Changes in production performance, antioxidant capacity and immune status of meat ducks under different rearing systems

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Research

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Abstract

Background

As potential substitutes of traditional free-range rearing, floor rearing system (FRS) and net rearing system (NRS) are currently predominant dryland rearing systems. In this study, a total of 720 Nonghua ducks were assigned to a 2×3 factorial arrangement with two rearing systems (FRS and NRS) and three ages (4w, 8w, and 13w) to study the effects of FRS and NRS on production performance, antioxidant capacity and immune status.

Results

The production performance was mainly affected at 8w. Body weight, average daily gain, eviscerated weight and semi-eviscerated weight were higher in NRS at 8w, but carcass yield was decreased at 8w and 13w ($P < 0.05$). Lipid deposition was enhanced in NRS with higher sebum and abdominal fat yield at 8w and 13w ($P < 0.05$). NRS resulted in the liver developmental retardation at 4w and decreased gizzard index at all ages ($P < 0.05$). Antioxidant capacity was not affected by rearing systems ($P > 0.05$), but a tendency of better antioxidant capacity in NRS at 4w was found, while glutathione peroxidase (GSH-Px) activity was higher in NRS at 13w ($P < 0.05$). Spleen and thymus were promoted in NRS and immune cytokines were extensively affected by rearing system ($P < 0.05$), exhibiting general higher profiles of IFN- γ , IL-1 β , IL-4, immunoglobulins in NRS. Serum biochemical parameters (AST, ALT, and ALP) indicated NRS was better for liver health, and in liver, ALP was higher in FRS at 13w, while ALP and IFN- γ in 13w-FRS were both higher than 4w-FRS and 8w-FRS ($P < 0.05$).

Conclusions

Compared with FRS, NRS was conducive to production performance and duck liver health to some extent but it had defects in visceral organ development and lipid deposition. Though antioxidant capacity was not significantly affected, NRS ducks might have better antioxidant capacity at the early stage of breeding, while GSH-Px activity was increased for scavenging excess free radicals at the later one. NRS increased the levels of IFN- γ , IL-1 β , IL-4, immunoglobulins in serum and promoted the development of thymus and spleen to improve duck immune function. These results revealed the physiological impacts of FRS and NRS on ducks and provided a reliable reference for rearing system selection.

Background

China, as the major producer and consumer of broiler ducks in the world [1], has embarked on the evolution from the free-range rearing to intensive rearing in order to improve production efficiency [2], which is also a transformation of duck husbandry from water-associated to terrestrial [3]. Compared with the demand of extensive waters in traditional rearing system [1, 4], dryland rearing systems have common merit in minimizing the need of waterbodies to alleviate environmental issues, such as rivers and lakes, which could also prevent ducks from the incidence of intestinal diseases caused by pathogens in waters [5].

Dryland rearing systems are generally classified into the cage-rearing system (CRS), floor-rearing system (FRS) and net-rearing system (NRS). Though CRS makes full use of the breeding house space, ducks were vulnerable as individual activities are much constrained [1]. Whereas, FRS is usually to lay a thick pad of 5–10 cm by straw, sawdust or wood shavings on a cement floor [6–8], which could absorb excess moisture but has defects in rearing management. Plastic floor net-rearing and wire floor net-rearing are national wide applied NRS. Due to the materials, stainless wires are more durable than plastic floors though sharp edges should be avoided. Both of them can create better hygiene than other rearing systems as ducks have less contact with their excreta [2, 9]. Floor-type had either positive or negative correlations with the environment and physiological characteristics of ducks during rearing [10]. Previous studies on this variable indicated multifaceted effects on ducks. Study on Pekin ducks showed that dryland rearing ducks had higher body weight (BW) than equipped with a swimming pool in intensive rearing system [11]. Study on NRS and FRS also demonstrated production performance was significantly affected [9, 10, 12]. Based on higher levels of final BW, average daily gain (ADG), average daily feed intake (ADFI) and lower feed conversion rate (FCR) in NRS ducks, Zhang et al. [9] concluded Chaohu ducks had better growth performance in NRS than in FRS. By comparing plastic floor net-rearing and litter floor-rearing, Pekin ducks were also found higher ADG in NRS [10]. However, study on Cherry Valley ducks reported higher ADG and lower FCR in FRS, and concluded FRS was one of the best duck rearing systems compared with NRS [12].

Furthermore, animal health status is another important aspect of modern poultry production, especially in intensive rearing systems [13, 14]. Health status is generally associated with individual immunity and antioxidant capacity. Research has reported that the rearing system could significantly influence duck immune response to avian influenza vaccine of different breeds [15], and alter the key immune genes expression, such as toll-like receptor 7 (TLR7) in bursa, lung and intestinal tissues of indigenous ducks [16]. Study on Shaoxing ducks also reported an

impact of NRS on duck health [5]. Meanwhile, waterfowls in FRS suffered from fluctuated temperature [9, 12], either heat or cold stress caused by temperature change would possibly lead to oxidative stress of ducks causing the changes in antioxidant defense system and a threat to health [17–19]. However, few studies had been conducted to investigate the potential physiological effects of FRS and NRS on duck immune status and antioxidant capacity.

Therefore, the objectives of this paper were to study the effects of FRS and NRS on production performance, antioxidant capacity and immune status of Nonghua ducks, which is one of the most economically valuable broiler duck breeds with superior meat quality and strong disease resistance in Southwest China. Comparisons were made at different ages to explore general changes on Nonghua ducks, which would reveal the physiological impacts of predominant dryland rearing systems on meat ducks, and provide a reliable reference for rearing system selection in modern production.

Material And Methods

Animals and experiment design

The present study was performed at Sichuan Agricultural University (Sichuan, China). This study constituted a 2×3 factorial arrangement with 2 different rearing systems (FRS and NRS) and 3 different slaughtering ages (**4w**, appropriate time for meat-type ducks breeding; **8w**, market age; **13w**, market age). Nonghua duck is a new high-quality meat-type Chinese local Sheldrake crossbreed, which has been bred through five generations. This Sheldrake strain has a merit of roughage tolerance, excellent growth performance and carcass traits. A total of 1000 one-day-old Nonghua ducks from one commercial hatchery were wire floor brooded with a commercial starter diet (Table 1) for two weeks. At 14-day-old, 720 ducks with a male/female ratio of 1:1 were selected by similar initial BW, then ducks were randomly allocated into FRS and NRS (10 replicates of 18 ducks each) with males and females separated (namely, 180 ducks in each group). Ducks in FRS were reared on concrete floor with sawdust bedding of 5 cm, bedding materials were added appropriately during the experiment period and cleaned weekly. Ducks in NRS were reared on stainless wire mesh bed with 1.0 cm diameter mesh holes at a height of 50 cm above the ground, the excreta was cleaned weekly. All groups of ducks were reared in a well-ventilated house with same environmental conditions of natural light in the experimental waterfowl breeding farm of Sichuan Agricultural University (Sichuan, China). The temperature was set at 31 °C at first week and gradually decreased to 25 °C until 14 d of age. Afterward, it was kept at approximately 15 to 20 °C. The stocking density was kept at a consistent 5 ducks/m². The experimental diets (Table 1) were formulated in accordance with NY/T 2122 – 2012 [20]. All diets and water were provided *ad libitum* to ducks during the experimental period.

Table 1
Ingredients and nutrients composition of basal diets (% , as fed)

Items	0 to 2 W	3 to 13 W
Ingredients		
Corn	52.10	48.30
Soybean meal	32.90	23.10
Wheat middling	4.00	10.00
Rice bran	7.10	9.00
Wheat bran	-	6.00
Calcium phosphate	1.67	1.40
Limestone powder	0.88	0.90
Vitamin and mineral premix ¹	1.00	1.00
NaCl	0.35	0.30
Total	100	100
Nutrients		
Metabolizable Energy, Mcal/kg	2.85	2.80
Crude Protein	19.50	17.00
Crude fat	3.64	4.00
Crude fiber	3.59	3.88
Crude ash	6.53	6.05
Calcium	0.90	0.80
Total Phosphorus	0.73	0.70
Available phosphorus	0.42	0.38
Lysine	1.00	0.80
Methionine	0.42	0.38
Methionine + Cystine	0.74	0.67
Threonine	0.74	0.63
Tryptophan	0.27	0.23
¹ Vitamin and mineral premix provided the following per kg of diet: Vitamin A (retinyl acetate), 10,000 IU; vitamin D ₃ (cholecalciferol), 2,250 IU; vitamin E (DL- α -tocopheryl acetate), 20 IU; vitamin K ₃ (menadione sodium bisulfate), 2.5 mg; vitamin B ₁ (thiamine mononitrate), 2.5 mg; vitamin B ₂ , 8 mg; vitamin B ₆ (pyridoxine hydrochloride), 3.4 mg; vitamin B ₁₂ (cobalamin), 0.024 mg; choline chloride, 1000 mg; calcium-d-pantothenate, 25.5 mg; nicotinic acid, 50 mg; folic acid, 1 mg; biotin, 0.25 mg; Cu (CuSO ₄ ·5H ₂ O), 10.4 mg; Fe (FeSO ₄ ·7H ₂ O), 60 mg; Mn (MnSO ₄ ·H ₂ O), 70 mg; Zn (ZnO), 35 mg; Se (NaSeO ₃), 0.30 mg; I (KI), 0.35 mg		

Sample collection and production performance measurement

All ducks were weighed to record one-day-old weight. At the end of 4th, 8th and 13th week, three ducks from each replicate (namely, 30 ducks from each group) were randomly selected and fast for 12 h, and then live BW was measured and ADG was calculated. Blood samples from selected ducks were collected at approximately 9:00 am by using venipuncture method with 0.7*25 mm disposable venous blood sampling needles (Jiangxi FUERKANG Industrial Group Co., Ltd., Jiangxi, China). Around 5 mL of blood was collected by using 5 mL-vacuum anticoagulant-free tubes (Jiangsu Kangjian Medical Apparatus Co., Ltd., Jiangsu, China). The plasma was placed stagnant for 24 h for stratification. The supernatant phase were collected and stored at -20°C for further determination of duck antioxidant capacity and immune status.

After slaughtered on the same day, a total of 10 carcass traits were measured according to NY/T 823–2004 [21], including carcass weight (**CW**), semi-eviscerated weight (**SEW**), eviscerated weight (**EW**), sebum fat weight (**SFW**), abdominal fat weight (**AFW**), carcass yield (**CY**), semi-eviscerated yield (**SEY**), eviscerated yield (**EY**), sebum fat yield (**SFY**), abdominal fat yield (**AFY**). After separating the visceral organs and immune organs, thymus, spleen, and bursa of Fabricius were collected to evaluate duck immune status, while visceral organs parameters of heart weight (**HW**), heart index (**HI**), liver weight (**LW**), liver index (**LI**), gizzard weight (**GW**), gizzard index (**GI**), proventriculus weight (**PW**) and proventriculus index (**PI**) were measured as part of production performance, in which organs indexes was calculated as organ weight divided by BW. After the determination of production performance, 20 g of liver samples of male ducks were collected for gene expression analysis.

Determination of serum biochemical parameters and antioxidant capacity indicators

7 out of 30 serum samples from each group were used for serum parameters analysis. The biochemical parameters including alanine aminotransferase (**ALT**), aspartate aminotransferase (**AST**), alkaline phosphatase (**ALP**), total proteins (**TP**), albumin (**ALB**) and globulin (**GLOB**) [22] were measured by automatic biochemical analyzer (Chemray 240, Shenzhen Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China). The serum antioxidant capacity indicators including the content of malondialdehyde (**MDA**) and the activity of superoxide dismutase (**SOD**) and glutathione peroxidase (**GSH-Px**) were measured by using commercial kits (Nanjing Jincheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. Briefly, MDA content was measured by using thiobarbituric acid (TBA) colorimetry. SOD activity was determined by using xanthine oxidase method (hydroxylamine method) with 10 µL of each sample, the corresponding amount of SOD when the SOD inhibition rate reached 50% per mL of reaction solution was defined as one SOD activity unit (U). As for GSH-Px activity, after the sample was diluted three times, 200 µL of each sample was used for enzymatic reaction and following color reaction. After deducting the non-enzymatic reactions, the unit of enzyme activity (U) of GSH-Px was defined as the GSH concentration reduced by 1 µmol/L in each reaction system involved in 0.1 mL of serum.

Determination of immune status

Immune status was evaluated by immune organs development and serum immune cytokines profiles. A total of 6 immune organs parameters were determined, including spleen weight (**SW**), spleen index (**SI**), thymus weight (**TW**), thymus index (**TI**), bursa of Fabricius weight (**BFW**) and bursa of Fabricius index (**BFI**). Serum immune cytokines in current study comprised immune globulin-A (**IgA**), immune globulin-G (**IgG**), immune globulin-M (**IgM**), interleukin-1β (**IL-1β**), interleukin-4 (**IL-4**), interleukin-6 (**IL-6**) and interferon-γ (**IFN-γ**). They were determined by using commercial ELISA kits (Wuhan Chundu Biological Technology Co., Ltd., Wuhan, China) with double-antibody sandwich ELISA method in accordance with the manufacturer's instructions.

Total RNA extraction and cDNA synthesis

Each 4 samples from male ducks in different rearing systems at different ages were used to determine the expressions of genes related to immune function and antioxidant capacity. Total RNA was isolated from the frozen liver tissues using Trizol reagent (TaKaRa Bio Inc, Dalian, China) following the manufacturer's instructions strictly. Extracted RNA concentrations and integrity were measured by electrophoresis and NanoDrop 2000. Based on the clear bands of 5S rRNA, 18S rRNA and 28S rRNA in vision and the A260:A280 ratio range within 1.8 ~ 2.1, the extracted RNA was accepted for reverse transcription. Reverse transcription was conducted by using PrimerScript™ RT reagent Kit with gDNA Eraser (TaKaRa Bio Inc, Dalian, China) according to the manufacturer instructions. Products of cDNA were stored at -20°C before quantitative real-time PCR.

Table 2
Primer pairs used for real-time PCR in this study

Gene ¹	Primer sequences (5'-3')	GenBank accession	Amplicon, bp	Annealing temperature,°C
<i>β-actin</i>	Forward: GCTATGTCGCCCTGGATTTC	NM_001310421	168	60
	Reverse: CACAGGACTCCATACCCAAGAA			
<i>GAPDH</i>	Forward: AAGGCTGAGAATGGGAAAC	XM_027449740	254	54
	Reverse: TTCAGGGACTTGTCATACTTC			
<i>SOD1</i>	Forward: TGTCTGATGGAGATCATGGCTTCC	KU048808	271	68
	Reverse: CCTTTGCCAGGTCGTCGCTTTT			
<i>ALB</i>	Forward: CTACAATGATTTGAAGGAGGAG	XM_005012746	664	62.5
	Reverse: CTGCGAATGAAGCCTTGGGATA			
<i>IFN-γ</i>	Forward: TCATACTGAGCCAGATTGTTAC	AF087134	129	56
	Reverse: CTTGTAGTCATTGGAAAGGGTATT			
<i>GOT1</i>	Forward: CTA CTGGGATGCCGCCAAGAGG	XM_027460734	273	67.5
	Reverse: CTCAAAGCCCTCGGAGACAAAG			
<i>ALP</i>	Forward: GCTCTGCTTGGCATCGCTGGTC	XM_027443431	335	70
	Reverse: GGCTTTGACACGCAGAGGTAGGC			
¹ <i>GAPDH</i> : glyceraldehyde 3-phosphate dehydrogenase; <i>SOD1</i> : superoxide dismutase-1; <i>ALB</i> : albumin; <i>IFN-γ</i> : interferon-gamma precursor; <i>GOT1</i> : glutamic-oxaloacetic transaminase-1; <i>ALP</i> : alkaline phosphatase				

Quantitative real-time PCR analysis

Primers were designed according to GenBank sequences using Primer Premier 5.0, in which *β-actin* and *GAPDH* were referred to Li et al. [23]. Primers were obtained from Beijing Tsingke Biotechnology Co., Ltd. (Sichuan, China) and shown in Table 2, of which *β-actin* and *GAPDH* were regarded as reference genes.

Quantitative real-time PCR was performed on a CFX96™ Real-Time PCR detection system (Bio-Rad, CA, U.S.A.) by using SYBR Premix ExTaq™ II (TaKaRa Bio Inc, Dalian, China). 12.5 μL of PCR reaction volume was consisted of 6.25 μL of the SYBR Premix ExTaq™ II, 4.25 μL of ddH₂O, 1 μL of cDNA template and 0.5 μL of forward/reverse primer. PCR conditions consisted of 3 min pre-denaturation at 95°C; followed by 40 cycles of 10 sec denaturation at 95°C, 30 s annealing at primers optimum reaction temperature, and 30 s extension at 72°C. Melting curve was conducted to verify the amplified PCR product was single. Every sample was tested for 3 times and adopted with standard deviations of threshold cycle (CT) below 0.5. Quantification data was calculated by using the 2^{-ΔΔCT} method against reference genes.

Statistical analysis

The experimental data was analyzed by using general linear model (GLM) procedure in SPSS 22.0. The statistical model comprised the main effects of rearing system (RS), age, as well as their interactions. Duncan's multiple-range comparison was conducted among 6 groups (4w-FRS, 4w-NRS, 8w-FRS, 8w-NRS, 13w-FRS and 13w-NRS) to test the significance of variance. Values were presented as means and SEM. Values of *P* < 0.05 were considered statistically significant.

Results

Table 3
Effects on growth performance and carcass traits of Nonghua ducks at different ages

Items ¹	4w		8w		13w		SEM	P-value ²		
	FRS	NRS	FRS	NRS	FRS	NRS		RS	Age	RS × Age
BW, g	1195.305 ^c	1186.281 ^c	2448.283 ^b	2663.672 ^a	2679.520 ^a	2650.098 ^a	39.156	0.021	< 0.001	< 0.0001
ADG, g	42.824 ^b	42.228 ^b	42.925 ^b	46.767 ^a	28.973 ^d	28.659 ^d	0.443	0.019	< 0.001	< 0.001
CW, g	1077.867 ^d	1026.614 ^d	2153.051 ^c	2318.103 ^b	2407.288 ^a	2354.237 ^{ab}	33.729	0.367	< 0.001	< 0.001
CY, %	89.379 ^a	86.878 ^{bc}	87.592 ^b	86.537 ^c	89.225 ^a	88.747 ^a	0.142	< 0.001	< 0.001	0.005
EW, g	866.950 ^d	839.271 ^d	1845.333 ^c	2013.167 ^b	2087.458 ^a	2058.169 ^{ab}	30.583	0.064	< 0.001	< 0.001
EY, %	71.595 ^c	70.966 ^c	75.041 ^b	75.460 ^b	77.855 ^a	77.635 ^a	0.191	0.566	< 0.001	0.207
SEW, g	972.117 ^d	933.432 ^d	1995.085 ^c	2185.167 ^b	2269.833 ^a	2230.153 ^{ab}	32.604	0.088	< 0.001	< 0.001
SEY, %	80.220 ^c	78.942 ^d	81.266 ^b	81.325 ^b	84.275 ^a	84.085 ^a	0.162	0.060	< 0.001	0.061
SFW, g	201.503 ^d	199.034 ^d	410.153 ^c	512.997 ^a	481.553 ^b	509.339 ^{ab}	8.317	< 0.001	< 0.001	< 0.001
SFY, %	23.162 ^{cd}	23.662 ^{bc}	22.117 ^d	25.455 ^a	23.079 ^{cd}	24.671 ^{ab}	0.184	< 0.001	0.520	0.004
AFW, g	7.532 ^e	8.064 ^e	25.102 ^d	44.305 ^b	38.139 ^c	50.502 ^a	1.106	< 0.001	< 0.001	< 0.001
AFY, %	0.858 ^d	0.937 ^d	1.217 ^c	1.983 ^b	1.791 ^b	2.376 ^a	0.042	< 0.001	< 0.001	< 0.001
The data was displayed as means (n = 60)										
¹ BW, body weight; ADG, average daily gain; CW, carcass weight; CY, carcass yield; EW, eviscerated weight; EY, eviscerated yield; SEW, semi-eviscerated weight; SEY, semi-eviscerated yield; SFW, sebum fat weight; SFY, sebum fat yield; AFW, abdominal fat weight; AFY, abdominal fat yield; FRS, floor rearing system; NRS, net rearing system; RS, rearing system										
² When P-values were below 0.001, the specific values were omitted and uniformly expressed as < 0.001										
^{abc} Different lowercase letters indicated that the difference between NRS and FRS corresponding group was significant (P < 0.05).										

Effects of FRS and NRS on growth performance, carcass traits and visceral organs

Results of growth performance and carcass traits were shown in Table 3, BW and ADG were affected by RS and the interactions between RS and age ($P < 0.05$). BW increased with ages, and together with ADG were higher in NRS ducks than FRS ducks at 8w ($P < 0.05$). As for carcass traits, CY, EY and SEY of Nonghua ducks exhibited a consistent tendency of higher than 85%, 70% and nearly 80% at different ages in both FRS and NRS. CW, SFW, SFY, AFW and AFY were affected by RS ($P < 0.05$). CY, EW, EY, SEW, SFW, SFY, AFW and AFY were affected by the interactions between RS and age ($P < 0.05$). CY was lower in NRS ducks at 4w and 8w ($P < 0.05$), while CW, EW and SEW were higher in NRS ducks at 8w ($P < 0.05$). SFW, SFY, AFW and AFY were significantly higher in NRS ducks at 8w ($P < 0.05$), and SFY, AFW, AFY were also higher at 13w ($P < 0.05$).

Table 4
Effects on visceral organ development of Nonghua ducks at different ages

Items ¹	4w		8w		13w		SEM	P-value ²		
	FRS	NRS	FRS	NRS	FRS	NRS		RS	Age	RS × Age
HW, g	8.188 ^c	7.880 ^c	15.873 ^b	16.735 ^a	17.223 ^a	16.769 ^a	0.236	0.864	< 0.001	0.010
HI, %	0.682 ^a	0.667 ^{ab}	0.651 ^{abc}	0.636 ^{bc}	0.651 ^{abc}	0.631 ^c	0.005	0.066	0.003	0.972
LW, g	40.203 ^c	34.620 ^d	55.621 ^a	57.947 ^a	49.655 ^b	51.180 ^b	0.627	0.523	< 0.001	< 0.001
LI, %	3.345 ^a	2.942 ^b	2.274 ^c	2.213 ^c	1.878 ^d	1.972 ^d	0.037	0.006	< 0.001	< 0.001
GW, g	43.897 ^b	39.988 ^c	60.876 ^a	59.693 ^a	58.322 ^a	46.690 ^b	0.611	< 0.001	< 0.001	< 0.001
GI, %	3.661 ^a	3.389 ^b	2.484 ^c	2.276 ^d	2.240 ^d	1.751 ^e	0.042	< 0.001	< 0.001	0.020
PW, g	5.435 ^c	5.395 ^c	7.563 ^{ab}	7.708 ^{ab}	8.098 ^a	7.426 ^b	0.107	0.295	< 0.001	0.154
PI, %	0.453 ^a	0.456 ^a	0.309 ^b	0.293 ^b	0.302 ^b	0.283 ^b	0.006	0.186	< 0.001	0.440
The data was displayed as means (n = 60)										
¹ HW, heart weight; HI, heart index; LW, liver weight; LI, liver index; GW, gizzard weight; GI, gizzard index; PW, proventriculus weight; PI, proventriculus index; FRS, floor rearing system; NRS, net rearing system; RS, rearing system										
² When P-values were below 0.001, the specific values were omitted and uniformly expressed as < 0.001										
^{abc} Different lowercase letters indicated that the difference between NRS and FRS corresponding group was significant (P < 0.05).										

Results of visceral organs were shown in Table 4, LI, GW and GI were affected directly by RS ($P < 0.05$), while HW, LW, LI, GW and GI were affected by the interactions between RS and age ($P < 0.05$). Notably, LW and LI were significantly higher in FRS ducks at 4w ($P < 0.05$). GW was higher in FRS ducks at 4w and 13w ($P < 0.05$), and GI was higher in FRS ducks at all ages ($P < 0.05$).

Table 5
Effects on antioxidant capacity indicators of Nonghua ducks at different ages

Items ¹	4w		8w		13w		SEM	P-value ²		
	FRS	NRS	FRS	NRS	FRS	NRS		RS	Age	RS × Age
GSH-Px, umol/L	588.742 ^{ab}	618.678 ^{ab}	493.945 ^b	491.684 ^b	493.214 ^b	633.214 ^a	17.702	0.102	0.029	0.203
SOD, U/mL	171.125 ^d	191.544 ^{cd}	267.989 ^a	224.180 ^{bc}	279.485 ^a	252.010 ^{ab}	6.951	0.127	< 0.001	0.054
MDA, nmol/mL	7.986 ^a	6.697 ^{ab}	5.168 ^b	5.063 ^b	5.693 ^b	6.408 ^{ab}	0.257	0.637	0.001	0.240
The data was displayed with means (n = 14)										
¹ GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; FRS, floor rearing system; NRS, net rearing system; RS, rearing system										
² When P-values were below 0.001, the specific values were omitted and uniformly expressed as < 0.001										
^{abc} Different lowercase letters indicated the difference between NRS and FRS corresponding group was significant (P < 0.05)										

Effects of FRS and NRS on serum biochemical parameters and antioxidant capacity indicators

Results of serum biochemical parameters was depicted in Fig. 1, where ALT, ALP, TP, ALB and GLOB were affected by RS ($P < 0.05$), while TP, ALB and GLOB were also affected by the interactions between RS and age ($P < 0.05$). As for liver health biomarkers, ALP was significantly lower in NRS ducks at 4w and 8w ($P < 0.05$), while ALT was lower in NRS ducks at 8w and 13w ($P < 0.05$). TP and GLOB were significantly higher but ALB was lower than FRS ducks at 13w ($P < 0.05$). According to Table 5, though neither RS nor the interactions indicated a significant impact on antioxidant capacity indicators, a trend of lower MDA content but higher GSH-Px and SOD activities were found in NRS ducks at 4w ($P > 0.05$). The activity of SOD was decreased in NRS ducks at 8w ($P < 0.05$), but the activity of GSH-Px was increased in NRS ducks at 13w ($P < 0.05$).

Table 6
Effects on immune organ development of Nonghua ducks at different ages

Items ¹	4w		8w		13w		SEM	P-value ²		
	FRS	NRS	FRS	NRS	FRS	NRS		RS	Age	RS × Age
SW, g	1.287 ^c	1.188 ^c	1.895 ^b	1.840 ^b	1.898 ^b	2.576 ^a	0.049	0.050	< 0.001	< 0.001
SI, ‰	1.079 ^a	0.986 ^a	0.772 ^b	0.684 ^b	0.726 ^b	1.001 ^a	0.021	0.450	< 0.001	< 0.001
TW, g	4.019 ^c	4.148 ^c	8.502 ^b	10.358 ^a	2.963 ^d	2.500 ^d	0.214	0.088	< 0.001	0.002
TI, ‰	3.338 ^b	3.397 ^{ab}	3.475 ^{ab}	3.876 ^a	1.107 ^c	0.962 ^c	0.093	0.503	< 0.001	0.238
BFW, g	2.478 ^b	2.197 ^b	3.193 ^a	2.955 ^a	1.367 ^c	1.128 ^c	0.059	0.005	< 0.001	0.954
BFI, ‰	2.076 ^a	1.824 ^b	1.309 ^c	1.099 ^d	0.499 ^e	0.405 ^e	0.043	< 0.001	< 0.001	0.515
The data was displayed as means (n = 60)										
¹ SW, spleen weight; SI, heart index; TW, thymus weight; TI, thymus index; BFW, bursa of Fabricius weight; BFI, bursa of Fabricius index; FRS, floor rearing system; NRS, net rearing system; RS, rearing system										
² When P-values were below 0.001, the specific values were omitted and uniformly expressed as < 0.001										
^{abc} Different lowercase letters indicated the difference between NRS and FRS corresponding group was significant ($P < 0.05$)										

Effects of FRS and NRS on immune organ development and serum immune cytokines profiles

Results of immune organs and immune cytokines were shown in Table 6 and Fig. 2, respectively. As for immune organs, SW, BFW and BFI were significantly affected by RS ($P < 0.05$), while SW, SI and TW were significantly affected by the interactions ($P < 0.05$). BFI was significantly lower in NRS ducks at 4w and 8w ($P < 0.05$), while TW was significantly higher in NRS ducks at 8w ($P < 0.05$). SW and SI were significantly higher than FRS ducks at 13w ($P < 0.05$). In terms of immune cytokines, all indicators we detected were significantly affected by RS ($P < 0.05$), and IgG was also affected by the interactions ($P < 0.05$). Notably, IL-1 β exhibited a consistent trend of higher profiles in NRS ducks at all ages ($P < 0.05$). IL-4 was significantly higher but IL-6 was lower in NRS ducks at 4w and 13w ($P < 0.05$), while IFN- γ , IgA and IgG were only higher than FRS ducks at 4w ($P < 0.05$).

Effects of FRS and NRS on liver gene expression

The results of gene expressions were depicted in Fig. 3. *ALB* was affected directly by RS ($P < 0.05$), and exhibited a consistent trend of lower expression in NRS ducks at different ages. Other genes were neither affected by RS, nor affected by the interactions ($P > 0.05$). *ALB* in FRS ducks was significantly higher than NRS ducks at 4w ($P < 0.05$), while *ALB* in 4w-FRS was also higher than 8w-FRS and 13w-FRS ($P < 0.05$). *ALP* in FRS ducks was significantly higher than NRS ducks at 13w ($P < 0.05$), while *ALP* in 13w-FRS was also higher than 4w-FRS and 8w-FRS ($P < 0.05$). *IFN- γ* was higher in 13w-FRS than 4w-FRS and 8w-FRS ($P < 0.05$).

Discussion

Production performance is one of the major concerns in poultry production as it is closely associated with the profits of animal products, while increasing focus on poultry health status, a reflection of physiological status, is not only due to its impact on production performance but also because of the raising attention to animal welfare issues. In this study, we compared the effects of FRS and NRS on production performance and health status of Nonghua ducks at different ages. Based on the higher final BW, ADG and lower FCR of waterfowls in NRS,

studies on Yangzhou geese and Chaohu ducks reported waterfowls in NRS exhibited better growth performance than in FRS [9, 24]. Similarly, in Moulard ducks, though FCR was not significantly affected, the higher final BW and ADG in NRS in comparison to sawdust-FRS and sand-FRS also revealed that NRS resulted in a better growth performance [25]. However, with a lower ADG but a higher FCR of Cherry Valley ducks in NRS, Chen et al. [12] reached an opposite conclusion of ducks in FRS exhibited better growth performance. These inconsistent conclusions may result from different breeds and slaughtering ages. To clarify the effects of FRS and NRS on meat ducks in different breeding stages, the current study selected three key weeks of age to study the interactions between RS and age. Results showed that BW and ADG were higher in NRS at 8w while no significant results were found at 4w and 13w, indicating a better growth performance of NRS ducks before the market age.

As for carcass traits, the average CY, EY and SEY of Nonghua ducks at 8w and 13w under either FRS or NRS were higher than 85%, 75% and 80%, indicating outstanding carcass traits compared with Pekin ducks, Cherry Valley ducks, White Muscovy ducks and Jingjiang ducks (a native Sheldrake breed in China) at similar ages [26–29]. Previous study on Chaohu ducks noticed the lower EY but higher AFY in NRS [9]. Similar to the current study, results showed that CY of NRS ducks was lower than FRS ducks at 4w and 8w, while CW, EW, SEW, SFW, AFW, SFY, and AFY were higher in NRS at 8w, SFY and AFY were also higher in NRS at 13w, indicating that NRS was conducive to carcass traits to some extent. With the age increasing, the majority of significances in carcass traits faded, but the lipid deposition was enhanced consistently in NRS. This was in agreement with Liu et al. [24], who reported Yangzhou geese in NRS exhibited higher subcutaneous fat thickness and higher AFY than in FRS. The visceral organ development is closely associated with duck growth, development, and health [30]. Results showed GW and GI were generally lower in NRS at different ages, indicated that FRS ducks had better gizzard development than NRS ducks. This was in agreement with Wang et al. [6], who also found a better gizzard development of birds in FRS compared with CRS and NRS. The reason can be concluded as NRS ducks cannot intake any grain of sand from the floor, because adding large particles and structural components to diets could stimulate poultry gizzard function and development [31, 32]. Moreover, LW and LI of NRS ducks were lower than FRS ducks at 4w, indicating a liver developmental retardation at the early stage of breeding in NRS. Therefore, NRS improved the carcass weight before the market age but had defects in carcass yield. FRS ducks exhibited better gizzard and liver development at the early stage of breeding, while NRS enhanced the lipid deposition at the later one.

Since the liver is the major site of metabolism and detoxification, this retardation in NRS ducks may influence the serum biochemical parameters of Nonghua ducks. Serum biochemical parameters of AST, ALT, and ALP were biomarkers to evaluate the health status of liver, because hepatocytes would secrete these enzymes into the blood once the liver was damaged [33, 34]. Results showed these parameters were coherently lower in NRS ducks at different ages, demonstrating NRS was better for duck liver health despite the lipid deposition enhancement and liver developmental retardation. And this also showed an advantage over CRS, as ducks suffered liver injury once they were just put into cages [35]. Furthermore, antioxidant capacity prevents cell damage from free radicals and other reactive oxygen species (ROS) to avoid oxidative stress (the imbalance between ROS and antioxidants) [36, 37]. The concentration of MDA and activities of SOD, GSH-Px are effective indicators to reflect the antioxidant status of ducks [38, 39]. A study on Shaoxing ducks showed rearing systems did not have significant impacts on the activity of MDA, SOD, CAT, T-AOC, and GSH-Px in the liver [35]. Another study though only determined the total antioxidant capacity (T-AOC) in serum, results showed no significant difference between FRS and NRS [5]. Similarly in the current study, rearing system did not significantly affect MDA, SOD, and GSH-Px, however, it exhibited a trend of lower MDA content and higher activities of SOD and GSH-Px in NRS at 4w, which indicated that NRS ducks might have a better antioxidant capacity than FRS ducks at this early stage of breeding. The activity of SOD decreased in NRS at 8w and maintained relatively lower than FRS ducks at 13w, while GSH-Px activity was significantly higher in NRS and MDA content was also relatively higher than FRS ducks at 13w. These results implied GSH-Px was responsible for scavenging excess free radicals at the later stage of breeding.

As for immune function, Xi et al. [40] reported that there was no significant difference in immune organ indexes of Cherry Valley ducks between NRS and FRS. Differently in current study, NRS ducks were higher in TW at 8w as well as SW and SI at 13w, which indicated a promotion on immune organ development in NRS. Whereas, the decreased BFI in NRS ducks at 4w and 8w implied a faster degradation of bursa. Current study also showed a trend of higher TI at 4w and 8w though the results were not significant, which was similar to Zhao et al. [5], who reported NRS ducks had lower mortality rate and higher TI than FRS ducks. Previous study also reported IL-1 β and IgG profiles of Shaoxing ducks were not significantly affected by NRS [5], while immune cytokines were extensively affected by RS in current study. Generally, the tendency of higher profiles of IFN- γ , IL-1 β , IL-4, immunoglobulins in NRS ducks indicated a better immune status, IL-1 [41] and IL-4 [42] contribute to T helper type-2 (Th2) cell activity, whereas IFN- γ contributes to T helper type-1 (Th1) cell activity [43]. Higher levels of IFN- γ , IL-1 β , and IL-4 during the experiment suggested the activity of Th1 and Th2 cells may be promoted in NRS. The enlargement of thymus at 8w may be associated with escalated IL-1 β because IL-1 can act as a growth factor for thymocytes [41]. Faster degradation of bursa and lower IL-6 content suggested NRS ducks may have lower immunological requirements. This could be explained by the direct dropping of duck feces in NRS, which makes ducks less opportunity to contact with their excreta. Wang et al. [44] and Almeida et al. [8] reported that FRS had higher microbial contamination and higher concentrations of NH₃ and CO₂ in the air than NRS, hence the better air quality in NRS also helped reduce the burden on duck immune system. Moreover, the elevation of immunoglobulins in NRS may be associated with escalated IL-1 β , due

to its promotion on IL-2 to enhance the secretion of immunoglobulins. In terms of serum proteins, NRS ducks showed higher GLOB and TP but lower ALB at 13w, this was in agreement with the study on Chaohu ducks [9], which also reported serum TP content was improved by NRS in comparison to FRS. Serum proteins change can be concluded as follows: First, FRS ducks took more exercise during rearing, which would enhance protein synthesis causing TP loss in blood [14]. Second, due to the enhancement of lipid deposition in NRS, ALB would be used for lipoprotein synthesis leading to the lower ALB serum profile in NRS [45]. Third, higher levels of immunoglobulins in NRS contributed to the change of GLOB in serum, especially the consistent elevation in IgA, IgG, and IgM in NRS at 13w. Based on the liver functions of synthesizing serum proteins including albumins [46], a coherent trend of lower *ALB* expression in NRS ducks at different ages might be responsible for the significantly lower serum ALB profiles at 13w. There was no significant change in *SOD1*, indicating this antioxidant was not significant in the change of two different rearing systems. IFN- γ is a pro-inflammatory cytokine, which participated in multiple inflammatory responses [43]. Hence, the higher expression of *IFN- γ* as well as *ALP* in 13w-FRS than 4w-FRS and 8w-FRS indicated a potential more intense environmental stress in liver at the later stage of breeding in FRS. However, the specific regulatory mechanism needs further study to elucidate. Therefore, NRS could improve duck immune function by increasing the levels of certain immune cytokines profiles and promoting the development of immune organs, while liver gene expression indicated that ducks might suffer from a potential higher environmental stress in FRS.

Conclusion

In conclusion, compared with FRS, NRS was conducive to improve growth performance and carcass traits to some extent but it had defects in visceral organ development and lipid deposition. According to lower serum profiles of ALT, AST, and ALP as well as liver gene expression in NRS, NRS could be better for duck liver health compared with FRS. Antioxidant capacity was not significantly affected by rearing system, but indicators showed NRS ducks may have better antioxidant capacity at the early stage of breeding, while at the later one, GSH-Px activity was increased for scavenging excess free radicals in NRS. NRS increased the levels of IFN- γ , IL-1 β , IL-4, immunoglobulins in serum and promoted the development of thymus and spleen to improve duck immune function, which would promote the activities of Th1 and Th2 cells.

Abbreviations

CRS: cage rearing system; FRS: floor rearing system; NRS: net rearing system; BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion rate; 4w: 4th week; 8w: 8th week; 13w: 13th week; CW: carcass weight; CY: carcass yield; EW: eviscerated weight; EY: eviscerated yield; SEW: semi-eviscerated weight; SEY: semi-eviscerated yield; SFW: sebum fat weight; SFY: sebum fat yield; AFW: abdominal fat weight; AFY: abdominal fat yield; HW: heart weight; HI: heart index; LW: liver weight; LI: liver index; GW: gizzard weight; GI: gizzard index; PW: proventriculus weight; PI: proventriculus index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; TP: total proteins; ALB: albumin; GLOB: globulin; MDA: malondialdehyde; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; SW: spleen weight; SI: heart index; TW: thymus weight; TI: thymus index; BFW: bursa of Fabricius weight; BFI: bursa of Fabricius index; IgA: immune globulin-A; IgG: immune globulin-G; IgM: immune globulin-M; IL-1 β : interleukin-1 β ; IL-4: interleukin-4; IL-6: interleukin-6; IFN- γ : interferon- γ ; RS: rearing system; ROS: reactive oxygen species; Th1: T helper type-1; Th2: T helper type-2

Declarations

Ethics approval and consent to participate

All protocols used in current study were authorized by the Institutional Animal Care and Use Committee of Sichuan Agricultural University (Approval No. DKY-B20141401).

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed in the present study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SH and JW designed and coordinated this study; YG, YW, ZL, XG, YD, QO and BH collected the samples, carried out the experiments and helped the data analyses. YG and YW visualized the data and composed the first draft of manuscript; LL, HH, LX and RZ contributed to project administration; HL, SH and JW reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figures

Figure 1

Items ¹	P-value ²		
	RS	Age	RS x Age
ALT, U/L	<0.001	<0.001	0.333
AST, U/L	0.117	0.003	0.156
ALP, U/L	<0.001	<0.001	0.100
TP, g/L	0.039	<0.001	0.010
ALB, g/L	0.027	0.263	0.028
GLOB, g/L	0.001	<0.001	<0.001

Figure 2

Effects of FRS and NRS on serum biochemical parameters of Nonghua ducks at different ages. Data was displayed as “Means ± SEM” in figures, the P-values of main effects of RS, age and their interactions were displayed in the table. Abbreviations: FRS, floor rearing system; NRS, net rearing system; RS, rearing system. 1 ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TP, total proteins; ALB, albumin; GLOB, globulin. 2 When P-values were below 0.001, the specific values were omitted and uniformly expressed as <0.001. abc Different lowercase letters indicated the difference between NRS and FRS corresponding group was significant (P<0.05), for both FRS and NRS corresponding groups, n=14.

Figure 2

Figure 4

Effects of FRS and NRS on serum immune cytokines of Nonghua ducks at different ages. Data was displayed as “Means ± SEM” in figures, the P-values of main effects of RS, age and their interactions were displayed in the table. Abbreviations: FRS, floor rearing system; NRS, net rearing system; RS, rearing system. 1 When P-values were below 0.001, the specific values were omitted and uniformly expressed as <0.001. abc Different lowercase letters indicated the difference between NRS and FRS corresponding group was significant (P<0.05), for both FRS and NRS corresponding groups, n=14.

Figure 3

Figure 5

Effects of FRS and NRS on liver gene expressions of Nonghua ducks at different ages. Data was displayed as “Means $\pm$ SEM” in figures, the P-values of main effects of RS, age and their interactions were displayed in the table. Abbreviations: FRS, floor rearing system; NRS, net rearing system; RS, rearing system. abc Different lowercase letters indicated the difference between NRS and FRS corresponding group was significant (P<0.05), for both FRS and NRS corresponding groups, n=4.