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RESEARCH PAPER

Effects of the Injection of Chicken Gonadotropin-Releasing Hormone I on Egg Fertility and Hatching Traits

Hüseyin Baki Çiftci[1,*]

[1]Selçuk University, Faculty of Agriculture, Departman of Animal Science, 42130, Konya, Turkey

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***Corresponding Author**
Tel: 05394126862
E-mail: hbciftci@selcuk.edu.tr

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Abstract

The aim of this study was to measure the effect of chicken GnRH-I injection of roosters on egg fertility, hatching traits, and performance in layer breeder. Sixteen male and 160 female breeder chickens were divided into four experimental groups. Each group is consisting of 4 males and 40 females with four replications. The study comprised a control group, a negative control group (received 200 µl phosphate-buffered saline, PBS), a low hormone group (received 200 µl PBS + 10 µg cGnRH-I) and a high hormone group (received 200 µl PBS + 50 µg cGnRH-I). Injection of 50 µg cGnRH significantly increased the fertility percentage of laid eggs over control, PBS and low hormone injected groups after period I. Injection of either 10 or 50 µg cGnRH significantly increased the relative fertility percentages of eggs following period IV. Injection of 50 µg cGnRH induced a significant increase in the hatchability of set eggs and the hatchability of fertile eggs after periods I and II. Further research is needed to measure the impact of cGnRH on fertility and hatching rates by injecting both male and female of same breed and age with the same doses.

Introduction

Gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus causes pituitary secretion of the gonadotropins (Kaiser et al., 1995). Studies have shown that sexually active birds express a greater number of immune-reactive GnRH neurons than their sexually inactive counterparts (Sharp et al., 1990; Hahn and Ball, 1995; Parry et al., 1997; Cho et al., 1998). It has been reported that two different variants of GnRH exist in chicken: chicken GnRH-I and II (Millar and King, 1984; Miyamoto et al., 1984; Sherwood et al., 1988). The former is considered to constitute the biologically active neuropeptide that controls gonadotropin secretion (Sharp et al., 1990). Decreased hypothalamic GnRH-I secretion in older roosters correlates with lower pituitary expression of mRNA for LH and FSH compared to expression in young roosters (Avital-Cohen et al., 2013). Studies have shown that plasma testosterone concentrations diminish in aging roosters (Weil et al., 1999a; Ottinger et al., 2002). As a result, testes' weight, semen volume, and sperm concentration are generally lower in older roosters (Avital-Cohen et al., 2013). It was reported that the fertility of young roosters reached a peak of 96.2 ± 3.9% at 37 weeks of age and then their fertility began to decline. At 72 weeks, their fertility was much lower than when they were 37 weeks old (Weil et al., 1999a).

Hypothalamic secretion of GnRH is a potential regulator of pituitary FSH secretion and has a half-life of 9 minutes (Reddy et al., 2013). Reduced blood concentrations of FSH in aging roosters have been deemed responsible for diminished daily sperm production. Because the number of Sertoli cells is

stimulated by FSH and the number of sperm produced is dependent on the number of the Sertoli cells hosting spermatogonia (Weil et al., 1999b; Avital-Cohen et al., 2013). Decreased sperm output is in turn strongly associated with decreased egg fertility (Rosenstrauch et al., 1994; Vizcarra et al., 2010). Egg fertility constitutes an economically important factor in the poultry industry due to its effect on chick hatching. Layer breeder roosters tend to become less fertile at about 70 weeks of age and this leads to their removal from the flock (Rosenstrauch et al., 1998; Weil et al., 1999b). However, male broiler breeders tend to see a dramatic reduction in fertility earlier about 45-55 weeks of age (Rosenstrauch et al., 1994; Weil et al., 1999b). Studies have shown that this occurs due to the reduction in sperm number in the ejaculate, although Sertoli cells may be crowded with excessive numbers of spermatids, which become trapped within the cytoplasmic extension owing to malformations in the Sertoli cells' ectoplasmic specialisations (Weil et al., 1996), as well as structural changes in the Leydig cells (Rosenstrauch et al., 1998). According to Sarabia Fragoso et al. (2013), a reduction in fertility occurs due to the decrease in plasma LH and testosterone concentrations. According to Weil et al. (1999a), alongside the decrease in roosters' fertility, reductions also occur in testicular weight, sperm production and testosterone levels. Similar results were obtained from another study, in which older roosts' fertility declined due to reductions in testes size, sperm concentrations and serum testosterone concentrations (Lagares et al., 2017). The reduction in aged roosters' fertility leads to diminished hatching rate and its subsequent removal from the flock. However, the poultry ındustry is currently suffering from a shortage of breeder roosters. Therefore, keeping genetically superior breeder roosters within the flock for a longer period of time, without any decrease in fertility, is economically important. The purpose of this study was therefore to determine whether injecting roosters with GnRH-I would boost egg fertility and hatching rate.

Materials and Methods

Animal, Feeding and Grouping

This study was conducted in a chicken house equipped with a fan pad cooling system, in the poultry unit of the Research and Exploration Centre, Faculty of Agriculture, Çukurova University. Sixteen male and 160 female Atak-S (BAR1, RIR1) breeder chickens were divided into 16 groups. In each group, chickens were housed in floor pens (5 m^2) under 16L: 8D light-dark cycle. Each group contained one 81-week-old male with an average body weight of 2,846 g, and ten 81-week-old females with an average body weight of 1,973 g. These groups were randomly divided into four experimental groups with four replications, comprising a control group (received no injection), a negative control group (received 200 µl phosphate-buffered saline, PBS), a low-hormone group (received 200 µl PBS + 10 µg chicken gonadotropin-releasing hormone-I (cGnRH-I) and a high-hormone group (received 200 µl PBS + 50 µg cGnRH-I. Each group consisted of 4 males and 40 females. In each group, only males were subcutaneously injected under the wing. The chickens were fed *ad libitum* with a diet containing 17% crude protein (CP) and 2,750 kcal/ kg metabolic energy (ME). Water was also provided ad libitum.

Preparation of PBS and cGnRH-I

Two PBS tablets (Cat; P4417-100AB, Sigma-Aldrich Co.,3050 Spruce Street, St. Louis, MO 63103 USA) were dissolved in 400 ml of deionized water (pH: 7.4) and subsequently sterilized by filtering through a Corning 500 ml bottle-top 0.22 µm vacuum filter (Cat: 430521, Corning Incorporated, Corning. NY 14831, USA). Five mg cGnRH-I (Cat; LHRH-012A, CPC scientific, 1245 ream wood avenue Sunnyvale, CA 94089, USA) was dissolved in PBS and its concentration was arranged to 10 or 50 µg/200 µl with PBS. In our previous study, we injected male quails with 5 or 20 µg cGnRH and we have a significant increase in blood testosterone level in 5 µg/100 µl cGnRH injected group over PBS injected group (P=0,036) (Çiftçi et al., 2018). Chickens are 3-4 times heavier than quails, and this was the reason I have chosen the above GnRH doses (10 or 50 µg) for the injections. The amino acid sequence of cGnRH-I was Pyr-His-Trp-Ser-Tyr-Gly-Leu-Gln-Pro-Gly-NH_2 (trifluoroacetate salt). This was different from the mammalian GnRH-I at position 8, arginine being substituted by glutamine (King and Millar 1982).

Injection Procedure

Following a one-week adaptation period, roosters from each group were subcutaneously injected (under the wing) for four weeks on Thursdays at 11:00 am (one injection per week). Birds were injected with 200 µl PBS (Cat; P4417-100AB, Sigma-Aldrich Co.,3050 Spruce street, St. Louis, MO 63103 USA), 200 µl PBS containing 10 or 50 µg cGnRH-I (Cat; LHRH-012A, CPC Scientific, 1245 Ream wood Avenue Sunnyvale, CA 94089, USA).

Measurement of Fertility and Hatchability

Chicken GnRH affects pituitary secretion of gonadotropins (LH and FSH), which in turn affects the Leydig cell secretion of androgen and Sertoli cell secretion of androgen binding proteins as well as transforming beta like peptides (TGF-βs) affecting the sperm formation, maturation and consequently fertilizing ability of male gametes (Weil et al. 1999a). Hatchability depends on whether an egg was fertilized or not therefore hatchability was also measured.

One week after the last injection, eggs were collected daily and stored at 18°C and 75% relative humidity until the incubation. The time span between incubation and hatching was referred as a period. The times of incubation and hatching and the number of eggs collected and incubated in each period are displayed in Table 1.

Once hatching was complete, the fertility (fertile eggs divided by set eggs), hatchability of set eggs (hatched chicks divided by set eggs), and hatchability of fertile eggs (hatched chicks divided by fertile eggs) were determined.

Measurement of Egg Production, Feed Consumption and Female Ratio

Egg production and bird mortality were recorded daily throughout the eight-week experimental period. Feed consumption was measured every week using a digital scale (Kern DS 30K 0.1, Nuremberg, Germany), and was calculated by subtracting the remaining feed from the total feed. The sex of a day-old chick was determined according to the colour of the feathers. The female ratio was expressed as a percentage divided by the number of total hatched chicks.

Statistical Analysis

Data were analysed by analysis of variance (ANOVA) by using Minitab statistical software. Pairwise comparisons were carried out according to Tukey test with 95% confidence intervals.

Results

The effects of cGnRH injection on egg fertility are presented in Table 2. Injection of 50 µg cGnRH caused a significant increase ($P<0.05$) in the fertility of the eggs relative to the other three groups for period I, while injection of 10 µg cGnRH did not cause any significant changes relative to the control and PBS injected groups. The coefficients of variations for fertility within and between groups range between 2.29-4.39% and 2.40-4.49% respectively. In period II, 10 µg cGnRH injections induced a significant increase in egg fertility over PBS-injected group, but there were no significant differences in 10 µg cGnRH groups relative to control and 50 µg cGnRH-injected groups. For period IV, injection of 10 and 50 µg cGnRH significantly increased the egg fertility relative to control and PBS-injected groups. The egg fertility rate was significantly higher in the control and 50 µg-cGnRH-injected groups relative to the PBS- and 10 µg-GnRH-injected groups for period VI. In periods III, V and VI fertility rates were lower in PBS-injected group relative to control.

The fertility rate, for period-VI, was the lowest in 10 µg-cGnRH-injected groups. The effects of cGnRH injection on the hatchability of set eggs are displayed in Table 3.

Table 1. Incubation and hatching times and the number of eggs collected and incubated in each period

Periods	Incubation Time	Hatch Time	Egg Collected	Egg Incubated
Period-I	14.05.2018	04.06.2018	446	320
Period-II	21.05.2018	11.06.2018	646	320
Period-III	29.05.2018	19.06.2018	567	542
Period-IV	06.06.2018	26.06.2018	517	321
Period-V	11.06.2018	02.07.2018	513	320
Period-VI	20.06.2018	10.07.2018	440	320

Table 2. The fertility rates of eggs collected from experimental groups enclosed with roosters received no injection (Control), injected with PBS, 10 or 50 µg cGnRH (Mean $^{\pm}$ SE)

Fertility rates (%)					
Periods	Control	PBS	10 µg cGnRH	50 µg cGnRH	P-values
Period-I	73.84^{b} ± 2.00	76.22^{b} ± 1.36	72.47^{b} ±1.51	92.50^{a} ± 1.44	0.000
Period-II	78.78^{ab} ± 0.90	75.00^{b} ± 0.00	79.97^{a} ± 0.41	76.34^{ab} ± 1.96	0.029
Period-III	87.33^{a} ± 0.66	77.83^{b} ± 0.98	88.69^{a} ± 0.96	84.68^{a} ± 1.35	0.000
Period-IV	80.21^{b} ± 0.48	76.25^{b} ± 1.25	86.23^{a} ± 1.29	88.83^{a} ± 0.83	0.000
Period-V	78.65^{a} ± 1.47	66.27^{b} ± 2.17	76.28^{a} ± 0.93	76.22^{a} ± 1.36	0.001
Period-VI	85.11^{a} ± 1.74	73.85^{b} ± 1.86	65.26^{c} ± 1.21	84.94^{a} ± 2.02	0.000
Periods I-VI	80.65^{b} ± 0.83	74.24^{c} ± 0.51	78.15^{b} ± 0.60	83.92^{a} ± 0.35	0.000

*Data with different superscripts are statistically different

*SE: Standard error

Table 3. The hatchability of set eggs from experimental groups, enclosed with rooster received no injection (Control), injected with PBS, 10 or 50 µg cGnRH (Mean ± SE)

Hatchability of set eggs (%)					
Periods	Control	PBS	10 µg cGnRH	50 µg cGnRH	P-values
Period-I	63.77^{b} ± 0.76	61.27^{b} ± 0.77	59.95^{b} ± 0.82	82.50^{a} ± 1.44	0.000
Period-II	63.77^{b} ± 0.76	63.75^{b} ± 1.25	61.27^{b} ± 0.77	70.09^{a} ± 1.43	0.001
Period-III	78.02^{a} ± 1.06	67.17^{c} ± 1.92	74.22^{ab} ± 1.04	70.08^{bc} ± 0.50	0.000
Period-IV	72.88^{b} ± 0.88	66.25^{c} ± 1.25	64.95^{c} ± 0.72	78.79^{a} ± 0.56	0.000
Period-V	72.49^{a} ± 1.03	53.75^{c} ± 0.72	67.52^{b} ± 1.08	64.96^{b} ± 0.72	0.000
Period-VI	77.53^{a} ± 1.17	61.23^{c} ± 0.45	46.10^{d} ± 1.36	72.52^{b} ± 0.85	0.000
Periods I-VI	71.41^{a} ± 0.23	62.24^{b} ± 0.67	62.33^{b} ± 0.81	73.16^{a} ± 0.49	0.000

*Data with different superscripts are statistically different

*SE: Standard error

The hatchability of set eggs was significantly higher in the 50 µg-cGnRH-injected group compared to the other treatment groups in periods I and II. The coefficients of variations within and between the groups ranged between 2.45-3.22 and 2.51-3.65%, respectively. The number of hatched set eggs was the lowest in the PBS-injected group relative to the other groups in periods III and V. Injection of PBS significantly reduced the number of hatched set eggs relative to the other groups in period V. The number of hatched set eggs was highest in the 50 µg-cGnRH-injected groups in period IV. The number of hatched set eggs fell in period VI in the PBS- and 10 µg-cGnRH-injected groups as compared with control and 50 µg-cGnRH-injected groups.

The effects of GnRH on the number of hatched fertile eggs are displayed in Table 4. The number of hatched fertile eggs increased (P<0.05) in the 50 µg cGnRH-injected groups relative to the PBS-injected groups in period I. The highest hatchability rate of fertile eggs was observed in the 50 µg cGnRH-injected group in period II. In period III, the number of hatched fertile eggs was significantly higher in the control group than in the GnRH-injected groups. In period IV, the injection of 10 µg cGnRH caused a significant reduction in the number of hatched fertile eggs compared with the other treatment groups. The number of hatched fertile eggs in period V was significantly higher in the control group compared with the PBS-injected group. In period VI, the number of hatched fertile eggs fell significantly in the 10 µg cGnRH-injected groups relative to other groups due to the reduction in the hatchability of fertile eggs.

Through the periods I-VI, injection of PBS decreased fertility (Table 2), as compared with other groups, but PBS injection did not negatively affect the hatchability of those fertile eggs (Table 4), while injection of 10 µg cGnRH did not cause important differences in fertility rates as compared with the control group but caused a decrease in the hatchability those fertile eggs as compared with other groups (Table 2 and 4). The data indicating the effect of cGnRH on female ratios are displayed in Table 5. According to the analysis of variance, female ratios significantly fell (P<0.05) in the 50 µg-cGnRH-injected group compared with the PBS- and 10 µg-cGnRH-injected groups in period I. In period II, the female ratio was the highest in the 50 µg-cGnRH-injected groups. The female ratio was the highest in the 10 µg-cGnRH-injected groups and the lowest in the 50 µg-cGnRH-injected groups in period III. The female ratio was the highest in the 50 µg-cGnRH-injected group compared with the control and PBS-injected groups, after period IV. The female ratio was the highest in the 10 µg-cGnRH-injected group relative to the other treatment groups in period V, and the differences were not statistically significant (P=0.179) in period VI. Within group and between group coefficients of variation for female ratio ranged between 2.68-8.42 and 3.17-8.73%, respectively. The differences in daily egg production and feed consumption were not statistically significant (Tables 6 and 7).

In overall, injection of GnRH caused some degree of increase in fertility rate and hatchability of fertile eggs. Injection of 50 µg GnRH caused better effect than the effect of 10 µg GnRH, while there were no differences in female ratios, egg production and daily feed consumption (g/bird) in period 1 and 2 (Table 5, 6 and 7). Injection of PBS caused some degree of adverse effect on fertility rate, hatchability of set eggs and hatchability of fertile eggs.

Discussion

In all vertebrate species including poultry, reproduction is determined by the hypothalamo-pituitary-gonadal axis. This axis controls the fertility of an individual, crucial to the economic viability of the industry (Ippala et al., 2016). According to a study, reduction in fertility occurs due to the decrease in plasma LH and testosterone concentrations (Weil et al., 1999a), which is under the control of hypothalamic GnRH secretion. Decrease in fertility of the older roosters might be a result of the decreased secretion of GnRH.

Table 4. The hatchability of fertile eggs weekly collected from groups enclosed with roosters received no injection (Control) or injected with PBS, 10 or 50 µg cGnRH (Mean ± SE)

Hatchability of fertile eggs (%)					
Periods	Control	PBS	10 µg cGnRH	50 µg cGnRH	*P-values*
Period-I	86.55^{ab} ± 2.63	80.49^{b} ± 2.20	82.81^{ab} ± 1.76	89.18^{a} ± 0.17	0.034
Period-II	80.94^{bc} ± 0.31	85.00^{b} ± 1.67	76.62^{c} ± 1.18	91.87^{a} ± 1.46	0.000
Period-III	89.34^{a} ± 0.75	86.28^{ab} ± 1.94	83.68^{b} ± 0.73	82.81^{b} ± 1.17	0.013
Period-IV	90.89^{a} ± 1.53	86.87^{a} ± 0.21	75.37^{b} ± 1.19	88.71^{a} ± 0.30	0.000
Period-V	92.31^{a} ± 2.80	81.27^{b} ± 1.58	88.54^{ab} ± 1.61	85.28^{ab} ± 1.39	0.010
Period-VI	91.18^{a} ± 1.70	83.05^{a} ± 1.90	70.72^{b} ± 3.09	85.52^{a} ± 2.22	0.000
Periods I-VI	88.53^{a} ± 0.78	83.82^{b} ± 1.11	79.62^{c} ± 0.99	87.23^{ab} ± 0.60	0.000

*Data with different superscripts are statistically different
*SE: Standard error

Table 5. Female ratios following the incubations of eggs collected from the groups enclosed with roosters received no injection (Control) or injected with PBS, 10 or 50 µg cGnRH (Mean±SE)

Female ratios (%)					
Periods	Control	PBS	10 µg cGnRH	50 µg cGnRH	P-values
Period-I	46.96^{bc} ± 2.53	55.13^{a} ± 2.01	52.10^{ab} ± 1.22	43.93^{c} ± 1.21	0.004
Period-II	49.04^{b} ± 1.84	41.19^{c} ± 1.82	50.96^{b} ± 0.96	57.14^{a} ± 0.00	0.000
Period-III	45.26^{b} ± 0.91	45.44^{b} ± 0.90	56.48^{a} ± 0.77	41.67^{c} ± 0.00	0.000
Period-IV	47.50^{b} ± 0.83	47.25^{b} ± 2.33	53.71^{ab} ± 3.46	60.33^{a} ± 0.77	0.003
Period-V	55.14^{b} ± 0.92	51.14^{b} ± 2.18	68.54^{a} ± 1.51	53.71^{b} ± 3.46	0.001
Period-VI	43.54^{a} ± 1.36	48.86^{a} ± 1.94	48.47^{a} ± 2.79	50.13^{a} ± 2.00	0.179
Periods I-VI	47.90^{b} ± 2.31	48.17^{b} ± 2.80	55.04^{a} ± 3.78	51.15^{ab} ± 3.75	0.001

*Data with different superscripts are statistically different
*SE: Standard error

Table 6. Daily egg production per hen in control and treatment groups throughout the eight weeks' experimental period (Mean ± SE)

	Egg production (hen/day, %)				
Weeks	Control	PBS	10 µg cGnRH	50 µg cGnRH	P-values
W-0	41.79^{a} ±5.57	40.36^{a} ± 3.61	39.29^{a} ± 3.76	41.79^{a} ± 3.10	0.966
W-1	55.71^{a} ± 2.92	58.57^{a} ± 6.25	57.14^{a} ± 4.84	59.29^{a} ± 8.76	0.976
W-2	53.57^{a} ± 2.64	50.36^{a} ± 6.32	46.43^{a} ± 2.89	52.14^{a} ±7.34	0.790
W-3	48.93^{a} ± 6.10	50.00^{a} ± 6.32	44.29^{a} ± 2.54	51.43^{a} ± 1.01	0.775
W-4	47.86^{a} ± 6.81	47.86^{a} ± 1.89	45.36^{a} ± 0.90	42.14^{a} ± 6.74	0.821
W-5	40.00^{a} ± 5.56	45.36^{a} ± 5.42	39.64^{a} ± 4.79	37.50^{a} ± 5.80	0.754
W-6	41.07^{a} ± 4.96	46.07^{a} ±2.13	39.64^{a} ± 3.89	35.36^{a} ± 3.57	0.299
W-7	36.79^{a} ± 6.10	45.71^{a} ± 3.45	41.07^{a} ± 4.72	35.00^{a} ± 3.38	0.313
W-8	37.86^{a} ± 7.39	37.86^{a} ± 7.20	41.43^{a} ± 2.02	37.86^{a} ± 6.71	0.969
W0-8	44.84^{a} ± 4.11	46.91^{a} ± 4.46	43.81^{a} ± 2.58	43.61^{a} ± 3.96	0,925

*Data with different superscripts are statistically different
*SE: Standard error

Table 7. Daily feed consumption by per bird, a week after the last injection of experiment (Mean ± SE)

	Daily feed consumption (g/bird)				
Weeks	Control	PBS	10 µg cGnRH	50 µg cGnRH	P-values
W-1	121.07^{a} ± 1.97	123.28^{a} ± 1.49	120.71^{a} ± 3.87	121.27^{a} ± 2.92	0.908
W-2	120.84^{a} ± 2.03	120.65^{a} ± 2.74	116.69^{a} ± 3.40	120.03^{a} ± 3.36	0.731
W-3	113.57^{a} ± 2.66	110.73^{a} ± 1.72	115.70^{a} ± 3.96	117.95^{a} ± 6.29	0.639
W-4	110.69^{a} ± 0.66	105.26^{a} ± 1.29	115.80^{a} ± 1.77	114.33^{a} ± 5.68	0.124
W-5	111.15^{ab} ±2.93	110.67^{ab} ±3.76	114.29^{a} ± 0.90	104.12^{b} ± 1.36	0.081
W-6	101.74^{a} ± 2.54	107.78^{a} ± 5.38	112.22^{a} ± 3.69	109.59^{a} ± 4.39	0.365
W-7	106.98^{a} ± 1.86	111.44^{a} ± 2.64	113.38^{a} ± 3.26	111.35^{a} ± 3.65	0.493
W-8	115.75^{a} ± 1.25	117.43^{a} ± 0.87	117.37^{a} ± 3.99	113.60^{a} ± 1.64	0.615
W 1-8	112.73^{a} ± 1.68	113.41^{a} ± 0.89	115.77^{a} ± 2.36	114.03^{a} ± 2.35	0.715

*Data with different superscripts are statistically different

*SE: Standard error

Therefore, 80 weeks old roosters were injected with 10 or 50 µg cGnRH. In overall, injection of 10 or 50 µg cGnRH caused significant increases in egg fertility over PBS-injected groups (P<0.05). The fertility rates were slightly decreased in 10 or 50 µg cGnRH-injected groups as compared to control group in periods V and VI due to the high amount of experimental error. In period VI, the fertility rate was the lowest in 10 µg cGnRH group due to the higher amount of error. Injection of 50 µg cGnRH caused better beneficial effect on fertility rate and as well as on hatchability of set eggs (Table 2 and 3). The decreases in hatchability of set eggs in periods V and VI were because of the higher experimental errors (Table 3). Close results were obtained following the broiler roosters' injection with a short-acting GnRH analogue (Buserelin acetate, 0.0042 mg/mL) by Hezarjaribi et al. (2016). According to their results, GnRH stimulated significant increases (P<0.05) in the percentages of fertile eggs and hatchability over the control group (Hezarjaribi et al. 2016). In this study, the control group (natural group) was not injected in the same way as the control groups in Hezarjaribi et al. (2016) study. Indeed, the key differences between the two studies comprise the dose and form of GnRH applied. Here we can state that the injection of GnRH (especially in high doses) increased the egg fertility rate. In this study, the injection of PBS caused a significant decrease in egg fertility after period V, relative to the control group. Indeed, PBS decreases the motility of sperm cells. In a study, different diluents (Such as saline, PBS, autologous prostate secretion or a modified TRIS buffer) were used to dilute canine semen in order to find out the best one that influences sperm motility and viability at a lower extent (Schäfer-Somi and Aurich, 2007). Dilution of canine semen with PBS caused a significant decrease in total motility as compared with sperm cells diluted in saline and in other diluents (Schäfer-Somi and Aurich, 2007). The motility of sperm cells is one of the important factors affecting the fertility potential of an individual. Hatching is primarily dependent on whether an egg is fertilized or not. Therefore, it is a broadly male-dependant phenomenon. This is the reason why commonly observed hatching losses in broiler breeds after 45 weeks of age isoften associated with the fertility of roosters (Sarabia Fragoso et al. 2013). In one study, the reproductive performances of young (35 weeks old) and aging (73 weeks old) broiler roosters were compared. It was reported that testes' weight, semen volume, sperm concentration, hypothalamic mRNA expression of GnRH-I, pituitary expression of mRNA for LH, FSH and plasma testosterone concentration were all lower in ageing roosters relative to their younger counterparts (Avital-Cohen et al., 2013). Thus, aging is associated with diminished GnRH secretion, resulting in reduced hatching rate.

In this study, injection of 50 µg cGnRH increased the number of hatched set eggs (Table 3). This was partly due to the increase in egg fertilization rate owing to the cGnRH injection (Table 2). In this study, 81-weeks- old male layer breeders were subcutaneously injected with 10 or 50 µg cGnRH. The dose, application route and form of GnRH all varied. Hezarjaribi et al. (2016) have also reported an increase in hatchability following injection of 60-weeks-old broiler roosters with 0.3 mL GnRH analogue buserelin acetate (0.0042 mg/mL). The results of the present study demonstrate that the hatchability of set eggs significantly increased (P<0.05) following the injection of 50 µg cGnRH (Table 3). Injection of 50 µg cGnRH induced a substantial increase in the hatchability of fertile eggs relative to the PBS-injected group and other groups in periods I and II, respectively (Table 4). Here, it is likely that GnRH has a positive impact on hatching. In mammals, it was reported that the treatment of hypophysectomised mice with a GnRH agonist increased the hatching rate (Yang et al., 1995). In this study, it was found that injection of 10 µg cGnRH caused a better effect in female ratio as compared to PBS and control groups (Table 5).

The coefficient of variation within and between groups ranged between the 2.68-8.42 and 3.17-8.73% respectively. It is difficult to compare the results of this study with those of similar studies due to the general lack of the data presenting the effect of cGnRH on sex ratio in avian species. Nevertheless, a relationship between hormone and secondary sex ratio has been reported in Holstein dairy cows (Emadi et al., 2014). It is physiologically accepted that the amount of estradiol produced in domestic mammals is relative to the amount of GnRH reaching the pituitary gland. Therefore, a cow's phenotypic response to estradiol may in turn be related to its response to GnRH. According to Emadi et al. (2014), estradiol benzoate administration increased the likelihood of calves being male. When day length shortens, melatonin secretion in domestic mammal increases and this increase is accompanied by an increase in GnRH secretion. According to one study, male-to-female ratio was higher in cows conceived in the summer, autumn, and winter than in cows conceived in the spring (Youssefi et al., 2013). This, in turn, indicates the relationship between GnRH and sex ratios. However, we cannot conclusively state that GnRH injection has a positive or negative impact on female ratios owing to different animal species and the administration of different hormones in different form, dose and application route.

In this study, no significant differences have been observed, in terms of daily egg production ($P>0.05$, Table 6), as a result of response to the cGnRH injections. This is because only roosters were injected. In literature, there are plenty of reports indicating the presence of a positive relation between the blood concentration of GnRH and the egg-laying performance of hens (Sharp et al., 1990; Ippala et al., 2016; Cowan et al., 2014). Also, no differences have been observed, in daily feed consumption per bird, between cGnRH-injected groups and others, (Table 7). Hence, the appetite of birds was not altered by GnRH injections.

Conclusion

The results demonstrate that the injection of older roosters with cGnRH (especially in high dose, 50 µg) has positive impacts on egg fertility and hatching rates. The decrease in fertility of older rooster results with decrease in fertility and hatching rates, which cause the removal of the roosters from the flock. But poultry industry is toughly suffering from the shortage of supplying the breeder rooster on time at required number. Thus, keeping the genetically superior breeder rooster within the flock longer, without any decrease in fertility, is economically important.

It is quite early to say that injection of older rooster with cGnRH-I extents the duration of their fertility. Further research needed to measure the impact of cGnRH on fertility and hatching rates by injecting both male and female of same breed and age with the same doses.

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PROOF

RESEARCH PAPER

Exposing Hatching Quail Eggs to High Light Intensity and Its Effect on Post-Hatch Testicular Histology

Hüseyin Baki Çiftci[1,*], İsa Coşkun[2], Ali Aygün[1]

[1]Selçuk University, Faculty of Agriculture, Departman of Animal Science, 42130,Konya, Turkey
[2,] Ahi Evran University, Faculty of Agriculture, Department of Animal Science, Kırşehir, Turkey

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***Corresponding Author**
Tel: +05394126862
E-mail: hbciftci@selcuk.edu.tr

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Abstract

The objective of this study was to monitor the effect of exposing hatching quail eggs to high light intensity on testicular function Quail eggs were distributed into four groups; dark application during the 18-day incubation period (C), light application during the 18-day incubation period (L), 5 days of light + 13 days of darkness (5L), and 5 days of darkness + 13 days of light (5K) and incubated. After the hatch, 40 chicks were selected from each group and placed in rearing cages. On day 42 after hatching the quails were weighed and their testes were removed and then embedded in paraffin. The blood glucose level of the 5K group was higher than the blood glucose level of the L group, but there was no statistically important difference ($P<0.05$) between the L, C, and 5L groups. The diameters of seminiferous tubules were bigger in the 5L group as compared with the L group. The groups treated with light had bigger tubule diameters than the control group. The number of Sertoli cells was significantly lower in the C group. Incubating quail eggs under five days of darkness and thirteen days of light have a favorable effect on post-hatch male fertility potential.

Introduction

Light is one of the important environmental factors affecting reproduction via the hypothalamic-pituitary-gonadal axis (Olanrewaju et al., 2006). Day length, light intensity, and light colors affect the anatomic, metabolic, and reproductive function of quail (Oishi and Lauber, 1973; Olanrewaju et al., 2006; Deep et al., 2012). Many studies have been conducted to show the impact of light on hatching rate, hatchability, and embryonic mortality (Özkan et al., 2012; Kaya and Aygün, 2019; Maman et al., 2018). But, the data related to the effect of light intensity on reproduction is limited. Therefore, there is a growing trend of studies on the application of different light intensities and colors to increase reproductive performance (Retes et al. 2017). According to a study, exposing the eggs for 12 and 24h/day under 250 lx light intensity caused an increase in hatching rate and hatchability (Riaz et al., 2021). Exposing eggs to light has been reported to relieve stress on embryos (Huth and Archer 2015) and help them hatch. Riaz et al. (2021) have also reported that stress susceptibilities such as heterophil to lymphocyte ratios were significantly lower in eggs exposed 12 h/day under 250 lx light intensity. Therefore, during the incubation, exposing the eggs to light relieves the stress and causes the hatched chicks to start their life with better self-confidence (Archer and Mench 2014).

The effect of exposing hatching quail eggs to high light intensity on blood glucose levels has not been studied in quail. But, some studies were performed to

measure the effect of the lightning regime on blood glucose levels in broiler breed hens. Broiler eggs were incubated totally under darkness, 12 h of lightness and 12 h of darkness, and 24 h of lightness. It was reported that blood glucose concentration (mmol/L) was increased by 12 h and 24 h lightness as compared to eggs incubated in darkness. The glucose concentration in the 24 h lighting group was also significantly high over the 12 h lighting group (Yameen et al., 2020).

The effects of light intensity on reproductive performance and hormone concentrations have been largely studied in poultry. But, not enough studies have been performed on quail, especially on the performance of male quail. In a study, male quail were exposed to six different types of lamps (incandescent, white fluorescent, or blue, white, red, or green light-emitting diode (LED). It was reported that testicular weight was higher in compact fluorescent and red LED bulbs as compared to other groups. In white LED groups, the area of the seminiferous tubules was higher, while no differences were seen in sperm concentration, motility, or fertility rates (Retes et al. 2017). Retes and his colleagues (2017) performed this study on one day old male Japanese quails after hatch. But, we do not know the impact of light on incubating eggs and its effect on the reproductive and metabolic function of hatched male quail after the incubation. Therefore, the objective of this study was to monitor the effect of exposing hatching quail eggs to high light intensity on testis volume, the number of Sertoli cells, and blood glucose level.

Materials and Methods

Eggs and Grouping

A total of 660 hatching quail eggs were used in this study, which were purchased from a private farm in Konya. The eggs were randomly distributed into four groups (C, L, 5L, and 5K). Each group consisted of 160 eggs and a separate tray was designated for each group. Experimental groups are displayed in Table 1.

Table 1. The number of incubated eggs and light application, in each group, during the incubation

Groups	The number of incubated eggs	Applications
C	160	18 days of darkness
L	160	18 days of light
5L	160	5 days of light and 13 days of darkness
5K	160	5 days of darkness and 13 days of light

Light Source and Intensity

White Light-emitting diode (LED) bulbs were used as a light source. The white LED was placed approximately 10 cm above the eggs.The light intensity was measured at different points on egg trays, ranging from 5000 to 6000 lux, and it was manually fixed by adjusting the distance to the LED.

Temperature and Humidity

For the first 14 days of incubation, the temperature and humidity within the incubator were set to 37.5 °C and 55-60% respectively. For the last three days of incubation, the temperature, and humidity were adjusted to 37.2 °C and 75%.

Chick Rearing and Feeding

After the hatch, 40 chicks were randomly selected from each group and placed in rearing cages. Each rearing cage consisted of 5-layer plastic structures and each layer had a width of 60 cm and a length of 120 cm (60x120). Stocking density was adjusted to ten centimeters area per chick. During the 42 days of the growing period, chicks were fed with a diet providing 24% crude protein and 2900 kcal/kg metabolic energy. In the first week, the ambient temperature is set at 30-33 °C. Every week, the temperature was reduced by 3 °C until it reached 21 °C. The lightning program is set to continuous light for the entire rearing period.

Measurements of Blood Glucose Levels

After the ten-day growing period, six chicks were randomly selected from each group and weighted by using precision balances (Radwag, USA). Blood glucose levels were measured using a blood glucose meter (IME-DC, Germany). A drop of blood, from the wing, was placed on the test strip of the blood glucose meter, and the blood glucose level was measured.

Measurement of Body Weight, Testes Weight, and Its Fixation

Quails were weighed on day 42 after hatching using a balance, with 0.01 sensitivity and then slaughtered. After the slaughter, their testes were carefully removed and weighed. Testes weights were plotted in g and expressed as a percentage of bodyweights. Dissected testes were kept in 10% formalin for tissue examination.

Measurements of Diameter of Seminiferous Tubules and the Number of Sertoli Cells

Tissue samples were fixed in 10% formalin for 24 hours and dehydrated with graded alcohol then embedded in paraffin. Embedded tissues were cut at 5 µm thickness and placed on glass slides and stained with hematoxylin and eosin solutions by using standard paraffin-embedding methods.

The diameters of seminiferous tubules and the number of Sertoli cells were differentiated (Figure 1) and measured by using an image processing and analysis system (ZEN 2012 SP2).

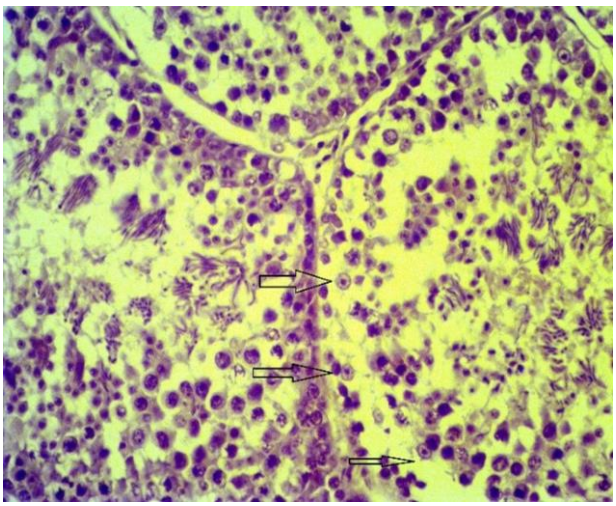

Figure 1. Photomicrograph of Sertoli cells, indicated with arrows, differentiated by a big white nucleus and dark small nucleolus

Statistical Analysis

For the statistical analysis of the data, initial assumptions regarding parametric tests were questioned. For this purpose, the Kolmogorov-Smirnov and Levene tests were used to determine normality and variance homogeneity, respectively. After meeting the parametric test assumptions for all variables, the hypothesis tests were conducted using variance analysis. When the difference between groups was found to be statistically significant, Tukey's multiple comparison test was used to identify its cause. All statistical analyses employed a significance level of 0.05 and were conducted using Minitab software.

Results

The data relating to body weights, testicular weights (g and %), and blood glucose levels in the C, L, 5L, and 5K groups were displayed in Table 2. The C, L, 5L, and 5K groups had testes weights of 5.86 g, 4.71 g, 5.62 g, and 5.94 g, respectively. But, no statistically significant differences between the groups were seen. Testis weights, as a percentage of bodyweight, were determined as 3.37%, 2.89%, 3.17%, and 3.46% in the C, L, 5L, and 5K groups, respectively, with no statistically significant variations between the groups. The blood glucose level of the 5K group (313 mg/dL) was higher than the blood glucose level of the L group (272 mg/dL), but there was no statistically important difference ($P<0.05$) between the L group, C (291 mg/dL) and 5L (289 mg/dL) groups. The differences in blood glucose levels between the 5K group (313 mg/dL), group C (291 mg/dL), and group 5L (289 mg/dL) were also not statistically important (Table 2).

Data showing the impact of light intensity on tubule diameter and Sertoli cell number were displayed in Table 3 and Figure 2. The diameters of seminiferous tubules were greater in the 5K group than the diameter of tubules in other groups ($P<0.05$, Table 3, Figure 2). The diameters of seminiferous tubules were significantly smaller in the C group as compared to the others ($P<0.05$). The diameters of seminiferous tubules were bigger in the 5L group as compared with the L group ($P<0.05$, Table 3, Figure 2). The groups treated with high light had bigger tubule diameters than the control group ($P<0.05$). The number of Sertoli cells was significantly lower in the C group than the number of Sertoli cells in the other groups ($P<0.05$, Table 1).

Discussion

Light intensity during incubation, as well as light colour, affect the bodyweight of birds. In a study, quail eggs were incubated under white LEDs, green LEDs, red LEDs, and darkness (control). Growth rates, after the hatch, were higher ($P<0.05$) in birds from eggs incubated under dark and white LED (Coelho et al., 2021). In another study, the positive effect of continuous lightning during incubation on body weight at hatching was reported by Farghly and Mahrose (2012). According to their report, birds produced from eggs exposed to light during the incubation period had significantly higher ($P\leq0.05$) post-hatch daily weight gain (Farghly and Mahrose 2012). In another study, the effect of different light sources on the post-hatch bodyweight of female Japanese quail was also studied. It was reported that; the lowest body weight score was obtained under compact fluorescent light (Bobadilla-Mendez et al., 2016). In another study, the effect of different types of lambs on the bodyweight of post-hatch male Japanese quail (Coturnix coturnix japonica) was studied. Higher body weight scores were observed with incandescent lam 35 days after hatching (Retes et al., 2017). In this study, the light intensity did not affect the body and testes weights 42 days after hatching. Here, we use the light intensity of 5000 to 6000 lux during the incubation and we measured body weight at 42 days of age. This might be the reason why we could not see significant differences.

Exposure of quails to different light intensities and colors can influence testiscular development. According to a study, higher testicular weights were observed in quails reared under a compact fluorescent bulb at 35 days of age. But at 57 days of age, the highest testicular weights were observed in quails reared under white LED (Retes et al., 2017). According to another study, conducted on three weeks old sexually immature male Japanese quails, testicular volume and seminiferous tubule diameters were significantly higher in quails

Table 2. Body and testes weights and blood glucose levels in experimental groups

Group	Bodyweight (42 d; g)	Testes weight, g	Testes weight (%)	Glucose (mg/dL)
C	173.48	5.86	3.37	291[ab]
L	162.79	4.71	2.89	272[b]
5L	176.78	5.62	3.17	289[ab]
5K	171.78	5.94	3.46	313[a]
SEM	5.932	0.407	0.202	9.230
P	0.418	0.150	0.217	0.042

[a-b]Data with different superscripts in the same column are statistically different (P<0.05)

Table 3. The diameters of seminiferous tubules and the number of Sertoli cells in testes of quails in different experimental groups

Groups	Diameters of seminiferous tubules (µm)	Count of Sertoli cell
C	86.10[d]	3.60[b]
L	119.19[c]	4.57[a]
5L	152.70[b]	4.67[a]
5K	169.91[a]	5.07[a]
SEM	3.68	0.16
P	0.001	0.012

[a-d] Data with different superscripts in the same column are statistically different (P<0.05)

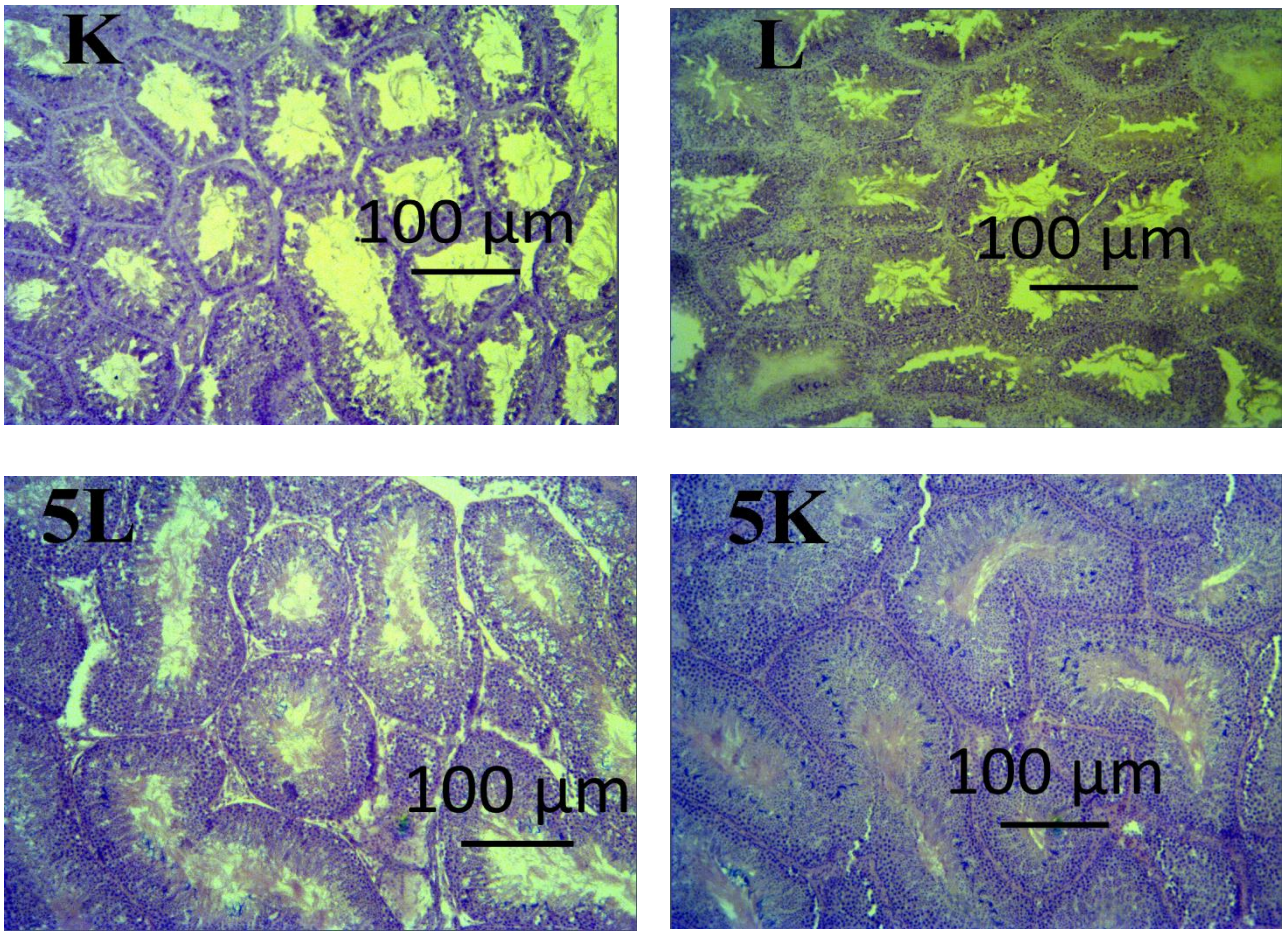

Figure 2. Photomicrograph of seminiferous tubules. The smallest diameters of seminiferous tubules were measured in the control group (K). Lightning increased the diameter of seminiferous tubules as compared with that of the control group. The biggest diameters of tubules were measured in the 5K group as compared with other groups.

exposed to 100 lux white fluorescent light as compared with quails exposed to 30 lux white or blue LEDs (Yadav and Chaturvedi, 2015). According to Coelho et al. (2021), no difference in the anatomical and histological characteristics of the testes due to the light treatment during the incubation except for the diameter of the seminiferous tubules, which was greater ($P < 0.05$) in the dark and in the white LED groups. According to Retes et al. (2017), semen volume and sperm concentrations were not statistically different due to the light treatment, except for sperm motility, which was higher ($P < 0.05$) in birds from eggs incubated in different colors of light.

According to our results, exposing the incubating eggs to 5000-6000 lux white LED increased post-hatch seminiferous tubule diameter and the number of Sertoli cells at 42 days of age as compared to that of chicks hatched from eggs incubated in the dark. Even though we used different intensities of light, our results are partly in parallel with the results of Coelho et al. (2021). In a study, broiler eggs were incubated under darkness, under 12 h of lightness, and under 24 h of lightness. Blood glucose concentrations, post-hatch, were significantly increased under 12 and 24 h of lightness as compared with eggs incubated in the dark (Yameen et al., 2020). Our results show that incubation of the egg under light significantly increased post-hatch blood glucose concentration as compared with the post-hatch blood concentrations of chicks hatched from eggs incubated in darkness. Even though the species of animal, application, and intensity of lightning are different our results are rare partly in agreement with the results of Yameen et al. (2020). In this study, our expectation was to see increases in the diameter of seminiferous tubules and related increases in the number of Sertoli cells by light application. This expectation was partly met as the diameter of seminiferous tubules increased by light application as compared with the control group. But more research is required to monitor the effect of light application during the incubation period on the hatchability of eggs produced during the breeding period.

Conclusion

Incubating quail eggs under five days of darkness and thirteen days of light have a favourable effect on post-hatch male fertility potential.

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PROOF
RESEARCH PAPER

POULTRY STUDIES

Detection of MDA Titer of Infectious Bronchitis Virus and Comparison of Antibody Titer Produced by Two Different Infectious Bronchitis Vaccine

Mirza Mienur Meher[1], Mosharof Hossain[2] Golam Haider[2,*]

[1]Bangabandhu Sheikh Mujibur Rahman Agricultural University, Faculty of Veterinary Medicine and Animal Science, Department of Microbiology and Public Health, Gazipur-1706, Bangladesh
[2]Bangabandhu Sheikh Mujibur Rahman Agricultural University, Faculty of Veterinary Medicine and Animal Science, Department of Pathobiology, Gazipur-1706, Bangladesh

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***Corresponding Author**
Tel: +8801712642948
E-mail: ghaider@bsmrau. edu.bd

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Abstract

The infectious bronchitis (IB) is divesting infectious diseases of poultry. Hence, this study determined the maternally derived antibody (MDA) titer of IBV and comparison of two IB vaccine in layer. A total of 1600 birds were equally allocated into two groups (group-A and group-B). The group-A and group-B were vaccinated by two different vaccines having the strain "B1-Strain of ND+Massachusetts B-41 Strains of IBV" and "Hitchner-B1 Strain of ND+Massachusetts H120 Strains of IBV" respectively. Blood samples were collected and antibody titer was detected by indirect ELISA test. Results showed that both groups had the protective level of MDA titer and both the vaccines significantly ($p<0.01$) increased the antibody mean titer than the MDA. Between the groups, the highest antibody mean titer (11544.43±177.51) was observed in group-B at 5 weeks age and had significant ($p<0.01$) difference with group-A (10222.11±96.96). The effect size of the antibody titer was higher in group-B (14.25; CI: 9173.82-9564.39; n=46 at 5 weeks birds) than group-A (9.54; CI: 8580.19-9131.68; n=46 at one-day-old birds) respectively. Shortly, the MDA mean titer was in protective condition and both the IB vaccinated groups had the significantly ($p<0.05$) higher antibody mean titer than the protective level of antibody titer (>853).

Introduction

Poultry farming is the faster growing and important subsector, assists in upgrading the financial condition as well as contribute to human nutrition (Kim et al., 2018). However, it is the matter of regret that there are several factors that hinder the progression in poultry sector. Among them, increased prevalence of disease caused by reemerging pathogens due to naturally recombination of virus and frequent use of live vaccine (Ali et al., 2015). One of the most economical important diseases of poultry is infectious bronchitis virus (IBV) (Uddin et al., 2016). It is highly contagious and causing devastating economic losses to chickens (Bwala et al., 2018). The Infectious bronchitis (IB) is a highly contagious viral disease of the chickens, usually demarcated as an acute, contagious disease of chickens characterized primarily by respiratory signs.

Especially in laying hens, the IB leads to nephritis and affects the reproductive tract, causing poor quality of egg and loss of egg production with misshapen ova (Z. A. Bhuiyan et al., 2018; Ignjatovic and Sapats, 2000). Infectious bronchitis (IB) is caused by Infectious bronchitis virus (IBV), a single-stranded, positive-sense RNA virus of genus *Gammacoronavirus* member of the *Coronaviridae* family and is an enveloped virus with a single-stranded positive-sense RNA genome (Cavanagh, 2007). The active control of this highly contagious disease could be achieved mainly through mass vaccination and strict biosecurity ((Sjaak) de Wit et al., 2011). In spite of regular vaccination, there may be still risk in vaccinated flock, because it does not raise the protection against different serotype and variant strain of this virus (Callison et al., 2006). Though, the

different vaccine strains are currently using, but the conferred defense also depends on different factors related to vaccine type, including vaccination procedures, schedule and also route of administration (Jordan, 2017). The layers are commonly vaccinated at approximately 8 weekly intervals with live attenuated vaccines and with inactivated vaccines after commencement of lay (Ignjatovic and Galli, 1995). The vaccination timing against this disease has particular debates. Basically, the optimal vaccination time depends upon the maternally derived antibody (MDA) level of the chicks (Block et al., 2007). Because, the high maternal antibodies interfere with reproduction of live vaccines and diminish the level of immunity production in the chicks. The application of live vaccines during the 1st week of hatch in chicks against diseases whose MDA still persist in the body of the chick will result in defusing of antigen and active immunity may not be delivered by the vaccine (Pitcovski et al., 2003). On the other hand, the humoral immunity induced by early vaccination may not be optimal, possibly because of interfering with MDA, thus remaining the chicks unprotected against the field viruses' strain and supporting immuno-escape as well as recombination (Saiada et al., 2018). Despite this indication, this practice has been observed towards the hatchery administration and also recently in intensive farming (Abdul-Cader et al., 2018; Franzo et al., 2016). Particularly, the traditional methods suggest the interval between subsequent administrations of vaccine for a better immune response, due to let the poultry for recovery of the tracheal epithelium (van Ginkel et al., 2015).

In recent, many countries are practicing the combined application of multiple vaccines at hatchery level to recover this problem (Abdul-Cader et al., 2018; Franzo et al., 2016). Different serological methods are available to detect the maternal antibody and the antibody provided by the vaccine. Among the different serological methods, indirect enzyme linked immunosorbent assay (indirect ELISA) is used most commonly as it is highly sensitive, specific and quantitative. Commercial indirect ELISA kits are available to detect antibodies of IBV from sera samples (Wang et al., 2008; Martinez-Torrecuadrada et al., 2000). Though the several studies on the detection of IBV antibodies, were performed in Bangladesh (Khan et al., 2009; Meher et al., 2017), but limited number of studies on the detection of IBV MDA and antibody developed after the vaccination. Additionally, the real-time information of humoral response to vaccination is essential to develop and incorporate the mapping tools for veterinary services to control and prevent the contagious diseases (García et al., 2021).

Moreover; regular monitoring of serum antibody from IB vaccinated flocks for IB antibody titres may help to specify the intensity of vaccine response. Hence, this study was designed to determine the MDA titer against IBV, along with the compare of antibody titer developed by two different IB vaccines in layer chickens.

Materials and Methods

Ethical Approval

The study was completed in accordance with the research ethics and strategies as well as the animal care followed by the Department of Microbiology and Public Health, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh.

Therefore, the aproval number is BSMRAU/FVMAS/MPH/20(Ethical Approval)/2020/04, Date: 17-02-2021.

Study Design, Sample Collection, Transportation and Processing

In this study, a total of 1600 birds were equally assigned into two groups (group-A and group-B). all the birds were originated from Novogen Brown. The commercially available two vaccines had different strains namely "B1 Strain of ND+ Massachusetts B 41 Strains of IBV" and "Hitchner-B1 Strain of ND+ Massachusetts H120 Strains of IBV" were administered to the birds at 21-28 days of age and then every 2-3 months interval. Particularly, the group-A were vaccinated by the "B1 Strain of ND+ Massachusetts B 41 Strains of IBV" and group-B were vaccinated by "Hitchner-B1 Strain of ND+ Massachusetts H120 Strains of IBV" respectively. Both the vaccines were administered through the drinking water. All the birds in this study were originated from IB vaccinated breeders. The commercially available feeds were ad libitum to feed the birds. The birds of both groups were raised under measured states based on the regulations of national animal welfare. The birds of both groups were vaccinated at the age of one weeks.

Sample Collection

A total of 368 blood samples were randomly collected from the birds in which 184 blood samples from each group (group-A and group-B). The blood samples of 46 in number were collected in each time, at the age of one day old (before vaccination), 5 weeks, 10 weeks and 15 weeks old birds of each group to estimate the antibody titer. The blood samples were taken from the large vein under the wing (brachial vein) of live birds without anticoagulant to obtain the serum or antiserum. Blood samples were collected with consideration of the animal welfare policy. Then, the blood samples were sent immediately to the Sufian agro care Lab, Birujuli, Kapasia, Gazipur in ice box with ice for serological test. After that, the serum samples were obtained by processing according to the methods followed by another author (Meher et al., 2021). In brief, after clotting of blood, the serum was exposed to spin at

3000 rpm for 5 min to remove the remaining clots, red blood cells, and other insoluble materials. Lastly, stored at −20℃ for execution the indirect ELISA.

Detection of Pre and Post Vaccinated Antibody Titer by Serological Test (indirect ELISA)

The antibody titre of serum samples was measured by indirect enzyme-linked immunosorbent assay test (indirect ELISA). It is a numerical test for the recognition of specific antibodies from serum samples. The market available ELISA test kit (ID Screen® IBV Indirect, ID. Vet, Grabels, France) containing IBV antigen-coated plates were used to measure the antibody titers. All this test (indirect ELISA test) was performed according to the manufacturer's instructions. Briefly, at first the serum samples were diluted at 1:50 dilution in dilution buffer, followed by 1:10 dilution, and 1:500 was ultimate dilution, used as working sample for indirect ELISA. Then, the A1, B1 and C1, D1 wells of antigen coated plate were added by 100 µl of negative and positive controls respectively. Remaining 92 wells were filled with 100 µl of diluted serum samples and the plate was allowed to incubate for 30 min at 21°C (±5℃) in dark condition. Meanwhile, the conjugate and wash solutions were arranged as per manufacturer's guidelines. After incubation, each well was enunciated and washed 3 times with around 300 µl of the wash solution to avoid drying of the wells between washes. Then, each well of microtiter plate was filled by 100 µl of conjugate and incubated for 30 min at 21°C (±5℃). As per previous methods, the plate was washed with wash buffer. After that, 100 µl substrate solutions were added to each well of microtiter plate and kept at 21℃ (± 5° C) for 15 min ± 2 min. After incubation, 100 µl stop solutions was added to stop the reaction.

Finally, the optical density value of each sample was determined at 405 nm within 15 min after adding stop solution, and noted by assessing sample to positive (S/P) ratio and antibody titer. The result was authorized based on the manufacturer's guideline that "mean OD value of the Positive Control (OD PC) must be greater than 0.250, and the ratio of the mean values of the positive and negative Controls (ODPC and ODNC) must be greater than 3".

Calculation of Results

For each sample, S/P ratio and antibody titer were calculated using the following formulas:

$$\mathrm{S/P} = \frac{\text{OD of sample} - \text{OD of negative control}}{\text{OD of positive control} - \text{OD of negative control}}$$

Antibody titer for IBV: $\text{Log}_{10}(\text{titer}) = 1.0 \times \log_{10}(\mathrm{S/P}) + 3.63$;
$\text{Titer} = 10^{\log_{10}(\text{titer})}$

Interpretation of Results

S/P Value	ELISA Antibody Titer	IBV Immune Status
S/P ≤ 0.2	Titer ≤ 853	Negative
S/P > 0.2	Titer > 853	Positive

Statistical Analysis

Data were inserted into SPSS software version 25 to perform the statistical test. Therefore, data were compared between the group-A and group-B by carrying out the independent t test. The antibody mean titer within the groups were compared by Repeated measure Analysis of Variance (ANOVA) followed by Bonferroni test to assess the mean effect among the different ages of each group. All the separate samples of group-A and group-B were considered to perform one sample t test to compare the antibody mean titre of each group to the marginal level of protective antibody titre (>853). Before execution all the test, all assumption for the specific statistical test were measured and found too good. The p value <0.05 were assumed too statistically significant. The effect size of one sample t test was measured by using the following formula.

$$\text{Effect size} = \frac{\mathrm{t}}{\sqrt{\mathrm{N}}}$$

Here, N= Sample size and t= t value of one sample t test.

Results

In this study, the antibody titers of layer birds were recorded by the indirect ELISA. Both, the group A and group B showed that the antibody mean titer were significantly ($p<0.01$) increased and decreased according to their age. Even though, the antibody mean titer (after vaccination) were significantly ($p<0.01$) increased than the MDA mean titer. Between the two groups, only at the age of 5 weeks had significant differences in the antibody mean titer. The highest antibody mean titer (11544.43±177.51) was observed in Group-B at 5 weeks of age and was significant ($p<0.01$) difference than the antibody means titer (10222.11±96.96) of Group-B at this stage. The MDA mean titer was higher in Group-B and the antibody titer (just after vaccination) hit the peak and then gradually decreased than the group-A (Figure. 1).

Among the two groups, the lowest antibody titer was MDA, 3054 in Group-A and the highest was 13659 at the age of 5 weeks in Group-B (table 2). The individual samples antibody titer is presented in the Figure 2 and 3 for the birds of Group-A and Group-B respectively.

Table 1. Infectious Bronchitis vaccine antibody titer (Mean±SEM) at different ages birds of Group-A and Group-B.

Group Level	Antibody titer (Mean ± SEM) Age of the Birds				F value	P value	LS
	1 day old	5 Weeks	10 Weeks	15 Weeks			
Group-A	6890.2[a]±171.16	10222.11[b]±96.96	10665.89[a]±155.53	8025.65[a]±199.84	128.14	<0.001	**
Group-B	9708.93[a]±136.9	11544.43[a]±177.51	8950.8[a]±194.83	6832.24[a]±169.13	134.45	<0.001	**
F value	0.918	13.764	3.580	1.195			
P value	0.341	<0.001	0.062	0.277			
LS	NS	**	NS	NS			

[a, b, c:] Row values with same letters do not differ significantly; ** Level of significance at 1% (p<0.01), NS: Insignificant; LS = Level of significance; SEM: Standard Error of Mean.

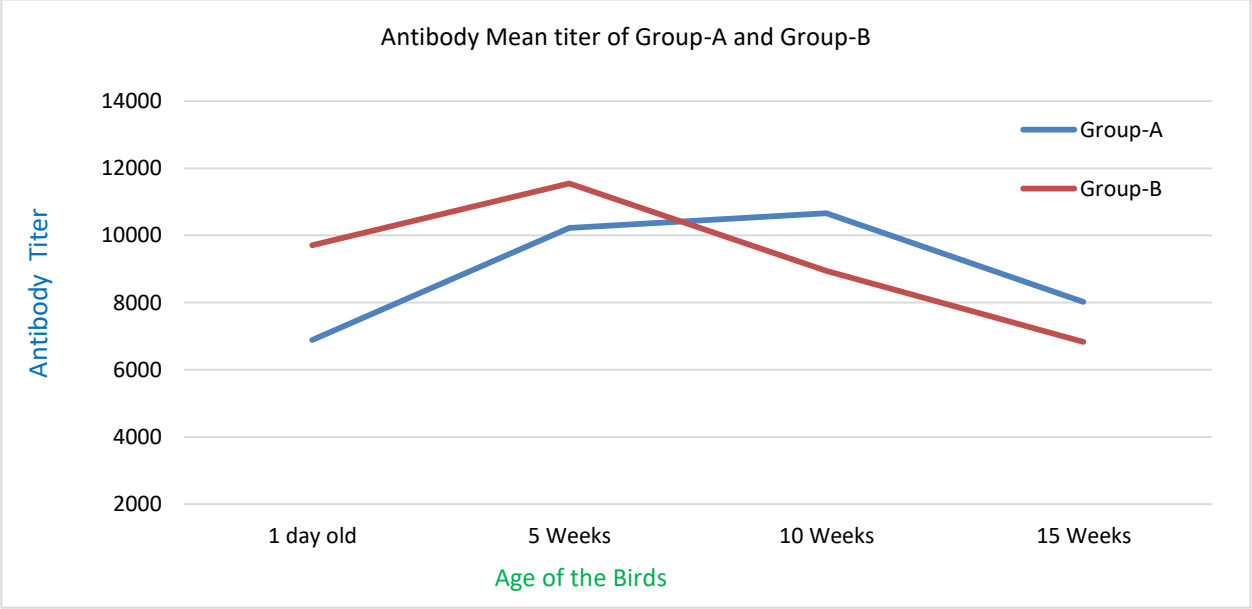

Figure 1. Antibody titer of Infectious Bronchitis (IB) vaccine at different ages of Layer Birds in group-A and group-B**.**

The IB antibody titer of group-A birds showed that their MDA titer fluctuated rapidly within the samples than the antibody titer of other ages. However, the samples of 5 weeks aged birds showed almost similar trend with little variation. Satisfactorily, all the samples of all ages showed the protective titer (>853), but among them, the MDA titer was lower than the titer of other ages. The highest trend of antibody titer was observed in samples of 10 weeks old birds. The mean titer of different ages birds was significantly higher than the protective antibody titer (>853). The effect size of the antibody titer was higher at 5 weeks aged birds (14.25; CI: 9173.82-9564.39; n=46), but the mean titer was highest at 10 weeks aged bird (9812.89; CI: 9499.64-10126.14, n=46). The antibody titer of group-B birds at 15 weeks of age had more fluctuation with lower trend than the others, even though the MDA titer was higher. In this group (B) the upper trend of antibody titer with in the samples was also observed at 5 weeks of age. Surprisingly, the highest effect size size (9.54; CI: 8580.19-9131.68; n=46) of antibody titer was recorded at one day old birds (MDA). But the antibody mean titer (10691.43; CI: 10333.91-11048.96, n=46) was higher in the birds of 5 weeks age in Group-B. Like the group-A, the antibody mean titers of all age's birds were also significantly (p<0.01) higher than the protective antibody titer (>853) in the birds of group-B.

Discussion

In this study, all the groups showed that the maternally derived antibody (MDA) were in protective level with an increased amount. These findings are consistent with previous reports of other authors (Michell et al., 2009), who reported that the offspring of the vaccinated breeders would have high titers of passive immunity at hatching. After the vaccination, in group-B the antibody mean titer was increased just after 5 weeks then decline than the MDA.

Table 2. Infectious Bronchitis vaccine antibody titer range (Maximum - Minimum) at different age's birds of Group-A and Group-B.

Group level	Antibody titer (Maximum - Minimum)			
	Age of the Birds			
	1 day old	5 Weeks	10 Weeks	15 Weeks
Group –A	3054-9542	8864-11620	7716-12421	4466-10506
Group -B	6816-11509	8578-13659	6210-11487	4496-8774

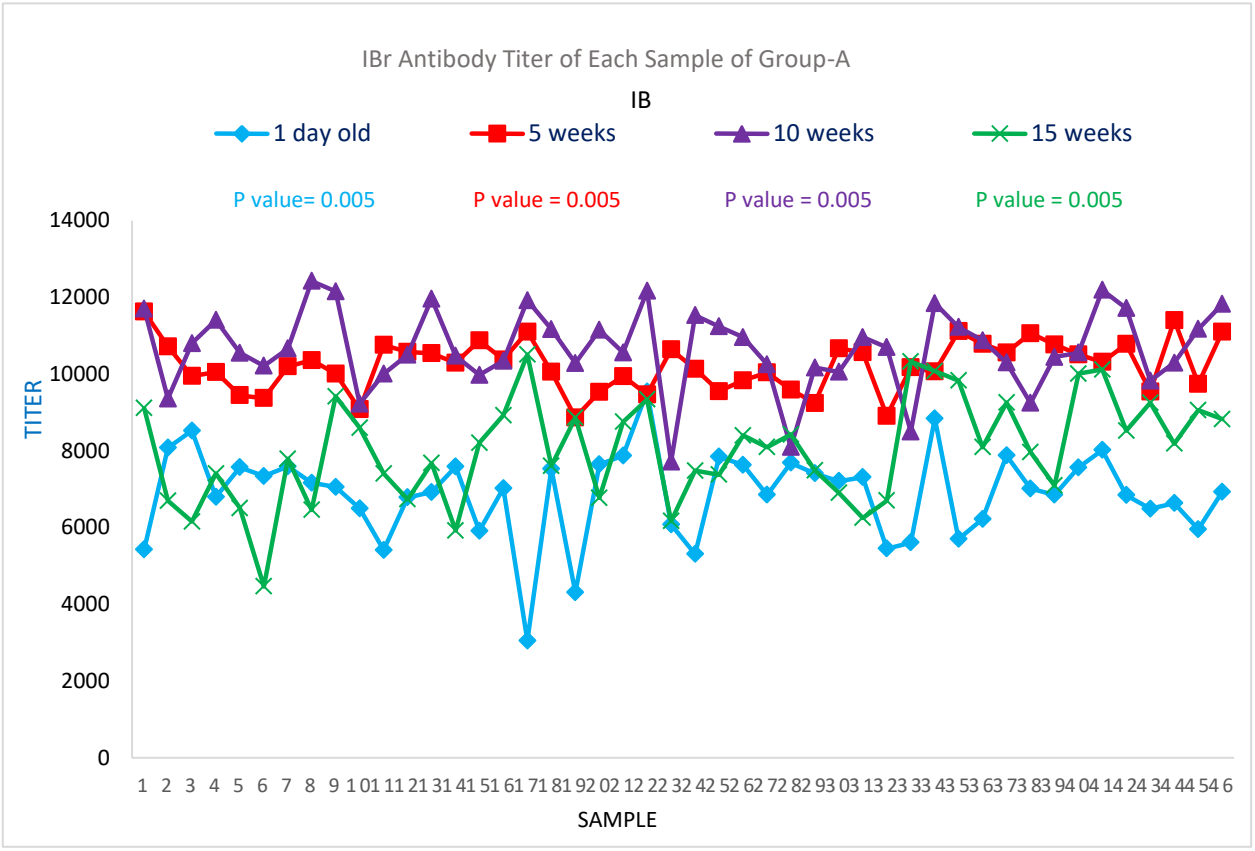

Figure 2. Antibody titer of Infectious Bronchitis (IB) vaccine at different ages of Layer Birds in group-A.

Table 3. Comparison of mean antibody titer of each age of different groups birds with the positive antibody titer (>853)

Test Value = > 853							
Variable		t value	Effect Size	P value (2-tailed)	Mean Difference	95% Confidence Interval	
Category	Level					Lower	Upper
Group-A	1 Day Old	35.27	5.20	0.000	6037.20	5692.45	6381.94
	5 Weeks	96.63	14.25	0.000	9369.11	9173.82	9564.39
	10 Weeks	63.09	9.30	0.000	9812.89	9499.64	10126.14
	15 Weeks	35.89	5.29	0.000	7172.65	6770.15	7575.15
Group-B	1 Day Old	64.69	9.54	0.000	8855.93	8580.19	9131.68
	5 Weeks	60.23	8.88	0.000	10691.43	10333.91	11048.96
	10 Weeks	41.56	6.13	0.000	8097.80	7705.40	8490.21
	15 Weeks	35.35	5.21	0.000	5979.24	5638.60	6319.88

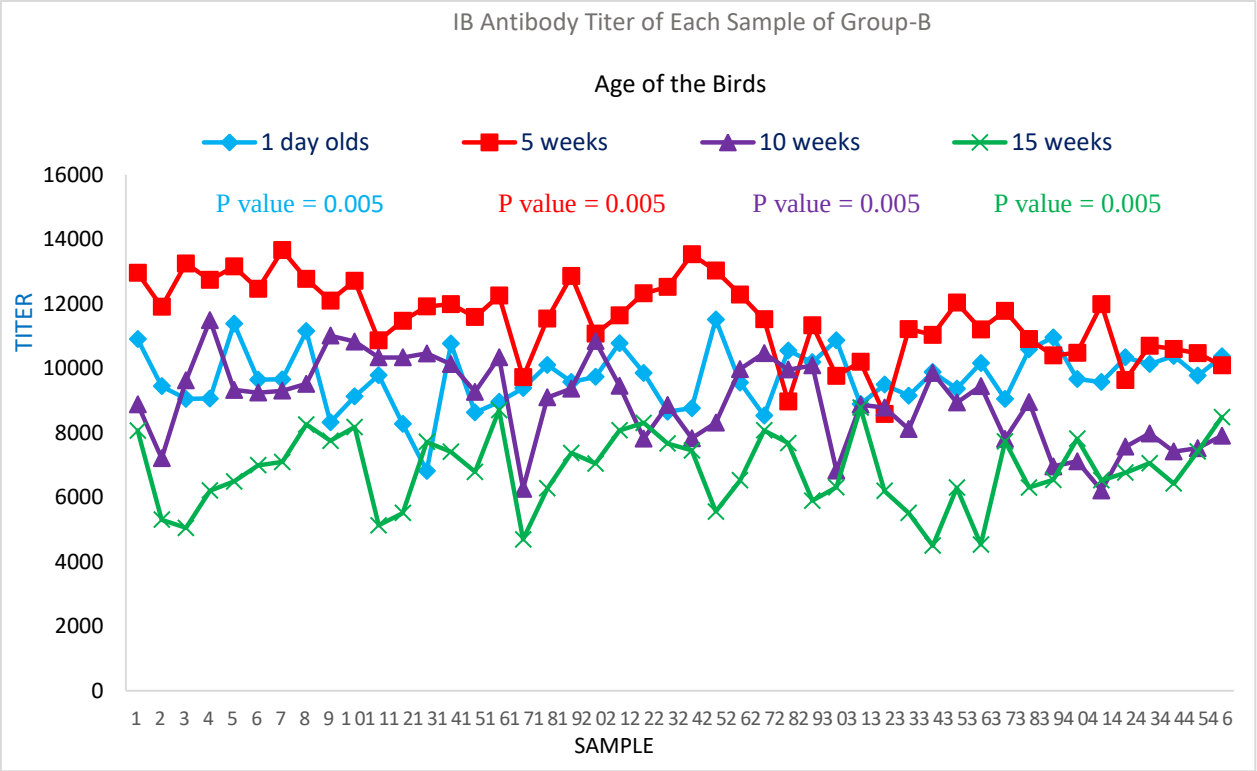

Figure 3. Antibody titer of Infectious Bronchitis (IB) vaccine at different ages of Layer Birds in group-B.

This might be due to the presence of high MDA titer because the authors (Wilson et al., 2014) reported that the MDA-positive vaccinated dogs had decreasing antibody titers following the first vaccination, but surges after the second vaccination. Interestingly, though the birds were originated from same breeder, but the MDA titer was high in group-B. This result suggested by the authors (Coakley et al., 2014) who reported that the transfer of comparative number of antibodies to offspring can vary between females with significantly and consistently. Generally, the MDA of IB remains up to 2 weeks of age, and subsequently, it decreases up to 7 weeks of age (Z. Bhuiyan et al., 2019).

In addition, the increasing trend of IB antibody due to infection is comparatively higher in aged birds especially at 63-73 weeks ages of layer birds (Meher et al., 2017). However, both the group-A and group-B was vaccinated by the Massachusetts serologically related types of Infectious Bronchitis and strains of Newcastle disease virus. These may have resulted the significantly higher IB antibody titer in group-B at 5 weeks, due to secondary effect of suppression of Newcastle disease (ND) virus. The vaccine of ND specially the clone vaccine can produce better immunity (Meher et al., 2021) against the Newcastle disease. The vaccine used in Group-A generate the protective immunity with more stable antibody mean titer and had the increasing trends up to 10 weeks than the vaccine used in group-B. In this study, the route of administration of the two IB vaccines were same (drinking water method).However, the different vaccine strategies like spray and gel administration having no any significant differences and both are less impactful on body temperature (Legnardi et al., 2021). Therefore, the development of protective immunity within the short time to protect against newly emerging IBV strains could be use of polyvalent vaccines IB vaccine via different vaccination strategies (Shao et al., 2020). Both groups had the significantly higher antibody mean titer than the protective level of antibody titer (>853). These might be due to efficacy of vaccine along with some factors that was practiced regularly. The factors like vaccine manufacturers guidelines for storage, timing, and due dates, consult veterinarians and health status monitoring before vaccine administration to the birds (Fesseha, 2020).

Conclusions

This study revealed that all the freeze-dried live vaccines of IB are able to produce optimum amount of protective antibody titer. Even though, the antibody mean titer (after vaccination) were significantly ($p<0.01$) increased than the MDA mean titer. The MDA mean titer was in protective condition and did not

hamper the generation of antibody titer after immediately vaccination. Bothe the IB vaccines had the significantly higher antibody mean titer than the protective level of antibody titer (>853).

Therefore, though the "Hitchner-B1 Strain of ND+ Massachusetts H120 Strains of IB" vaccine can produce higher antibody titer than "B1 Strain of ND+ Massachusetts B 41 Strains of IB" vaccines at the beginning, but the antibody titer of "B1 Strain of ND+ Massachusetts B 41 Strains of IB" vaccine decreases slower than "Hitchner-B1 Strain of ND+ Massachusetts H120 Strains of IB" vaccine, even though antibody titer was higher for "B1 Strain of ND+ Massachusetts B 41 Strains of IB" vaccine at the last stage. However, the further study could be the compare of different polyvalent IB and IBD vaccines and the detection of antibody titer weekly do determine the effective vaccination regiments.

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PROOF
RESEARCH PAPER

Broiler Chickens' Growth, Haematological Indices, Guts Microbiota, Carcass and Meat Analysis in Response to Dietary Supplementation with *Anacardium occidentale* Leaf Powder and A Mix of Prebiotic, Probiotic and Acidifier

Olugbenga D. Oloruntola[1,*], Simeon O. Ayodele[2], Samuel A. Adeyeye[3] Deborah A. Oloruntola[4], Clement O. Osowe[5], Oluwagbemiga S. Fasuhami[6]

[1]Animal Science Department. Adekunle Ajasin University, Akungba Akoko, Nigeria
[2]Department of Agricultural Technology, The Federal Polytechnic, Ado Ekiti, Nigeria.
[3]Department of Animal Health and Production Technology, The Federal College of Agriculture, Akure, Nigeria
[4]Department of Medical Laboratory Science, University of Medical Sciences, Ondo, Nigeria.
[5]Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria.
[6]Department of Biochemistry, The Federal University of Technology, Akure, Nigeria.

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***Corresponding Author**
Tel: +2348035841626
E-mail: olugbenga.oloruntola@aaua.edu.ng

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Abstract

This 42-day study evaluates the broiler chickens' response to dietary supplementation with *Anacardium occidentale* leaf powder (ALP) and a mix of prebiotic, probiotic and acidifier (PPA). Two baseline diets were compounded for the starter phase (age 1-21 days) and finisher phase (age 22-42 days) and divided into four parts: Diet 1(control), Diet 2 (250mg/kg PPA), Diet 3 (2,500mg/kg ALP), and Diet 4 (250mg/kg PPA+2,500mg/kg ALP). 240 Cobb 500 broiler chicks were distributed randomly to the experimental diets (6 replicates/diet; 10 birds/replication). The relative growth rate of the birds fed diets 3, and 4 were similar (P>0.05) to those fed diet 2; but higher (P<0.05) than those fed diet 1. The packed cell volume, haemoglobin concentration and red blood cells were improved (*P<0.05*) by the dietary supplements. Meat catalase and glutathione peroxidase activities dressed weight and gut lactic acid-producing bacteria improved (P<0.05) by the treatments. Meat cholesterol level was reduced (P<0.05) by diet. Conclusively, 250mg/kg PPA and 2,500 mg/kg ALP improved the growth rate, dressed weight, erythrogram values, and gut's lactic acid-producing bacteria population, meat catalase, meat glutathione peroxidase of the broiler chickens.

Introduction

Broiler farming has been highlighted as a feasible solution to Africa's acute animal protein shortfall (Hatab et al., 2019). However, due to the negative impact of climate change on livestock output in the tropics, this may not be possible. In the tropics, for example, a 2°C–6°C increase in average ambient temperature by 2100 will increase heat stress impact and pose a considerable challenge to economical and sustainable broiler production (Sylla et al., 2016).

Heat stress caused by a climatic challenge has been demonstrated to harm growth, meat quality, immunity (Kpomasse et al., 2021), blood parameters (Altan et al., 2000), gut health (Rostagno, 2020) and carcass (Zeferino et al., 2016) of broiler chickens and other domestic animals. Antioxidant supplementation in the diet has been found to help birds cope with heat stress. As a result, dietary adjustments or alterations are made in reaction to climatic variations in the tropics (Attia and Hassan, 2017). Prebiotics, probiotics,

phytobiotics, and acidifiers have all been investigated as nutritional supplements to assist offset the detrimental effects of elevated environmental temperature and heat stress (Awaad et al, 2018; Awad et al., 2020). When used as dietary supplements, a variety of tropical botanicals or phytogens show antioxidant action (Manuelian et al., 2021; Oloruntola et a., 2022). Prebiotics has been associated with an increase in the beneficial microbial population, whereas probiotics have been connected to a reduction in antioxidative stress in animals (Zhang et al., 2011; Awad et al., 2020). Acidifiers work as antioxidants by preventing the generation of oxygen radicals. (Awaad et al, 2018).

Anacardium occidentale L, a tropical tree with components that could be used in animal production, is a prospective plant. According to a preliminary investigation by Oloruntola (2021), *Anacardium occidentale* leaf powder has nutritional profile, bioactive components and antioxidant properties, which qualifies it as a phytogenic supplement in an animal feeding system. As a result, the purpose of this study is to determine how supplementing *Anacardium occidentale* leaf powder and a mixture of prebiotics, probiotics, and acidifiers to broiler chickens' feed affects their growth, blood indices, gut microorganisms, carcass and meat.

Materials and Methods

Approval of Animal Protocols, Experimental Site and Dietary Supplements

The Research and Ethics Committee at Adekunle Ajasin University's Department of Animal Science in Akungba Akoko, Nigeria, approved the animal protocols. Between February and March 2021, the feeding trial was conducted at Adekunle Ajasin University's Teaching and Research Farm in Akungba Akoko, Nigeria.

The site is located between 7°28' and 7°0' north latitude and 5°44' and 5°0' east longitude of the Greenwich meridian. The average ambient temperature was 30.13℃ and the relative humidity was 68.27 percent.

The *Anacardium occidentale* leaves were collected, processed to *Anacardium occidentale leaf* powder (ALP) and analyzed as reported by Oloruntola (2021). The mix of prebiotic, probiotic, and acidifier (PPA) was prepared Xvet, GMBH, 22529, Hamburg, Germany. The PPA is composed of *Bacillus licheniformis*+*Bacillus subtills* ($4x10^9$ CFU); *Lactobacillus acidophilus* ($5x4x10^9$ CFU); *Saccharamyces cerevisae* (40.00%); *Enterococcus foecium* ($1×4×10^9$ CFU); Magnesium (5.00%); Citric acid (2,000 mg); Formic acid (9,000mg); Ortho-phosphoric acid (3,000 mg); and Lactic acid (3,000 mg).

The Experimental Diets and Design

Two separate basal diets (Table 1) were devised and compounded for the starter phase (age 1-21 days) and finisher phase (age 22-42 days), taking into account the nutritional needs of the birds at the two phases of broiler chicken production. At each stage of production, the baseline diet was divided into four equal parts and called diets 1 to 4.

Diet 1: No supplement
Diet 2: 250mg/kg PPA
Diet 3: 2,500mg/kg ALP
Diet 4: 250mg/kg PPA+2,500mg/kg ALP

In a completely randomised design, 240 Cobb 500 broiler chicks weighing 34.98±1.18 g were randomly distributed to the experimental diets (6 replicates per diet; 10 birds per replication). The floor of the experimental pen, which measured 2m x 1m and was covered in dry wood shavings to a depth of 3.5cm, was kept at 31 ±3 degrees Celsius for seven days, and then decreased by 2 degrees Celsius each week until it reached 26 ±3 degrees Celsius. The lights were left on for 24 hours on the first day, and then for 23 hours on successive days.

Collection of Data, Blood Samples and Data Analysis

The body weights of broiler chickens were measured every seven days. The relative growth rate (RGR) was calculated using the following formula:

$$RGR= [(w2-w1)/ ((w1+w2))/2] *100$$

w1= Bodyweight when the trial began; w2= Bodyweight at the conclusion of the study.

At the end of the sixth week of the feeding research, four birds were randomly selected, tagged, weighed, and slaughtered by cutting their jugular vein using a clean, sharp knife. The blood of the birds was allowed to flow into a blood sample bottle containing Ethylenediaminetetraacetic acid (EDTA) for haematological analysis. The haematological tests were completed within 120 minutes of the blood collection as outlined by Shastry (1983).

Following that, the sacrificed birds' breast meat was collected and packed aerobically in an oxygen-permeable bag. The samples were kept in the freezer for 20 days at 18 degrees Celsius. The disappearance of H_2O_2, as measured by a decrease in absorbance at 240 nm, was used to determine the beef catalase activity (Muhlisin et al., 2016). The glutathione peroxidase activity (Cichoski et al. (2012) while the thiobarbituric acid (TBA) assay method was used to determine the amount of lipid oxidation in the meat (Tokur et al. 2006). Using commercial kits (Asan Pharm. Co., Ltd. Seoul, Korea), the cholesterol concentrations were measured

Table 1. Experimental diets' make-up

Components (g/kg)	Starter feed	Finisher diet
Fish meal	30.00	30.00
Soybean meal	300.00	240.00
Maize bran	70.20	0.00
Rice bran	0.00	60.30
Maize	523.30	593.20
Soy oil	30.00	30.00
Limestone	5.00	5.00
Bone meal	30.00	30.00
Salt	3.00	3.00
Premix*	3.00	3.000
Lysine	2.50	2.50
Methionine	3.00	3.00
Analyzed composition (g/kg)		
Crude protein	221.30	200.60
Crude fat	44.40	39.80
Crude fibre	35.20	36.10
Calculated composition (g/kg)		
Metabolizable energy (KJ/kg)	12631.12	13004.46
Lysine	13.80	12.60
Methionine	6.90	6.60
Available phosphorus	4.50	4.00
Calcium	10.10	9.90

*Premix composition: 2.5 kg of premix contains: Vitamin K3 (2000 mg), Vitamin E (12000 iu), Vitamin D3 (2500000 iu), Vitamin B1(2000 mg), Vitamin A (10000000 iu), Vitamin B6 (1500 mg), Niacin (15000 mg), Vitamin B12 (10 mg), Biotin (20 mg), Folic Acid (600 mg), Panthothenic Acid (7000 mg), Iron (40000 mg), Chlorine Chloride (150000 mg), Manganese (80000 mg), Copper (10 mg), Magnesium (100 mg), Selenium (150 mg), Iodine (1000 mg), Zinc (60000 mg), Ethoxyquine (500 g), BHT (700 g).

spectrophotometrically as described by de Almeida et al. (2006). The slaughtered birds were de-feathered, eviscerated and dressed. Following that, the carcass yield and carcass percentage were calculated. The heart, liver, gizzard, lung, and spleen were removed, weighed, and stated as a percentage of the slaughtered weight.

The caecal content of the sacrificed birds was collected for further serial dilution investigation of bacterial populations. Before collecting samples, the culture mediums were prepared and put into petri dishes 24 hours in advance. To cultivate the total aerobic bacterial counts, nutrient agar was utilized. Coliforms and intestinal lactose-negative bacteria were cultured on MacConkey agar. The lactic acid bacteria (LAB) were grown on de Man, Rogosa and shape agar (Seidavi and Simoes 2015; Oloruntola et al., 2020).

The model: $Dey = \mu + ae + bey$, was used in this experiment, where *Dey* is the response variable; e = the overall average; ae = the eth dietary effect (*D*= diets 1, 2, 3, and 4); and *bexy* = random error due to the investigation. In SPSS, all of the data were subjected to one-way ANOVA. To discover differences between the treatment averages, the SPSS Duncan multiple range tests were employed (P<0.05).

Results

Relative Growth

The effects of *Anacardium occidentale* leaf powder (ALP) and a commercial mix of prebiotic, probiotic, and acidifier (PPA) on the relative growth rate of broiler chicken are shown in Figure 1.

The relative growth rate of the birds fed diets supplemented with 2,500mg/kg ALP (Diet 3); and 2,500mg/kg ALP and 250mg/kg PPA (Diet 4) were similar (P>0.05) to those fed 250mg/kg PPA supplemented diet (Diet 2); but significantly (P<0.05) higher than those fed the control (Diet 1).

Blood Indices

The blood indices were significantly (P<0.05) affected by the dietary supplements in this study, except for the mean cell haemoglobin (Table 2). The packed cell volumes (PCV), red blood cell count (RBC) and haemoglobin concentration (HbC) of the experimental birds were enhanced by the ALP and PPA dietary supplementation. The PCV and HbC of the birds fed diets 2 and 4 were similar (P>0.05) but significantly higher than diet 1.

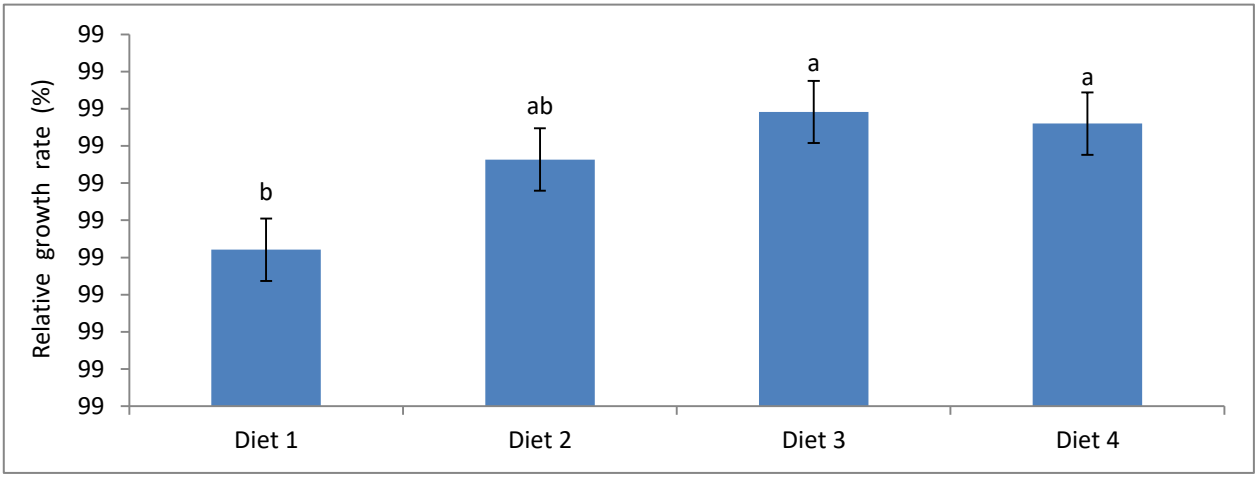

Figure 1. The effects of *Anacardium occidentale* leaf powder and PPA on the relative growth rate of broiler chickens; PPA: Commercial mix of prebiotic, probiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg PPA; Diet 3: 2,500mg/kg *Anacardium occidentale* leaf powder; Diet 4: 2,500mg/kg *Anacardium occidentale* leaf powder and 250mg/kg PPA.

Table 2 . The effects of *Anacardium occidentale* leaf powder and PPA on blood indices of broiler chickens

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Packed cell volume (%)	30.50^{c}	37.00^{a}	33.50^{bc}	35.50^{ab}	1.46	0.01
Red blood cell ($x10^{6}$/l)	1.70^{b}	2.35^{a}	2.50^{a}	2.31^{a}	0.18	0.01
Haemoglobin conc. (g/dl)	10.20^{c}	12.30^{a}	11.20^{bc}	11.8^{ab}	0.48	0.02
White blood cells ($x10^{9}$/l)	2.96	2.79	2.85	2.51	0.13	0.72
Granulocytes ($x10^{9}$/l)	1.20	1.29	1.18	1.24	0.02	0.15
Lymphocytes ($x10^{9}$/l)	1.16	1.14	1.12	1.21	0.07	0.98
Monocytes ($x10^{9}$/l)	0.34	0.36	0.55	0.06	0.03	0.48

Means in a row without a common superscript letter differ (*P<0.05*); PPA: mix of prebiotic, probiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg PPA; Diet 3: 2,500mg/kg *Anacardium occidentale* leaf powder; Diet 4: 2,500mg/kg *Anacardium occidentale* leaf powder and 250mg/kg PPA; SEM Standard error of the mean.

The RBC significantly (P<0.05) increased in birds fed diets 2, 3 and 4, compared to the control. The White blood cells (WBC) count and differentials (granulocyte count and monocyte counts) were stable (P>0.05) among the birds fed the various experimental diets.

Meat antioxidant activities, meat cholesterol, carcass traits and internal organ weight

When compared to the control diet, the catalase activity and glutathione peroxidase activity of broiler meat were considerably (P<0.05) greater in broiler birds fed diets 2, 3, and 4. When compared to the control, dietary supplements tend to lower the extent of lipid oxidation in broiler meat (P=0.06). However, as compared to the control diet, the meat cholesterol in the birds fed diets 2, 3, and 4 was considerably (P<0.05) lower (Table 3). Diets 2, 3, and 4 significantly (P<0.05) increased the dress weight of the birds compared to diet 1. Across the diet groups, the dressed percentage, and relative weight of the heart, liver, gizzard, lung and spleen were similar (P>0.05).

Gut Microflora

Table 5 shows the effects of ALP and PPA on gut microflora of broiler chickens. The aerobic bacteria, and coliform bacteria counts were similar (P>0.05) across the diets. However, the lactic acid bacteria count of birds fed diets 2 and 4 were similar (P>0.05) to those fed diet 3, but significantly (P<0.05) higher than those fed the control diet.

Discussion

The increased growth rate observed in broiler chickens fed diets supplemented with 250 mg/kg PPA and 250 mg/kg ALP in this study reflects the growth-promoting characteristics of the aforementioned dietary supplements. The antioxidant capabilities of ALP, probiotics, prebiotics and acidifiers (Awaad et al, 2018; Awad et al., 2020; Oloruntola, 2021), the activities of bioactive substances (tannin, flavonoids, alkaloids, phenols, saponins, etc.) could have improved the growth of experimental chickens (Oloruntola, 2021).

Table 3. The effects of *Anacardium occidentale* leaf powder and PPA on meat antioxidant enzymes and cholesterol of broiler chickens

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Catalase (kU)	16.43[b]	36.69[s]	38.23[s]	39.80[s]	3.31	0.01
Glutathione peroxidase (mg/ml)	188.23[b]	267.48[s]	288.18[s]	284.81[s]	9.44	0.01
Lipid oxidation (mgMDA/g)	0.50	0.29	0.17	0.13	0.06	0.06
Cholesterol (mg/dl)	42.09[a]	19.65[b]	17.28[b]	21.96[b]	3.45	0.01

Means in a row without a common superscript letter differ (*P<0.05*); PPA: mix of prebiotic, probiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg PPA; Diet 3: 2,500mg/kg *Anacardium occidentale* leaf powder; Diet 4: 2,500mg/kg *Anacardium occidentale* leaf powder and 250mg/kg PPA; SEM Standard error of the mean.

Table 4. The effects of *Anacardium occidentale* leaf powder and PPA on carcass trait and internal organ relative weight (% SW) of broiler chickens

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Carcass weight (g/bird)	1859.33[b]	2097.67[a]	2122.00[a]	2083.66[a]	37.07	0.01
Carcass percentage (%)	73.20	75.44	76.54	74.50	0.54	0.16
Heart	0.35	0.33	0.31	0.33	0.04	0.65
Liver	1.33	1.15	1.42	1.39	0.09	0.08
Gizzard	1.94	2.03	1.77	1.88	0.11	0.21
Lung	0.41	0.38	0.42	0.38	0.05	0.75
Spleen	0.08	0.10	0.08	0.07	0.01	0.37

Means in a row without a common superscript letter differ (P<0.05); PPA: mix of prebiotic, probiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg PPA; Diet 3: 2,500mg/kg *Anacardium occidentale* leaf powder; Diet 4: 2,500mg/kg *Anacardium occidentale* leaf powder and 250mg/kg PPA; SEM Standard error of the mean.

Table 5. The effects of *Anacardium occidentale* leaf powder and PPA on gut microflora (log 10 CFU/g) of broiler chickens

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	SEM	P value
Aerobic bacteria	7.82	7.96	8.39	8.22	0.21	0.11
Coliform bacteria	8.25	8.32	8.79	8.68	0.64	0.80
Lactic acid bacteria	7.76[b]	9.92[a]	9.02[ab]	9.74[a]	0.33	0.04

Means in a row without a common superscript letter differ (P<0.05); PPA: mix of prebiotic, probiotic, and acidifier; Diet 1: Control, Diet 2: 250mg/kg PPA; Diet 3: 2,500mg/kg *Anacardium occidentale* leaf powder; Diet 4: 2,500mg/kg *Anacardium occidentale* leaf powder and 250mg/kg PPA; SEM Standard error of the mean.

Flavonoids, for example, could boost chicken growth by modulating growth hormone (GH) and enhancing insulin-like growth factor (IGF-1) production. Furthermore, flavonoids stimulate growth by stimulating muscle protein synthesis and epiphyseal cartilage proliferation (Ouyang et al., 2016, Setiawan et al., 2018). Additionally, acidifiers, prebiotics, and probiotics have been shown to enhance growth by preventing micronutrient shortages, improving gut health, reducing infection, and improving zinc, vitamin B_{12}, and calcium absorption (Scholz-Ahrens et al. 2007; Pearlin et al., 2020).

Blood indices can be used to determine an animal's health state (Agbede et al., 2019), and nutrition has a major impact on haematological indices and performance (Oloruntola et al., 2018). The improved haematocrit value, red blood cell count and haemoglobin concentration of birds fed the supplemented diets (diets 2, 3 and 4) in this study suggest the supplements under study enhanced the adequate ingestion, absorption or utilization of essential nutrients that are needed in the processes involved in erythropoiesis (Beckman et al., 2010; Oloruntola et al., 2022). This is advantageous since the nutritional supplements help the birds maintain their regular overall health. The potential for phytogenic feed supplements to promote nutrient absorption from the intestine and, as a result, improve performance and blood indices has been documented (Alaeldein et al., 2018; Oloruntola et al., 2022). This result concurred with Sjofjan et al., (2022), who reported improved red blood cell counts in birds fed probiotics supplemented diets. Dietary acidifiers and probiotics, on the contrary, had no effect on haematocrit, red blood cell count, or haemoglobin concentration (Ogunwole et al., 2017; Aguihe et al., 2018). The fundamental role of white blood cells is to protect the bodily system from infections. In addition, the phytogens', probiotics',

prebiotics' and acidifiers' immunomodulatory activities were reported (Rajput et al., 2013; Oloruntola et al., 2016; Pearlin et al., 2020).

As a result, the similar white blood cell count and differentials across the dietary treatments in this study indicate that dietary supplements did not have an unfavourable effect on the birds' immunological condition. The principal peroxide-removing enzymes found in the cytosol are glutathione peroxidase and catalase (Utama et al., 2016). When superoxide dismutase scavenges superoxide anions by producing hydrogen peroxide, catalase safely decomposes hydrogen peroxide to water and oxygen, whereas glutathione peroxidase decomposes both hydrogen peroxide and lipoperoxides generated during lipid oxidation (Terevinto et al., 2010). This study's findings of improved meat catalase and glutathione peroxidase activities, as well as a tendency for decreased lipid oxidation in broiler birds that were fed diets fortified with ALP and PPA, are similar to those of Hosseindoust et al., (2020), who reported increased antioxidant status, as well as a decreased lipid peroxidation in the meat of broiler chickens that were fed diets supplemented with astaxanthin. Phytosupplements, for example, increase antioxidant activity in meat and reduce lipid oxidation, resulting in an extended meat storage period (Valenzuela-Grijalva et al., 2017). A previous study found that after prebiotic absorption and systemic circulation, poultry meat has substantial antioxidant activity.

Additionally, the antioxidant component in the prebiotic prevents the oxidation of broiler breast meat (Biswas et al., 2021). In another study, Wang et al. (2020) credited dietary acidifiers with improving nutrient digestion, antioxidant capacity, and meat quality. As a result of the increased meat catalase and glutathione peroxidase activities seen in broiler chickens fed ALP and PPA supplemented diets in this study, these supplements may be used in an attempt to improve the meat quality of broiler chickens. Producers' interest in producing animal products or proteins with lower cholesterol content has grown as the demand for lowering human dietary cholesterol consumption has become more urgent (Ponte et al., 2004). This is because eating a lot of high-cholesterol meat raises blood cholesterol levels and increases the risk of coronary heart disease. (Oloruntola et al., 2018). As a result, this study's findings of lower meat cholesterol levels in chicken that were fed ALP and PPA enriched diets are intriguing. Earlier, phytosupplements (Valenzuela-Grijalva et al., 2017; Oloruntola et al., 2022) and prebiotics (Biswas et al., 2021) have been shown to have hypocholesterolemic properties. For instance, tannin, one of the bioactive compounds found in PPA, has been shown to have a retarding effect on lipid absorption in the intestine and hence regulate excess lipid accumulation in the blood and tissues (Oloruntola et al., 2022).

Some phytochemicals and feed supplements have recently been postulated to have comparable effects on animal metabolism as anabolic steroids, acting as growth enhancers by altering animal metabolism in favour of building muscle tissue (Valenzuela-Grijalva et al., 2017). The increased dressed weight observed in the birds fed the supplemented feed followed the same pattern as the broiler chickens' relative growth rate in this study. This is consistent with a prior study that identified live weight as one of the elements influencing animal dressing weight, and that heavier and larger animals have higher dressing weight (Boler, 2014; Oloruntola et al., 2021). Furthermore, dietary constituents can affect the weight of animals' internal organs (Ayodele et al., 2016). Therefore, the fact that relative heart, gizzard, liver, lung and spleen weights remained consistent throughout dietary regimens suggests that the supplements employed in this investigation are nutritionally safe for broiler chicken production. The chickens' capacity to realise their genetic potential is influenced by their microbiome makeup and health. Phytogenic, prebiotic, and probiotic supplements have been shown to impact the microbial profile and health condition of broiler chicken guts in recent investigations (Oloruntola et al., 2019; Emami et al., 2020). The stable broiler chickens' gut aerobic bacteria and Coliform bacteria population across the dietary treatments is of health benefit because the inhibition of pathogen proliferation and enhanced performance require the preservation of normal gut microbiome and/or increase of non-pathogenic gut flora (Oloruntola et al., 2019).

Furthermore, the increased gut population of Lactic acid-producing bacteria found in birds fed supplemented diets reveals further nutritional benefits of the supplements under study in broiler production by encouraging the multiplication of beneficial and non-pathogenic gut microbes. Some harmful bacteria have the potential to stick to the gut epithelium; however, lactic acid-forming bacteria prevent these bacteria from clinging to the gastric epithelium by competitive and direct inhibition and exclusion, eliminating harmful microbes (Servin and Coconnier, 2003; Emami et al., 2020).

Conclusion

Conclusively, the 250mg/kg PPA and 2,500 mg/kg ALP, either singly or in combination improved the growth rate, dressed weight, erythrogram values, and gut's lactic acid-producing bacteria population of the broiler chickens. In addition, the dietary supplements improved the antioxidant status of the broiler meat by increasing its catalase, and glutathione peroxidase concentration. The PPA and ALP have hypocholesterolemic properties and decreased the broiler meat cholesterol content.

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PROOF
RESEARCH PAPER

POULTRY STUDIES

Effect of Chick Quality on Viability, Performance Traits and Meat Quality Characteristics of Broiler Chickens

Doğan NARİNÇ[1, *]

[1]Faculty of Agriculture, Department of Animal Science, Akdeniz University, 07070, Antalya, Turkey

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***Corresponding Author**
Tel: +905065057659
E-mail: dnarinc@akdeniz.edu.tr

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Abstract

The chick quality in broiler production is a major factor that has a direct impact on the profitability of both hatcheries and producers. In recent years, there has been considerable interest in the correlations between chick quality, which is measured by quantitative or qualitative methods, and yield characteristics. This study aims to compare the viability, performance traits, and meat quality characteristics of broiler chickens classified into two chick quality categories based on the Tona score method. In the study, broilers in the first-grade group had a lower mortality rate of 1.67%, while the average mortality rate of those with poor chick quality was 23.33% ($P<0.05$). There were no statistically significant correlations between post-hatch performance and chick quality characteristics such as Tona score, chick weight, chick length (all $P>0.05$). According to the Tona score, there were no differences between the mean values of body weight, feed efficiency, Gompertz growth curve parameters, slaughter carcass traits, and meat quality characteristics of first- and second-grade broiler chickens (all $P>0.05$). Although there is no difference between high- and low-quality chicks in terms of performance characteristics, it is possible that the use of low-quality chicks in conventional broiler production will increase the general mortality of the flock. Due to their superior yield potential, it is recommended that low-quality chicks be utilized in more suitable production systems without culling, rather than in conventional broiler production.

Introduction

Commercial hatcheries that produce broiler chicks aim to generate a large quantity of high-quality chicks for maximum profit. A quality chick must exhibit optimal development during incubation, high viability, good development after hatching, and yield qualities that conform to industry standards (Decuypere et al., 2002; Decuypere and Bruggeman, 2007; Reijrink et al., 2010). High-quality chicks should have bright eyes, no abnormalities or scars on the body, a totally closed navel, be free of remnants of membranes and shells, and be entirely separated from the yolk. These chicks should be able to react. There should be no edema, lesion, or similar swelling in their bodies. They should react to external sounds or different stimuli. They should be active and related to their environment. There should be no edema, lesion, or similar swelling in their bodies (Tona et al., 2005a; Narinç and Aydemir, 2021a). Evaluation of chick quality is quite difficult, and there are a variety of quantitative and qualitative measurement techniques available. Morphological metrics, such as chick weight, chick length, leg length, body circumference, and shank diameter are quantitative methods used to evaluate chick quality (Tona et al., 2004b).

Chick weight is not necessarily a reliable predictor of chick quality since it may include yolk residue unrelated to chick development. Therefore, evaluating the quality of chicks based on their body weight is difficult and might be deceiving. Using yolk-free body weight instead of chick weight to measure the quality of a chick may provide more precise findings. Different studies have shown a positive relationship between yolk-free body weight and the subsequent performance of the bird (Molenaar 2018; Narinç and Aydemir 2021a). The length of a chick is measured by extending it along a ruler and determining the distance from its beak to its mid-nose. Numerous studies have shown that the length of a chick correlates with its yolk-free body weight and predicts its long-term performance. Determined as the ratio of chick weight to initial egg weight, the yield percentage assesses weight loss during incubation. Yield percentage and chick weight are unreliable measures of chick quality because they do not account for the quantity of yolk-free body mass (Molenaar et al., 2018).

In qualitatively determining the quality of chicks, each criterion is objectively determined, therefore these methods are more dependable (Boerjan, 2002; Tona et al., 2005b). To determine the quality of chicks, visual evaluation methods such as the Pasgar score and the Tona score are utilized. Using the agility, belly condition, leg, and beak structural criteria of newly hatched and dried one-day-old chicks, the Pasgar score is determined by removing one point for each unfavorable circumstance out of 10. The Tona score considers the general activity and look of newly hatched and dried one-day-old chicks, as well as the existence and quantity of yolk residue, the condition of the eyes, umbilical region, and legs, the presence and quantity of residual membranes, and yolk absorption. The Tona score is a qualitative method with a 100-point evaluation (Tona et al., 2005b).

In industrial broiler production, chick quality, which is evaluated by a quantitative or qualitative method, is used to classify chicks according to their physical characteristics (Tona et al., 2004a). The quality of chicks produced at hatcheries is assessed, and day-old chicks of a specific quality (first-grade) are sold to producers. Chicks of lesser quality are culled and removed from the production line. The hatchery industry would benefit from information on the emergence of second-grade chicks, as these chicks are culled shortly after hatching and consequently constitute a direct economic loss. The percentage of second-grade chicks obtained from breeder flocks has fluctuated between 0.25% and 20.6% in scientific studies (Tona et al., 2004a; Lourens et al., 2005; Reijrink et al., 2010; Van de Ven et al., 2012). Although most hatcheries collect data on second-grade chicks, they save this information for internal use and do not disseminate it. The primary reason for this is to avoid society's reaction to the culling. According to some researchers, second-grade chicks must be culled because their chances of survival are so low (Nowak et al., 2019). In addition, they stated that the presence of these birds on the farm could lower their production indices and reduce the amount of stocking density and feed available for quality chicks. It has been claimed that such chicks may also enhance the risk of disease transmission by creating a pathogen reservoir (Muhammad et al., 2009; Nowak et al., 2019).

Many genetic and environmental factors influence the deterioration of chick quality, and numerous studies have been done on this topic. The performance characteristics of these chicks are just as intriguing as the causes for their inferior status. Only one study has been carried out to determine whether these chicks actually have a decreased chance of survival and perform sub-optimally in conventional broiler production (Van de Ven et al., 2012). In the study conducted by Van de Ven et al. (2012), the performances of first- and second-grade chicks hatched using conventional incubation and patio systems were compared. Researchers claimed that second-grade chicks had a high mortality rate (65.16%), that their slaughter weight was 258 g lower, and that the general practice of culling these chicks in the hatchery was justifiable. However, the primary worry in their study was the potential for a deterioration in performance characteristics owing to the high mortality rate in the patio system and the absence of chick quality determination. However, the patio system is not commonly used in modern commercial broiler production, and it is of interest to examine the performance characteristics of second-grade chicks in the conventional broiler production system. The purpose of this study is to compare the mortality, growth, feed efficiency, slaughter-carcass, and meat quality characteristics of first-and second-grade broiler chickens reared in the conventional system.

Material and Methods

The study was carried out at the Faculty of Agriculture, Animal Production Facilities of Namık Kemal University, Tekirdağ, Türkiye. Animal material for the study comprised of broiler chickens obtained from a commercial company's hatchery operation. Utilizing the Tona score method, the quality of 7,870 one-day-old chicks from the 42-week-old Ross 308 breeder flock held by a commercial company was evaluated. Table 1 describes the procedure of the Tona score method used to determine the quality of the chicks in the study (Tona et al., 2003). During the classification of the chicks' quality, those with 95 to 100 points were considered first-grade, and those with lower scores were deemed second-grade. As a result of the chick quality evaluation, a total of 284 chicks with a Tona score below 95 were identified (second-grade chick rate 3.63%). Sixty chicks from the first grade and sixty chicks from the second grade were randomly selected and transported to the experimental facilities. The chicks were housed in environmentally controlled chambers with a

Table 1. Criteria for determining chick quality in Tona score method

Quality criterion	Determination Conditions	Score
Activity	Activity is assessed by laying the chick on its back to determine how quickly it returned to its feet. A quick spring back on to its feet was regarded as good, but trailing back on to its feet or remaining on its back was assessed as weak.	6-0
Down and appearance	The chick body was examined for dryness and cleanness. It was regarded as normal if it is dry and clean. If it is wet or dirty or both then it is not good.	10-8-0
Retracted yolk	The chick was put on its back obliquely on the hand palm until the abdominal movement totally stopped. The height of its abdomen was estimated. The consistency of the abdomen to touch was then estimated. If the height of the abdomen was estimated to be higher and harder to touch than normal, then yolk retracted was regarded as large and consistent.	16-12-8-4-0
Eyes	The chick was put on the legs, and its eyes were observed. The state of brightness and wideness of the gape of the eyelids were estimated.	16-8-0
Legs	The chick was put on its feet to determine if it remained upright well. The toes were examined for their conformation. If the chick remained upright with difficulty, articulations of the knees were examined to detect signs of inflammation or redness or both.	16-8-0
Navel area	Navel and surrounding areas were examined for the closure of the navel and its coloration. If the color was different from the skin color of the chick, then it was regarded as bad.	12-8-4-0
Remaining membrane	Observation of the navel area allowed estimation of the size of any remaining membrane. The size of any remaining membrane was classified as very large, large, or small.	12-6-0
Remaining yolk	Observation of the navel area allowed estimation of the size of any remaining yolk. The size of any remaining yolk was classified as very large, large, or small.	12-0

temperature of 34 °C. Then the temperature was decreased gradually to 22 °C at the end of the study. The wing numbers of day-old chicks were attached so that each measurement could be recorded individually. In the study, 6 floor pens with a stocking density of 10 broilers/m^2 were used, with shavings serving as the deep litter material. The temperature regime, lighting schedule, and feed were administered in accordance with the broiler company's guideline. The chicks were fed a broiler starting diet comprising 3000 kcal of ME/kg and 22% CP from day 1 to day 14. They were fed a grower diet comprising 3100 kcal of ME/kg and 20% CP from day 14 to day 28. Beginning on day 28, a diet containing 3200 kcal of ME/kg and 19% CP was provided. To obtain the estimates of individual growth curve parameters, all birds were weighed weekly from hatching to 6 weeks of age. The Gompertz nonlinear regression model (1) was used to estimate the growth curve of each chick.

$$\boldsymbol{y_t = \beta_0 e^{(-\beta_1 e^{-\beta_2 t})}} \quad (1)$$

Where yt is the weight at age t, β_0 is the asymptotic (mature) weight parameter, β_1 is the scaling parameter (constant of integration), and β_2 is the instantaneous growth rate (per day) parameter (Narinç and Genç, 2021). Parameter estimations were performed by the NLIN procedure of SAS 9.3 software (SAS Institute Inc., Cary, NC). The Gompertz model is characterized by an inflection point in a manner such that β_0/e of the total growth occurs before it and the remainder occurring after (Alkan et al., 2012). The coordinates of the point of inflection, weight (2) and time (3) at inflection point (IPW), were obtained as follows (Narinc et al., 2017).

$$\mathbf{IPW = \beta_0/e} \quad (2)$$
$$\mathbf{IPT = \ln(\beta_1)/\beta_2} \quad (3)$$

In the study, the feed consumption of the chickens was measured weekly in each floor-pen. The weekly feed conversion coefficients were determined individually by proportioning with the individual body weight gain.

All broilers were weighed at six weeks of age, eight hours after their feed was withdrawn, then slaughtered in an experimental processing facility. The birds were manually cut, bled, scalded (55 °C, 2 minutes), defeathered, manually eviscerated, and the abdominal fat pad (from the proventriculus surrounding the gizzard to the cloaca) was removed, refrigerated in an ice-water tank, and drained. The following day, upon dissection of the carcasses, the breast with bone and remaining abdominal fat were weighed using an electronic digital balance with a precision of 0.01 g. Slaughtering and dissection were carried out by the same experienced operators. The yields of cold carcass, breast, leg, wing, and total fat pad were evaluated in relation to 6-week-old body weight (Narinc et al., 2014). At the carcass dissection processing (about 24 h post-mortem), the ultimate pH values (pHU) were obtained by inserting the pH meter's electrode directly into the anterior part of the left Pectoralis major muscle. On the medial surface (bone-side) of each right breast fillet, the color of the breast flesh was evaluated using a Minolta Chromameter (CR-300). The CIE L*a*b* system was utilized, where L* represents the meat's lightness, a* its redness, and b* its yellowness (Narinç et al., 2013). Each right fillet was weighed, packaged, and frozen at -18 ° C for 28 days. At the end of the storage period, breast samples were thawed in a refrigerator (+4 °C) for 24 hours before being taken from their bags, wiped with paper, and weighed (thawed weight). After this, all muscle samples were immersed in plastic bags in an 80 °C water bath until they reached an internal temperature of 70 °C, then chilled, wiped, and weighed (cooked weight). Thawing loss (TL) was calculated as a percentage of the weight lost after thawing relative to the original muscle weight. The cooking loss (CL) was determined as the difference between the cooked and uncooked weights (Narinç et al., 2013). Texture Profile Analyzer (TA-XT plus Stuble Microsystems, Godalming, Surrey, UK) device with Warner-Bratzler (WB) shearing knife was used to measure the toughness of breast meat. Vertical samples (1x2x2 cm) were obtained from the fibers of cooked muscle. These samples were cut using a WB shearing knife and the shear force (WB) was measured in kilograms (Narinç et al., 2013).

First, parametric test assumptions for performance characteristics, growth curve parameters, slaughter-carcass traits, and meat quality characteristics were assessed. After confirming variance homogeneity and normal distribution for all variables, the One Sample T test was performed as a hypothesis test to compare the chick quality groups. SAS 9.4 software was used to conduct statistical analyses.

Results

The mean values of chick length, weekly live weight, feed conversion, and mortality of broiler chickens in two different chick quality groups, and statistical analysis results are shown in Table 2.

In terms of the mean values of chick length, chick weight, live weights at 35 and 42 days of age, there were no statistical differences between the first- and second-grade chick quality groups (all P>0.05). A similar situation is also valid for cumulative feed conversion ratios at 35 and 42 days of age, there were no statistically significant differences between the mean values of the experimental groups (both P>0.05). The cumulative mortality rate for second-grade broilers was 23.33%, while the cumulative mortality rate for first-grade broilers was 1.67% (P<0.05). The difference between mortality rates of broiler chickens from different classes of chick quality was statistically significant (P<0.05). The phenotypic correlation between day-old chick weight and day-old chick length was statistically significant and fairly strong (r = 0.68; P<0.05) for all chicks (data not shown in any table). The study revealed no relationships between Tona score and day-old chick weight (r=0.16; P>0.05) or length (r=0.05; P>0.05).

Table 3 presents the growth curve parameters estimated by the Gompertz function of chickens in two different chick quality classes. In terms of the model parameters and inflection point coordinates of the Gompertz growth curve model, there were no statistically significant differences between the means of the first- and second-grade chick quality groups (all P>0.05). Figure 1 shows the growth curves derived by the Gompertz function for broilers in the first- and second-grade chick quality groups. The mean values of carcass yield, carcass part percentages, ratios of edible internal organs and abdominal fat of chickens in two different chick quality classes and statistical analysis results are presented in Table 4. While the carcass yield of the chickens in the first-grade chick quality group was 70.99%, the mean of the other group was 71.14%, and there was no statistical difference between the groups (P>0.05). Similarly, there were no statistical differences between the groups in terms of the mean values for ratios of carcass parts (all P>0.05). In the study, the mean values for ratios of abdominal fat and edible inner organs of chickens in the first-grade chick quality group were 1.90% and 5.37%, respectively, compared to 1.74% and 5.45% in the second-grade chick quality group. There were no statistically significant differences between the groups regarding the means of both characteristics (both P>0.05). Table 5 and Table 6 provide the mean values of pH, color, thawing loss, drip loss, cooking loss, water holding capacity, and shear force characteristics of breast muscle samples from broiler chickens classified into groups based on chick quality, and statistical analysis results. There were no statistically significant differences between the groups of first- and second-grade chicks for any of the aforementioned meat quality characteristics.

Table 2. Some performance characteristics of broilers from different classes of chick quality

Chick Quality	Chick Length (mm)	Chick Weight (g)	BW 35 (g)	BW 42 (g)	FRC 35	FCR 42	Mortality (%)
Grade 1	176.11	44.44	1932	2675	1.73	1.83	1.67[b]
Grade 2	174.99	43.73	1897	2635	1.71	1.81	23.33[a]
SEM	8.64	0.85	26.93	39.20	0.11	0.12	2.29
P Value	0668	0.512	0.190	0.136	0.440	0.172	0.028*

BW: Body weight, FCR: Cumulative feed conversion ratio, *P<0.05

Table 3. The growth curve parameters estimated using the Gompertz function of broilers in two different chick quality classes

Chick Quality	β_0	β_1	β_2	IPW	IPA
Grade 1	6924	4.64	0.038	2547	40.92
Grade 2	6969	4.64	0.037	2563	41.51
SEM	456	0.35	0.005	168	1.71
P Value	0.522	0.778	0.885	0.522	0.716

β_0: Asymptotic body weight, β_1: Integration constant, β_2: Instantaneous growth rate, IPW: Body weight at inflection point, IPA: Age at inflection point

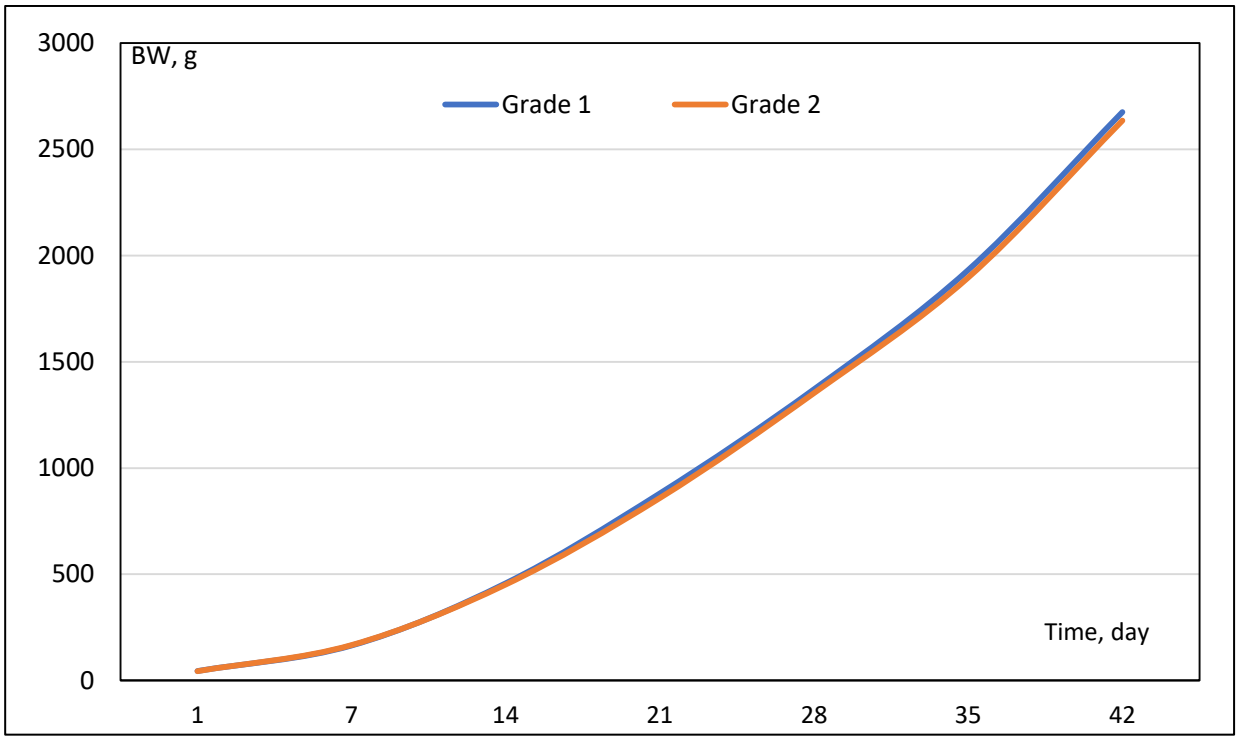

Figure 1. Gompertz growth curves of broiler chickens in different chick quality classes

Table 4. The slaughter-carcass characteristics of chickens from two different categories of chick quality

Chick Quality	CY	BP	LP	WP	AFP	EIOP
Grade 1	70.99	28.12	19.89	6.56	1.90	5.37
Grade 2	71.14	28.00	19.99	6.51	1.74	5.45
SEM	1.10	0.80	0.77	0.40	0.43	0.53
P Value	0.614	0.948	0.456	0.114	0.716	0.805

CY: Carcass yield, BP: Breast percentage, LP: Leg percentage, WP: Wing percentage, AFP: Abdominal fat percentage, EIOP: Edible inner organ percentage

Table 5. The pH and color characteristics of breast muscle of broiler chickens grouped according to chick quality

Chick Quality	pH_{15}	pH_U	L	a	b
Grade 1	6.46	5.79	51.26	3.81	4.98
Grade 2	6.51	5.83	52.21	3.57	4.57
SEM	0.23	0.18	0.98	0.38	0.41
P Value	0.784	0.485	0.741	0.814	0.111

Table 6. The meat quality characteristics of breast muscle of broiler chickens categorized by chick quality

Chick Quality	Thawing Loss (%)	Drip Loss (%)	Cooking Loss (%)	Water Holding Capacity (%)	Shear Force (kg)
Grade 1	2.01	2.01	21.32	74.86	2.14
Grade 2	1.94	1.92	21.88	74.45	2.07
SEM	0.08	0.05	1.18	0.82	0.12
P Value	0.721	0.856	0.888	0.524	0.511

Discussion

In the study, when all weekly body weight averages including hatching weight were taken into account, no differences were found between chick quality classes. Tona et al. (2004a) classified three broiler genotypes according to the Tona score method at one-day old in their study. At 41 days of age, the average live weight of chicks with a perfect score of 100 was between 6.29 and 8.05 % higher than that of chicks with the second-grade quality score.

It is thought that the inconsistency between the results of this report and those of the current study is due to the small sample size (12 chickens per replication and 48 chickens in total per genotype) and the failure to account for mortality rates. Willemsen et al. (2008) and Van de Ven et al. (2012) reported that the relationship between qualitative chick quality scores and post-hatch performance is not significant using the Tona score unless a significant percentage of second-grade chicks are included. Van de Ven et al. (2012) suggested that the efficacy of qualitative chick quality indicators for post-hatch performance can be questioned if poor quality chicks are not culled from the flock in the hatchery. In the current study, it was determined that the use of second-grade quality chicks in production did not cause a significant difference in live weight. In addition, there was no difference between the feed conversion ratios of broilers from different classes of chick quality. There is no study in the literature on the relationship between chick quality and feed efficiency. Some researchers have claimed that each of the methods for measuring chick quality-chick weight, chick length, Tona score, and Pasgar score-can predict chick's growth potential at one day of age (Hill, 2001; Tona et al., 2003; Wolanski et al., 2006; Molenaar et al., 2008; Mukhtar et al., 2013).

Although a strong correlation (r=068) was found between chick weight and length in this study, statistically insignificant and weak correlations (between 0.05 and 0.21; data not shown in table) were determined between the chick quality criteria and the body weight of broilers at 35 or 42 days. According to a recent study (Narinç and Aydemir 2021b), the heritability estimates of qualitative chick quality traits are quite low. The genetic and phenotypic correlations between qualitative chick quality traits and post-hatch performance traits were found insignificant by researchers. In their study, in which genetic parameter estimations for qualitative chick quality traits were performed for the first time in the scientific literature, researchers noted that the environmental factors for these traits were rather significant. Tona et al. (2005b) stated that differences in growth potential amongst chicks of the same quality indicate that other unknown factors impact growth performance. Thus, it can be predicted that these factors may have changed the physiology of the embryo by causing changes in gene expression. The present study's findings are consistent with those of Narinç and Aydemir (2021b) and Tona et al. (2005a).

Most of the studies (Tona et al., 2004a, Willemsen et al., 2008; Tona et al., 2005a) comparing chicks of different quality classes did not focus on survivability. In the present study, the mortality rates of second-grade quality chicks (23.33%) were significantly higher than those of first-grade quality chicks (1.67). In addition, this value is far greater than commercial production standards. When the birds were divided into two groups based on the length of the chick, which is one of the quantitative methods used to measure chick quality, the mortality rates were found to be 11.15 and 12.25%. When categorizing according to the weight of one-day-old chicks, a variation in mortality was detected. This demonstrates that low-viability chicks can be culled from a flock more efficiently with the Tona score method than with quantitative methods. In a study conducted by Van de Ven et al. (2012), it was determined that the cumulative mortality rate for chicks

with second-grade quality was quite high. If chick culling is not implemented in systems where all chicks are included in the production process after hatching, such as the patio system, the mortality rate may exceed the standards. While the average mature weight parameter of the chickens in the first-grade chick quality group was 6924 g, it was determined as 6969 g in the second-grade group (P>0.05). These averages were found to be compatible with the averages (5797-6974 g) reported by Narinç et al. (2007), Topal and Bolukbasi (2008), Demuner et al. (2017), and Koushandeh et al. (2019). In addition, the β_0 parameter averages estimated in the current study were found to be higher than the averages (2691-3472 g) reported by Mignon-Grasteau et al. (2000), Roush et al. (2006), Norris et al. (2007), and Şekeroğlu et al. (2013). It is known that genetic or environmental manipulations carried out in some studies can change the shape of the growth curve without changing the body weight at the end of the experiment. However, in the current study, there were no statistical differences between broilers in different chick quality classes in terms of both end-of-trial body weights and Gompertz growth model parameters. In a study conducted by Narinç and Aydemir (2021b), it was determined that there were no relationships between the Tona score and the parameters, and the inflection point coordinates of the Gompertz growth model. The current study findings were consistent with the results reported by Narinç and Aydemir (2021b).

In the study, there were no statistical differences between different chick quality groups in terms of carcass yields and other slaughter-carcass characteristics of broiler chickens (all P>0.05). In a study performed by Narinç and Aydemir (2021b), it was found that Pasgar and Tona score values, which are qualitative chick quality assessment methods, lacked genetic and phenotypic correlations with carcass yield, ratios of breast, thigh, wing, and abdominal fat. The findings of the current study support the results of Narinç and Aydemir (2021b). Moreover, the same situation true for meat quality characteristics. All meat quality characteristics of broiler chickens from the first- and second-grade chick quality groups did not differ statistically significantly (all P>0.05). There is no study in the scientific literature that evaluates the relationships between the chick quality and the meat quality.

Conclusions

In numerous studies, the potential of chick quality, which is measured by qualitative and quantitative methods, to be a reliable predictor of subsequent performance of broilers has been evaluated. In this study, it was found that the correlations between chick quality and performance characteristics were quite weak. In addition, there were no differences in the performance characteristics of broilers categorized according to the chick quality. It has been determined that the Tona method for assessing the quality of chicks is an accurate predictor of viability. Due to the high mortality rate of broilers in the second-grade chick quality group, failure to cull is likely to increase the total flock mortality rate. It is not suggested to employ these birds in conventional broiler production, as ethical issues may develop if specific limits for conventional production under the applicable European Union legislation are surpassed. The use of broilers with low chick quality but high-performance features in alternative rearing systems when mortality rates are acceptable can be advised.

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PROOF
RESEARCH PAPER

Sexual Dimorphism in Body Size and Some Exterior Traits of Pigeon Breed Groups

Türker Savaş[1, *] Hakan Erdem[1,]

[1]Çanakkale Onsekiz Mart University, Animal Science, Çanakkale, Turkey

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***Corresponding Author**
Tel: +905442137064
E-mail: turkersavas65@gmail.com

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Abstract

In this study, sexual dimorphism in pigeons was investigated. Rensch's rule was evaluated across pigeon breeds. Body weight, body length, beak length, wingspan, tail length, and tarsus length were used for the analysis. We have divided the breeds in the five groups (form, frills and owls, tumbler and rollers, homer and highflyer, feral). The allometric relation in the traits between female and male measures were analyzed. The measure of sexual size dimorphism was measured for each trait as a simple male size divided by female size (Sexual Size Index, SSI). On average across traits, male birds have higher values than female birds, both across breed groups and overall. No deviation from isometric allometry can be observed except the tail length. No trends towards logarithmic female values according to the SSI could be identified. According to the results, the rules of Rensch for pigeon breeds are rejected. Sexual dimorphism exists between breeds and traits, mainly in favor of the male birds. In most cases, allometric relationships between breeds change, as do traits

Introduction

The sex-specific differences, especially in overall size and body parts, are a topic that biology has been dealing with for a long time. It is mostly about evolutionary questions across the species (Kappeler, 1990; Fairbairn, 1997; Teder, 2014; Janicke and Fromonteil, 2021). An earlier hypothesis, known as Rensch's rule, states that sex size dimorphism increases with species size in species in which males are larger and decreases in those in which females are larger (Rensch, 1950). Abouheif et al. (1997) report that Rensch's rule is generally valid across several taxa. Guillermo-Ferreira et al. (2014) confirm this for insects but not for the stoneflies they worked on.

The outstanding difference between wild and domestic animals are the wide variation in size, shape, and color. The variation mentioned is so great that it is not seen between some wild species. The variation between breeds of domestic animals even leads to sexual barriers, which as a factor leads to speciation (Kaneshiro, 1980). Even if there is such a large variation between the breeds of a species, they are all the same species. However, some scientists have attempted to evaluate in particular Rensch's rule on breeds of domestic animals. The motivation for these studies is likely to evaluate the difference in sexual selection between natural conditions and human care. For example, Polak and Frynta (2009) compared sexual dimorphism in wild sheep and goat species with domestic sheep and goat breeds. The authors found greater sexual dimorphism in wild goat and sheep species than in domestic goat and sheep breeds. However, the dimorphism was lowest in non-European

chamois, which are also wild species. The authors also conducted a similar study in subfamilies of the *Bovinae* species (Polak and Frynta, 2010). The results of this study state that water buffaloes are less dimorphic than domestic cows, yaks, and wild bovines, and draft breeds are less dimorphic than beef, dual-purpose, and dairy breeds.

In their study entitled "Morphometrics Within Dog Breeds Are Highly Reproducible and Dispute Rensch's Rule" Sutter et al. (2008) found that the differences in height at the withers of female and male dogs are proportionally equal among dog breeds. On the contrary, without the dog breeds specified as monomorphic in the FCI (Federation Cynologique Internationale) standard, Frynta et al. (2012) reported that the withers height in dog breeds confirms Rensch's rule.

Relatively high male dimorphisms were found in the body size of the chickens (live weight), which the jungle chickens showed to be extreme with an average difference of 68% (Remeš, Szekely, 2010). Geibel et al. (2016) found also great differences in favor of rosters in various chicken breed groups. In modern breeding programs that select for economically important traits, sex variation in traits is eliminated prior to selection. It can be assumed that in this way sexual selection loses its meaning. Since the mating decision is also made by humans when breeding domestic animals outside of breeding programs, sexual selection presumably plays a subordinate role here as well. The fact that dimorphism is more pronounced in draft cattle breeds reinforces these statements. Although the physical difference in females and males is smaller in domestic animals, artificial selection has not yet been able to eliminate physical sex dimorphism.

Therefore, in this study, we have investigated the extent to which sexual dimorphism in pigeons exists. We also evaluated Rensch's rule across breeds. How sexual dimorphism behaved in breeds selected for different traits was another question of the study. Furthermore, we investigated the extent to which sexual dimorphism differs in different body traits.

Material and Methods

The averages of female and male pigeons were collected from the literature shown in Table 1. Body weight, body length, beak length, wingspan, tail length, and tarsus length existed in most studies. Therefore, we used these traits for the analysis. We have grouped the breeds for analysis (Table 2). The grouping of the pigeon breeds was based on the grouping of the Association of German Show Pigeon Breeders. However, the "Homer and Highflyer" and "Feral" groups were created based on breed characteristics. Most are well-known breeds among international pigeon fanciers. However, some are local breeds but clearly defined in the literature. For some, on the other hand, only a small amount of open information can be obtained. If we briefly touch on these local pigeon breeds in the study, Denizli Azman pigeons are natives breed to the city of Denizli in western Anatolia. The breeding goals are primarily their shape, form, and color patterns and secondly their ability to fly. Halfbreed Baska is kept in and around Istanbul. This breed developed from a cross between Baska and Mısıri, which main keeping area is also Istanbul. Again, a breed bred for its shape, form, and color pattern and originating from the prince's city of Manisa is the Manisa Azman Breed. A small breed known with a very short beak and large eyes is the Mısıri Pigeon, which originated from Istanbul. The Turkish "Fleet pigeons" on the other hand are not a breed but rather a group of relatively large pigeons with good flight and navigation ability. Especially in Southeastern Anatolia, these birds are flown in large flocks, consisting of males. Driving the flocks is about attracting and catching as many "stranger" birds as possible from other flocks.

Firstly, the allometric relation in the traits (live weight, body length, beak length, wingspan, tail length, and tarsus length) between female and male measures were analyzed. Allometry, better known as growth allometry, is basically an equation that shows the exponential change of a dependent variable with respect to the independent variable. The allometric equation expressing a remarkable scaling symmetry is $y=\alpha+x^b$. It can also be expressed as:

$$\log y = \log \alpha + b \log x$$

which is again a simple regression between the logarithmic forms of the variables. In addition to the general allometric relation between females and males, we have also used it separately for the breed groups. The differences between the regression coefficients of the breed groups were tested using contrast in PROC GLM (SAS, 2002). A simple contrast (c) is the difference between two means

$$(H_0: \bar{x}_1 = \bar{x}_2;\ c_1 = 1,\ c_2 = -1)$$

Complex contrast can test differences between multiple means, between a single mean and combined means, or between combined means and other combined means. We know that contrast is essentially a difference between regression coefficients. It can be estimate sample contrast by using the means. We can test significance via an F-statistic calculated by dividing MS_c by MS_{error}. SS_c is also a mean square (MS) since all contrasts have 1 degree of freedom.

$$(SS_c = \frac{n \sum c_i \overline{x_i}}{\sum c_i^2})$$

For the regression, the mean for each value of the predictor is estimated as the corresponding point on the line, and the deviations from the line are used as the sum of the squared deviations. To test the difference of the slopes to 1 we created a dummy variable with b=1 and R^2=1. Then the contrast to this dummy variable was determined.

The measure of sexual size dimorphism, discussed at length in Lovich and Gibbons (1992), was measured for each trait as a simple male size divided by female size (Sexual Size Index, SSI). To see how SSI is related to trait size, we regressed SSI to female size, for overall and for the groups separately. The differences between the regression coefficients of the groups were tested using contrast in PROC GLM as described above.

Table 1. Origin of the data by breed

Breeds	Source
Rock Buhara Bombay Hungarian Giant Indian Fantail Indian Lotan Indigenous Koka Lahore Mookee Homer Turkish Clap Tumbler 1	Parés-Casanova, P. M., Kabir, A. (2020)
Turkish Clap Tumbler 2	Atasoy, F., Erdem, E., Hacan, Ö. G. (2013)
Scanderoon	Yıldırım, H., Doğan, U., Cımrın, T. (2018)
Crested Edremit Kelebek Non-Crested Edremit Kelebek	Erdem H, Konyalı, C, Savaş T., (2018)
Adana Dewlap	Özbaşer, F. T., Alaşahan, S., Narinç, D., Gündüz, Ö., Özkul, B. Y. (2018)
Bursa Roller	Balcı, F., Ardıçlı, S., Alpay, F., Dinçel, D., Soyudal, B., Mehlika, E. R. (2018)
Alabadem Roller	Erdem, E., Özbaşer, F. T., Gürcan, E. K., Soysal, M. İ. (2021)
Fleet Pigeons	Özbaşer, F. T., Atasoy, F., Erdem, E., Güngör, İ. (2016)
Turkish Clap tumbler 3	Özçelik, U. C. (2019)
Jalali	Bhowmik, N., M., M. M., Rahman, M. A., (2014)
White Galatz Roller Blue Bar Pied Galatz Roller	Ionescu, H., Oroian, T. E., Botha, M. (2015)
Turkish Donek	Özbaşer, F. T., Erdem, E., Gürcan, E. K., Soysal, M. İ. (2021)
Trace Roller	Soysal, M. İ., Gürcan, E. K., Alter, K., Akar, T., Genç, S. (2011)
Feral 1 (Blue Bar) Feral 2 (Checker) Feral 3 (Dark Checker)	Hetmański, T., Jarosiewicz, A. (2008)
Kendari	Harapin H, Napirah, A., Wanci, S., 2017
Hünkari (Old Fashioned Oriental Frill)	Turkish Official Journal, (2020). Breed description in Türkeş and Gündüz (2021)
Manisa Azman	Data collected by "Salihli Pigeon" (Serkan GÜNDÜZ) in Salihli/Manisa-Turkey for the registry report
Denizli Azman	Data collected by "Pigeon House Society" (İskender DAMGACI) in Denizli for the registry report
Halfbred Baska English Tippler	Unpublished data from the project "Studies on Side Effects of Traits Created or Conserved as a Result of Artificial Selection in Animals: Effects of Short Beak on Pigeon Biology"
Mısıri (Istanbul Owl)	Data collected by the "Committee of Mısıri, Turkish Pigeon Association" (Mehmet CEYLAN) in Istanbul for the registry report

Table 2. Breed by groups

Form	Frills and Owls	Tumbler and Rollers	Homer and Highflyer	Feral
Bombay	Denizli Azman	Alabadem Roller	Adana Dewlap	Feral 1 (Blue Bar)
Buhara	Hünkari (Old Fashioned Oriental Frill)	Blue Bar Pied Galatz Roller	English Tippler	Feral 2 (Checker)
Hungarian Giant	Halfbred Baska	Bursa Roller	Turkish Fleet	Feral 3 (Dark Checker)
Indian Fantail	Manisa Azman	Crested Edremit Kelebek	Homer	Rock
Kendari	Mısıri (Istanbul Owl)	Edremit Kelebek	Indian Indigenous	
Lahore		Indian Lotan	Jalali	
Scanderoon		Mookee	Kokah	
		Trace Roller		
		Turkish Clap tumbler 1		
		Turkish Clap tumbler 1		
		Turkish Clap tumbler 1		
		Turkish Donek		
		White Galatz Roller		

Results

The descriptive statistics of the traits by sex and breed groups are summarized in Table 3. On average across traits, male birds have higher values than female birds, both across breed groups and overall. However, this does not mean that in all breeds the female birds have lower average values. Not in all breed groups, but overall, across the traits, there are breeds where the female birds have higher values on average. Looking at the SSI in Table 3, while males are on average 8% heavier than females, other traits are between 2% to 8% higher in males on average. The average relative sexual size dimorphism varies also between breed groups. The largest body weight difference is observed in the form pigeons and the lowest in the feral birds. Body length could only be considered in the frills and owls and, tumbler and rollers breed groups, where in both groups the male birds are 2% larger than female birds on average. The males of the form, frills and owls, tumblers and rollers, homers and highflyer pigeons have 4%, 2%, 1% and 6% longer beaks than the female birds, respectively. A difference of 2% between the sexes in favor of the males can be observed for the wingspan, also only considered in two groups, frills, and owls as well as tumblers and rollers. While the form pigeons show no difference between the sexes in the length of the tail, it is 3% in favor of the male pigeons in the frills and owls as well as the tumblers and rollers. The cocks of the form pigeons have 11% longer tarsus than the hens. In addition, the tarsus of male birds is in the frills and owls 4%, and tumblers and rollers 3% longer. Figure 1 shows the allometric relationship of the male values to the female values in all traits. A clear deviation from the isometric relationship to positive allometry was observed in tail length ($P<0.0435$).

No deviation from isometric allometry can be observed in other traits ($P\geq0.1291$). The regression coefficients of SSI to logarithmic values of female birds can be shown in figure 2. No trends towards logarithmic female values according to the SSI could be identified ($P>0.05$). The results shown in Figure 2 clearly reject Rensch's rule for the pigeon breeds. Interesting results are presented in Table 4 for female-to-male allometry by trait and breed groups. Except for tail and tarsus length, there were no significant differences in the slopes between the breed groups for other traits. While isometry between body weights of the sexes can be observed in form pigeons as well as homer and highflyer pigeons, frills and owls showed negative allometry, feral pigeons positive allometry. In contrast, no relation between the sexes is observed in body weights of tumblers and rollers pigeons. In body length, where only two groups could form, show negative allometry between males and females in frill and owl birds, while the slope for the group of tumbler and roller birds are not different from 1. Significant but not significantly different slopes from 1 were observed for all groups in beak length. Another trait, the wingspan, for which only two groups could form, no trend can be observed in frills and owls.

However, the slope in tumblers and rollers birds shows a highly significant isometry. In tail length, a highly significant positive allometry was observed for the slope of female to male values in form pigeons, negative allometry in frills and owls, and an isometry in tumblers and rollers. The slope of female to male values in tarsus length shows also positive allometry in form pigeons. However, no trend can be observed in frills and owls. Furthermore, the female to male slope for tarsus length

Table 3. Descriptive statistics of the traits by sex and breed groups

Groups	$\bar{x}$ ± SD (min-max)	Live Weight (g)	Body Length (cm)	Beak Length (mm)	Wingspan (cm)	Tail Length (cm)	Tarsus Length (mm)
Form	Female	432.51±114.782 (310.0-680.0)	-	21.46±5.095 (17.0-31.2)	-	12.68±2.066 (9.9-16.4)	25.83±5.672 (22.0-29.0)
	Male	481.00±119.315 (330-690)	-	22.12±5.104 (16.0-31.9)	-	12.86±2.925 (8.7-17.4)	28.83±5.67 (22.0-39.0)
	SSI	1.12±0.113 (1.01-1.32)	-	1.04±0.097 (0.92-1.20)	-	1.00±0.083 (0.88-1.08)	1.11±0.125 (1.0-1.3)
	n	7	-	7	-	7	6
Frills and Owls	Female	288.15±29.137 (244.4-312.4)	30.84±0.898 (29.6-31.7)	12.83±2.400 (9.7-15.1)	60.31±1.14 (59.0-61.8)	11.30±0.458 (10.7-11.8)	26.54±0.491 (26.1-27.1)
	Male	306.14±21.61 (275.6-327.8)	31.59±0.376 (31.1-32.0)	13.09±2.514 (9.9-15.5)	61.76±0.386 (61.3-62.1)	11.65±0.201 (11.4-11.8)	27.65±0.694 (26.9-28.3)
	SSI	1.06±0.038 (1.00-1.03)	1.02±0.018 (1.01-1.05)	1.02±0.014 (1.00-1.06)	1.02±0.016 (1.01-1.04)	1.03±0.024 (1.00-1.06)	1.04±0.024 (1.02-1.06)
	n	5	4	5	4	4	3
Tumblers and Rollers	Female	319.46±22.198 (296.3-369.6)	33.29±2.821 (26.2-35.2)	18.82±4.034 (12.3-26.2)	64.39±2.953 (58.9-68.2)	12.51±1.448 (10.0-14.4)	24.24±3.564 (19.5-31.9)
	Male	343.68±31.612 (280.0-420.0)	33.98±2.817 (27.2-36.3)	18.9±4.002 (11.9-25.7)	65.46±3.395 (59.2-69.5)	12.85±1.569 (10.0-14.7)	24.81±3.213 (20.1-31.7)
	SSI	1.08±0.113 (0.90-1.40)	1.02±0.030 (0.94-1.05)	1.01±0.030 (0.95-1.06)	1.02±0.015 (0.98-1.04)	1.03±0.0216 (1.00-1.06)	1.03±0.058 (0.93-1.14)
	n	13	10	13	10	11	10
Homer and Highflyer	Female	384.17±125.457 (241.3-600.0)	-	19.37±1.119 (17.9-20.3)	-	-	-
	Male	409.88±112.041 (261.7-550.0)	-	20.50±2.304 (16.9-24.0)	-	-	-
	SSI	1.08±0.111 (0.92-1.29)	-	1.06±0.089 (0.95-1.20)	-	-	-
	n	7	-	6	-	-	-
Feral	Female	362.25±28.826 (320.0-385.0)	-	-	-	-	-
	Male	379.00±46.224 (310.0-407.0)	-	-	-	-	-
	SSI	1.04±0.051 (0.97-1.08)	-	-	-	-	-
	n	4	-	-	-	-	-
Overall	Female	354.43±89.224 (261.7-690.0)	33.22±3.043 (26.2-39.5)	18.60±4.437 (9.7-31.2)	63.87±3.405 (58.9-70.2)	11.97±1.806 (8.0-16.4)	24.77±2.997 (19.5-31.9)
	Male	381.96±92.048 (241.3-680.0)	33.99±3.079 (27.2-41.1)	19.07±4.617 (9.9-31.9)	65.16±3.685 (59.2-72.6)	12.44±1.877 (8.7-17.4)	26.59±4.154 (20.1-39.0)
	SSI	1.08±0.098 (0.90-1.40)	1.02±0.026 (0.94-1.06)	1.03±0.062 (0.92-1.20)	1.02±0.015 (0.98-1.04)	1.04±0.082 (0.88-1.31)	1.08±0.142 (0.93-1.60)
	n	36	17	32	17	26	22

Single-breed groups were excluded from the analyses. However, not in the analysis as overall. n: Number of Breeds in groups.

shows slightly negative allometry in tumblers and rollers. Table 5 shows the regression of female logarithmic values to SSI by traits and breed groups. No trend in the above values could be observed *(P*<0.05), except for form pigeons in tail and tarsus length. While the relationship between female logarithmic tail length in form pigeons is almost one-to-one, in tarsus length, the SSI value increases about twice than the logarithmic female value. Since the slopes of the frills and owls and tumblers and rollers do not deviate significantly from zero, the significant differences between the groups' slopes in the tail and tarsus length have practically no meaning.

Discussion

As expected, the form pigeons are the heaviest breed group on average (Table 3). Form pigeons are followed by homer and highflyer pigeons, feral pigeons, tumbler and roller pigeons, and frill and owl pigeons, in order. The biggest difference between hens and cocks in body size can also be seen in form pigeons (SSI=1.12). SSI ranges from 1.04 to 1.08 in other breed groups. Although few, female biased dimorphism is also found in the data collected from the pigeon literature. This female-biased dimorphism can be based on small sample sizes.

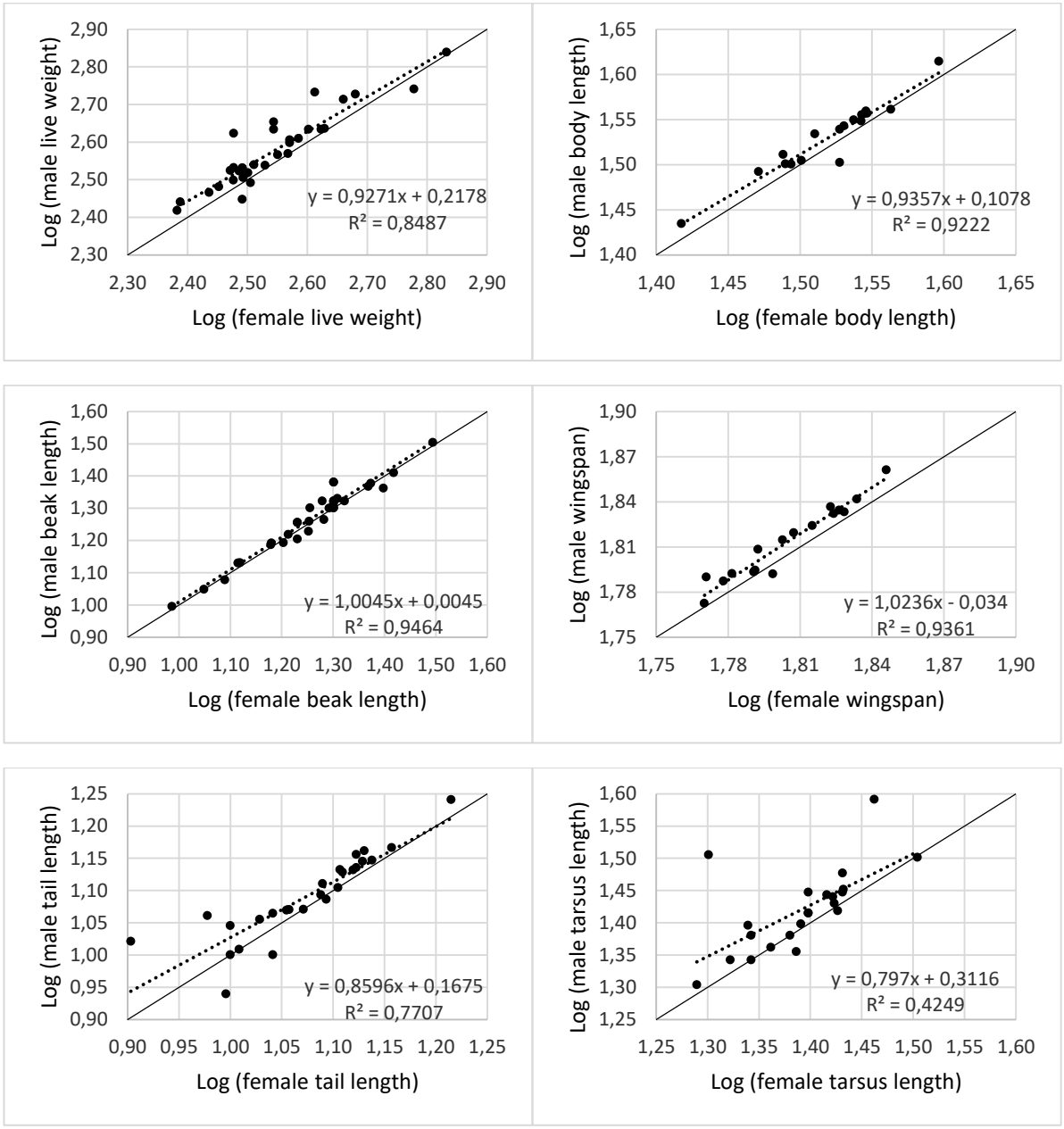

Figure 1. Allometric relations between male and female traits (all slopes were significant different from 0 by P<0.05; no significant differences were between the slopes of the traits (P>0.05). The straight line with a slope of 1.0 defines isometry, a size ratio of 1.0)

Therefore, the results must be viewed with caution. Fairbairn (1997) reported an SSI range between 3% and 128% in male-biased bird species, in which the smallest was in Passerines and the highest in Galliformes. In contrast to domesticated chicken breeds, sexual dimorphism is modest in the pigeon breeds in our study (Remeš and Szekely, 2010; Geibel et al., 2016). It is known that Galliformes are polygamous, but most of the Passerines are monogamous, also the Columbidae. Male sexual competition is expected to be fiercer in polygamous species than in monogamous species. This probably explains the low dimorphism in body size of the pigeons in contrast to the chickens.

It is no wonder that in pigeon breeds with larger cocks, hens are also larger (Figure 1). This does not change for other traits, and there are also no significant differences between the slopes of the traits. The slight differences in the slopes could probably be considered as measurement errors. Also, the distances between the slopes and b=1 of the traits are not significant, except for the tail length. This means that the characteristics of females and males of the breeds increase in a ratio of 1:1, i.e., there is an isometric relationship. However, when it comes to tail length, the ratio is hypoallometric (negative allometry).

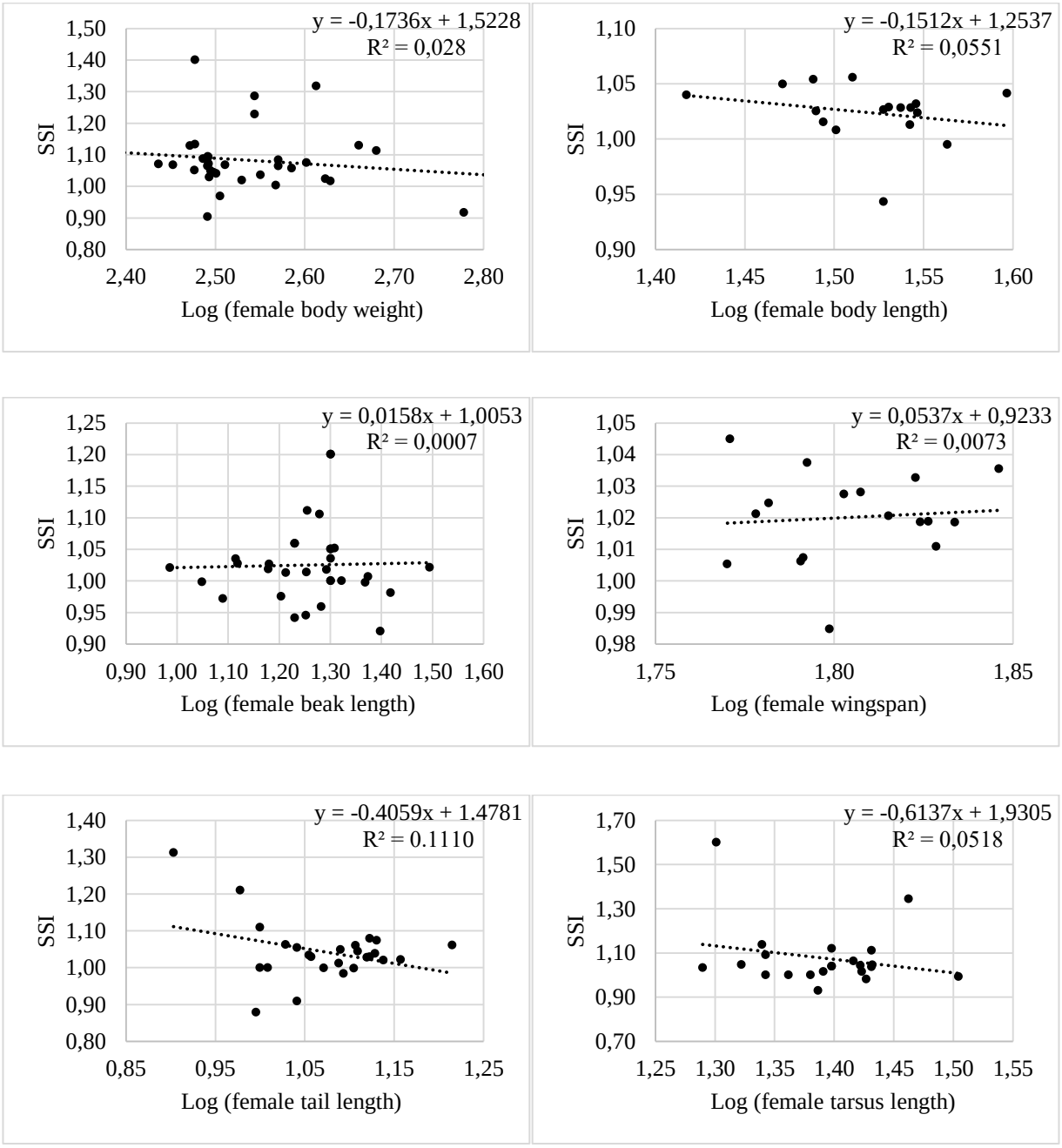

Figure 2. Relation between logarithmic female trait measures and sexual size dimorphism index (SSI) (P>0.05).

Parés-Casanova and Kabir (2020) also found hypoallometry in body mass, neck thickness, and wing length in pigeons. But the sample size is relatively small, the results should be viewed with caution. The authors further stated that the slopes in other morphological traits were not significantly different from 1. According to Sutter et al. (2008), the sex ratios of dog breeds do not differ in terms of withers height and body length. In contrast, Polak and Frynta (2010) reports that the significant positive allometric slopes for body mass in domestic cows change almost to isometry at the wither height. The results summarized in Figure 2 refute Rensch's rule for the pigeon breeds used in this study (Rensch, 1950).

Corroborate of Rensch's rule has been reported in domestic cattle as well as in domestic sheep and goats (Polak and Frynta, 2009, 2010). In studies conducted on dog breeds, on the other hand, no clear results can be seen with regard to Rensch's rule (Sutter, 2008; Frynta et al., 2012). In rare studies with domestic avian species, for example chicken, geese, and pigeons, Rensch's rule is usually refuted (Remeš and Szekely, 2010; Parés-Casanova, 2014; Parés-Casanova and Kabir, 2020). However, Remeš and Szekely (2010) found agreement with Rensch's rule in wild Galliformes. Perhaps a one-to-one comparison of wild gallinaceous birds and domestic breeds is not entirely correct. Ultimately, one deals with the variation between species while the other deals with

Table 4. Regression coefficients (b), their standard errors (SE), and *P* values between logarithmic male to logarithmic female values by traits and breed groups

Groups	Traits	Body Weight	Body Length	Beak Length	Wingspan	Tail Length	Tarsus Length
Form	b	0.86	-	0.92	-	1.44[a]	1.84[a]
	SE	0.117	-	0.130	-	0.092	0.263
	P[1]	<0.0001	-	<0.0001	-	<0.0001	0.0001
	P[2]	0.2697	-	0.5308	-	0.0007	0.0131
Frills and Owls	b	0.68	0.40	1.03	0.22	0.40[b]	0.62[ab]
	SE	0.038	0.030	0.026	0.124	0.072	0.856
	P[1]	<0.0001	0.0002	<0.0001	0.1558	0.0052	0.5438
	P[2]	0.0002	<0.0001	0.3725	0.0033	0.0011	0.7012
Tumblers and Rollers	b	0.34	0.91	1.00	1.09	1.06[b]	0.81[b]
	SE	0.280	0.077	0.028	0.078	0.038	0.088
	P[1]	0.2363	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P[2]	0.0277	0.2729	0.8724	0.2548	0.1618	0.0512
Homer and Highflyer	b	0.85	-	1.39	-	-	-
	SE	0.089	-	0.481	-	-	-
	P[1]	<0.0001	-	0.0209	-	-	-
	P[2]	0.1257	-	0.4516	-	-	-
Feral	b	1.56	-	-	-	-	-
	SE	0.111	-	-	-	-	-
	P[1]	<0.0001	-	-	-	-	-
	P[2]	0.0071	-	-	-	-	-

[1]H_0: b=0; [2]H_0: b=1 [ab] The regression coefficients of the breed groups according to traits denoted by different letters differ significantly

Table 5. Regression coefficients (b), their standard errors (SE), and *P* values between logarithmic female trait measures to sexual size dimorphism index (SSI)

Groups	Traits	Body Weight	Body Length	Beak Length	Wing-span	Tail Length	Tarsus Length
Form	b	-0.35	-	-0.21	-	0.99[a]	2.22[a]
	SE	0.374	-	0.285	-	0.190	0.739
	P	0.3623	-	0.4780	-	<0.0001	0.0101
Frills and Owls	b	-0.80	-1.42	0.06	-1.85	-1.43[b]	-0.92[ab]
	SE	1.08	1.216	0.396	0.996	1.055	6.006
	P	0.4675	0.2703	0.8834	0.0933	0.1947	0.8800
Tumbler and Rollers	b	-1.74	-0.21	-0.01	0.21	0.13[b]	-0.44[b]
	SE	0.976	0.224	0.201	0.235	0.194	0.369
	P	0.0859	0.3753	0.9541	0.3842	0.5119	0.2507
Homer, Highflyer and Wattle	b	-0.35	-	0.90	-	-	-
	SE	0.291	-	1.165	-	-	-
	P	0.2431	-	0.4502	-	-	-
Feral	b	1.32	-	-	-	-	-
	SE	1.586	-	-	-	-	-
	P	0.4137	-	-	-	-	-

[ab] The regression coefficients of the breed groups according to traits denoted by different letters differ significantly

the variation within a species. It is expected that the difference in biology between species belonging to the same order or family is much greater than that of intraspecific breeds.

The breed groups differ strongly in terms of breeding characteristics. This difference is reflected in the sex allometry between groups and traits partially (Table 4). But no significant differences were found between the slopes of breed groups within the traits. This is probably due to the relatively small sample sizes of the individual groups. On the other hand, the largest group tumblers and rollers show no trend in body weight. Possibly it is the result that in this group one breed has a very high dimorphism (SSI=1.40), whereas in another breed the female is larger (SSI=0.90). In the case of the feral pigeons, the dimorphism is not large (in one breed even the hen is larger than the cock), but a trend towards positive allometry (hyperallometric) can be observed. In this breed group, it seems that Rensch's rule applies. However, the relatively small sample does not allow a clear statement. As with body mass, body length shows negative allometry (hypoallometric) in frills and owls. So, in this group sexual dimorphism in relatively larger breeds decreases. This group includes the smallest breeds (Table 3). Smaller breeds are known to have reduced dimorphism or non (Sutter et al., 2008; Frynta et al., 2012). The hen and cock allometry in beak length does not deviate from 1 in all groups. While frills and owls have short beaks, the beaks of form pigeons appear to be enormously large (Table 3). However, a proportional consideration of beak length to body mass shows that the beak is slightly larger in tumbler and roller pigeons. The slope of wingspan in frills and owls no differ from 0. Therefore, the significant deviation of the slopes from 1 has no meaning. There is simply no connection to allometry. On the other hand, there is clear isometric allometry in the tumblers and rollers group. In the tail length of the three breed groups, the allometric relationships of the sexes behave differently. While the allometric relationship of the sexes is positive in the form pigeon breeds, it is negative in the frills and owls. The tumblers and rollers pigeons, on the other hand, show isometric allometry. What can be responsible for this? Although the difference between average tail lengths between breed groups is not large, the variation within breed groups differs. The form pigeons show the greatest variation, the smallest can be seen in the frills and owls. The variation in tail length in the tumblers and rollers pigeons is between the other two groups. It is questionable whether the positive allometry in the form pigeons can be interpreted as a confirmation of Rensch's rule. In some breeds, such as the Indian Fantail, there is targeted breeding for an impressive tail, which could lead to the lengthening of the tail, which is not the case with other breeds. Furthermore, probably because of the shape-oriented breeding, there are also relatively large female-biased breeds. Probably, from tail lengths of larger in females to larger in males led to the positive allometric slope. As with tail length, allometry from female to male for tarsus length is similar in form and tumblers and rollers. It seems possible to explain this situation in a similar way. On the other hand, female-to-male allometry has no meaning in frills and owls.

The regression coefficients of the logarithmic measures for female traits to SSI show no significance in all groups, except for tail and tarsus length in form pigeons (table 5). Since the regression coefficients of the other groups are not significant, a discussion of the significant differences in regression coefficients between the groups in tail and tarsal length is omitted. The significant slopes between log female values to SSI in the tail and tarsus length support the results presented in Table 4.

Conclusion

The results can be concluded in 3 articles. First, according to the overall analysis, there is sexual dimorphism between breeds and traits, mainly in favor of the male birds. Secondly, even if there seems to be a connection to Rensch's rule for some breed groups and characteristics, it is rejected in general consideration of the results. Third, in most cases, allometric relationships between breeds change, as do traits.

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