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Original Article

Morphology, Blood Chemistry and Behavioral Response of Crossbred Chickens Reared Under Alternative Production Systems

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Alternative production systems; antibody response; genotypes; morphometrics; serum chemistry.

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ABSTRACT

In the present study, the second generation of two genotypes RNN (Rhode Island Red × Naked Neck) and BNN (Black Australorp × Naked Neck) obtained by two self-crosses (RNN × RNN =RR and BNN × BNN= BB) and two reciprocal crosses (RNN × BNN = RB and BNN × RNN = BR) were evaluated in three alternative production systems (conventional cages, enriched cages, and aviary). In total 480 birds, comprising 240 pullets with 240 cockerels (60 pullets & 60 cockerels from each crossbred) were used during rearing phase (17-21 weeks). Higher body weight, shank circumference, body and drumstick length were noticed in RB and BR genotypes. Among different production systems, chickens reared in enriched cages showed higher body weight and body lengths than other production systems. Comparing the behavior of chickens, higher feeding, jumping and dust bathing were observed in chickens reared in aviary systems than in other production systems while the maximum perching behavior was showed by the chickens reared in enriched cages. The highest glucose level was observed in RR genotypes when reared in aviary systems. Antibody titers against ND and IB were highest in chickens reared in aviary systems. In conclusion, RB and BR genotypes had better performance in terms of morphometrics and blood biochemistry when reared under enriched cages and aviary systems.

INTRODUCTION

The increasing world population is tremendously demanding food production and measures to increase animal protein sources. In many countries, different strategies have been applied to develop a dual-purpose rural chicken breed with further improvement in its production traits (Mallia, 1999). The genetic improvement can be achieved through crossbreeding and selection. However, cross breeding is better in terms to instigate improvements in growth rate, reproductive traits and feed conversion efficiency without disturbing potential of acclimatization ultimately reducing production costs (Adebambo *et al.,* 2011). Better productive performance and adaptability traits of naked neck can be exploited through heterosis led by its crossbreeding with Rhode Island Red and Black Australorp which have genes of better egg production and meat yield with higher potential of economic returns helping to develop a crossbred with improved production potential and maintained acclimatizing abilities.

In association with the genetic improvement, better management and provision of suitable environment is necessary for the exploitation of genetic potential (Menge *et al.,* 2005). Traditional conventional cages have been long perceived as the most proficient and efficient system for egg type chicken but the negative effect of cage system on welfare of chicken is also being considered widely (Craig & Swanson, 1994). It has been observed that confinement and complexity in conventional cages restricts the birds to limited natural habitat which eliminates the expression of natural behaviors like roosting, nesting and scratching (Baxter, 1994). Banning of conventional cages by European Union and to overcome the lack of natural behaviors, the alternative housing systems are gaining strength day by day to cope with the consumer's demand. It is assumed that alternative systems can help the birds not only to express their natural behavior but also enhance the economical production traits.

Blood biochemistry is an ideal evaluator of health profile and is frequently used to assess immune status by avian pathologists. Among different parameters of blood biochemistry, serum proteins are useful in estimating the quality of dietary proteins (Alikwe *et al.,* 2010). Similarly, glucose and triglycerides are useful in drawing inference about energy requirement for the body's biochemical functions and physiological response (Kral & Suchy, 2000). Information regarding the immune status is mandatory to understand the level of disease resistance against infectious diseases. Indigenous chickens can be used as the most efficient model to study immune status against different viral and bacterial infections (Haunshi *et al.,* 2011). The availability of data regarding the performance of different crossbreds of Naked Neck, Black Australorp and Rhode Island Red under aviary and enriched cages is limited and need to be explored. So, its need of the time to work on developing new genotypes with improved growth, better immune response and better acclimatization to local climatic condition and to evaluate their genetic potential under alternative production systems.

MATERIALS AND METHODS

The present project was planned to evaluate the performance of different crossbreds of Naked Neck (NN), Black Australorp (BAL) and Rhode Island Red (RIR) under alternative production systems i.e., Conventional Cages, Aviary systems and Enriched Cages. The study was conducted at the Indigenous Chicken Genetic resource Centre (ICGRC), Department of Poultry Production, UVAS, Ravi Campus Pattoki, Pakistan. Pattoki city is located at 73º50'60E and 31°1'0N with the altitude of 610 ft (186 m) and normally experiences tropical hot and humid climate where the temperature ranges between 12°C to 45°C.

Population Size

The present study was in the continuation of the previous research project in which the performance of progeny (F1) taken from Rhode Island Red × Naked Neck (RNN) and Black Australorp × Naked Neck (BNN) was evaluated (Ahmad *et al.,* 2019). In the present study, the second generation (F2) of these crossbreds (BNN and RNN) were evaluated in reciprocal crosses. For this purpose, a total of 200 heterozygous partial feather chickens (discarding homozygous full feathered and homozygous naked neck) comprising 50 birds (10 [redacted] × 40 [redacted]) from each crossbred of first generation i.e.; Crossbred I (BNN [redacted] × BNN [redacted]), Crossbred II (BNN [redacted] × RNN [redacted]), Crossbred III (RNN [redacted] × RNN [redacted]) and Crossbred IV (RNN [redacted] × BNN [redacted]) were used to take more than 1200 hatching eggs in the 33rd week of age (Figure 1). The birds were arranged according to a Completely Randomized Design (CRD). the birds were fed commercial breeder ration formulated according to the recommendations of Leeson & Summer (2005) (Table 1, 2). The chicks were brooded

Table 1 – Specification for each production system.

Specifications	Conventional cage (Separate for each sex)	Enriched cage (Separate for each sex)	Aviary (Straight run)
Dimensions (Length × Depth × Height) (ft)	2 × 2.5 × 2	3 × 3 × 3	10 × 10 × 10
Stocking Density	5 birds/ cage	5 birds/cage	50 birds / aviary
Floor space per bird	1.08 ft^2 (931.9 cm^2)	1.75 ft^2 (1672 cm^2)	2 ft^2 (1858 cm^2)
Dimensions of Dust bathing Area (Length × width) (ft)	-----------------------	1.79 × 0.83	4.5 × 4.5
Dust bathing Space/bird	-----------------------	0.3 ft^2 (276 cm^2)	0.4 ft^2 (376 cm^2)
Perches			
Number	-----------------------	1	2
Material	-----------------------	Wooden	Wooden
Shape	-----------------------	Round	Round
Diameter (cm)	-----------------------	1.8	4.2
Length		4 ft	10 ft
Height from floor		1.5 ft	3 ft

Table 2 – Weekly feed allowance (g) during growing phase.

Age (weeks)	Daily feed allowance (g)
17	44
18	46
19	48
20	50
21	52

under standard managemental conditions up to 6 weeks of age. During brooding phase, the birds were vaccinated against IB and ND according to the local area schedule. After brooding, the birds were divided into three different production systems (conventional cages, enriched cages and aviary systems). A total of 480 birds comprising of 240 pullets with 240 cockerels (60 pullets and 60 cockerels from each crossbred) were used to evaluate the performance during rearing phase (17-21 weeks).

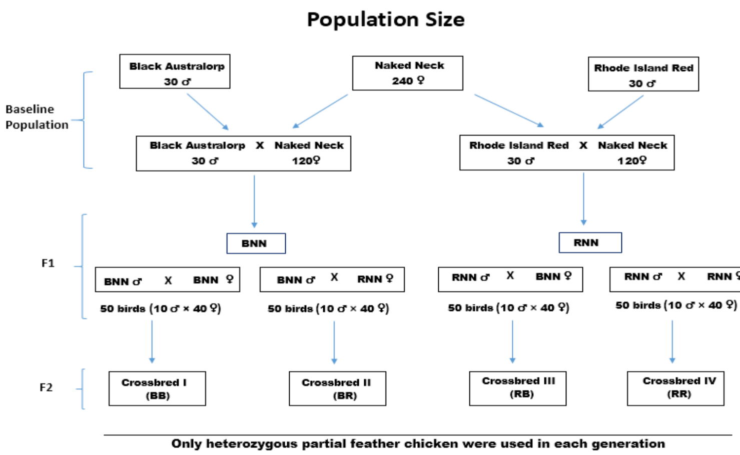

Figure 1 – Population size.

Production systems

Chickens reared in enriched cages and aviary systems were provided with perches and dust bathing area. Open sided windowed enclosures ventilated with ceiling fans were used for aviaries and enriched cages. Galvanized round feeders and plastic manual drinkers were used in aviaries and enriched cages. The physical characteristics of each system is explained in Table (3). Birds reared in conventional cage systems were maintained in environmental controlled poultry shed equipped with galvanized three-tiered battery cage system, automatic manure belt, automatic water nipple line and feed trolley (FACCO, Poultry Equipment-C3). Supply of ad libitum fresh water was ensured.

Housing conditions

The duration of the experiments was 5 weeks (June and July) during which the minimum to maximum temperature and humidity were maintained between the range of 28°C to 35°C and 62 % to 77 % respectively inside open sided enclosures while in environmentally control houses the minimum and maximum temperature and humidity ranged between 26°C to 32°C and 66 % to 74 % respectively. Rice husk was used as litter material in both enriched cages and aviaries. Approximately 4 inches depth of bedding material was maintained and racking on daily basis was ensured to uphold its better condition.

Parameters evaluated

Data were collected for morphometric measurements on a weekly basis during rearing phase from 17- 21 weeks in terms of body, keel, drumstick and shank length, wing spread and drumstick and shank circumference with the help of measuring tape. To evaluate serum chemistry, 3ml of blood collected from the brachial vein of 3 birds per treatment group was centrifuged to collect serum in Eppendorf tubes and stored at -20°C (Gunes *et al.,* 2002). The serum was analyzed by the method adopted by Kumar & Kumbhakar (2015). Antibody response against NDV was evaluated by Hemagglutination inhibition technique (HI) (Xie *et al.,* 2008).

Table 3 – Nutrition value and composition of experimental ration during growing phase.

Feed Ingredient (%)	Grower (17-21 weeks)	Nutritive value	Grower (17-21 weeks)
Corn	59	CP (%)	15.46
Soybean Meal	7	ME (Kcal/Kg)	2913
Rice tips	8.4	Ca (%)	1
Soybean Oil	1.2	Av. P (%)	0.42
Wheat grain	5	Lysine (%)	0.69
Wheat bran	5	Methionine (%)	0.39
Canola Meal	10		
Limestone	2.4		
Feather Meal	1.1		
NaCl	0.30		
Methionine	0.12		
Total	100		

Behavior

To evaluate the behavioral and welfare related traits, the birds from each treatment groups were observed keenly,11:00 AM to 01:00 PM on a weekly basis. Welfare traits were included dust bathing, jumping, perching, sitting and walking (Costa *et al.,* 2012).

Statistical Analysis

The effect of production systems and genotypes were evaluated on morphometric measurements and carcass traits. Two-way ANOVA technique was used for analysis assuming production systems and genotypes as main effects. Tukey's HSD test (Tukey, 1953) was employed to compare treatment means at significance level of $p \leq 0.05$. Mathematical model is given.

$$Y_{ijk} = \mu + P_i + G_j + (P \times G)_{ij} + \varepsilon_{ijk}$$

Where,

Y_{ijk} = Observation of dependent variable recorded on i^{th} Production System and j^{th} Genotype

μ = Population mean

P_i = Effect of i^{th} Production System (i = 1, 2, 3)

G_j = Effect of j^{th} Genotype (j = 1, 2, 3, 4)

$(P \times G)_{ij}$ = Interaction between production system and Genotype

ε_{ijk} = Residual error of k^{th} observation on i^{th} Production system and j^{th} genotype NID ~ 0, σ^2

RESULTS

Morphometric measurements

The effect of genotypes, production system and their interaction on body weight and morphometric measurements for males and females are explained in Tables 4 and 5. RB and BR genotype chickens (male $p<0.0001$ and female $p=0.0028$) were heavier than RR and BB genotype chickens. Females reared in Enriched cages were heavier ($p<0.0001$) than females reared in Aviary and Conventional cages while males from enriched cages and conventional cages showed higher ($p<0.0001$) body weights than males reared in aviary system. The interaction between the genotypes and production systems showed the highest body weight of females ($p<0.0001$) from BR genotype reared in Enriched cage system while males ($p<0.0001$) of BB genotype reared in aviary system showed the lowest body weight than other interaction groups.

Table 4 – Male morphometric measurements at 21 weeks of age.

GT	PS	BW	BL	SL	SC	DL	DC	KL	WS
RB		1927.82±32.60ᵃ	71.32±1.80ᵃ	10.61±0.17	4.17±0.04ᵃ	15.18±0.23ᵃ	9.20±0.22	13.55±0.20	10.48±0.16
RR		1787.92±37.09ᵇ	64.19±3.04ᵃᵇ	10.08±0.18	3.81±0.12ᵇᶜ	14.39±0.26ᵃᵇ	8.63±0.14	12.85±0.29	10.25±0.20
BR		1881.78±24.35ᵃ	69.34±2.05ᵃ	10.34±0.18	4.08±0.09ᵃᵇ	14.90±0.33ᵃᵇ	9.07±0.25	13.09±0.27	10.32±0.16
BB		1733.26±34.02ᵇ	57.26±0.90ᵇ	10.22±0.23	3.62±0.12ᶜ	14.06±0.21ᵇ	8.50±0.14	12.45±0.70	10.20±0.22
	CC	1859.77±21.55ᵃ	66.55±2.38ᵃᵇ	10.21±0.15	4.13±0.06ᵃ	14.57±0.19	8.80±0.21	12.20±0.22ᵇ	10.09±0.13
	AV	1716.71±33.83ᵇ	61.86±1.95ᵇ	10.35±0.13	3.72±0.10ᵇ	14.40±0.21	8.68±0.14	13.74±0.11ᵃ	10.47±0.14
	EC	1921.61±21.56ᵃ	68.17±1.94ᵃ	10.37±0.21	3.92±0.10ᵃᵇ	14.93±0.30	9.08±0.17	13.02±0.53ᵃᵇ	10.37±0.19
RB	CC	1974.89±34.05ᵃ	73.70±3.44ᵃᵇ	10.40±0.31	4.21±0.08ᵃ	14.85±0.24	9.11±0.54	12.77±0.34	10.24±0.34
RB	AV	1830.43±73.44ᵃᵇ	72.99±3.64ᵃᵇᶜ	10.28±0.24	4.16±0.07ᵃᵇ	14.83±0.28	8.93±0.23	14.00±0.21	10.44±0.18
RB	EC	1978.14±32.45ᵃ	67.26±1.69ᵃᵇᶜᵈ	11.14±0.24	4.16±0.10ᵃᵇ	15.87±0.48	9.57±0.36	13.88±0.22	10.76±0.28
RR	CC	1803.58±24.46ᵃᵇ	63.81±7.33ᵃᵇᶜᵈ	9.72±0.20	4.19±0.13ᵃ	14.47±0.53	8.62±0.20	11.94±0.63	9.84±0.20
RR	AV	1638.23±42.43ᵇᶜ	57.32±2.50ᵈ	10.54±0.40	3.39±0.19ᵇ	14.09±0.29	8.53±0.21	13.35±0.07	10.42±0.45
RR	EC	1921.94±43.51ᵃ	71.45±3.55ᵃᵇᶜᵈ	9.98±0.27	3.85±0.14ᵃᵇ	14.61±0.54	8.76±0.34	13.27±0.45	10.48±0.36
BR	CC	1863.57±33.55ᵃ	70.98±1.46ᵃᵇᶜᵈ	10.04±0.36	4.18±0.16ᵃ	14.96±0.36	9.03±0.56	12.23±0.54	9.94±0.33
BR	AV	1807.20±21.26ᵃᵇ	60.30±2.66ᵇᶜᵈ	10.46±0.19	3.81±0.14ᵃᵇ	14.79±0.69	8.87±0.43	1378±0.31	10.56±0.16
BR	EC	1974.57±32.00ᵃ	76.74±0.65ᵃ	10.53±0.37	4.26±0.12ᵃ	14.96±0.73	9.33±0.40	13.26±0.24	10.46±0.30
BB	CC	1797.01±29.22ᵃᵇ	57.73±0.94ᶜᵈ	10.68±0.26	3.93±0.14ᵃᵇ	14.00±0.36	8.45±0.38	11.86±0.20	1035±0.15
BB	AV	1591.00±57.75ᶜ	56.83±1.66ᵈ	10.13±0.21	3.53±0.25ᵃᵇ	13.89±0.20	8.38±0.19	13.82±0.24	10.46±0.36
BB	EC	1811.77±18.69ᵃᵇ	57.23±2.18ᵈ	9.86±0.60	3.40±0.22ᵇ	14.27±0.55	8.66±0.13	11.68±2.09	9.80±0.56
SOV		ANOVA							
GT		< 0.0001	< 0.0001	0.2950	0.0004	0.0165	0.0394	0.2588	0.7490
PS		< 0.0001	0.0355	0.7681	0.0043	0.2514	0.2414	0.0085	0.2473
GT × PS		< 0.0001	< 0.0001	0.1820	0.0002	0.2424	0.4542	0.1484	0.6199

Note: Superscripts on different means within column differ significantly at $p \leq 0.05$.

GT = Genotype; RB = RNN × BNN; RR = RNN × RNN; BR = BNN × RNN; BR = BNN × RNN; BB = BNN × BNN; PS = Production System; CC = Conventional Cages; AV = Aviary; EC = Enriched Cages; SOV = Source of Variation; BW = Body weight (g); BL = Body length (cm); SL = Shank length (cm); SC = Shank circumference (cm); DL = Drumstick length (cm); DC = Drumstick circumference (cm); KL = Keel length (cm); WS = Wing spread (cm); ANOVA = Analysis of Variance.

Table 5 – Female morphometric measurements at 21 weeks of age.

GT	PS	BW	BL	SL	SC	DL	DC	KL	WS
RB		1524.07±2863[a]	67.18±1.73	8.90±0.56	3.45±0.11	14.31±0.32[a]	8.62±0.23[a]	10.84±0.32	10.02±0.21
RR		1390.12±54.21[ab]	62.00±3.02	8.25±0.39	3.29±0.18	13.39±0.30[ab]	7.82±0.22[ab]	10.13±0.33	9.53±0.21
BR		1465.09±58.87[a]	65.82±2.37	8.82±0.25	3.57±0.12	13.98±0.46[ab]	8.43±0.25[ab]	11.12±0.38	9.96±0.20
BB		1317.69±36.57[b]	60.22±0.89	8.03±0.40	3.10±0.06	12.82±0.30[b]	7.63±0.31[b]	10.34±0.36	9.70±0.20
	CC	1398.48±39.96[b]	64.97±2.09[ab]	8.37±0.35	3.44±0.11	13.70±0.29	8.20±0.18	10.40±0.28	9.54±0.20
	AV	1319.84±35.17[b]	59.71±1.79[b]	8.74±0.38	3.26±0.14	13.33±0.39	7.80±0.21	10.74±0.26	10.06±0.20
	EC	1554.41±36.76[a]	66.73±1.57[a]	8.38±0.36	3.35±0.09	13.85±0.28	8.37±0.29	10.68±0.38	9.80±0.12
RB	CC	1542.62±48.47[abc]	69.70±3.41[ab]	8.88±0.81	3.28±0.32[ab]	14.38±0.44	8.77±0.22	11.14±0.67	9.76±0.21[ab]
RB	AV	1499.21±32.63[abc]	68.36±3.43[ab]	9.75±1.13	3.52±0.13[ab]	13.96±0.61	8.15±0.58	10.40±0.44	10.89±0.32[a]
RB	EC	1530.36±70.33[abc]	63.48±1.68[ab]	8.07±1.02	3.56±0.13[ab]	14.59±0.67	8.95±0.30	10.98±0.61	9.42±0.22[ab]
RR	CC	1318.21±92.91[bc]	61.54±7.30[ab]	7.16±0.88	3.68±0.13[a]	13.52±0.31	7.87±0.19	10.26±0.76	9.17±0.53[b]
RR	AV	1233.21±37.28[c]	55.10±2.52[b]	9.26±0.52	2.62±0.30[b]	12.93±0.46	7.69±0.21	9.54±0.31	9.48±0.20[ab]
RR	EC	1618.95±17.40[ab]	69.36±3.24[ab]	8.34±0.19	3.57±0.28[ab]	13.71±0.76	7.90±0.68	10.59±0.60	9.93±0.26[ab]
BR	CC	1455.47±83.32[abc]	68.12±1.38[ab]	7.96±0.18	3.79±0.16[a]	14.05±0.67	8.55±0.28	10.53±0.41	9.76±0.45[ab]
BR	AV	1301.13±89.87[c]	55.42±3.44[b]	8.62±0.21	3.76±0.26[a]	13.92±1.29	7.88±0.52	11.21±0.24	10.28±0.38[ab]
BR	EC	1638.67±84.28[a]	73.92±0.82[a]	9.88±0.39	3.16±0.13[ab]	13.98±0.41	8.87±0.44	11.61±1.06	9.83±0.17[ab]
BB	CC	1277.61±33.93[c]	60.54±0.92[ab]	9.49±0.34	3.02±0.13[ab]	12.83±0.73	7.64±0.49	9.66±0.22	9.47±0.40[ab]
BB	AV	1245.81±39.99[c]	59.96±1.57[ab]	7.36±0.65	3.15±0.13[ab]	12.52±0.58	7.47±0.41	11.82±0.45	9.59±0.39[ab]
BB	EC	1429.65±80.43[abc]	60.18±2.23[ab]	7.24±0.63	3.12±0.06[ab]	13.11±0.22	7.77±0.77	9.54±0.58	10.04±0.28[ab]
SOV					ANOVA				
GT		0.0028	0.0669	0.4024	0.0770	0.0245	0.0223	0.2001	0.2986
PS		< 0.0001	0.0187	0.7185	0.5488	0.4875	0.1805	0.7051	0.1291
GT × PS		< 0.0001	0.0009	0.0360	0.0035	0.4785	0.2838	0.0859	0.0833

Note: Superscripts on different means within column differ significantly at $p \leq 0.05$.

GT = Genotype; RB = RNN × BNN; RR = RNN × RNN; BR = BNN × RNN; BR = BNN × RNN; BB = BNN × BNN; PS = Production System; CC = Conventional Cages; AV = Aviary; EC = Enriched Cages; SOV = Source of Variation; BW = Body weight (g); BL = Body length (cm); SL = Shank length (cm); SC = Shank circumference (cm); DL = Drumstick length (cm); DC = Drumstick circumference (cm); KL = Keel length (cm); WS = Wing spread (cm); ANOVA = Analysis of Variance

Males of RB and BR genotype showed higher (p<0.0001) body lengths than BB and RR genotypes while non-significant differences (p 0.0669) were observed in body lengths of females among different genotypes. Regarding production systems, chickens reared in enriched cages (male p=0.0355 and female p=0.0187) showed longer bodies than chickens reared in aviary and conventional cages. Chickens of BR genotypes (Males p<0.0001 and females p=0.0009) reared in Enriched cage systems showed significantly higher body lengths than other interaction groups.

Higher (p=0.0004) shank circumference was observed in males of RB genotype followed by males of BR, RR and BB genotypes and conventional cage system (p=0.0043) followed by Enriched cage and aviary system. Shank circumference of females did not differ significantly among the genotypes (p=0.0770) and production systems (p=0.5488). Significant interactions in shank circumference of both sexes (male p=0.0002 female p=0.0035) were noticed.

The drumstick length (male p=0.2514, female p=0.4875) and circumference (male p=0.2414, female p=0.1805) did not differ significantly among birds reared in different production systems. However, longer drumsticks in chickens of RB genotype (male p=0.0165, females p=0.0245) were observed. Females of RB genotype showed higher drumstick circumference (p=0.0223) than other genotypes while males showed non-significant differences (p=0.0394) in drumstick circumference among different genotypes.

Keel length of both the sexes showed non-significant differences (male p=0.2588, females p=0.2001) in all genotypes. Males reared in aviary systems showed higher (p=0.0085) keel length than other males reared in enriched cage and conventional cage systems while females showed no influence (p=0.7051) of production system on keel length. Non-significant differences in wing spread (male p=0.7490, females p=0.2986) and shank length (male p=0.2950, females p=0.4024) of different genotypes and wing spread (male p=0.2473, females p=0.1291) and shank length (male p=0.7681, females p=0.7185) of different production systems were observed. Non-significant interactions were found in the drumstick length (male p=0.2424, females p=0.4785), drumstick circumference (male p=0.4542, females p=0.2838), keel length (male p=0.1484, females p=0.0859), shank length (male p=0.1820, females p=0.0360) and wing spread (male p=0.6199). However, females showed significant differences (p=0.0833) in wing spread among interaction groups.

Antibody response and serum chemistry

Serum chemistry and antibody response of Naked Neck, Black Australorp and Rhode Island Red crossbreds were studied under alternative production systems (Table 6). Among different genotypes, highest glucose level (p=0.0006) was observed in RR genotypes followed by RB, BR and BB genotypes. Among different production systems, the highest glucose level (p<0.0001) was observed in the chickens reared in aviary system followed by conventional cages and enriched cages. Antibody titer against ND (p=0.0235) and IB (p=0.0004) were highest in the chickens reared in aviary systems followed by enriched cages and conventional cages. Significant interactions between genotypes and production systems were observed in glucose (p<0.0001) and total proteins (p=0.0176) among serum chemistry parameters. Similarly, significant interactions in antibody titer against IB (p=0.0073) was observed.

Table 6 – Serum chemistry of experimental birds.

GT	PS	GLU	TP	ALB	GLB	UA	CRT	CHO	ND	IB
RB		168.57±5.93^{ab}	4.15±0.14	2.78±0.08	1.57±0.06	6.87±0.45	0.59±0.03	141.15±2.66	4.88±0.09	3704.53±70.35
RR		178.27±4.88^{a}	4.60±0.13	2.65±0.09	1.59±0.06	6.94±0.46	0.58±0.05	136.07±3.55	4.61±0.08	3660.36±69.61
BR		158.36±7.78^{bc}	4.34±0.10	2.66±0.07	1.50±0.04	6.79±0.52	0.59±0.04	138.84±3.24	4.82±0.09	3777.93±66.97
BB		150.51±6.26^{c}	4.41±0.16	2.79±0.05	1.59±0.05	7.80±0.33	0.51±0.01	131.45±5.71	4.77±0.08	3640.27±54.34
	CC	163.89±5.44^{b}	4.48±0.15	2.69±0.07	1.55±0.05	7.21±0.31	0.54±0.03	137.30±2.62	4.62±0.05^{b}	3547.71±40.92^{c}
	AV	182.06±3.74^{a}	4.42±0.09	2.69±0.06	1.58±0.04	7.14±0.53	0.55±0.03	141.56±3.90	4.93±0.09^{a}	3845.61±52.06^{a}
	EC	145.83±4.66^{c}	4.22±0.09	2.78±0.06	1.56±0.05	6.95±0.30	0.61±0.03	131.77±3.44	4.76±0.07^{ab}	3694.00±51.91^{b}
RB	CC	181.20±6.51^{ab}	3.94±0.24^{c}	2.71±0.21	1.66±0.15	7.40±0.58	0.63±0.01	132.87±2.73	4.57±0.14	3443.11±73.02^{d}
RB	AV	176.06±3.56^{ab}	4.34±0.20^{bc}	2.91±0.10	1.55±0.10	6.32±1.28	0.56±0.07	149.56±1.89	5.10±0.04	3865.49±93.90^{ab}
RB	EC	148.45±11.17^{cd}	4.18±0.30^{bc}	2.72±0.13	1.51±0.10	6.88±0.27	0.58±0.07	141.02±4.51	4.96±0.18	3804.99±74.29^{abc}
RR	CC	178.25±4.15^{ab}	5.13±0.18^{a}	2.47±0.13	1.58±0.11	6.71±1.04	0.58±0.12	131.79±6.46	4.58±0.09	3674.49±70.27^{abcd}
RR	AV	194.56±4.78^{a}	4.39±0.12^{bc}	2.57±0.14	1.58±0.13	7.42±0.86	0.52±0.09	145.61±4.10	4.72±0.19	3735.72±150.43^{abcd}
RR	EC	162.01±6.75^{bc}	4.29±0.02^{bc}	2.91±0.14	1.60±0.11	6.71±0.69	0.62±0.07	130.80±5.90	4.53±0.17	3570.86±145.30^{bcd}
BR	CC	160.81±8.45^{bc}	4.53±0.20^{abc}	2.63±0.05	1.37±0.06	7.47±0.54	0.45±0.03	138.45±6.03	4.60±0.14	3558.52±98.00^{bcd}
BR	AV	185.19±5.74^{a}	4.12±0.19^{c}	2.63±0.14	1.62±0.09	6.79±1.48	0.59±0.10	141.17±8.14	4.99±0.23	3971.75±50.23^{a}
BR	EC	129.08±5.88^{d}	4.37±0.11^{bc}	2.73±0.20	1.52±0.02	6.10±0.44	0.73±0.03	136.91±3.19	4.87±0.07	3803.52±91.75^{abc}
BB	CC	135.31±4.29^{d}	4.32±0.35^{bc}	2.95±0.06	1.60±0.02	7.27±0.37	0.51±0.04	146.10±3.06	4.75±0.09	3514.71±64.61^{cd}
BB	AV	172.44±10.69^{ab}	4.85±0.08^{ab}	2.66±0.08	1.57±0.02	8.04±0.71	0.52±0.01	129.90±12.07	4.89±0.22	3809.47±101.70^{abc}
BB	EC	143.78±6.77^{cd}	4.05±0.24^{c}	2.77±0.07	1.61±0.17	8.09±0.68	0.50±0.03	118.35±8.64	4.68±0.07	3596.65±50.30^{bcd}
SOV						ANOVA				
GT		0.0006	0.1496	0.4543	0.6651	0.3809	0.4790	0.3348	0.1694	0.3222
PS		<0.0001	0.2796	0.5503	0.9211	0.8894	0.3545	0.1295	0.0235	0.0004
GT × PS		<0.0001	0.0176	0.3388	0.8789	0.8392	0.4009	0.0667	0.1695	0.0073

Note: Superscripts on different means within column differ significantly at p≤0.05.

GT = Genotype; RB = RNN × BNN; RR = RNN × RNN; BR = BNN × RNN; BR = BNN × RNN; BB = BNN × BNN; PS = Production System; CC = Conventional Cages; AV = Aviary; EC = Enriched Cages; SOV = Source of Variation; GLU = Glucose (mg/dL); TP=Total protein (mg/dL); ALB = Albumin (mg/dL); GLB = Globulin (mg/dL); UA = Uric acid (mg/dL); CRT = Creatinine (mg/dL); CHO = Cholesterol (mg/dL); ND = Newcastle Disease (HI titer); IB = Infectious Bronchitis (ELISA titer); ANOVA = Analysis of Variance.

Behavioral Parameters

Significant differences were observed in behavioral pattern of males and females among different production systems (Table 7, 8). As far as males are concerned, higher dust bathing (p<0.0001) was observed in BB genotype than RR, RB and BR genotypes. The rest of the observing parameters including feeding (p=0.9315), jumping (p=0.1032), perching (p=0.9623), sitting (p=0.0928) and walking (p=0.3486) remained the same among the chickens of different genotypes. Among different production systems, higher dust bathing (p<0.0001), feeding (p<0.0001), jumping (p<0.0001) and walking (p<0.0001) were observed in the males reared in aviary system than in the males reared in other production systems. Perching behavior (p<0.0001) was observed to be higher in the chickens reared in enriched cages than in the aviary system. Sitting behavior (p<0.0001) was highest in chickens reared in conventional cages followed by enriched cages and aviary system. Significant interactions among genotypes and production system were observed in the behavioral traits of males.

As far as behavioral parameters of females are concerned, non-significant differences were observed in the behavior of chickens from different genotypes. Among different production systems, higher dust bathing (p<0.0001), feeding (p<0.0001), jumping (p<0.0001), and walking (p<0.0001) were observed

Table 7 – Behavioral response of male birds.

GT	PS	Dust bathing	Feeding	Jumping	Perching	Sitting	Walking
RB		13.69±0.84[b]	16.20±0.61	0.78±0.14	15.72±1.13	14.75±2.20	10.77±2.68
RR		13.64±0.81[b]	16.05±0.63	0.74±0.13	15.82±1.13	14.67±2.23	10.69±2.67
BR		13.59±0.84[b]	16.20±0.61	0.72±0.13	15.72±1.13	14.75±2.20	10.77±2.68
BB		14.22±0.85[a]	16.28±0.65	0.73±0.13	15.82±1.11	14.31±2.30	10.86±2.68
	CC	--	13.98±0.18[c]	0.03±0.00[c]	--	23.66±0.09[a]	0.22±0.00[c]
	AV	16.29±0.07[a]	19.13±0.27[a]	1.25±0.01[a]	12.42±0.13[b]	3.36±0.12[c]	24.31±0.07[a]
	EC	11.28±0.07[b]	15.45±0.16[b]	0.94±0.01[b]	19.12±0.12[a]	16.77±0.12[b]	7.74±0.08[b]
RB	CC	--	14.09±0.30[cd]	0.04±0.00[d]	--	23.56±0.21[a]	0.21±0.00[d]
RB	AV	16.21±0.05[b]	19.15±0.59[a]	1.29±0.04[a]	12.36±0.31[b]	3.71±0.12[c]	24.29±0.10[a]
RB	EC	11.18±0.12[d]	15.38±0.29[bc]	1.01±0.04[b]	19.08±0.24[a]	16.98±0.22[b]	7.79±0.15[bc]
RR	CC	--	13.20±0.06[d]	0.03±0.00[d]	--	23.58±0.05[a]	0.23±0.00[d]
RR	AV	16.07±0.09[b]	18.98±0.13[a]	1.26±1.01[a]	12.45±0.29[b]	3.47±0.27[cd]	24.19±0.14[a]
RR	EC	11.22±0.10[d]	15.97±0.09[b]	0.93±0.02[bc]	19.18±0.29[a]	16.97±0.09[b]	7.66±0.18[bc]
BR	CC	--	14.09±0.30[cd]	0.03±0.00[d]	--	23.79±0.26[a]	0.21±0.00[d]
BR	AV	16.12±0.08[b]	19.15±0.59[a]	1.22±0.05[a]	12.33±0.24[b]	3.35±0.34[cd]	24.43±0.13[a]
BR	EC	11.05±0.08[d]	15.38±0.29[bc]	0.92±0.02[c]	19.11±0.17[a]	16.82±0.25[b]	7.52±0.06[c]
BB	CC	--	14.53±0.46[cd]	0.03±0.00[d]	--	23.71±0.16[a]	0.23±0.01[d]
BB	AV	16.76±0.08[a]	19.23±0.84[a]	1.23±0.03[a]	12.53±0.30[b]	2.91±0.13[d]	24.36±0.18[a]
BB	EC	11.68±0.17[c]	15.08±0.46[bc]	0.92±0.01[bc]	19.12±0.31[a]	16.33±0.30[b]	8.00±0.17[b]
SOV		ANOVA					
GT		<0.0001	0.9315	0.1032	0.9623	0.0928	0.3486
PS		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
GT × PS		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Note: Superscripts on different means within column differ significantly at $p \leq 0.05$.

GT = Genotype; RB = RNN × BNN; RR = RNN × RNN; BR = BNN × RNN; BR = BNN × RNN; BB = BNN × BNN; PS = Production System; CC = Conventional Cages; AV = Aviary; EC = Enriched Cages; SOV = Source of Variation; ANOVA = Analysis of Variance.

Table 8 – Behavioral response of female birds.

GT	PS	Dust bathing	Feeding	Jumping	Perching	Sitting	Walking
RB		15.01±0.92	14.65±0.47	0.75±0.13	17.89±1.00	15.81±2.31	9.89±2.37
RR		15.23±0.85	14.62±0.40	0.74±0.13	17.87±1.00	15.74±2.32	9.95±2.37
BR		15.30±0.84	14.63±0.42	0.74±0.14	17.83±0.97	15.65±2.33	9.87±2.42
BB		15.15±0.92	14.67±0.54	0.74±0.13	17.85±1.00	15.69±2.32	9.98±2.41
	CC	--	12.93±0.06[c]	0.04±0.00[c]	--	25.08±0.08[a]	0.21±0.00[c]
	AV	17.80±0.04[a]	16.57±0.28[a]	1.25±0.02[a]	14.91±0.10[b]	4.14±0.06[c]	21.83±0.07[a]
	EC	12.54±0.13[b]	14.43±0.20[b]	0.94±0.01[b]	20.81±0.14[a]	17.94±0.08[b]	7.72±0.07[b]
RB	CC	--	12.95±0.14[c]	0.04±0.00[c]	--	25.10±0.19[a]	0.21±0.00[d]
RB	AV	17.74±0.07[a]	16.61±0.66[a]	1.24±0.03[a]	14.99±0.38[b]	4.29±0.22[c]	21.64±0.09[a]
RB	EC	12.28±0.34[b]	14.39±0.46[b]	0.97±0.02[b]	20.79±0.48[a]	18.03±0.22[b]	7.81±0.19[b]
RR	CC	--	12.88±0.08[c]	0.03±0.00[c]	--	25.15±0.15[a]	0.23±0.00[d]
RR	AV	17.77±0.05[a]	16.43±0.28[a]	1.24±0.03[a]	14.85±0.04[b]	4.21±0.08[c]	21.72±0.21[a]
RR	EC	12.68±0.12[b]	14.54±0.11[b]	0.94±0.03[b]	20.88±0.14[a]	17.85±0.15[b]	7.91±0.10[b]
BR	CC	--	12.91±0.07[c]	0.04±0.00[c]	--	25.10±0.22[a]	0.20±0.01[d]
BR	AV	17.81±0.12[a]	16.52±0.43[a]	1.26±0.06[a]	14.96±0.22[b]	4.01±0.04[c]	21.98±0.16[a]
BR	EC	12.79±0.18[b]	14.46±0.26[b]	0.92±0.04[b]	20.70±0.30[a]	17.83±0.19[b]	7.43±0.12[c]
BB	CC	--	12.98±0.22[c]	0.04±0.00[c]	--	24.97±0.09[a]	0.21±0.01[d]
BB	AV	17.89±0.05[a]	16.70±0.90[a]	1.25±0.02[a]	14.84±0.12[b]	4.04±0.03[c]	21.99±0.08[a]
BB	EC	12.41±0.35[b]	14.32±0.67[b]	0.92±0.02[b]	20.86±0.21[a]	18.06±0.13[b]	7.73±0.12[bc]
SOV					ANOVA		
GT		0.5108	0.9990	0.9529	0.9966	0.6331	0.6859
PS		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
GT × PS		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Note: Superscripts on different means within column differ significantly at $p \leq 0.05$.

GT = Genotype; RB = RNN × BNN; RR = RNN × RNN; BR = BNN × RNN; BR = BNN × RNN; BB = BNN × BNN; PS = Production System; CC = Conventional Cages; AV = Aviary; EC = Enriched Cages; SOV = Source of Variation; ANOVA = Analysis of Variance.

in the females reared in aviary system than in the females reared in other production systems. Perching (p<0.0001) was observed to be higher in females reared in enriched cages than in the females reared in aviary system. Sitting (p<0.0001) was highest in females reared in conventional cages followed by enriched cages and conventional cages. Significant interactions among genotypes and production system were observed in the behavioral traits of females.

DISCUSSION

Morphometric measurements

The present study revealed the effect of genotype and production systems on the growth, morphometric measurements, serum chemistry and antibody response. Body weight of both males and females were significantly higher in RB and BR genotypes when compared with RR and BB genotypes. This might be attributed to the additive gene action between genes of RNN and BNN genotypes. Similarly, both RB and BR genotypes showed significantly higher body length, shank length and drumstick lengths as compared to BB and RR genotypes. This might be ascribed by the body weight differences in genotypes as the positive association between body weight and body measurements is the well-known fact. However, non-significant differences were observed among genotypes in terms of wing spread. The effect of crossbreeding between Rhode Island Red, Black Australorp and Naked neck were also explained by Ahmad *et al.* (2019a) where they reported the higher body weight, shank circumference and keel length of progeny obtained when Rhode Island Red and Black Australorp were crossed with naked neck chicken. Variations in morphological traits among genotypes has also been explained by Qureshi *et al.* (2018) where they reported the differences in varieties of Aseel chicken of Pakistan. Adekoya *et al.* (2013) reported dissimilarities in morphometric traits among different chicken genotypes of Nigeria. As far as the production system is concerned, higher body weight and body lengths were observed in enriched cage system when compared with aviary and conventional cage systems. Higher keel length of males was observed in aviary system whereas non-significant differences were observed in the drumstick length and drumstick circumference. The lowest body weight of chickens in the aviary system might be attributed to their vigorous activities, as birds have enough space to do more exercise thus utilizing more nutrients and burning of calories. Higher body lengths associated with higher body weights in enriched cage system might be due to less stress and more countenance of natural behavior. Availability of perches and dust bathing in enriched cages provides stress free compact space less than aviary to prevent nutrient wastage and more than conventional cage in which no materials were provided to express natural behavior. Higher shank circumference was observed in the conventional cage system which showed the negative association of shank circumference and perching behavior as conventional cages were not equipped with any perching material. Similar findings have also been observed by Ahmad *et al.* (2019a) who found lower body weight of chicken reared in free range system compared with semi intensive and intensive systems in which chickens were 9- 14 % heavier. The present findings are also in accordance with the observations of Olaniye *et al.* (2012) and Rehman *et al.* (2016). Lower body weight of slow growing broilers reared in free range system has also been explained by Stadig *et al.* (2016).

Serum chemistry and antibody response

In the present study, higher glucose level was observed in BB genotype than in RR, RB and BR genotypes which might be attributed to the specific genetic makeup of BB chickens. Another possible reason behind the higher glucose level in BB genotype might be the insulin inhibition due to higher fat contents. However, in previous studies, non-significant effect of genotype on plasma glucose level has been reported (Ahmad *et al.,* 2019a). In the present study, higher plasma glucose level was observed in chickens reared in aviary system followed by chickens reared in conventional cages and enriched cage. Similar findings have also been reported in previous studies in which significant variations has been observed in plasma glucose level of chickens reared in different production systems (Gunes *et al.,* 2002, Rehman *et al.,* 2016, Ahmad *et al.,* 2019a). Non-significant variations in albumin, globulin, uric acid and blood cholesterol level was observed in the chickens of different production systems. Negligible effect of production systems on blood cholesterol level has been reported in previous studies (Elerogly *et al.,* 2011, Diktas *et al.,* 2015, Elerogly *et al.,* 2015). As far as antibody response is concerned, the highest antibody titer against ND and IB was observed in chickens reared in aviary system followed by enriched cages and conventional cages. The highest antibody response in chickens reared in aviary system revealed its potential of acclimatization and survival in harsh climatic conditions. This might be attributed to stress free environment with better expression of natural behaviors in aviary system which may enhance the robustness against stressors and fighting ability against disease causing agents. This showed that increased stress in conventional cages inhibits the immune response against diseases while natural behaviors enhance the welfare traits and boosts immunity in aviary system. Ahmad *et al.* (2019a) also reported the higher antibody response in chickens reared in free range system when compared with those reared in semi intensive and intensive housing systems.

Behavioral Parameters

In the present study, higher dust bathing was observed in the males of BB genotype than in RR, BR and RB genotypes which might be due to its specific genetic adaptation. As far as production systems are concerned, dust bathing, feeding, jumping and walking behavior was higher in the aviary system than in the enriched cages and conventional cages. This might be due to the availability of ample space which provoked explorative behavior and stimulated foraging. Chickens were provided with the opportunity to express natural doings which enhanced the comfort zone with reduced stress and encouraged activities. In previous studies, Ponte *et al.* (2008) observed increased foraging behavior of commercial broilers in free range area when compared with confined area. Similarly, increased feeding behavior of commercial layers was noticed when provided with free range access (Shimmura *et al.,* 2008). Restricted explorative behaviors in confinement has been explained by Irfan *et al.* (2016) who reported higher immobility of turkeys (Maleagris gallopavo) in confinement than free range chickens. Mench *et al.* (2001) found that frequency of perching, leg stretching and wing flapping was increased when broilers were provided with outdoor access. Rehman *et al.* (2018) reported increased walking of indigenous Aseel chicken in part time free range. In the present study, sitting behavior was noticed to be highest in the conventional cages than in the enriched cages and aviary system which might be attributed to less moving capacity in conventional cages which dampens the activities and bird movements which were restricted to sitting and stimulated short duration expressions. Similar findings have been reported by Ahmad *et al.* (2019b) who found higher aggressive, standing and sitting behavior of chickens in intensive cage system. Rehman *et al.* (2018) reported higher standing and sitting behavior of Aseel chickens in confinement. Irfan *et al.* (2016) observed more frequent standing and sitting behavior of turkeys in intensive system than in free range system.

CONCLUSIONS

It can be concluded that RB and BR genotypes had better body weight and body length. Among genotypes, chickens reared in enriched cage system had better morphological traits while plasma glucose level and antibody response were more pronounced in aviary production system along with more explorative natural behavior.

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CONFLICT OF INTEREST

No potential conflict of interest was found by the authors.

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