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Original Article

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Effect of Egg Weight and Patio Strategies on Hatching Traits and Subsequent Performance of the Broilers

ABSTRACT

Objectives of the present study were to assess the hatchery based interventions and their impact on hatching traits and subsequent performance of broilers. In total, 3000 eggs of Hubbard broiler breeders were equally divided into Small (50-55 g), Medium (56-61 g) and Large (62-70 g) eggs. The eggs from each category were distributed into 20 trays each containing 50 eggs. Each tray was considered as a replicate. At the end of the 18th day of incubation, the eggs of each weight category were divided into four different patio strategies i.e. PO, P3, P5 and P7 where feed and water were provided to the chicks for 0, 3, 5 and 7 days in the hatcher followed by conventional rearing. Data were collected for hatch window, hatching traits, growth, physical asymmetry, welfare and blood profile in broilers. Statistical analysis showed that the smaller eggs had significantly (P<0.05) shorter hatch window compared to larger and medium eggs. Hatching traits were significantly (p<0.05) better in medium eggs but the embryonic mortalities did not differ (p>0.05). The post-hatch performance was significantly (p < 0.05) better in P3 birds from medium eggs. Physical asymmetry and scores of feather and gait were not affected by both treatments (p>0.05). Blood profile was also significantly (p<0.05) better in P3 birds from medium-size eggs. It was recommended that the eggs of 56-61g should be selected for better hatchability and the chicks should be provided with feed and water within the hatcher for at-least three days for optimum performance on rearing facility.

INTRODUCTION

Poultry production is one of the major industries fulfilling the protein requirement all over the world presenting 35% of overall meat production (Statista, 2019). Among different segments of the poultry industry, broiler production is considered as an integral element of the agribusiness because the meat from broilers has a notable contribution in today's food consumption (Bell et al., 2002). The poultry business profitability is highly dependent on hatchery operations and on-farm management practices. Recent advances in broiler production have enabled the poultry producers to get the two kilograms live weight of the broilers within 35 days (Saxena & Kolluri, 2018). But, this situation has led to the deterioration of many welfare and health-related traits. These problems are related to not only higher growth rate but also parent flock history (Saxena & Kolluri, 2018). The on-farm practices have been so much optimized that there is very little or no choice to focus on other aspects of broilers' production. However, there is enough room to enhance the profitability by improving hatchery based operations and by facilitating the broilers with a better environment in early life. Egg weight has been reported to play a notable role in getting optimum



Effect of Egg Weight and Patio Strategies on Hatching Traits and Subsequent Performance of the Broilers

performance in terms of egg weight loss, embryonic mortality (Olutunmogun *et al.*, 2018), chick yield and body weight (Duman & Şekeroğlu, 2017).

Generally, chicks are taken out of the hatcher after 21 days (504 hours) of incubation and it comprises of hatch window around 36-48 hours (Careghi et al., 2005). Many factors affect the hatch window but most important included age of parent stock, egg storage time and incubation conditions (Decuypere et al., 2001). So, the chicks that hatch in early hours of hatch window face high stocking density, variations in conditions due to multiple opening and closing of hatcher machine and dehydration which ultimately resulted in low-quality chicks (Bamelis et al., 2005). Previous studies (Gonzales et al., 2003) showed that late collection of chicks from the hatcher resulted in a bodyweight loss of around 2.6 % and 2.3 % in males and female respectively as the absence of feed and dehydration during their initial hours caused in lowering down of their growth rate. This happened because yolk sac feeding is not sufficient and chicks need exogenous nutrients to maintain their high growth rate. Similarly, Tong et al. (2015) estimated that 70 % of chicks must have to wait around 20-35 hours in the hatcher before hatch pull. Moreover, Bergoug et al. (2015) reported a negative impact of late hatching on early growth describing that the late hatchers grew less as compared to early hatchers.

To avoid these risks, Patio hatchery system is a new trend in the commercial poultry industry. In it, hatching chicks are brooded in the hatchery for a week (Van de Ven *et al.*, 2009), and this system reported better results in terms of body weight, chick quality and decreasing mortality rate in broilers (Van de Ven *et al.*, 2009). Keeping in mind the adverse effects of prolonged hatch window and recent trends to tackle this problem, this study was planned to check the effect of eggs of different weight category and different patio time on hatching traits, subsequent growth performance, blood metabolites and welfare aspects of birds.

MATERIALS AND METHODS

Study site and ethical statement

The experiment was conducted at Avian Research & Training Centre, Department of Poultry Production, University of Veterinary & Animal Sciences, Lahore, Pakistan (UVAS). The study was approved from by the Office of the Research and Innovation, UVAS, Lahore, Pakistan.

Experimental design

In total, 3000 eggs of Hubbard broiler breeders were procured from a commercial renowned breeder company and were divided into three weight categories i.e., Small (50-55 grams), Medium (56-61 grams) and Large (62-70 grams). The eggs from each category were distributed into 20 trays each having 50 eggs. Each tray was considered as a replicate. The egg trays were subsequently placed in the setter portion of the hatching machine (Victoria Incubator made in Italy) for 18 days. On the 18th day, the eggs were candled and cleared eggs were removed and the eggs with embryonic growth were sent to the hatcher section. In the hatcher, the eggs of each weight category were divided into four different patio strategies that were designed for the chicks. Each patio strategy had five trays from each egg weight category. These patio strategies were:

Control = conventional hatching, no feed and water provision in the hatcher, transfer of the chicks to rearing shed at the end of the 21st day of incubation.

P3 = provision of feed and water to the chicks for three days in the hatcher (18 to 21st day of incubation, the temperature in hatcher 36.5°C), then transfer to the rearing shed.

P5 = provision of feed and water to the chicks for five days in the hatcher (18 to 23^{rd} day of incubation, the temperature in the hatcher 36.5°C from 19 to 21^{st} day, 36°C at 22^{rd} , 35.5°C at 23^{rd} day), then transfer to the rearing shed.

P7 = provision of feed and water to the chicks for seven days in the hatcher (18 to 25^{th} day of incubation, the temperature in the hatcher was 36.5° C from 19 to 21^{st} day, 36° C at 22^{nd} , 35.5° C at 23^{rd} day, 35 C at 24^{th} and 34.5 at 25^{th} day), then transfer to the rearing shed.

Post-hatch management of the birds

Chicks obtained in each patio strategy from each egg weight category were sent to the rearing facility after they completed the assigned duration in the hatcher. The rearing shed was furnished with replication units made of stainless steel wire. The chicks were kept according to 3×4 factorial arrangement and each experimental unit had separate manual feeders. The water was provided with nipple drinking systems. The initial brooding temperature was maintained at 33°C until the 10th day of the birds' age. Gradually, the temperature of the house was reduced on a daily basis to 26°C with the advancement of the age. Broiler starter (day 1 to day 10), grower (day 11 to 22) and



finisher (23 to 35 days of age) diets were offered to the birds *ad-libitum* (Table 1). Vaccination of the birds

was performed following the recommendations of the Pakistan Poultry Association (Table 2).

 Table 1 – Ingredient composition of rations for experimental birds.

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Ingredients	Starter	Grower	Finisher	Premix	Unit /kg	Starter	Grower	Finishe
Maize	61.03	63.29	63.99	А	IU	11000	10000	9000
Soybean Meal 46%	36	33.72	30.92	D3	IU	5000	4500	3600
Soybean oil		0.32	2.68	E	mg	25	20	20
Lime stone	1.06	0.96	0.84	К	mg	3.2	3.0	2.2
MCP	0.36	0.22	0.1	B1	mg	3.2	3.0	2.2
Salt	0.3	0.3	0.3	B2	mg	8.0	6.5	5.4
DLM	0.29	0.27	0.24	Pyridoxin	mg	4	3	2
L-Lysine Hcl	0.23	0.24	0.24	B12	mg	0.016	0.011	0.011
L-therionine	0.09	0.08	0.08	Folic acid	mg	1.4	1.4	1.4
Sodabicarconate	0.15	0.15	0.15	Pantothenate	mg	12	10	8
Choline Chloride 60%	0.07	0.03	0.04	Niacin	mg	55	50	45
Phytase	0.02	0.02	0.02	Biotin	mg	0.20	0.16	0.12
Premix	0.4	0.4	0.4	Copper	mg	18	18	18
Crude Protein	22.09	21.2	20	Ferous	mg	50	50	50
Metabolizable Energy (Kcal/Kg)	2872	2930	3090	Manganese	mg	120	120	120
				Zinc	mg	100	100	100
				lodine	mg	1.1	1.1	1.1
				Selenium	mg	0.3	0.3	0.3

Table 2 – Vaccination s	chedule of e	xperimental birds.
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Day	Vaccine	Rout of Administration
0 (at hatchery)	ND + IB	Sub cut
7	ND	Eye Drop
13	IBD	Drinking Water
18	ND	Drinking Water

PARAMETERS EVALUATED

Hatch window and hatching traits

Hatch window was calculated in hours by noting the complete hatch time of each treatment. For egg weight/moisture loss, eggs were weighed on the 1st and 18th day of incubation and difference was termed as egg weight/moisture loss. The break-out analysis was done after the completion of the hatch window, and dead germs, dead in shells, total chicks obtained, culled chicks, and chicks with no defects were counted. Their results were presented in percentage of total eggs sett. Fertility % was derived by dividing the total fertile eggs (sum of total chicks, dead germs and dead in shells) with the total eggs sett. Hatch of fertile was calculated by dividing the total hatched chicks by total fertile eggs. Chick weight of each treatment was calculated by measuring 10% of the sample using a weighing scale which can measure up to 0.01 gm.

Post-hatch growth performance

In growth performance, daily feed intake was calculated by using the following formula.

Feed intake (g) = Feed offer (g) – Feed refusal (g)

Body weight of the birds was calculated on a weekly basis by using a weighing scale (Company name) which can weigh up to 0.5 g.

Body weight gain (g) = Finale weight (g) – initial weight (g)

The feed conversion ratio was calculated on a weekly basis till the market day (35th day) of bird, by using the following formula.

$$FCR = \frac{Feed consumed (g)}{Body weight (g)}$$

Livability was noted on the 35th day by the following formula.

Livability % =
$$\frac{\text{no. of birds placed}}{\text{no. of live birds}} \times 100$$

Welfare Traits

Welfare parameters were assessed on 15 birds (35 days old) from each replicate and an average value was used for statistical analysis. Feather scores were assigned from 0-2 (0= no feather loss: 1= moderate feather loss: 2= severe feather loss) following the Welfare Poultry Protocol of the Netherland. For gait scoring, walking the birds for five feet was assessed for any signs of lameness and score was assigned from 0 to 2 where 0= perfect, 1= obvious signs and 2= severe signs of lameness.

Physical asymmetry

Physical asymmetry of 05 birds per replicate was measured as per Archer *et al.* (2009) on the 35th day



Effect of Egg Weight and Patio Strategies on Hatching Traits and Subsequent Performance of the Broilers

of age. For this purpose, metatarsal length, width and middle toe length (mm) were measured of both left (L) and right (R) legs, by using Vernier Caliper. The composite asymmetry score was calculated by taking the sum of the absolute value of the left minus the right of each trait, then dividing by the total number of traits.

Composite asymmetry score = $\frac{\{L-R(mt)\} + \{L-R(mt)\} + \{L-R(mt)\}$

Where, mtl = Middle toe length (mm) ml = Metatarsal length (mm) mw = Metatarsal width (mm) No. of traits = 3

Blood biochemical profile

On the 35th day of age, blood samples of 2ml each were collected from four birds per replicate to study blood metabolites. These samples were sent to the University Diagnostic Laboratory, UVAS, Lahore, Pakistan where the samples were analyzed using commercially available specific kits for, glucose (mmol/L), total protein (g/dL), albumin (g/dL), globulin (g/dL), uric acid(mg/dL), cholesterol (mg/dL) and creatinine (mg/dL).

Statistical Analysis

Data for hatch window and hatching traits were analyzed through one-way ANOVA using SAS software and significant treatment means were separated through Least Significant difference test by assigning the egg weight categories as a source of treatment. Post-hatch growth performance, livability, welfare traits, physical asymmetry and blood biochemical profile were analyzed through factorial ANOVA (twoway analysis of variance) by assigning egg weight categories and patio strategies as the main effect and significant means were compared through Duncan's Multiple Range test (Duncan, 1955).

RESULTS

Hatch window

Figure 1 describes the overall hatching pattern for small, medium and large-sized eggs under study. It is clear from the figure that the small-sized eggs hatched more at 471 hours (p<0.05) compared to medium-sized eggs and took significantly less time for hatch completion. Maximum hatchability was noted at 491 hours post-placement in setter in small eggs compared to larger eggs (p<0.05). On an overall basis, medium

and larger sized eggs took significantly more time than small-sized eggs as shown in figure 1, which shows that the chicks from medium and large-sized eggs were yet to come even after 504 hours (p<0.05).

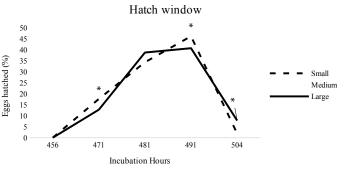


Figure 1 – Hatch window of different egg weight categories in broiler breeder. *Shows the significant differences among small, medium and larger size eggs.

Hatching traits

Table 3 shows the mean values of egg weight loss and hatching traits of broiler breeder's eggs of different weight categories. Statistical analysis showed a significant impact of egg weight on egg weight loss and most of the hatching traits (p < 0.05) except embryonic mortalities. Medium-sized eggs encountered with a significantly higher loss (10.16±0.21) compared to large-sized eggs (9.10±0.16). However, the weight loss in small eggs (9.70±0.37) was not significantly different from medium and large eggs (p>0.05). Hatchability % was significantly higher in medium-sized eggs (89.60±1.27) compared to large eggs (81.70±2.35) whereas the small eggs' hatchability (86.10 ± 1.57) was comparable to both the medium and large size eggs. A similar trend was observed for fertility results where medium-sized eggs were more fertile (97.70±0.68) than larger eggs (94.00±0.95) and small-sized eggs (96.20±0.74) were non-significantly different from medium and large-sized eggs. On the other hand, the hatch of fertile was similar among all three egg weight categories (p>0.05). Moreover, embryonic mortality patterns (early, mid and late mortalities) were not different among the experimental egg weight groups. Culled chicks were significantly higher in larger eggs (2.08±0.88) than medium-sized eggs (0.00±0.00), however, smaller sized eggs produced a comparable number of culled chicks (0.61±0.31) to that of medium and large size eggs. The number of chicks with no defects was significantly higher in mediumsized eggs (100.00±0.00) compared to large eggs (97.92±0.88) while small eggs produced a comparable number of defect-less chicks (99.39±0.31) to that of medium and larger eggs. There was a significant effect of egg weight on the weight of the chicks (p < 0.05).



Larger sized eggs produced significantly heavier chicks (44.27 ± 0.40) than medium-sized eggs (41.78 ± 0.28)

and the lowest chick weight was noted in small-sized eggs (38.40±0.28).

Table 3 – Hatching traits as affected by different egg weights.

E. Wt.	E.Wt. loss	Hatch	HOF	Fertility	EEM	MEM	LEM	Cull	No defect	Chick Wt
					(%)					(g)
Small	9.70 ± 0.37^{ab}	86.10 ± 1.57^{ab}	89.40±1.10	96.20 ± 0.74^{ab}	4.00±0.50	2.50±0.54	4.00±0.71	0.61 ± 0.31^{ab}	99.39±0.31 ^{ab}	38.40±0.28 ^c
Medium	10.16±0.21ª	89.60±1.27ª	91.68±0.98	97.70±0.68ª	4.00±0.58	2.50±0.81	4.50±1.18	0.00 ± 0.00^{b}	100.00±0.00 ^a	41.78±0.28 ^b
Large	9.10 ± 0.16^{b}	81.70±2.35 ^b	86.81±2.08	94.00 ± 0.95^{b}	3.40±0.54	2.50±0.79	5.50±1.00	2.08±0.88ª	97.92±0.88 ^b	44.27 ± 0.40^{a}
<i>p</i> -value	0.0217	0.0108	0.0731	0.0070	0.6681	1.000	0.5504	0.0241	0.0241	<0.0001

E.Wt is egg weight; Hatch is hatchability; HOF is hatch of fertile; EEM is early embryonic mortality; MEM is mid embryonic mortality; LEM is late embryonic mortality; Chick Wt is chick weight.

Post-hatch growth performance

Statistical analysis showed that the feed intake of the broilers (Table 4) was significantly affected by egg size (p<0.05). The birds from small eggs consumed significantly less feed (3337.81±22.81) than those from medium (3475.88±45.23) and large eggs (3498.17±35.37). Patio strategies significantly affected the feed intake of the birds. Minimum feed intake (3354.17±26.40) was noted when the birds were conventional hatching practice i.e control (P0) whereas maximum feed consumption (3528.44±54.50) was recorded when the birds provided feed and water for five days inside the hatcher (P5). Concerning interactions, the lowest feed intake was found in the birds from the smaller eggs reared under P0 (3341.29 ± 26.95) while the highest feed intake was noted in the birds from the medium-sized eggs subjected to P5 (3662.33 ± 111.20).

Broilers' body weight gain was also affected significantly (p<0.05) by the egg weight (Table 4). The birds from medium and larger size eggs gained significantly higher weight (2139.99±31.66) than the birds from the small-size eggs (2024.33±15.87). Patio strategies significantly affected the body weight gain of the birds. The birds under P3 had significantly higher

Table 4 – Growth performance and livability of broilers from three different egg weight categories reared under different patio strategies.

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	F.I	B.W.G	FCR	Livability
Egg Wt				
Small	3337.81±22.81 ^b	2024.33±15.87 ^b	1.65±0.01 ^b	92.51±1.00
Medium	3475.88±45.23ª	2139.99±31.66ª	1.63±0.01 ^c	92.68±0.94
Large	3498.17±35.37ª	2090.27±27.63ª	1.68±0.01ª	93.60±0.66
<i>p</i> -value	0.0016	0.0021	<.0001	0.1547
Patio strategy				
PO	3354.17±26.40 ^b	2004.87±16.31 ^b	1.67±0.01ª	90.92±0.49°
РЗ	3460.98 ± 48.26^{ab}	2144.60±34.20 ^a	1.61±0.01 ^c	94.81±0.51 ^b
Р5	3528.44±54.50°	2144.30±37.06 ^a	1.65±0.01 ^b	96.34±0.34ª
Р7	3405.57±33.93 ^b	2045.68±20.23 ^b	1.66±0.01 ^{ab}	89.64±1.23°
p-value	0.0113	0.0002	<.0001	<.0001
Interaction				
Small×P0	3341.29±26.95 ^d	1994.07±22.21°	1.68±0.01 ^{abc}	91.52±0.99 ^{cd}
Small×P3	3294.16±20.46 ^d	2034.96±28.19°	1.62±0.02 ^{de}	94.88 ± 0.83^{ab}
Small×P5	3357.95±67.57 ^{cd}	2040.63±47.29°	1.65±0.01 ^{bcd}	97.07±0.50ª
Small×P7	3357.86±59.13 ^{cd}	2027.66±30.52°	1.66±0.01 ^{bcd}	86.57±1.19 ^e
Medium×P0	3311.40±28.17 ^d	2023.19±19.12 ^c	1.64±0.01 ^{cd}	91.70±0.73 ^{cd}
Medium×P3	3492.65±50.12 ^{abcd}	2202.46±50.36 ^{ab}	1.59±0.02 ^e	95.88±0.77 ^{ab}
Medium×P5	3662.33±111.20 ^a	2255.57±67.89 ^a	1.62±0.00 ^{ed}	96.16±0.42 ^{ab}
Medium×P7	3437.15±85.47 ^{bcd}	2078.72±53.58 ^{bc}	1.65±0.01 ^{bcd}	86.99±1.23 ^e
Large×P0	3409.82±67.25 ^{bcd}	1997.35±42.51°	1.71±0.01 ^a	89.54±0.48 ^d
Large×P3	3596.13±98.73 ^{ab}	2196.38±66.61 ^{ab}	1.64±0.02 ^{cd}	93.69±0.91 ^{bc}
Large×P5	3565.03±46.57 ^{abc}	2136.69±42.17 ^{abc}	1.67±0.03 ^{abc}	95.80±0.76 ^{ab}
Large×P7	3421.70±20.13 ^{bcd}	2030.67±9.26 ^c	1.69±0.01 ^{ab}	95.35±0.80 ^{ab}
p-value	0.0014	0.0002	<.0001	<.0001

FI is feed intake; BWG is body weight gain; FCR is feed conversion ratio; P0 is patio feeding for 0 day; P3 is patio feeding for 3 days; P5 is patio feeding for 5 days; P7 is patio feeding for 7 days.



weight gain (2144.60 \pm 34.20) whereas the birds under P0 showed the lowest gain in weight (2004.87 \pm 16.31). Significant interactions among egg weight and patio strategies were also noted for body weight gain. When the broiler chicks from the medium-sized eggs were reared under P5 they presented the highest weight gain (2255.57 \pm 67.89) while the birds from the smallsized eggs reared under conventional hatching had the lowest weight gain (1994.07 \pm 22.21).

When FCR was compared, significant variations (p<0.05) were noted among the birds from three egg weight categories (Table 4). Overall FCR was better in the broilers from medium-sized eggs (1.63 ± 0.01) followed by those from smaller eggs (1.65±0.01) and the poor FCR was recorded for those from the larger eggs (1.68±0.01). Patio strategy significantly impacted the FCR of the birds (p < 0.05). FCR of the birds under P3 was better (1.61±0.01) followed by those under P5 (1.65±0.01) and the poor FCR (1.67±0.01) was noted in the birds under conventional hatching system i.e. PO. Regarding interaction, the birds from medium-size eggs reared under P3 showed better FCR (1.59±0.02) whereas the birds from the larger eggs under PO showed poor FCR (1.71±0.01) among all the possible interactions.

Livability

Egg weight did not exert any impact on livability percentage (p>0.05) of the birds. However, patio strategies resulted in significant differences in the livability of the birds (Table 4). The birds under P5 survived more (96.34±0.34%) followed by P3 and the least survival rate was recorded in the birds under P7 (89.64±1.23). Livability differed significantly when the interactions among the egg weight categories and patio strategies were evaluated. The broilers from the smaller eggs reared under P5 showed the highest livability (97.07±0.50) whereas the birds from small egg size under P7 had the lowest survival rate (86.57±1.19).

Physical asymmetry and welfare traits

Statistical analysis showed no effect (p>0.05) of egg weight and patio strategies on physical asymmetry and gait scores of the broilers (Table 5). However, feather score significantly differed among the broilers from different egg weight categories (p<0.05). The birds from the larger eggs had significantly cleaner feathers (lower feather score, 0.18±0.00) than those from the medium-sized eggs which had dirty feathers and showed poor feather score (0.47±0.07). Patio strategies did not affect the feather score (p>0.05). However, egg weight significantly interacted with patio strategies (p<0.05). The chicks from small and large size eggs, when provided with feed and water for seven days within the hatcher (P7), had cleaner feathers with adequate feather cover than any other treatment both showing the lowest feather scores (0.00±0.00). On the other hand, the birds from the medium-sized eggs under conventional hatching and rearing process (P0) showed a poor feather score (0.80±0.14).

Table 5 – Effect of egg weight and patio strategies onphysical asymmetry and welfare traits of broilers.

	Physical asymmetry	Gait Score	Feather Score
Egg weight			
Small	0.42±0.29	0.42±0.07	0.37 ± 0.07^{ab}
Medium	0.26±0.28	0.35±0.06	0.47±0.07ª
Large	-0.34±0.34	0.33±0.07	0.18 ± 0.0^{b}
<i>p</i> -value	0.1793	0.6429	0.0049
Patio strategies			
PO	0.17±0.41	0.36±0.07	0.31±0.08
Р3	0.12±0.32	0.47±0.09	0.40±0.08
Р5	-0.13±0.34	0.29±0.07	0.40±0.08
P7	0.28±0.34	0.36±0.07	0.24±0.06
<i>p</i> -value	0.8634	0.4301	0.3337
Interaction			
Small×P0	0.68±0.73	0.40±0.13	0.53 ± 0.17^{ab}
Small×P3	0.59 ± 0.59	0.40±0.13	0.40 ± 0.13^{b}
Small×P5	-0.76±0.78	0.27±0.12	0.00±0.00 ^c
Small×P7	-0.27±0.40	0.47±0.17	0.20 ± 0.11^{bc}
Medium×P0	0.49±0.60	0.40±0.13	0.80±0.14ª
Medium×P3	0.15±0.65	0.53±0.17	0.20±0.11 ^c
Medium×P5	0.73±0.53	0.33±0.13	0.33±0.13 ^{bc}
Medium×P7	0.25±0.61	0.27±0.12	0.33±0.13 ^{bc}
Large×P0	-1.37±0.53	0.27±0.12	0.53 ± 0.17^{ab}
Large×P3	0.52±0.60	0.47±0.13	0.40 ± 0.13^{b}
Large×P5	-0.29±0.40	0.33±0.13	0.33±0.13 ^{bc}
Large×P7	0.63±0.72	0.27±0.12	0.00±0.00 ^c
<i>p</i> -value	0.1880	0.9483	0.0013

Different superscript within a column show significant differences.

Blood biochemical profile

Table 4 shows the mean values of glucose, total protein, albumin, globulin, uric acid, cholesterol and creatinine. Statistical analysis showed significant (p<0.05) impact of egg weight and patio strategies on the blood glucose levels of broilers (Table 6). Higher glucose contents were noted in the birds from large eggs (13.63±0.52) while the lower glucose was observed in the blood of the birds from the medium eggs (11.08±0.48). Among the patio treatments, significantly higher glucose level was observed in the birds from P7 group (14.95±0.60) that was non-significantly different from P5 and the lower glucose was noted in P0 (10.28±0.40) that was non-significant to P3. A significant interaction was noted among the



Effect of Egg Weight and Patio Strategies on Hatching Traits and Subsequent Performance of the Broilers

egg weight and patio strategies (p<0.05). Highest glucose in broilers (16.05±1.02) was recorded when the chicks from the large eggs were offered feed and water inside the hatcher for seven days (P7). While small × P3 treatment presented the lowest glucose contents (8.30±0.54) than any other experimental group.

There was no effect of egg weight categories and patio strategies, nor was any interaction on total protein contents in the blood of the broilers (p>0.05). There was a significant influence (p < 0.05) of egg weight and patio strategies on albumin contents of the broilers. Significantly higher albumin was found in the broilers from large eggs (1.45±0.03) compared to those from medium eggs (1.36 ± 0.03) . Among the feeding strategies, providing the chicks with feed and water inside the hatcher for seven days (P7), resulted in significantly higher blood albumin contents (1.49±0.04) whereas the birds from conventional routine practice (PO) were lowest in this parameter (1.27±0.03). Regarding interaction, significantly highest albumin contents (1.64±0.04) were noted in the birds from larger eggs (p < 0.05) and PO group and the lowest glucose (1.20±0.06) was recorded when the chicks from the small eggs were kept in the

hatcher for three days (P3). Regarding globulin, egg weight and patio strategies resulted in non-significant differences in blood globulin contents of the broilers. However, there was significant interaction (p < 0.05) showing the highest contents of globulin (2.08±0.17) in the broilers from medium eggs and P7 patio strategy and the lowest (1.31±0.13) were found in the bids from large eggs and P7 group. There was a significant effect of egg size and patio strategies on blood uric acid. Among the egg categories, the birds from the larger eggs had significantly (p < 0.05) higher uric acid contents (3.52±0.09) in their blood compared to those from the smaller eggs (3.23±0.08). In terms of patio strategies, P7 group presented the highest contents of uric acid (4.05±0.06) while the birds from P0 had the lowest in uric acid contents (2.44±0.06). As far as interaction is concerned, significant differences (p<0.05) were observed among the treatment group. The chicks from large eggs under P5 regime had significantly highest uric acid contents (4.27±0.10) at 35 days of age while those from the smaller eggs under PO presented the lowest contents of uric acid (2.53±0.10) in their blood. There were significant differences in blood cholesterol level of the broilers from different egg weight categories (p < 0.05). Highest

Table 6 – Effect of egg weight and patio strategies on blood constituents of broilers.

	Glucose mmol/L	Total protein g/dL	Albumin g/dL	Globulin g/dL	Uric acid mg/dL	Cholesterol mg/dL	Creatinine mg/dL
Egg weight							
Small	13.34±0.42 ^a	3.02±0.06	1.43±0.03 ^{ab}	1.59±0.07	3.23±0.08 ^b	124.08±2.14 ^b	0.61±0.02
Medium	11.08±0.48 ^b	3.14±0.07	1.36±0.03 ^b	1.79±0.08	3.33±0.09 ^b	130.03±1.69ª	0.66±0.02
Large	13.63±0.52ª	3.03±0.06	1.45±0.03ª	1.57±0.07	3.52±0.09 ^a	130.91±1.82ª	0.64±0.03
<i>p</i> -value	<0.0001	0.3168	0.0402	0.0657	0.0005	0.0014	0.1637
Patio strategies							
PO	10.28±0.40 ^b	3.00±0.07	1.27±0.03 ^b	1.73±0.08	2.44±0.06 ^d	115.68±1.81°	0.48±0.02 ^c
РЗ	11.65±0.49 ^b	3.07±0.07	1.44±0.04 ^a	1.63±0.08	3.17±0.07℃	121.63±1.79°	0.65±0.03 ^b
P5	13.83±0.54ª	3.10±0.08	1.45±0.04 ^a	1.65±0.10	3.76±0.07 ^b	133.58±1.65 ^b	0.68±0.03 ^{ab}
Р7	14.95±0.60 ^a	3.09±0.07	1.49±0.04 ^a	1.60±0.08	4.05±0.06 ^a	142.45±1.81ª	0.73±0.02ª
<i>p</i> -value	<0.0001	0.7445	<0.0001	0.7186	<0.0001	<0.0001	<0.0001
Interaction							
Small×P0	11.45±0.61 ^{def}	3.02±0.11	1.35±0.04 ^{cd}	1.67±0.13 ^{ab}	2.53±0.10 ^f	107.45±2.85 ⁹	0.50±0.03 ^e
Small×P3	8.30±0.54 ⁹	2.86±0.13	1.20±0.06 ^d	1.66±0.16 ^{ab}	2.31±0.09 ^f	120.15±3.00 ^{ef}	0.60±0.03 ^{cd}
Small×P5	11.10±0.73 ^{ef}	3.11±0.15	1.25±0.06 ^{cd}	1.86±0.15 ^{ab}	2.49±0.10 ^f	119.45±2.85 ^{ef}	0.34±0.04 ^f
Small×P7	11.85±0.55 ^{cdef}	3.03±0.15	1.42±0.05 ^{bc}	1.60±0.15 ^{ab}	2.87±0.10 ^e	113.05±2.39 ^{fg}	0.63±0.05 ^{bc}
Medium×P0	9.70±0.78 ^{fg}	3.21±0.11	1.62±0.06 ^a	1.59±0.12 ^{ab}	3.11±0.09 ^e	122.05±2.39 ^{de}	0.56±0.05de
Medium×P3	13.40±0.98 ^{abcde}	2.99±0.09	1.29±0.06 ^{cd}	1.70±0.13 ^{ab}	3.55±0.10 ^d	129.80±3.33 ^{cd}	0.77±0.03ª
Medium×P5	14.25±0.83 ^{abc}	2.86±0.10	1.43±0.06 ^{bc}	1.44±0.13 ^{ab}	3.71±0.13 ^{cd}	134.65±3.11 ^{bc}	0.58±0.03de
Medium×P7	13.30±0.88 ^{bcde}	3.39±0.16	1.30±0.05 ^{cd}	2.08±0.17 ^a	3.63±0.10 ^d	138.75±2.61 ^{ab}	0.78±0.05ª
Large×P0	13.95±1.13 ^{abcd}	3.06±0.15	1.63±0.06 ^a	1.43±0.17 ^{ab}	3.96±0.12 ^{bc}	127.35±2.34 ^{cde}	0.70 ± 0.04^{ab}
Large×P3	15.80±0.96 ^{ab}	3.19±0.12	1.53±0.07 ^{ab}	1.66±0.13 ^{ab}	3.81±0.11 ^{bcd}	141.15±3.19 ^{ab}	0.74±0.03 ^{ab}
Large×P5	13.00±1.07 ^{cde}	3.13±0.13	1.30±0.06 ^{cd}	1.82±0.16 ^{ab}	4.27±0.10 ^a	139.15±3.02 ^{bc}	0.71 ± 0.02^{ab}
Large×P7	16.05±1.02ª	2.95±0.13	1.64±0.04 ^a	1.31±0.13 ^b	4.08±0.09 ^{ab}	147.05±3.04ª	0.75±0.04 ^{ab}
p-value	<.0001	0.0731	<0.0001	0.0141	0.0008	<0.0001	< 0.0001

Different superscripts on within a column show significant differences.



cholesterol levels (130.91±1.82) was noted in the birds from large eggs while the birds from the small eggs had the lowest cholesterol levels (124.08±2.14). Significant effect of patio strategies was also evident on blood cholesterol showing higher value in the birds from P7 group (142.45±1.81) followed by P5 (133.58±1.65), P3 (121.63±1.79) and the least value was recorded in PO (115.68±1.81). Concerning the interaction among egg weight categories and patio strategies, a significantly higher level of cholesterol (147.05±3.04) was observed in large \times P7 while the birds under Small \times P0 group showed the lowest cholesterol contents (107.45 ± 2.85) in their blood compared to the other treatment groups. Statistical analysis revealed no impact of egg weight on creatinine contents of the experimental birds (p>0.05). The patio strategies resulted in significant differences (p<0.05) among the treatments for blood creatinine of broilers. P7 strategy resulted in the highest creatinine (0.73±0.02) while conventional PO group had the lowest (0.48±0.02) creatinine level. There were significant interactions among the egg weight and patio strategies for creatinine contents. The chicks from the medium eggs under P7 strategy had the highest level of creatinine in their blood (0.78±0.05) while the least contents of creatinine (0.34±0.04) were observed in small \times P5 treatment.

DISCUSSION

Hatch window

Hatch window is an important criterion to assess the efficiency of the hatching process and to minimize the losses caused by post-hatch delayed feeding (Paul, 2015). In the current study, it was found that the small-sized eggs hatched more at 471 hrs and took significantly less time for hatch completion compared to medium-sized eggs. Maximum hatchability was noted from small-sized eggs at 491 hours postplacement in the setter. However, medium and larger sized eggs took significantly more time than small-sized eggs even after 504 hours. These findings are in line with the previous reports of Ulmer-Franco et al. (2010) who reported that lightweight eggs took a hatching time of 498 hours that was significantly less than average-sized and heavy sized eggs. Various factors can influence the hatching time of the eggs and the breeder age is the most important one of them (Ipek & Sozcu, 2015). The eggs from younger flocks contain more nutrient contents and there are more chances to obtain healthier chicks from these eggs which can easily manage their hatching process. On the other hand, older flocks are often deficient in essential

vitamins and minerals that can hinder and delay the hatching process (Vieira, 2007; Calini & Sirri, 2007). Possibly, the breeders' age had created a difference in total hatch time of the eggs under the study. This is the reason that the in-ovo practices are being applied to provide to feed for the chicks with essential micronutrients.

Hatching traits

Hatching traits are considered traits of economic importance for broiler breeders. The results of the present experiment showed a higher loss of weight in medium-sized eggs compared to large-sized eggs. Complementary reports were published earlier where lower egg weight loss was noted in larger eggs than medium eggs (Igbal et al., 2016) and can be attributed to higher egg albumen and greater water contents in larger sized eggs. This suggested that the embryonic metabolism in different egg weights was different from each other. Generally, an equal amount of water is produced from yolk fat stores during embryonic development and the amount of water excreted is dependent on shell thickness, shell membranes, egg size and incubation conditions (lqbal et al., 2016). However, the weight loss in small eggs was not significantly different from medium and large eggs (p>0.05). In terms of overall hatchability and fertility, medium-sized eggs were better than large eggs whereas the small eggs' hatchability and fertility were comparable to both the medium and large size eggs. This is similar to the findings of Duman & Şekeroğlu (2017) who reported significantly higher hatchability in medium weight eggs than in larger sized eggs. Igbal et al. (2016) reported higher fertility and hatchability in medium-sized eggs than in larger eggs. The hatch of fertile and embryonic mortality patterns (early, mid and late mortalities) were not different among the experimental egg weight groups (p>0.05). More number of culled chicks were observed from larger eggs than those from medium-sized eggs. Generally, the culled chicks are characterized by un-healed naval, week legs and other deformities. An earlier study from Duman & Sekeroğlu (2017) reported similar results showing a higher percentage of deformed chicks from larger sized eggs compared to medium-sized eggs but small-sized eggs were comparable to both egg weight categories in terms of deformed/culled chicks. Our results showed that the proportion of chicks with no defect/healthy chicks was significantly higher in medium-sized eggs compared to the large eggs. Duman & Şekeroğlu (2017) reported a significantly higher yield of healthy chicks from medium-sized eggs



compared to that from the larger sized eggs. The initial weight of the chick was found to be significant among the egg weight categories (p<0.05). Larger sized eggs produced significantly heavier chicks than the medium-sized eggs and the lowest chick weight was noted in the small-sized eggs. Our findings are in-line with previous studies where egg weight significantly affected the chick weight showing higher chick weight in the larger sized eggs (Ishaq *et al.*, 2015; Iqbal *et al.*, 2016; Duman & Şekeroğlu, 2017). Higher chick weight from the larger eggs could be due to higher residual egg yolk contents in the larger eggs compared to the medium and small eggs (Joseph *et al.*, 2006; Ishaq *et al.*, 2015).

Post-hatch growth performance

Feed intake of the broilers was significantly affected by egg size (p < 0.05). The birds from the small eggs consumed significantly less feed than those from the medium and large eggs. Iqbal et al. (2017) reported lower feed consumption in three-week-old male broilers from small eggs compared to those from medium and large-sized eggs. Ewonetu & Kasaye (2018) showed higher feed intake in white leghorn from larger size eggs compared to those from smaller eggs. The feed intake is highly dependent on the physiological needs and body size of the birds. Lower intake in the birds from smaller eggs might be due to lower weight gain. However, some previous studies showed nonsignificant differences in the birds from different egg weight categories (Vieira & Moran Jr, 1998; Ulmer-Franco et al., 2010). However, the chicks from smaller eggs reared under PO consumed significantly lower feed while highest feed intake was noted in the birds from the medium-sized eggs subjected to P5. Earlier to this, Proszkowiec-Weglarz et al. (2019) showed that post-hatch 48 hours of feed deprivation in broiler chicks resulted in significantly less intake compared to the birds having immediate access to the feed. The higher feed intake in P5 might be because the birds use this feed in body development including gastrointestinal tract (Lamot et al., 2014; Simon et al., 2014; Lilburn & Loeffler, 2015). Bodyweight gain was significantly different in the chicks from different egg weight categories (P<0.05). The birds from medium and medium-size eggs gained significantly higher weight than the birds from small size eggs. Similarly, Igbal et al. (2017) reported significantly higher three-week body weight in broilers from larger size eggs compared to those from smaller eggs. In another study, Alabi et al. (2012) reported similar results showing higher weight gain in 7 weeks old Potchefstroom Koekoek

chicks from the larger eggs compared to those from small eggs. This shows that the larger chicks obtained from larger eggs in this experiment maintained the advantage of their initial higher egg weight (Alabi et al., 2012). In the current experiment, the birds under P3 had significantly higher weight gain. Simon et al. (2015) reported higher body weight of the birds having access to the feed in the hatcher compared to those conventionally grown broilers. When the broiler chicks from medium-sized eggs were reared under P5, they presented the highest weight gain compared to those from small-sized eggs reared under conventional hatching. The lower weight gain in the chicks from smaller eggs might be due to small yolk reservoirs. The volk sac is considered as the main source of nutrients for newly hatched chicks and its deficiency may lead to Ketosis and losses in live performance if chicks are subjected to prolonged fasting (Vieira & Moran Jr, 1999).

Significantly different FCRs were noted among the birds from three egg weight categories. Overall FCR was better in the broilers from the mediumsized eggs followed by those from the smaller eggs and the poorest FCR was recorded for those from the larger eggs (p<0.05). Similarly, Ng'ambi et al. (2013) reported better FCR in the birds from the mediumsize eggs compared to those from the smaller eggs in indigenous chickens. Better FCR of birds from the medium-sized eggs can be attributed to higher weight gain. However, Duman & Şekeroğlu (2017) showed similar values of FCR among the birds from small, medium and large size eggs in broilers. Regarding FCR, the birds under P3 were better followed by those under P5 and the poor FCR was noted in the birds under conventional hatching system i.e PO. The chicks from the medium-size eggs reared under P3 showed significantly better FCR whereas the birds from the larger eggs under PO exhibited the poorest FCR among all the possible interactions. Similar to our findings, de Jong et al. (2017) revealed that delayed feeding of chicks for 72-hour post-hatch resulted in higher FCR from hatch from day 0 to 42. Contrary to our results, Hollemans et al. (2018) reported a non-significant effect of delayed feeding on the FCR of the broilers. de Jong et al. (2017) suggested that the exposure to exogenous feed in early 48 hours of the chick's life is important for the development of gastrointestinal tract that helps the chicks obtain better enzymatic activity and absorptive capacity. This might have led to better nutrient utilization and more weight gain ultimately resulting in better FCR of the early fed birds.



Livability

No effect of egg weight on livability in the current experiment is similar to the findings of Yousaf et al. (2018). However, patio strategies presented a higher survival rate in the birds under P5 followed by P3 and the least survival rate was recorded in the birds under P7. Findings of the present study are in-line with the report of de Jong et al. (2017) who performed a meta-analysis and reported that the feed and water deprivation up to 48 hours of post-hatch may lead to significantly higher total mortality than 0 or 24 hours feed and water post-hatch deprivation in broilers. Livability differed significantly when the interactions were evaluated among the egg weight categories and patio strategies. The survival rate was the highest in the chicks from the smaller eggs reared under P5 whereas higher mortality was noted in the chicks from the small egg size under P7 and P0. Early feeding had been reported to stimulate the development of immune organs such as gut associate lymphoid and hence a higher lymphocyte production can be expected (Bigot et al., 2001) which might have played a role to protect the birds in P5 group compared to P0. P7 birds were forced to stay in the hatcher section for a longer period than P5 and P3 which might have created a stressful situation due to little space and relatively poor ventilation in that section. It is suggested that a stay in the hatcher more than five days is not suitable for further performance and survival rate of the birds.

Physical asymmetry and welfare traits

Symmetric traits (relative as well as fluctuating asymmetry) are related to the welfare especially the gait score (Caplen *et al.*, 2012) and compatibility of the birds to the environment. Under optimum environmental conditions, these traits grow symmetrically (Mitchell & Kettlewell, 1998; Duman & Şekeroğlu 2017). In the current experiment, physical asymmetry and gait scores did not differ among the chicks from different egg weight categories and patio strategies. Our findings suggest the absence of any environmental stress on the experimental birds as there was no difference in the asymmetry of the broilers. This might have led to similar gait scores among the treatment groups (Caplen *et al.*, 2012).

The birds from larger eggs had significantly cleaner feathers than those from the medium-sized eggs that had dirty and damaged feathers and showed poor feather score. The broilers from the small and large size eggs under P7 had the cleaner feathers with adequate feather cover compared to any other treatment both showing the lowest feather scores. On the other hand, the birds from the medium-sized eggs under P0 showed the poorest feather score. The data regarding the effect of early feeding on feather conditions is very limited. Although, we were unable to find the literature, however, feed restriction has been found to have a detrimental effect on feathers cover in broilers resulting in poor welfare. Chronic hunger may create stressful conditions for the birds leading to frustration, aggressive and abnormal behaviour and injurious feather pecking in chickens (De Jong & Guemene 2011; Nicol *et al.*, 2017).

Blood biochemistry

Statistical analysis showed a significant effect of egg weight and patio strategies on the blood glucose levels of broilers. Higher glucose contents were noted in the birds from large eggs while the lower glucose was observed in the blood of birds from medium eggs. However, Contrarily, Duman & Sekeroğlu (2017) reported non-significant differences among the birds from light, medium and heavy eggs. Among the patio treatments, a significant effect was noted. Highest glucose in broilers was recorded in the chicks from the large eggs under P7. While small × P3 treatment presented the lowest glucose contents compared to any other experimental group. Similarly, van de Ven et al. (2011) reported significantly higher blood glucose in the chicks from patio systems compared to those under the conventional system. In another study, Wang et al. (2014) reported a significant effect of hatching time and early feeding on the glucose contents in broilers. This can be attributed to the increased level of corticosterone that possibly had increased gluconeogenesis in the chicks from the patio system as compared to the conventional hatcher (Joseph & Ramachandran, 1992; Piestun et al. 2008).

Total protein contents were not affected by egg weight categories and patio strategies (P>0.05) which is similar to the findings of Kang *et al.* (2018) who reported no effect of early feeding inside the hatcher on the total protein contents of broilers. Several factors affect the serum protein contents in chickens and of these, nutrition is the major one. Comparable serum protein in the current study could be attributed to similar crude protein in the diet of the experimental birds (Liu *et al.,* 2015). However, in a previous study, significantly higher total protein contents were reported in the chicks accessing the feed within 4 hours of hatching than those who were provided feed after 24 and 48 hours post-hatch (Adeleye *et al.,* 2018).



There was a significant influence of egg weight and patio strategies on albumin contents of the broilers. The chicks from the larger eggs had significantly higher serum albumin compared to those from the medium eggs. P7 resulted in significantly higher albumin contents whereas the birds under P0 were lowest in this parameter. However, contrary to our findings, the chicks provided with a pre-starter diet and those under conventional hatching did not differ in albumin contents in the blood (Abousekken *et al.*, 2017).

The interaction of egg weight and patio strategies influenced the serum globulin contents which showed a significantly (p < 0.05) lower globulin values in chicks from larger eggs under P7 suggesting a better utilization of protein in the diet (Adeleye et al., 2018). This could be attributed to the size of the ova released from the ovary. Previously, it has been described that the maximum transport of gamma globulin per square centimetre of yolk surface occurs in smaller ova (Patterson et al., 1962). There was a significant effect of egg size and patio strategies on uric acid contents in blood. Among the egg categories, the birds from the larger eggs had significantly higher uric acid contents in their blood compared to those from the smaller eggs. In terms of patio strategies, P7 group presented the highest contents of uric acid while the birds from PO were lowest in uric acid contents. In another study, the chicks that were offered feed 48 hours post-hatch presented significantly higher uric acid contents than those who were provided feed 4 hours after the hatching process (Adeleye et al., 2018). The chicks from the large eggs under the P5 regime had significantly highest uric acid contents at 35 days of age while those from the smaller eggs under PO presented the lowest contents of uric acid in their blood. Contrarily, in a previous study, patio hatching system resulted in comparable uric acid contents in newly hatched chicks to conventional hatching system (van de Ven et al., 2011). Regarding cholesterol, the highest cholesterol level was noted in the birds from the large eggs while the birds from the small eggs had the lowest cholesterol. Similarly, in earlier literature, a positive relationship between egg weight and cholesterol has been reported (Jiang & Sim, 1991). Possibly, the breeder age was a factor that affected the egg weight and subsequently the cholesterol contents in the blood (Wilson, 1978). Significant effect of patio strategies was also obvious on blood cholesterol showing higher value in the birds from the P7 group followed by P5, P3 and the least value was recorded in PO. These findings suggested that the egg weight and early feeding

in newly hatched chicks play an important role in modulating lipid metabolism. Contrary to our findings, cholesterol was not influenced by feed restriction in broilers (Afsharmanesh *et al.*, 2016). A significantly higher level of cholesterol was observed in large × P7 while the birds under Small×P0 group showed the lowest cholesterol contents in their blood compared to the other treatment groups. However, Kang *et al.* (2018) were unable to find significant differences in the broilers under patio feeding regimes.

Creatinine is an important criterion to assess the muscle/protein catabolism. No impact of egg weight on creatinine was noted, but the patio strategies resulted in significant differences among the treatments for blood creatinine of broilers. P7 resulted in the highest creatinine while conventional PO group was the lowest in creatinine level of the broilers. In a previous report, significantly higher creatinine was noted in the birds offered feed 48 hours post-hatch compared to those who had access to the feed just after 4 hours posthatch (Adeleye et al., 2018). The chicks from medium eggs under P7 strategy were highest in their blood creatinine while the least contents of creatinine were observed in small × P5 treatment. This suggested that the muscle/protein catabolism was lowest when chicks from small eggs were provided feed for five days within the hatcher. Attia et al. (2017) suggested that the difference in the blood creatinine could be due to growth, duration and intensity of the feed restriction. Contrary to our findings, Attia et al. (2017) reported no difference in creatinine level of feed restricted and non-restricted slow-growing chickens.

CONCLUSIONS

From the discussion of the findings of the current study, it can be concluded that the egg weight is an important criterion that can affect most of the hatching traits, hatch window and overall subsequent performance of the broilers. The early feeding strategies have a significant role in broilers' life and affect body weight, FCR and body function especially the carbohydrates, protein and lipid metabolism. Selecting the medium size eggs for hatching and providing the chicks from these medium eggs within the hatcher for three days (Medium × PS3) could be the best strategy to obtain optimum performance of the broilers.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.



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