



## Morphometric Traits, Serum Chemistry and Antibody Response of Three Chicken Genotypes under Free-Range, Semi-Intensive and Intensive Housing Systems

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### ■ Keywords

Antibody response, chicken genotypes, housing system, morphometrics, serum chemistry.



### ABSTRACT

The present study evaluated the effect of housing system on the morphometrics, serum chemistry and antibody response of dual-purpose chicken genotypes. A total of 156 pullets and 39 cockerels were randomly picked from 18 treatment block groups (3 housing system × 3 genotypes × 2 sexes) according to Randomized Complete Block Design (RCBD). Three genotypes, purebred Naked Neck (NN) and two crossbred Rhode Island Red × Naked Neck (RIR × NN = RNN) and Black Australorp × Naked Neck (BAL × NN = BNN), were compared. Morphometric traits were recorded during rearing period, thereafter, serum chemistry and antibody response were evaluated in pullets. Intensive and semi-intensive chickens were heavier (males,  $p=0.0012$ ; females,  $p<0.0001$ ) on week 21. Body length was maximum ( $p<0.0001$ ) for free-range female chicken. Maximum ( $p<0.0001$ ) keel length was found in semi-intensive female chickens. Regarding genotypes, RNN and BNN chickens were heavier than NN (males,  $p=0.0015$ ; females,  $p<0.0001$ ). Keel length was maximum ( $p=0.0002$ ) in BNN and NN female chickens. Drumstick circumference were maximum (males,  $p<0.0001$ ; females,  $p<0.0001$ ) in NN chickens, shank circumference was maximum ( $p=0.0150$ ) in RNN and BNN male chickens. Wingspan was maximum ( $p=0.0029$ ) in NN female chickens. Plasma glucose level was higher ( $p=0.0008$ ) in intensive female chickens whereas cholesterol levels was higher ( $p=0.0123$ ) in NN female chicken. Antibody titer against ND was higher ( $p=0.0204$ ) in RNN female chickens while higher ( $p=0.0001$ ) antibody titer against IB was found in free-range chickens. Overall, housing system did not impact morphometric traits or serum chemistry. Only a few differences were observed regarding body weight, body and keel length, plasma glucose, cholesterol and antibody response against ND and IB.

### INTRODUCTION

Crossbreeding is an effective tool for the development of modern-day commercial chickens and equally important for the improvement of rural chickens (Sheridan, 1981). There are different types of crossbreeding comprising two-way, three-way, and four-way rotational crosses or back crosses. Crossbreeding also maximizes the expression of hybrid vigor, improves fitness characteristics that are generally reflected in the resultant cross. Three-way or four-way crosses has to be employed in order to retain the heterosis in material traits (Hoffmann, 2005). In general, crossbreeding involves a two-way cross between an exotic breed and a local one. The aim of these crosses is to combine the characteristics of both genotypes and produce individuals that are more productive, have higher resistance to disease and better adapted to harsh climatic conditions than the parent genotypes (Khawaja *et al.*, 2013).



Despite having enormous potential, limited research work has been conducted for the improvement of indigenous chickens in developing countries. Some attempts have been made to improve the productive of indigenous chickens by crossbreeding or upgrading with known exotics breeds and then leaving the offspring to natural selection (Njenga, 2005). In Pakistan, a dual-purpose chicken genotype was developed by adopting four-way crossbreeding programs in which local chicken (desi = non-descript) was crossed with three exotic breeds: White Cornish, New Hampshire and White Leghorn. The resultant breed, named Lyallpur Silver Black (LSB), was developed that have better productive performance and livability in harsh climatic conditions (Siddiqi *et al.*, 1979).

Blood biochemical profile is generally considered as an ideal indicator of health status, and frequently applied by avian pathologists to determine birds' immune status and to obtain basic knowledge on specific poultry diseases. Regarding blood chemistry, total serum protein is useful to draw inferences about the quality of dietary protein (Bonadiman *et al.*, 2009; Alikwe *et al.*, 2010). Likewise, triglyceride and glucose level indicate the energy requirements for physiological responses and allow proper body biochemical functions (Kral & Suchy, 2000).

In order to understand infection outcomes and bird's performance, the knowledge of the immune response is essential. In this regard, indigenous poultry may be the most efficient model to study the immune response against bacterial and viral infections (Haunshi *et al.*, 2011). There are still limited data on the maternal effects or reference values of distinct crosses. The lack of reference serum chemistry levels and antibody response against diseases motivates scientists to establish these references for particular crossbreds. Therefore, present study aimed at investigating if there are differences in morphometric traits, serum chemistry and antibody response in dual-purpose chicken genotypes reared in free-range, semi-intensive or intensive systems.

## MATERIALS AND METHODS

This study was conducted under practical conditions at Indigenous Chicken Resource Centre (ICGRC), Department of Poultry Production, University of Veterinary and Animal Science (UVAS), Ravi Campus, A-Block, Pattoki, Pakistan. Pattoki is located at 31°1'0N, 73°50'60E and at an altitude of 186 m (610 ft). This city normally experiences hot and humid tropical climate, with maximum temperatures ranging from 13°C in winter and 43°C in summer.

### Ethics

Birds' care and use of bird were in accordance with the laws and regulations of Pakistan, and the experimental procedures were approved by the Committee of Ethical Handling of Experimental Birds (No. DR/124), UVAS, Pakistan.

### Experimental Birds

Four hundred and eighty one-day-old chicks hatched at Avian Research and Training (ART) Centre, UVAS, Lahore, Pakistan, were transported to ICGRC, A-Block, UVAS, Ravi Campus, Pattoki, Pakistan for evaluation. Chicks belong to the genotypes (160 birds each): Rhode Island Red × Naked Neck (RNN) crossbreds, Black Australorp × Naked Neck (BNN) crossbreds, and Naked Neck × Naked Neck (NN) purebreds.

Chicks were brooded in well-ventilated open-sided house, and submitted to standard management practices until six weeks old (June to July, 2018.) Birds were fed a commercial broiler breeder diet (16% crude protein, 2900 kcal metabolizable energy/kg). During the brooding period, birds were vaccinated against Newcastle disease and infectious bronchitis, according to local schedule of area.

At 6 weeks of age, 60 (30 males and 30 females) from each genotype (RNN, BNN and NN) were transferred to three housing systems (free-range, semi-intensive or intensive), totaling 360 birds (3 genotypes × 3 housing systems × 2 sexes × 20 birds = 360). Weekly body weight and behavioral repertoires were recorded for the duration of 10 weeks (6 to 16 weeks).

At 16 weeks of age, out of the 260 birds (156 ♀ and 104 ♂), 52 pullets and 13 cockerels from each genotype were used in rearing phase (17 to 21 weeks). For this, 156 females and 39 males were randomly picked from 18 treatment groups (3 genotypes × 3 housing systems × 2 sexes) according to Randomized Complete Block Design (RCBD). Furthermore, males were reared separately.

### Free-Range, Semi-intensive and Intensive Systems

All the experimental birds were individually tagged. In the free-range and semi-intensive systems, birds were kept in open sided shed (6.1 m L × 6.1 m W × 3.66 m H) oriented east to west. A range area of fertile land (10 m L × 2.99 W, at a stocking density of 0.23m<sup>2</sup> / bird) located in front of the shed was used. Free range area enriched with grasses and platns [Mung (*Vigna radiate L.*), Black eyed Pea (*Vigna unguiculata L.*), French Pea (*Phaseolus vulgaris L.*) and Lucerne



(*Medicago sativa L.*) (Table 1), which was divided in two rows were made by fishing nets (one for the free-range and one for the semi-intensive system). Fresh water was provided *ad libitum* in manual drinkers. For the protection of the birds, a 2.44-m high wire-mesh enclosure was installed surrounding the range area.

**Table 1** – Proximate analysis of legumes cultivated at range area.

Proximate Analysis (%)	Mung ( <i>Vigna radiata L.</i> )	Black Eyed Pea ( <i>Vigna unguiculata L.</i> )	French Peas ( <i>Phaseolus vulgaris L.</i> )	Lucerne ( <i>Medicago sativa L.</i> )
Dry Matter	18.60	12.12	10.12	18.20
Crude Protein	18.04	26.84	30.80	22.50
Crude Fiber	17.75	21.58	16.52	24.00
Ether Extract	2.13	2.02	1.79	1.70
Ash	9.40	12.26	15.16	12.40

The birds under intensive system were maintained in well-ventilated poultry shed equipped with three-tiered battery cage system (FACCO, Poultry Equipment-C3, Italy), during rearing phase, 17 cages were used comprising four birds each; 0.14 m<sup>2</sup>/bird floor space was provided. Birds were offered a broiler breeder developer diet formulated according to the recommendations of the NRC (1994) (Table 2) and daily feed allowance was increased corresponding to their growth and requirement (Table 3).

**Table 2** – Composition of the feed supplied during the rearing phase.

Feed Ingredient (%)	Rearing Phase (17-21 weeks)
Corn	59.00
Wheat grain	5.00
Rice tips	8.40
Wheat bran	5.00
Soybean Meal	7.00
Fish Meal	--
Canola Meal	10.00
Feather Meal	1.10
Soybean Oil	1.20
Dicalcium phosphate	--
Limestone	2.40
NaCl	0.30
Methionine	0.10
Total	100
Nutrient Levels	
Dry matter	89.8
Crude Protein	15.46
ME (kcal/kg)	2913
Calcium	1.00
Phosphorus	0.42
Lysine	0.69
Methionine	0.35

(Leeson & Summers, 2005).

The birds under free-range and semi-intensive systems were given access to the vegetation and drinking water from 06:00 to 18:00 and 06:00 to 12:00, respectively. Birds in the semi-intensive systems were offered 50% of the developer feed in the evening, whereas free-range birds did not receive any feed (Table 1).

**Table 3** – Weekly feed allowance (g) during the rearing phase (17-21 weeks).

Age (Week)	Housing System		
	Free-range	Semi-intensive	Intensive
17	0	22	44
18	0	23	46
19	0	24	48
20	0	25	50
21	0	26	52

(NRC, 1994; Leeson & Summers 2005).

## Parameters Studied

### Morphometric traits

Morphometric traits were weekly measured, including body weight, beak length, drumstick length, shank length, drumstick circumference, shank circumference, body length and wing spread.

### Serum Chemistry and Antibody Response

At the end of the experiment (21 weeks of age), 3 mL of blood were collected from the brachial wing vein of three females per treatment using a syringe with anticoagulant. After blood centrifugation, the serum was collected in Eppendorf tubes and stored at -15°C to -20°C until analyses (Gunes *et al.*, 2002). Serum was analyzed for albumin, globulin, uric acid, glucose, total protein, creatinine and cholesterol contents using serum analysis kits (Kumar and Kumbhakar, 2015). One week prior to slaughter, birds were vaccinated against Newcastle Disease and Infectious Bronchitis and antibody titer were evaluated at the end of experimentation (Xie *et al.*, 2008).

### Statistical Analysis

Collected data regarding morphometric traits, serum chemistry and antibody response were analyzed by two-way analysis of variance assuming



genotypes and housing systems as the main effects. Morphometric trait data were analyzed separately for males and females to assess the treatment effect within sex, whereas serum chemistry was evaluated only in females. GLM procedures of SAS software (Version 9.1, 2002-2004) was used, and significant treatment means were compared by Tukey-Kramer test (Tukey, 1953), considering a significance level of  $P \leq 0.05$ . The following mathematical model was used:

$$Y_{ijk} = \mu + \beta_i + \tau_j + (\beta \times \tau)_{ij} + \epsilon_{ijk}$$

Where,

$Y_{ijk}$  = Observation of dependent variable recorded on  $j^{\text{th}}$  Housing System in  $i^{\text{th}}$  Block

$\mu$  = Overall population mean

$\beta_i$  = Effect of  $i^{\text{th}}$  Block ( $i = 1, 2, 3$ )

$\tau_j$  = Effect of  $j^{\text{th}}$  Housing System ( $j = 1, 2, 3$ )

$(\beta \times \tau)_{ij}$  = Interaction between block and housing system

$\epsilon_{ijk}$  = Residual error of  $k^{\text{th}}$  observation on  $j^{\text{th}}$  treatment in  $i^{\text{th}}$  block NID  $\sim 0, \sigma^2$

## RESULTS

### Morphometric traits

Morphometric traits differed among genotypes, housing system and their interaction (Tables 4, 5, 6).

**Table 4** – Effect of genotype and housing system on body weight, body length and keel length of chickens at 21 weeks of age.<sup>1</sup>

Genotype	Housing System	Body Weight (g)		Body Length (cm)		Keel Length (cm)	
		Male	Female	Male	Female	Male	Female
RIR × NN <sup>2</sup>		1817.25 <sup>a</sup> ± 45.32	1425.17 <sup>a</sup> ± 18.35	69.64 ± 1.81	63.27 ± 1.67	11.68 ± 0.37	10.04 <sup>b</sup> ± 0.35
BAL × NN <sup>2</sup>		1811.17 <sup>a</sup> ± 63.10	1456.22 <sup>a</sup> ± 25.26	68.49 ± 2.39	62.27 ± 1.78	12.27 ± 0.36	10.89 <sup>a</sup> ± 0.32
NN		1616.05 <sup>b</sup> ± 30.99	1256.79 <sup>b</sup> ± 34.92	69.41 ± 0.30	65.29 ± 0.76	12.27 ± 0.29	11.58 <sup>a</sup> ± 0.17
	Free-range	1619.60 <sup>b</sup> ± 20.88	1273.80 <sup>c</sup> ± 31.76	69.53 ± 1.39	65.49 <sup>a</sup> ± 1.39	11.54 ± 0.28	9.86 <sup>c</sup> ± 0.31
	Semi-intensive	1774.89 <sup>a</sup> ± 57.21	1412.12 <sup>b</sup> ± 30.15	70.45 ± 1.57	66.76 <sup>a</sup> ± 0.71	12.64 ± 0.33	11.75 <sup>a</sup> ± 0.21
	Intensive	1849.97 <sup>a</sup> ± 56.20	1452.26 <sup>a</sup> ± 19.65	67.56 ± 2.44	58.58 <sup>b</sup> ± 1.85	12.03 ± 0.36	10.89 <sup>b</sup> ± 0.32
RIR × NN	Free-range	1639.84 <sup>bc</sup> ± 48.80	1558.90 <sup>b</sup> ± 9.62	69.50 ± 3.40	65.48 <sup>a</sup> ± 2.99	11.28 ± 0.53	8.30 <sup>d</sup> ± 0.44
RIR × NN	Semi-intensive	1875.10 <sup>a</sup> ± 53.89	1388.32 <sup>d</sup> ± 26.57	69.64 ± 2.16	66.06 <sup>a</sup> ± 1.50	12.07 ± 0.85	11.80 <sup>a</sup> ± 0.52
RIR × NN	Intensive	1936.80 <sup>a</sup> ± 30.57	1328.28 <sup>c</sup> ± 23.82	69.77 ± 4.44	58.28 <sup>b</sup> ± 3.57	11.68 ± 0.66	10.01 <sup>bc</sup> ± 0.57
BAL × NN	Free-range	1645.64 <sup>bc</sup> ± 28.13	1242.62 <sup>f</sup> ± 10.87	72.68 ± 1.64	68.63 <sup>a</sup> ± 2.32	11.37 ± 0.67	9.56 <sup>c</sup> ± 0.50
BAL × NN	Semi-intensive	1822.51 <sup>ab</sup> ± 132.75	1663.10 <sup>a</sup> ± 14.25	68.50 ± 3.16	67.49 <sup>a</sup> ± 1.21	13.16 ± 0.47	12.24 <sup>a</sup> ± 0.27
BAL × NN	Intensive	1965.38 <sup>a</sup> ± 90.98	1462.93 <sup>c</sup> ± 11.51	64.31 ± 6.22	50.70 <sup>c</sup> ± 3.19	12.27 ± 0.48	10.86 <sup>abc</sup> ± 0.64
NN	Free-range	1573.33 <sup>c</sup> ± 23.43	1019.88 <sup>h</sup> ± 8.68	66.41 ± 0.72	62.37 <sup>ab</sup> ± 1.62	11.98 ± 0.22	11.73 <sup>a</sup> ± 0.28
NN	Semi-intensive	1627.07 <sup>bc</sup> ± 63.94	1184.93 <sup>g</sup> ± 20.37	73.22 ± 2.93	66.73 <sup>a</sup> ± 0.94	12.70 ± 0.30	11.20 <sup>ab</sup> ± 0.24
NN	Intensive	1647.75 <sup>bc</sup> ± 70.30	1565.57 <sup>b</sup> ± 33.90	68.59 ± 5.15	66.76 <sup>a</sup> ± 1.08	12.14 ± 0.82	11.81 <sup>a</sup> ± 0.35
Source of Variation		<i>p</i> -value					
Genotype		0.0015	<0.0001	0.9044	0.2510	0.3783	0.0002
Housing System		0.0012	<0.0001	0.5539	<0.0001	0.0910	<0.0001
Genotype × Housing System		0.0009	<0.0001	0.6835	<0.0001	0.4278	<0.0001

<sup>a-h</sup> Means in the same column with no common superscript differ significantly at  $p \leq 0.05$ .

<sup>1</sup>Values are mean ± standard error.

RIR = Rhode Island Red; NN = Naked Neck; BAL = Black Australorp.


**Table 5** – Effect of genotype and housing system on drumstick, shank length and drumstick circumference of chickens at 21 weeks of age.<sup>1</sup>

Genotype	Housing System	Drumstick Length (cm)		Drumstick Circumference (cm)		Shank Length (cm)	
		Male	Female	Male	Female	Male	Female
RIR × NN <sup>2</sup>		13.69 ± 0.41	12.45 ± 0.49	8.00 <sup>b</sup> ± 0.20	6.70 <sup>b</sup> ± 0.22	11.05 ± 0.55	8.42 ± 0.34
BAL × NN <sup>2</sup>		14.37 ± 0.31	12.64 ± 0.42	8.01 <sup>b</sup> ± 0.39	7.03 <sup>b</sup> ± 0.24	11.10 ± 0.90	8.79 ± 0.44
NN		13.76 ± 0.51	13.15 ± 0.26	10.13 <sup>a</sup> ± 0.23	9.82 <sup>a</sup> ± 0.11	9.85 ± 0.27	9.46 ± 0.16
	Free-range	13.38 ± 0.42	12.56 ± 0.35	8.69 ± 0.44	7.56 ± 0.26	10.06 ± 0.33	8.66 ± 0.22
	Semi-intensive	14.53 ± 0.45	12.57 ± 0.39	8.70 ± 0.41	7.65 ± 0.31	11.35 ± 0.91	9.04 ± 0.39
	Intensive	13.92 ± 0.33	13.10 ± 0.46	8.75 ± 0.39	8.04 ± 0.27	10.60 ± 0.51	8.97 ± 0.38
RIR × NN	Free-range	13.09 ± 0.50	12.31 ± 0.80	8.19 <sup>b</sup> ± 0.51	6.94 <sup>b</sup> ± 0.30	10.04 ± 0.78	8.00 ± 0.43
RIR × NN	Semi-intensive	14.29 ± 1.05	12.01 ± 0.87	7.81 <sup>b</sup> ± 0.25	6.27 <sup>b</sup> ± 0.49	12.07 ± 1.28	8.79 ± 0.66
RIR × NN	Intensive	13.69 ± 0.49	13.02 ± 0.92	8.00 <sup>b</sup> ± 0.32	6.88 <sup>b</sup> ± 0.34	11.05 ± 0.62	8.47 ± 0.66
BAL × NN	Free-range	14.23 ± 0.70	12.07 ± 0.62	7.97 <sup>b</sup> ± 0.94	7.00 <sup>b</sup> ± 0.46	10.73 ± 0.34	8.55 ± 0.41
BAL × NN	Semi-intensive	14.51 ± 0.65	12.70 ± 0.71	8.05 <sup>b</sup> ± 0.70	6.87 <sup>b</sup> ± 0.39	11.47 ± 2.63	8.98 ± 0.97
BAL × NN	Intensive	14.38 ± 0.35	13.14 ± 0.85	8.01 <sup>b</sup> ± 0.55	7.22 <sup>b</sup> ± 0.39	11.10 ± 1.35	8.85 ± 0.83
NN	Free-range	12.80 ± 0.92	13.31 ± 0.24	9.90 <sup>a</sup> ± 0.48	9.62 <sup>a</sup> ± 0.15	9.40 ± 0.44	9.44 ± 0.20
NN	Semi-intensive	14.77 ± 0.79	13.00 ± 0.40	10.24 <sup>a</sup> ± 0.38	9.82 <sup>a</sup> ± 0.15	10.50 ± 0.44	9.34 ± 0.22
NN	Intensive	13.70 ± 0.85	13.14 ± 0.66	10.24 <sup>a</sup> ± 0.45	10.03 <sup>a</sup> ± 0.25	9.65 ± 0.46	9.59 ± 0.40
Source of Variation		p-value					
Genotype		0.4638	0.4550	<0.0001	<0.0001	0.4052	0.0939
Housing System		0.1755	0.5645	0.9879	0.3905	0.3391	0.7079
Genotype × Housing System		0.5830	0.8618	0.0039	<0.0001	0.7999	0.6373

<sup>a,b</sup> Means in the same column with no common superscript differ significantly at  $p \leq 0.05$ .

<sup>1</sup>Values are least square mean ± standard error.

<sup>2</sup>RIR = Rhode Island Red; NN = Naked Neck; BAL = Black Australorp.

**Table 6** – Effect of genotype and housing system on shank circumference and wingspan of chickens at 21 weeks of age.<sup>1</sup>

Genotype	Housing System	Shank circumference (cm)		Wingspan (cm)	
		Male	Female	Male	Female
RIR × NN <sup>2</sup>		4.25 <sup>a</sup> ± 0.21	3.56 ± 0.12	10.25 ± 0.48	8.30 <sup>b</sup> ± 0.34
BAL × NN <sup>2</sup>		4.06 <sup>a</sup> ± 0.10	3.56 ± 0.11	11.04 ± 0.46	8.94 <sup>ab</sup> ± 0.26
NN		3.58 <sup>b</sup> ± 0.11	3.35 ± 0.06	10.01 ± 0.23	9.62 <sup>a</sup> ± 0.14
	Free-range	3.98 ± 0.21	3.44 ± 0.10	10.10 ± 0.40	9.01 ± 0.24
	Semi-intensive	3.89 ± 0.18	3.67 ± 0.09	10.87 ± 0.47	8.96 ± 0.28
	Intensive	4.01 ± 0.10	3.36 ± 0.09	10.34 ± 0.38	8.90 ± 0.29
RIR × NN	Free-range	4.47 ± 0.51	3.57 ± 0.23	10.04 ± 0.89	8.53 ± 0.57
RIR × NN	Semi-intensive	4.03 ± 0.39	3.74 ± 0.19	10.47 ± 0.98	8.21 ± 0.62
RIR × NN	Intensive	4.25 ± 0.17	3.37 ± 0.19	10.25 ± 0.87	8.16 ± 0.62
BAL × NN	Free-range	3.98 ± 0.16	3.35 ± 0.19	10.46 ± 0.90	9.07 ± 0.40
BAL × NN	Semi-intensive	4.14 ± 0.21	3.96 ± 0.14	11.63 ± 0.99	8.95 ± 0.51
BAL × NN	Intensive	4.06 ± 0.16	3.37 ± 0.18	11.05 ± 0.60	8.80 ± 0.48
NN	Free-range	3.50 ± 0.17	3.40 ± 0.11	9.79 ± 0.23	9.42 ± 0.17
NN	Semi-intensive	3.51 ± 0.47	3.31 ± 0.09	10.51 ± 0.47	9.71 ± 0.22
NN	Intensive	3.73 ± 0.12	3.33 ± 0.11	9.72 ± 0.43	9.75 ± 0.34
Source of Variation		p-value			
Genotype		0.0150	0.1908	0.2312	0.0029
Housing System		0.8485	0.0641	0.4506	0.9631
Genotype × Housing System		0.2115	0.0748	0.7358	0.1213

<sup>a,b</sup> Means in the same column with no common superscript differ significantly at  $p \leq 0.05$ .

<sup>1</sup>Values are least square mean ± standard error.

<sup>2</sup>RIR = Rhode Island Red; NN = Naked Neck; BAL = Black Australorp.

RNN females. The interaction between factors showed that RNN and BNN females reared in the semi-intensive system and NN female chicken reared in the free-range and intensive systems had maximum keel length.

Drumstick and shank lengths were not influenced by housing system (males,  $p=0.1755$ ,  $p=0.3391$ ; females,  $p=0.5645$ ,  $p=0.7079$ ), genotype (males,  $p=0.4638$ ,  $p=0.4052$ ; females,  $p=0.4550$ ,  $p=0.0939$ ), or their



interaction (males,  $p=0.5830$ ,  $p=0.7999$ ; females,  $p=0.8618$ ,  $p=0.6373$ ). Larger drumstick circumference (males,  $p<0.0001$ ; females,  $p<0.0001$ ) in NN chickens than those of RNN and BNN chickens. The interaction between housing system and genotype determined the largest (males,  $p=0.0039$ ; females,  $p<0.0001$ ) drumstick circumference in NN chickens reared in the free-range and intensive systems. Larger shank circumference ( $p=0.0150$ ) was measured in RNN and BNN males compared with NN, but in females, shank length was not affected by the treatments. No differences in wingspan was determined in males ( $p>0.05$ ), however, NN females had longer wingspan ( $p=0.0029$ ) compare with RNN females.

### Serum Chemistry and Antibody Response

There were no differences in total protein, albumin, globulin, uric acid and creatinine blood levels among genotypes and housing systems and no significant interactions were detected (Table 7, 8).

There was no influence of housing system or genotype on total protein, albumin, globulin, uric acid or creatinine levels ( $p>0.05$ ). However, serum glucose and cholesterol levels, as well as antibody responses against ND and IB differed among treatments (Table 8). Serum glucose level was higher ( $p=0.0008$ ) in females reared in the intensive system relative to the semi-intensive and free-range systems, and in NN

birds than in RNN birds ( $p<0.0123$ ). The interaction between housing system and genotype showed the highest ( $p=0.0164$ ) plasma glucose level in NN females reared in the intensive system. Higher cholesterol levels ( $p=0.0123$ ) were detected in NN birds compared with BNN. The interaction between housing system and genotype was significant ( $p=0.0103$ ), with the highest cholesterol level measured in BNN birds reared in the intensive system. Relative to antibody titers against ND, higher ( $p=0.0204$ ) titer were determined in RNN birds than in BNN. Furthermore, higher ( $p=0.0001$ ) antibody titer against IB was found in free-range chickens followed by those reared in the semi-intensive and intensive systems. The interaction showed that NN birds reared in the free-range system had the highest ( $p=0.0067$ ) titer against IB.

### DISCUSSION

The present study aimed at comparing morphometric traits, serum chemistry and antibody response among different genotypes and housing systems.

When housing systems were compared, although no differences were detected in drumstick length and circumference, shank length and circumference, and wingspan, males were 9-14% and females were 11-14% heavier when reared in the intensive and semi-intensive systems at market age (21 weeks) compared

**Table 7** – Effect of genotype and housing systems on the serum chemistry of 21-week-old pullets.<sup>1</sup>

Genotype	Housing System	Glucose (mg/dL)	Total Protein (mg/dL)	Albumin (mg/dL)	Globulin (mg/dL)	Uric Acid (mg/dL)	Creatinine (mg/dL)
RIR × NN <sup>2</sup>		157.48 ± 8.55	4.30 ± 0.20	2.64 ± 0.10	1.61 ± 0.09	7.65 ± 0.54	0.59 ± 0.06
BAL × NN <sup>2</sup>		167.62 ± 9.31	4.23 ± 0.14	2.70 ± 0.10	1.50 ± 0.07	7.48 ± 0.36	0.52 ± 0.03
NN		157.71 ± 10.72	4.51 ± 0.07	2.80 ± 0.09	1.52 ± 0.04	6.37 ± 0.58	0.59 ± 0.05
	Free-range	138.43 <sup>b</sup> ± 5.63	4.51 ± 0.17	2.71 ± 0.07	1.50 ± 0.06	7.31 ± 0.41	0.55 ± 0.05
	Semi-intensive	158.93 <sup>b</sup> ± 10.11	4.21 ± 0.14	2.66 ± 0.12	1.64 ± 0.09	7.25 ± 0.68	0.55 ± 0.04
	Intensive	185.45 <sup>a</sup> ± 3.18	4.32 ± 0.11	2.78 ± 0.10	1.49 ± 0.05	6.93 ± 0.49	0.61 ± 0.06
RIR × NN	Free-range	130.74 <sup>c</sup> ± 6.76	4.81 ± 0.30	2.70 ± 0.19	1.51 ± 0.16	6.84 ± 0.90	0.56 ± 0.09
RIR × NN	Semi-intensive	154.60 <sup>abc</sup> ± 5.11	3.80 ± 0.28	2.60 ± 0.19	1.84 ± 0.13	8.74 ± 0.56	0.51 ± 0.10
RIR × NN	Intensive	187.10 <sup>ab</sup> ± 2.17	4.29 ± 0.20	2.63 ± 0.19	1.48 ± 0.10	7.36 ± 1.19	0.69 ± 0.14
BAL × NN	Free-range	152.11 <sup>abc</sup> ± 12.94	4.15 ± 0.33	2.77 ± 0.11	1.47 ± 0.12	7.87 ± 0.91	0.48 ± 0.03
BAL × NN	Semi-intensive	173.29 <sup>ab</sup> ± 2.588	4.28 ± 0.01	2.67 ± 0.31	1.61 ± 0.16	7.44 ± 0.43	0.62 ± 0.04
BAL × NN	Intensive	177.46 <sup>ab</sup> ± 4.18	4.27 ± 0.33	2.64 ± 0.12	1.42 ± 0.10	7.12 ± 0.65	0.46 ± 0.05
NN	Free-range	132.43 <sup>c</sup> ± 5.09	4.57 ± 0.21	2.64 ± 0.12	1.53 ± 0.01	7.21 ± 0.38	0.60 ± 0.11
NN	Semi-intensive	148.90 <sup>bc</sup> ± 19.21	4.55 ± 0.11	2.70 ± 0.17	1.45 ± 0.10	5.58 ± 1.58	0.51 ± 0.09
NN	Intensive	191.80 <sup>a</sup> ± 6.75	4.39 ± 0.05	3.06 ± 0.05	1.56 ± 0.05	6.32 ± 0.85	0.67 ± 0.09
Source of Variation				<i>p</i> -value			
Genotype		0.5290	0.3397	0.5382	0.4513	0.1994	0.5383
Housing System		0.0008	0.3003	0.7121	0.2269	0.8622	0.6474
Genotype × Housing System		0.0164	0.2056	0.7411	0.3118	0.4784	0.5769

<sup>a-c</sup> Means in the same column with no common superscript differ significantly at  $p\leq 0.05$ .

<sup>1</sup>Values are least square mean ± standard error.

<sup>2</sup>RIR = Rhode Island Red; NN = Naked Neck; BAL = Black Australorp.


**Table 8** – Effect of genotype and housing system on the cholesterol and antibody response of 21-week-old pullets.<sup>1</sup>

Genotype	Housing System	Cholesterol (mg/dL)	ND (HI titer)	IB (ELISA titer)
RIR × NN <sup>2</sup>		134.48 <sup>ab</sup> ± 3.50	5.10 <sup>a</sup> ± 0.06	3629.91 ± 53.88
BAL × NN <sup>2</sup>		127.11 <sup>b</sup> ± 5.85	4.70 <sup>b</sup> ± 0.10	3629.89 ± 70.91
NN		143.87 <sup>a</sup> ± 3.13	4.95 <sup>ab</sup> ± 0.11	3599.70 ± 87.39
	Free-range	128.96 ± 5.41	4.98 ± 0.10	3823.56 <sup>a</sup> ± 30.79
	Semi-intensive	138.01 ± 4.44	4.79 ± 0.14	3598.62 <sup>b</sup> ± 31.44
	Intensive	138.48 ± 4.21	4.97 ± 0.05	3437.32 <sup>c</sup> ± 65.58
RIR × NN	Free-range	131.84 <sup>abc</sup> ± 3.06	5.13 ± 0.07	3801.17 <sup>ab</sup> ± 51.87
RIR × NN	Semi-intensive	140.83 <sup>ab</sup> ± 4.92	5.08 ± 0.15	3588.95 <sup>abc</sup> ± 68.15
RIR × NN	Intensive	130.75 <sup>abc</sup> ± 9.09	5.07 ± 0.09	3499.61 <sup>c</sup> ± 59.20
BAL × NN	Free-range	112.83 <sup>c</sup> ± 9.41	4.80 ± 0.10	3801.14 <sup>ab</sup> ± 69.18
BAL × NN	Semi-intensive	123.11 <sup>bc</sup> ± 4.79	4.40 ± 0.13	3640.87 <sup>abc</sup> ± 22.34
BAL × NN	Intensive	145.38 <sup>a</sup> ± 4.69	4.92 ± 0.10	3447.66 <sup>c</sup> ± 154.03
NN	Free-range	142.21 <sup>ab</sup> ± 5.60	5.02 ± 0.28	3868.35 <sup>a</sup> ± 48.92
NN	Semi-intensive	150.09 <sup>a</sup> ± 1.04	4.89 ± 0.25	3566.04 <sup>bc</sup> ± 72.42
NN	Intensive	139.30 <sup>ab</sup> ± 7.34	4.94 ± 0.08	3364.70 <sup>c</sup> ± 140.52
Source of Variation			<i>p</i> -value	
Genotype		0.0123	0.0204	0.8858
Housing System		0.1274	0.2546	0.0001
Genotype × Housing System		0.0103	0.1001	0.0067

<sup>a-c</sup> Means in the same column with no common superscript differ significantly at  $p \leq 0.05$ .

<sup>1</sup>Values are least square mean ± standard error.

<sup>2</sup>RIR = Rhode Island Red; NN = Naked Neck; BAL = Black Australorp.

with those reared in the free-range system. This may be attributed to the active behavior of free-range chickens. In general, these birds do more exercise during their life span, ultimately spending more calories. These results are in agreement with the findings of Rehman *et al.* (2016) who found higher body weight of Aseel chicken varieties when reared under intensive and semi intensive housing systems. Likewise, Olaniyi *et al.* (2012) reported higher body weight of Harco black and Novogen cockerels when reared under deep litter system as compared to free range reared birds. Similarly, reduced body weight in slow-growing broilers exposed to free-range access was reported by Stadig *et al.* (2016). In the present study, the longest body length was measured in free-range females, and keel length females reared in the semi-intensive system.

Differences among genotypes were also detected. Both male and female RNN and BNN chickens were heavier at 21 weeks of age, and larger shank circumference than NN chickens. Longer keels were measured in BNN and NN females, whereas higher drumstick circumference and wingspan values were determined in NN chickens.

The observed differences in morphological traits agree with the findings of Qureshi *et al.* (2018), who found variation among different phenotypes of Aseel chickens in Pakistan. Similarly, Adekoya *et al.* (2013) and Fadare (2014) reported variation in morphological

traits among five indigenous chicken genotypes in Nigeria.

The higher glucose level obtained in female reared in the intensive system relative to semi-intensive and free-range systems are consistent with the reports of Gunes *et al.* (2002) and Rehman *et al.* (2016), who evaluated alternative housing systems and determined higher blood glucose levels in intensively-reared layers and in Aseel chickens, respectively. It is possible that the lower plasma glucose level determined in free-range chickens may be due to their intense exercise, which ultimately increases insulin level and stimulates glucose metabolism.

There was no influence of housing systems on cholesterol level, in agreement with other studies (Elerogly *et al.* 2011; Diktas *et al.* 2015; Eleroglu *et al.* 2015) that found negligible effects of housing system on cholesterol level among different chicken genotypes. However, higher cholesterol level was determined in NN than in BNN birds. This may be attributed to their specific genetic makeup.

Antibody titers against ND were not influenced by housing system, but higher titers were determined in RNN than in BNN birds. This result may be attributed to distinct genetic resistance against the disease, which was more pronounced in RNN chickens compared with BNN chickens. On the other hand, genotype did not affect antibody titers against IB, whereas higher titers were measured in free-range chickens followed



by those reared in the semi-intensive and intensive systems. Similar differences in antibody response against ND and IB among different chicken and duck genotypes were obtained by Shini (2003), Arbona *et al.* (2011), Shi *et al.* (2011), and Rehman *et al.* (2016).

## CONCLUSIONS

In general, morphometric traits and serum chemistry were not affected by housing system, except for a few differences observed regarding body weight, body and keel length, plasma glucose, cholesterol and antibody response against ND and IB. Therefore, alternative housing systems (semi-intensive and free-range) can successfully be adopted for dual-purpose chicken genotypes.

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## CONFLICT OF INTEREST

No potential conflict of interest was found by the authors.

## REFERENCES

- Adekoya KO, Oboh BO, Adefenwa MA, Ogunkanmi LA. Morphological characterization of five Nigerian indigenous chicken types. *Journal of Science Research and Development* 2013; 14: 55-66.
- Alikwe PCN, Faremi AY, Egwaikhide PA. Biochemical evaluation of serum metabolites, enzymes and haematological indices of broiler-chicks fed with varying levels of rumen epithelial scraps in place of fish meal proteins. *Research Journal of Poultry Science* 2010; 3: 27-31.
- Arbona DV, Anderson KE, Hoffman JB. A comparison of humoral immune function in response to a killed Newcastle's vaccine challenge in caged vs. free-range Hy-Line brown layers. *International Journal of Poultry Science* 2011; 10: 315-319.
- Bonadiman SF, Stratievsky GC, Machado JA, Albernaz AP, Rabelo GR, Damatta RA. Leukocyte ultrastructure, hematological and serum biochemical profiles of ostriches (*Struthio camelus*). *Poultry Science* 2009; 88: 2298-2306.
- Diktas M, Sekeroglu A, Duman M, Yildirim A. Effect of different housing systems on production and blood profile of slow-growing broilers. *Kafkas Universit Vet Fak Derg* 2015; 21: 521-526.
- Eleroglu H, Yalcin H, Yildirim A. Dietary effects of Ca-zeolite supplementation on some blood and tibial bone characteristics of broilers. *South African Journal of Animal Science* 2011; 41:319-330.
- Eleroglu H, Yildirim A, Duman M, Sekeroglu A. The welfare of slow growing broiler genotypes reared in organic system. *Emirates Journal of Food and Agriculture* 2015; 27: 454-459.
- Fadare AO. Morphometric and growth performance variations of Naked Neck, Frizzled Feathered and normal feathered crosses with exotic Giri-Raja chickens. *Jordan Journal of Agricultural Sciences* 2014; 10(4): 811-820.
- Gunes N, Polat U, Petek M. Investigation of changes in biochemical parameters of hens raised in alternative housing systems. *Uludag Univ Ver Fak Derg* 2002; 21: 39-42.
- Haunshi S, Niranjana M, Shanmugam M, Padhi MK, Reddy MR, Sunitha R, Rajkumar U, Panda AK. Characterization of two Indian native chicken breeds for production, egg and semen quality, and welfare traits. *Poultry Science* 2011; 90: 314-320.
- Hoffmann I. Research and investment in poultry genetic resources challenges and options for sustainable use. *World's Poultry Science Journal* 2005; 61: 57-70.
- Khawaja T, Khan SH, Mukhtar N, Parveen A, Fareed G. Production performance, egg quality and biochemical parameters of three way crossbred chickens with reciprocal F1 crossbred chickens in sub-tropical environment. *Italian Journal of Animal Science* 2013; 12: 127-132.
- Kral I, Suchy P. Haematological studies in adolescent breeding cocks. *Acta Vet Bmo* 2000; 69: 189-194.
- Leeson S, Summers JD. *Commercial Poultry Nutrition*. 3rd Ed. Nottingham University Press, Nottingham, England, 2005. p. 297-305.
- Njenga SK. Productivity and socio-cultural aspects of local poultry phenotypes in Coastal Kenya. MSc Thesis. Denmark: Department of Animal Breeding and Genetics, The Royal Veterinary and Agricultural University (KVL). 2005. p. 123.
- NRC. National Research Council. *Nutrient Requirement Table of poultry*. 9th Ed. Washington, D.C. National Academy Press. 1994.
- Olaniyi OA, Oyeniya OA, Sogunle OM, Akinola OS, Adeyemi OA, Ladokun OA. Free-range and deep litter housing systems: effect on performance and blood profile of two strains of cockerel chickens. *Tropical Subtropical Agroecosystem* 2012; 15: 511-523.
- Qureshi M, Qadri AH, Gachal GS. Morphological study of various varieties of Aseel chicken breed inhabiting district Hyderabad. *Journal of Entomology and Zoological Studies* 2018; 6(2): 2043-2045. 2018.
- Rehman MS, Mahmud A, Mehmood S, Pasha TN, Hussain J, Khan MT. Blood biochemistry and immune response in Aseel chicken under free-range, semi-intensive and confinement rearing systems. *Poultry Sciences* 2017; 96:226-233. 2017.
- SAS Institute. *SAS® Users Guide: Statistics*. Version 9.01.SAS Institute Inc., Cary, N.C. 2002-2004.
- Sheridan AK. Cross breeding and heterosis. *Animal Breeding*. Abstract. 1981; 19:131-144.
- Shi SH, Huang Y, Cui SJ, Cheng LF, Fu GH, Li X, Chen Z, Peng CX, Lin F, Lin JS, Su JL. Genomic sequence of an avian paramyxovirus type 1 strain isolated from Muscovy duck (*Cairina moschata*) in China. *Archive Virology* 2011; 156: 405-412.
- Shini S. Physiological responses of laying hens to the alternative housing systems. *International Journal of Poultry Science* 2003; 2: 357-360.
- Siddiqi MZ, Qazi MA, Siddique M. *Poultry industry in Pakistan* (Mimeo). Faisalabad: University of Agriculture. 1979.
- Stadig LM, Rodenburg TB, Reubens B, Aerts J, Duquenne B, Tuytens FAM. Effects of free-range access on production parameters and meat quality, composition and taste in slow-growing broiler chickens. *Poultry Science* 2016; 95:2971-2978.
- Tukey JW. The problem of multiple comparisons. In: *The Collected Works of John W. Tukey VIII. Multiple Comparisons*. Chapman and Hall, New York. 1953.
- Xie D, Wang ZX, Dong YL, Cao J, Wang JF, Chen JL, Chen YX. Effects of monochromatic light on immune response of broilers. *Poultry Sciences* 2008; 87: 1535-1539.