriction and achieving a similar final BW as compared to the free feeding group. The rapid growth was also found to result in some unwanted side effects in poultry, such as leg disease (Güz et al., 2020) and hepatic steatosis (Chen et al., 2019). The levels of ALT, AST, CK, and GGT are usually used as indicators of liver function, and increases are often associated with some metabolic diseases (Després and Lemieux, 2006; Tomizawa et al., 2014; Abro et al., 2018). Of note, the elevation of serum enzyme AST is thought to be due to release from injured liver. Compared to aminotransferases, CK is a more specific index of muscle injury (McMillan et al., 2011), whereas CK-adjusted aminotransferase level may present an even more accurate biomarker of liver damage (Wang et al., 2018). Therefore, in the present study, high levels of AST and CK in the serum of AA broilers indicated that the liver function of broilers may have been poor.

Chickens exhibit hyperglycemic characteristics during embryonic growth, early growth (Zhao et al., 2014), and the adult stage (Chen et al., 2012). In this study, the fasting blood glucose of all chickens fluctuated between 8 and 11 mmol/L, which was like the fasting blood glucose levels of other studies (Seki et al., 2003). We also noticed that AA broilers had a lower mean fasting blood glucose compared with Silky chickens. This might be attributed to genetic selection, i.e., the fast-growing strains of broilers had higher fasting insulin levels with slightly lower fasting glucose levels and similar glucose handling rates as compared to slow-growing strains (Simon et al., 2000). In addition, the genetically fat broilers had slightly higher plasma insulin but lower glucose and faster and more sensitive glucose handling than the lean broilers (Leclercq et al., 1988), similar to the fact that lean genotype sheep also had greater basal glucose concentrations than fat genotype sheep (Francis et al., 1999).

As the glucose reservoir, the liver helps to keep the circulating blood sugar levels constant, working together with the pancreas in a tightly controlled system. GLUT2 is a low-affinity glucose transporter and serves as the main transporter of glucose between liver and blood (Dai et al., 2020). PCK1 is a key gluconeogenic enzyme in liver (Hu et al., 2019). The high abundance of glucose regulatory genes, such as *PCK1* and *GLUT2*, in the liver of AA broilers suggested that AA broilers have a greater capacity for glucose absorption which may contribute to their fasting hypoglycemia and rapid growth.

Comparing with fasting blood glucose level, a GTT might be more efficient to reflect glucose disposal ability. In this study, the AA broilers (with fast growth) presented impaired glucose tolerance compared with Silky chickens, especially at 42 d, which may be attributed to impaired excretion by the pancreas. The pancreas plays an important role in nutrient metabolism and glucose homeostasis as the endocrine organ that excretes glucagon, insulin, somatostatin, and pancreatic polypeptide (Lavoie et al., 2010). In particular, insulin is a protein hormone and the only hypoglycemic hormone in the body (Zhao et al., 2017). In the current study, AA broilers had a lower relative weight and decreased pancreas islet proportion. Meanwhile the expression level of glucose regulatory genes, especially *INS*, was lower in the pancreas of AA broilers, which was line with our previous findings that when compared with Silky chickens, AA broilers had hyperinsulinemia, and the insulin receptor (IR) level was lower in the pancreas, meanwhile, they also presented with impaired

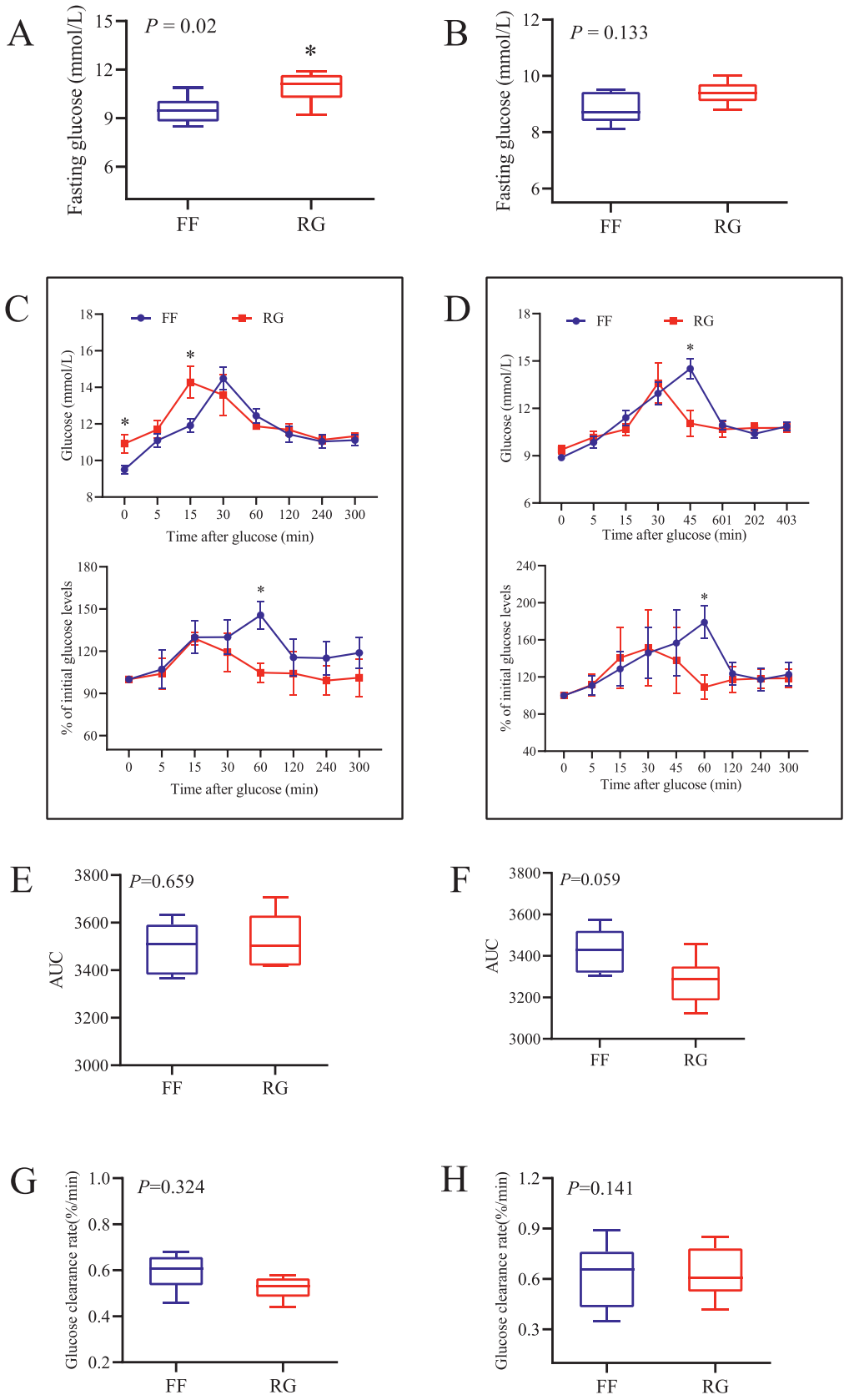

Fig. 4. Glucose metabolic response to restricted feeding in AA broilers. (A and B) Effect of restricted feeding on fasting glucose in 21 and 42 d AA broilers. The control group (FF) was fed ad libitum, and restricted feeding group (RG) was fed from 08:00 to 13:00 on d 7–21. (C and D) Glucose tolerance test (GTT) results and blood glucose change relative to initial value after glucose treatment at 21 and 42 d. Chickens in FF and RG were given oral glucose (2 g/kg body weight; 20% wt/vol H_2O) after 16 h of fasting. (E and F) The area under the curve of blood glucose (AUC) at 21 and 42 d. (G and H) Glucose clearance rate (%/min) at 21 and 42 d. Data are presented as means with standard deviation (both genders, $n = 6$, $^*P < 0.05$).

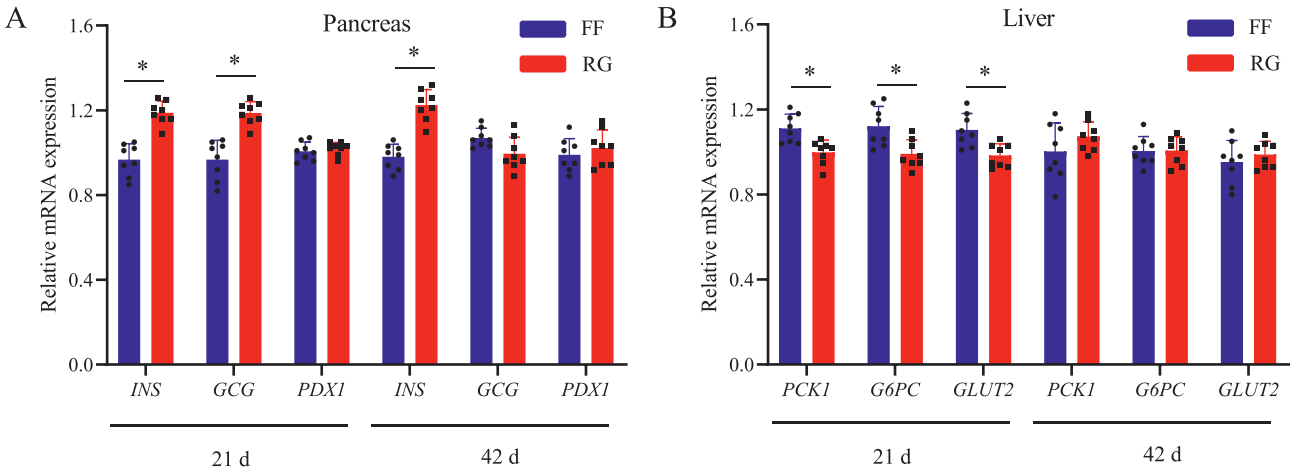

Fig. 5. Effects of restricted feeding on glucose-regulated genes in the pancreas and liver of broilers. The control group (FF) was fed ad libitum, and restricted feeding group (RG) was fed from 08:00 to 13:00 on d 7–21. (A) The mRNA expression of pancreas glucose regulatory genes at 21 and 42 d, including insulin (*INS*), glucagon (*GCG*), pancreatic and duodenal homeobox 1 (*PDX1*). (B) Transcription of phosphoenolpyruvate carboxy kinase 1 (*PCK1*), glucose-6-phosphatase catalytic (*G6PC*), glucose transporter 2 (*GLUT2*) in hepatic at 21 and 42 d. Mean values with an asterisk are significantly different from AA broilers (male, $n = 8$, $^*P < 0.05$).

regulation of blood glucose and serum insulin homeostasis under an insulin tolerance test (Ji et al., 2020).

It is well-known that relative hyperinsulinemia has been reported to result in increased triglyceride concentration independent of body fat content and distribution (Walton et al., 1995), whereas hyperinsulinism in chickens causes excessive lipid deposition in the aorta and elevation of blood lipids (Stout et al., 1973). Studies have shown that lipid levels are substantially influenced by glycemic control (Guy et al., 2009). Similarly, in the present study, the proportion of abdominal fat in AA broilers was lower than in Silky chickens, but the blood lipid levels (including TG, CHOL, HDL and LDL) were higher in AA broilers than Silky chickens, suggesting that blood lipids may be more sensitive in reflecting insulin tolerance than abdominal fat.

Similar to the blood glucose changes observed in the 2 breeds with high and low BW, the reduction in BW of broilers after feeding restriction was also accompanied by a significant increase in fasting blood glucose level at 21 d. The effect of early feed restriction on prompting serum glucose was still observed after the compensatory growth over 4 weeks. Besides, restricted feeding at d 7 to 21 also improved the response speed to oral glucose and glucose tolerance to some degree, especially at 21 d, which also reflects similar alterations in glucose-regulated genes in both the pancreas and liver of AA broilers at 21 and 42 d in response to feed restriction.

In summary, Silky chickens, which have a lower growth rate, displayed a stronger ability to regulate glucose homeostasis than AA broilers, as showed by the faster response to oral glucose. This might be associated with upregulated pancreatic glucose regulatory gene expression and reduced liver glucose regulatory gene mRNA abundance. Feed restriction could increase and decrease the expression of glucose-regulated genes in the pancreas and liver, respectively, to improve fasting blood glucose and the response to oral glucose. Our findings enhance the understanding of the glucose metabolism in chickens, which may be a good model for research in human obesity.

Author contributions

Pengfei Du: Animal trial, Laboratory and Statistical analysis, Writing; **Huanjie Wang**: Methodology, Laboratory analysis, Writing; **Xiuwen Shi:** Animal trial, Data collection and evaluation; **Xiangli Zhang:** Data evaluation, Manuscript review, Writing; **Yao Zhu:** Animal trial, Data evaluation; **Wen Chen:** Feed formulation, Data collection; **Huaiyong Zhang:** Data evaluation, Statistical analysis, Critical manuscript review; **Yanqun Huang**: Study design, Data evaluation, Critical manuscript review.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2023.02.005.

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