



Effect of Post-Hatch Transportation Duration on Growth Performance, Immune Function, Blood Profiles and Intestinal Bacteriology of Broiler Chicks

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Abstract | The purpose of this study was to determine the effect of different transportation periods (1.5, 3, 6 and 12 h) after hatching on production, carcass traits, immune response, and intestinal bacteriology of 308 Ross broiler chickens. A total of 256 unsexed chicks were removed from hatchery trays immediately and were randomly assigned to four groups. The first Group, transported to farm within 1.5 hours (T1), second group: transported to farm within three hours (T2), third group, transported to farm within six hours (T3), and fourth group: transported to farm within 12 hours (T4). In farm, these groups were reared in 16 pens based on a completely randomized designed (dimensions of each pen were 1×2 m²). Feed consumption, body weight (BW), and feed conversion ratio (FCR) were measured biweekly, while growth efficiency index (GEI), internal organ weights, immune function, and intestinal bacteriology were assessed at 42 days of age. Chicks transported during 1.5 and 3 h (T1, T2) had the highest body weight, feed consumption, and intestinal weight ($p < 0.05$). Growth efficiency index (GEI) was the lowest in T1, T2 when compared with T3 and T4 ($p < 0.05$). The chicks transported during 1.5-3 h also showed stronger immune responses to Newcastle Disease Vaccine (NDV) ($P < 0.05$). T1 and T2 showed the highest number of Lymphocytes (L) ($P < 0.05$), and the lowest stress index ($P < 0.05$). The relative bursa weight and small intestine of the T1 and T2 were heavier ($p < 0.05$). T1 and T2 were effective in reducing the numbers of pathogenic bacteria in the ileum and preserved the beneficial bacteria. Pathological bacteria in ileum were increased in T3, T4 ($p < 0.05$). We conclude that transportation periods of 1.5 to 3 hours post-hatch do not adversely affect production, immune response, or intestinal microbiota in broiler chicks. However, if the period is increased from 6 to 12, it may affect production, immune response, and intestinal microbiota. The findings of this study shed light on managerial strategies that could be used to reduce the side effects of transportation in the broiler industry.

Keywords | Broiler, Growth, Heath, Immune, Post-hatch, Transportation

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In conventional hatcheries, broilers face multiple stressors, including transportation, food and water deprivation (up to 36 hours post-hatch), sexing, counting, and packaging. Poultry is the only production animal that is transported very early in life, making this a critical stage for early life (Lamot *et al.*, 2014). Studies have demonstrated that the exposure to stress during the first days of life has been shown to have effects on growth performance later in life, which may be attributed to release of the corticosteroid hormones (Jacobs *et al.*, 2016; Boussaase *et al.*, 2020). Transportation periods exceeding 24 hours can induce metabolic changes (Van Dono 2012; Bergoug *et al.*, 2013; Jacobs *et al.*, 2016). Reported effects include impaired competence of the systemic immune system, depressed mucosal development in the gastrointestinal tract (GIT) and decreased BW (Uni *et al.*, 1998; Shira *et al.*, 2005). Furthermore, the preservative nutrients in the yolk after hatching are insufficient to meet the nutritional needs of neonatal chicks during the first days of life. Consequently, the neonatal chicks use gluconeogenesis to overcome this deficiency (Yahya *et al.*, 2016). Yolk sac is considered the main source for developing immune system and gastrointestinal tract, especially during early period of life (Panda *et al.*, 2015). As transport may exacerbate the depletion of reserves through excessive thermal regulation requirements and stress, the relationship between the depletion of yolks sacs and transportation period must be checked in order to determine the impact of transport duration on the welfare, health, and productivity of chicks (EFSA, 2011). Transportation of neonatal chicks for 8 hours reduces growth performance by day 21 compared to non-transported chicks (Bergoug *et al.*, 2013). Jacobs *et al.* (2016) indicated that weight yield and yolk mass were decreased in broiler chicks 8-10 hours after transportation compared to 1.5-3 hours of transportation. During this period, the intestinal mucosa and immune system need to profound amount of energy for reaching their full development (Lilburn and Loeffler, 2015). Additionally, transporting chicks over distances ranging from 50 to 300 km during the first seven days increased the mortality rate from 1.22% to 1.36% (Chou *et al.*, 2004). Experiments have indicated that late access to feed or prolonged transportation negatively affects growth, immune system, intestine morphology, microbiota, and development of gastro-associated lymphoid tissue (Panda *et al.*, 2015). These experiments have also revealed that immediate access to feed post-hatch and short transportation increase cell activity and muscle growth, as well as enhance the lymphoid organs and immune competency (Bhanja *et al.*, 2009; Hollemans *et al.*, 2018). Therefore, the first week performance of broiler chicks is used as indicator for improving their growth later in life (Hollemans *et al.*, 2018; Gawel *et al.*, 2022). Although there are several studies demonstrating that early life stress can lead to de-

lay the growth performance, during the first weeks of life, the chicks can recovery the growth performance after that (Shariatmadari, 2012). This paper seeks to fill a small, but significant gap in the extensive literature devoted to the research on the suitable transportation period of chicks post hatching on poultry production and health especially in tropical area. In addition, this study provided insight into whether transportation stress on growth and health during the rearing period is constant or temporary.

MATERIALS AND METHODS

ETHICAL CONSIDERATIONS

The Ethics Committee for Poultry Research at Al-Qasim Green University in Iraq approved the study protocol (code: Alqas-rec.2022-feb-EA989937). The following categories are concluded by the ethical guidelines: refinement and animal housing standards (housing, bedding, food, watering, temperature, humidity, and containment): (Animals should not be subjected to unnecessary burdens; they should be restrained and transported humanely; they should not be mutilated, and disposal of animal in a proper manner).

EXPERIMENTAL FORM

The study was conducted from 15 April to 28 March, 2023, using Ross 308 broiler chicks. The study was approved in accordance with the animal research committee of veterinary medicine college in Alqasim Green University.

ANIMAL AND HOUSING

For this study, four different transportation periods were assessed. The broiler chicks (Ross 308) were purchased from a local hatchery immediately after release from setter with an initial mean body weight 40 ± 5 g. After the mixed-sex chicks hatched (they were not sex-separated post-hatch), hatchery personnel administered the vaccines and visually assessed the chicks for down appearance, anomalies, and cleanliness. Downgraded chicks were excluded from the experiment. Standard boxes contain 16 chicks ($21 \text{ cm}^2/\text{chick}$) and were used for transportation to the experimental farm. The chicks ($n = 256$) were divided over 16 transportation boxes. These boxes were alternately stacked and divided 4 stacks of boxes, with in all stacks an equal number of boxes with chicks, 4 stacks per transportation treatment. Chicks were transported for 1.5, 3, 6, or 12 h, which respectively correspond to mean and maximum transportation durations in Iraq. Commercial transportation conditions were conducted by placing all boxes in one fully loaded commercial semi-trailer together with commercial chicks intended for other farms. The environmental conditions of the trailer were $28 - 30^\circ\text{C}$ temperature and 40% humidity, the speed was 80-90 km per hour. Numbered crates containing the experimental chicks were placed in 4 stacks in the truck. After transporting the 256 chicks in 16 boxes (4 boxes per treatment, 16 chicks per boxes), these experimental chicks

were housed in 16 pens (2m² on wood shavings, stocking density at slaughter age was 35 kg/m²). The chicks underwent 42 days of rearing in standard conditions for Ross 308 broiler chickens and designed as following into four groups, four replications, and 16 birds per replication based on a randomized completely design (CRD) with a 1×4 treated arrangement. groups included chicks transported for 1.5, 3, 6, and 12 hours to the farm (T1, T2, T3, T4) respectively (Figure 1). To minimize on-site bias among groups, the groups were placed in alternating sections in the farm for each replication such that each group was arranged four times in each of the four section. The ration was designed to meet national research council recommendation. The ration included a starter included 21% crude protein and 3000 kcal/kg metabolic energy from 1 to 10 day, grower ration containing 21% crude protein and 3100 kcal/kg feed metabolic energy from 11 to 24 day of age, and finisher ration from 25 to 42 (Table 1). The access to water and feed was freely. The temperature degree in farm was 35 °C on day one and gradually reduced until 21°C at third week of age. The 23 hours:1 dark was the lighting regimen.

on days 1, 14, 28, and 42 using a digital scale. Feed intake was also calculated on the same days, and the ratio of feed conversion was measured as the ratio of feed intake to body weight gain. The growth efficiency (GEI) index on day 42 was measured using the following equation: a

$$GEI = \frac{BW \times V}{FCR \times D} \times 100$$

where BW=body weight (kg), V equal to viability (%), FCR=feed conversion ratio, and D=the period of experiment (days) (Yahya *et al.*, 2022).

Table 1: Chemical composition of broiler of 308 Ross strain in different stage (day).

Composition	Starter (1 to 10 d)	Grower ((11 to 24 d)	Finisher (25 to 42 d)
materials (g Kg ⁻¹ as fed basis)			
maize	537.2	572.1	640.0
Soybean	393.0	360.0	290.0
Vegetable Oil	25.0	28.0	30.4
Calcium Di-phosphate	19.0	16.5	15.5
Calcium Carbonate	12.5	11.0	11.5
Food salt	3.9	3.9	3.7
DL-methionine	2.4	1.9	2.0
Lysine HCl	2.0	1.6	1.9
*Vit. + Min. Premix	5.0	5.0	5.0
Chemical Composition (g Kg ⁻¹)			
Metabolisable energy (kcal Kg ⁻¹)	2900	3000	3050
Crude protein	219	207	182
arginine	15.02	14.18	12.30
lysine	13.35	12.25	10.75
methionine	5.86	5.24	5.03
methionine + cystine	9.25	8.48	7.87
Threonine	8.85	8.43	7.90
Calcium salt (ca)	10.3	9.1	8.9
Phosphorus(P)	5.0	4.5	4.2
Sodium(Na)	1.7	1.7	1.6

*1kg of Premix containing: zinc 80 mg, manganese oxide, 100 mg, selenium, 10 mg, iron sulfate 80 mg, vitamin retinol, 11000 IU, vitamin D3, 5000 IU, vitamin E, 40 IU, vitamin K, 4 mg, riboflavin, 5 mg; vitamin B6, 4 mg, vitamin B12, 0.011mg, niacin, 50 mg, biotin, 0.01 mg, thiamine, 3 mg.

CARCASS TRAITS: On day 42, ten birds were taken from each group with approximately similar average body weight of ± 100 g and slaughtered using hallal method. Carcass yield was measured as a ratio of carcass weight to live BW. Immediately after slaughtering, the breast muscle, thigh, heart, liver, gizzard, and whole intestine were picked and their relative weights were measured as the percentage of

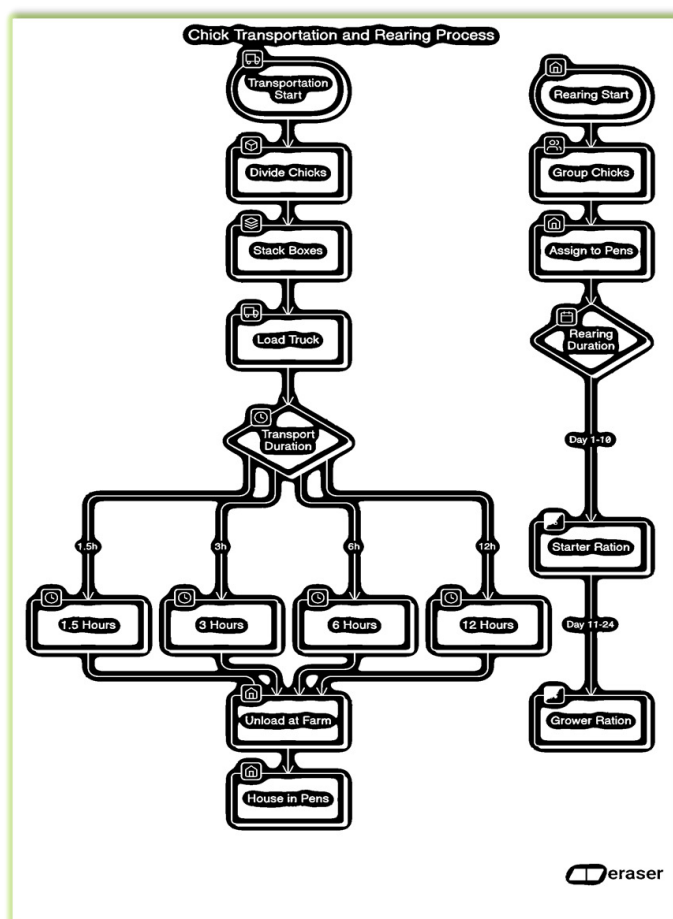


Figure 1: The experimental design consists of four main components for transportation duration after hatching.

TRAITS MEASURED

PERFORMANCE: The growth performance was calculated per pen, the chicks in each pen were collectively weighed

live BW. The non-stretchable thread and scale were used for measuring the lengths of small intestines from these slaughtered birds.

LYMPHOID ORGANS: The Bursa of fabricius and spleen(lymphoid organs) of the previous slaughtered birds were weighed as ratio (%) to carcass weight.

CELL MEDIATED IMMUNE TEST (CMI): The skin Basophils sensitive reaction to phytohemagglutinin compound (PHA) (Pahar®, Az-strmoon Co. Tehran, Iran) was used as indicator for measuring CMI. The phytohemagglutinin compound in 0.1 mL of sterile phosphate buffer saline on day 40 were intramuscular injected between 3rd and 4th skin sheet of digits in the right foot of ten chicks from each group. Skin thickness changes were measured using a digital micrometer before one day of inoculation and after two day (s) of the inoculation (Corrier and Deloach, 1990).

HUMORAL IMMUNITY TEST: Immunity titers against the sheep red-blood cells (SRBCs). 0.1 mL of S RBC (5%) in sterile phosphate buffer saline was inoculated in pictorial muscle of ten chicks per group on day 21 and 28 of life. 3mL of blood were suckled from the brachial vein before and after seven days of the first SRBC injection. Antibody titers values were calculated as log two of reciprocal heist dilution that reached to full agglutination (Wegman and Smithies, 1966).

ANTIBODY TITERS OF NDV: Ten chicks per group on day nine of age were vaccinated intra-ocularly against Newcastle virus (NDV, LostaNobilis® ND LaSota, Intervet Co, Intervet Lane PO Box 318 Millsboro, United States). 3mL of blood samples were obtained from the brachial vein before and after seven and 14 days of the first intraocular dropping. The antibody titers were measured using HI (hamoagglutination inhibition) method (Anon, 1971).

BLOOD PICTURES: On day 42, blood smears were taken from brachial vein and stained by Giemiza stain for differential count of Leukocytes (WBC) using a “cell-counter (Nihon CohdenCentax-MEK-6450K, Japan) (without making division into fraction) (Coles, 1989).

BACTERIA COUNT: The total count of bacteria, *Lactobacillus*, *Bifidobacterium*, *Coliform*, and *Clostridium* spp were aplied for measuring bacterial load in middle part of ileum digesta. The Plate count agar (PCA) (Merck KGaA, Darmstadt, Germany), De Man Rogosa -Agar (MRC) (BiolifeCompany, Viale Monza, 272, 20128 Monza MI, Italy), Mac-Conkey Agar (Merck KGaA, Darmstadt, Germany), and SPS (Merck KGaA, Darmstadt, Germany) were selected for isolation these bacteria respectively. On day 42, three grams of digesta were picked and homogenized with buffered peptone water and diluted of decimal (10⁻³

to 10⁻⁷). The bacterial incubation was divided into the anaerobic condition (anaerobic jar) which used for isolation of *Bifidiobacterium* and *Clostridium* spp for 48 hours, and the aerobic condition which applied for microbial population of total bacteria and coliform at 37°C for 24 hours and for *Lactobacillus* at 37°C for 48 hours. Bacterial numbers were represented as log 10 colony-forming units per gram of ilealdigesta (log cfu/g).

STATISTICAL TEST

The complete randomized design (CRD) with a 1×4 factorial arrangement (ANOVA) and SPSS/17 statistical package was applied to analysis of data. Duncan's multiple range test method was adopted to compare of the means, and the general linear model was applied to determine the main effects of factors.

$$y_{ijk} = \mu + a_i + e_{ijk}$$

where y_{ijk} is the response measured, μ is mean of the observation, a_i is the effect of transportation (+/-), and e_{ijk} is the error term. The pen means were considered the experimental unit of growth performance and the values of individual birds were considered the experimental unit for immune response, carcass parts and other measured items. The number of bacteria per milliliter or gram of sample will be obtained by the number of colonies divided by the dilution factor multiplied by the volume of specimen supplied to liquefied agar). CFUs = Numbers of bacteria / ml × invert dilution factor. All bacteria enumeration data were transformed to log₁₀ CFU/mL. Significant level of means was P ≤ 0.05 level.

RESULTS AND DISSCUSSION

GROWTH PERFORMANCE

Compared with Groups (T3, T4) the body weight (BW) and feed intake were the highest in birds transported for 1.5 and 3 hours during 28-42 days of age (P<0.05, Table 2). GEI was the lowest value in birds transported for 1.5 and 3 hours (Table 3).

Genetic selection and nutritional strategies make early post-hatch processing particularly significant for overall broiler growth performance. Several studies have established that first days of life of newborn chicks are a managerial control approach used to protect the developing newborn chick from acquiring the required nutrients before the digestive system matures. There are studies referred that chick growth performance can be compensated by resting (Obun et al., 2013). Additionally, the some events referred that the stress factors post hatching such as starvation for 36 hours and long transportation may be compensated after short periods of resting (Jessen, et al., 2021). Herein, our

results determined that short transported duration significantly increased BW and FI during the grower Period. The growth performance of short transportation duration (1.5-3 h) was superior to that of long transportation duration (6-12 h) which is consistent with findings of (Yahya *et al.*, 2015; Simon *et al.*, 2015).

Table 2: Influence of transportation periods after hatching on growth performance of Broilers¹ at different ages.

Treatments	Body weight (g)				Feed Intake (g)			
Transportation periods (hour)	14 day	28 day	42 day	1-14 day	15-28 day	29-42 day	1-42 day	
12	461	1494 ^b	2799	509	1672	2416 ^{bc}	4467	
6	482	1511 ^{ab}	2769	544	1690	2400 ^c	4516	
3	482	1523 ^{ab}	2788	552	1716	2441 ^{abc}	4602	
1.5	500	1597 ^a	2800	549	1714	2528 ^a	4669	
Pooled Sem	9.6	38.2	37.3	13.4	19.3	28.6	59.0	
Main effects Probability	ns	*	ns	ns	ns	ns	ns	

Values: 4 replicates out of 16 birds per pen. a,b,c Means within the same column without the resembled superscripts mean significant differences (P<0.05). **Pooled Sem:** Pooled Standard error of means; **ns:** Not Statistically significant; *: P<0.05.

Table 3: Influence of transportation periods after hatching on feed conversion and growth efficiency index (GEI) of Broilers¹ at different ages.

Treatments	FCR (g/ g)				GEI (42day)
Transport (hours)	1-14 day	15-28 day	29-42 day	1-40 day	
12	1.20	1.575	2.15	1.65	441.5 ^{bc}
6	1.24	1.561	2.07	1.6	482.7 ^a
3	1.276	1.56	2.3	1.67	430.8 ^c
1.5	1.26	1.58	2.3	1.68	435.8 ^c
Pooled Sem	0.020	0.028	0.049	0.014	9.6
Main Effect	ns	ns	ns	ns	ns

Values: 4 replicates out of 16 birds per pen. a-e Means within the same column without the resembled superscripts mean significant differences (P<0.05). **Pooled Sem:** Pooled Standard error of means, **ns:** not significant difference; *: P<0.05.

However, there were no significant differences in the FCR among groups during the overall period; nonetheless, the growth performance of short transportation duration was still better than long transportation duration revealing that compensatory growth occurred in the long transportation duration group but did not return to that of the short trans-

ported duration group. Similarly, Kornasio *et al.* (2011) and Van Dono (2012), growth performance due to early stress may not have been compensated during later life. The longer transportation duration of the chicken, the less immunity it was, and the longer the adjustment time required to correct for development. The intestinal development of newborn chicks plays a crucial role in growth performance. The retardation of intestinal development may be related to long transportation period and delay access to feed and water. According to our experience, broiler chicks transported for 6 to 12 hours after hatching may continue to deteriorate in growth till the end of experimental life.

CARCASS TRAITS AND LYMPHOID ORGANS

The birds transported for 1.5 to 6 hours had greater (P<0.05) bursa and intestinal weight than those transported for 12 hours (Table 4). Nevertheless, there were no statistically significant differences in relative weights of carcass yield and organs groups (P > 0.05). Post-hatch management promotes stimulating gastrointestinal development and broiler performance. The high weight of intestine attributed to increase the intestinal cell development and production of new cells caused from the low stress post hatching and early access to feed (Biloni *et al.*, 2013). Following chick hatching, the intestine length, weight, and digestive enzyme activity rapidly increase, whereas fasting can impair the growth and development of the intestine and hence limit food absorption. The gizzard organs weights did not change with long or short transportation period. The compensatory growth responses and experimental durations (42 days) have a crucial role in minimization of the adverse effects of early stress of transportation post-hatch on internal organs. This result was consistent with Yahya *et al.* (2015) where the response of internal organs of broiler chicks exposed to early life stress was compensated through last weeks of age. Abdel *et al.* (2011) found that the effects of early stress (e.g., fasting chicks post-hatching) on internal organs disappeared on days 21 and 42 of age. These results were inconsistent with the results from other studies showing that the liver weight was higher in animals with early transportation to farm and immediate access to feed after hatching than in animals experiencing 18 and 24 h delayed transportation (Saki, 2005; Hooshmand, 2006). The bursa of Fabricius is critical to the normal development of B lymphocytes and the antibodies they go on to produce. The enlargement of bursa weight indicated to development of lymphoid tissues and their function. Also, it is presumed that antibodies (IgM, IgG, B cells) are generated by the attachment of immune complexes to IgM bursal B cells because IgM IgG B cells are induced by antigen-dependent attachment of maternal IgG (Khaled *et al.*, 2018). Yahya *et al.* (2015) found that immediate access to feed increased the bursa weight. The compensatory growth may be the reason why transportation periods have no effect on internal organs.

Table 4: Influence of transportation periods after hatching on carcass traits and relative weights on carcass traits to body weight of Broilers.

Treatments	Carcass Yield (%)	Relative Weights of Carcass traits (%)								Length of Intestine (cm)
		Breast	Thigh	Liver	Heart	Gizzard	Bursa of Faricius	Spleen	Intestine	
Transport period (hrs)										
12	65.7	26.0	25.3	2.0	0.69	1.75	0.089 ^c	0.122	5.98 ^{bcd}	232.3
6	65.8	25.5	26.0	2.0	0.68	1.82	0.095 ^{bc}	0.122	5.49 ^d	229.3
3	65.6	27.1	24.6	2.09	0.67	1.87	0.119 ^{ab}	0.143	6.15 ^{abc}	242.9
1.5	64.4	25.8	24.6	2.21	0.70	1.93	0.117 ^{ab}	0.105	6.31 ^{abc}	236.4
Pooled sem	0.55	0.53	0.43	0.11	0.03	0.07	0.012	0.032	0.19	4.3
Effect	Probability									
	ns	ns	ns	ns	ns	ns	*	ns	*	ns

Values: 10 birds per treatments, a-d means within the same column without same superscripts mean significant differences. Pooled sem = Pooled Standard error of means, ns= not statistically significant, * means $P < 0.05$.

HUMORAL IMMUNITY AGAINST SRBCs

The highest titers against SRBC were recorded in transported for 1.5-3 h groups compared to 12 h group with primary and secondary response ($P < 0.05$) (Table 5). On the other hand, the lowest antibody titer was recorded for group with 12 hours transport time ($P < 0.05$). It is fact that the good management of newborn chicks promotes the absorption of leftover egg yolk in chicks, as well as the absorption and utilization of hydrophilic substances, thereby stimulating early gastrointestinal development and immune defenses. Additionally, early transport of the birds to farm has a positive effect on immune responses, it may enhance the immune capacity and increase lymphocyte cells colonization (Ao *et al.*, 2012). This result was in line with the findings from several studies showing that the post-hatch stress factors had a detrimental effect on antibody titer against SRBC (Panda *et al.*, 2015; Simon *et al.*, 2015). Similarly, Panda *et al.* (2010) indicated that 24 h deprivation had a negative impact on the development of immune competence through affecting immune responses, lymphoid organs, and cellular immunity against PHA inoculation. These findings were inconsistent with the results from the studies by Juul-Madsen *et al.* (2004); Engber *et al.* (2013); Shinde *et al.* (2015) which said delayed access to food for a short time (about 24 hours after hatching) had no effect on the normal development and function of the immune system. This adverse effect may be attributed to presence of corticosteroid hormones (stress hormones), which reduces the lymphoid organs and cell-mediated immune reaction (Lesson and Summer, 2001). This finding in this experiment indicated that early transportation post-hatch may have reduced the defect on lymphoid organ and immune competence of the chicks.

HUMERAL IMMUNITY AGAINST NDV

The transport periods did not affect the antibody titers (AB) ($P \geq 0.05$) at all the stages (Table 5). This result was in agreement with the findings by Yahya *et al.* (2016) and Simon *et al.* (2014) who found no significant effect of post-

hatch transportation periods on Ab titer against NDV after prolonged lifespan. This result was also consistent with the finding by Ao *et al.* (2012) and Yahya *et al.* (2016) who determined that immune competence of chicks exposed to certain stressors (e.g. transport) had an acceptable ability to modulate. Nnadi *et al.* (2010) argued that although no lymphatic organ change was observed, the earlier transportation of newborn chicks had 'immunologically wise' compared to their delayed transportation. According to the findings that early transportation of birds post hatching is considered as important strategies of immune development.

Table 5: Influence of transportation periods after hatching on immune function in Ross 308 Broilers.

Treatments	Web toe thickness (mm) After 24 or 48 h of PHA injection		Antibody-SRBC response (log 2) after 7 and 14 days of initial injection		Antibody-NDV response (log 2) after 7 and 14 days of initial ingestion	
	After 24hour	After 48hour	After 7 days	After 14 day	After 7 days	After 14 day
12 hours	1.50	4.26	1.96 ^c	2.933 ^c	3.86	3.58
6 hours	1.50	4.16	2.34 ^{bc}	3.21 ^{bc}	3.45	3.45
3 hours	1.40	4.35	2.92 ^a	3.69 ^{ab}	3.93	3.40
1.5 hours	1.30	4.18	2.53 ^{ab}	3.95 ^a	3.56	3
Pooled Sem	0.21	0.46	0.20	0.23	0.24	0.20

Values: 10 chicks per treatments. ^{a-c} letters within the same columns without the resembled superscripts mean significant differences, NDV means Newcastle virus vaccination, SRBC means Sheep red blood cells; **Sem:** Standard error of means, statistically significant; *: $P < 0.05$.

CELL MEDIATE IMMUNITY (CMI)TEST

PHA-P was not affected with transportation periods (Table 5). This result was in line with the study of Friedman and Bar-Shir, (2005) suggesting that the immune defect was eliminated after 14 days of life of birds exposed to early

stress. CMI response was not affected by early or delayed access to feed post-hatch (Yahya *et al.*, 2015). One possible explanation for our findings is that a negative immune response against PHA-P was caused by excessive body weight (Martin *et al.*, 1990) that meaning the body weight is associated with an immune response.

Table 6: Influence of transportation periods after hatching on blood profiles in Ross 308 Broilers.

Treatments Transportation periods hour	White blood Cell ($\times 10^4$)	Lym- phocytes (%)	Heter- ophil (%)	Heterophil/ Lymphocyte ratio
12	1.450	58.43 ^b	36.48 ^a	0.63 ^a
6	1.407	61.58 ^{ab}	34.19 ^{ab}	0.58 ^{ab}
3	1.357	62.20 ^a	32.37 ^b	0.53 ^{bc}
1.5	1.488	64.69 ^a	30.75 ^b	0.49 ^c
Pooled sem	0.091	1.26	1.35	0.03

Values: 10 chicks per treatments; a-b Means with different superscript litters in the same column indicate significantly different; **sem:** Standard error of means; statistically significant; *: $P < 0.05$.

BLOOD PROFILE

The result in Table 6, showed that the heterophil count of chicks transported for 6 and 12 hours was significantly increased ($P < 0.05$) compared with that of chicks transported for 1.5 or 3 hours. The Heterophil to Lymphocyte ratio (L:H) was reduced ($P < 0.05$) in chicks with transportation duration of 1.5 or 3 h. Lymphocyte count was significantly increased of transported broiler during 1.5 and 3 h post-hatch. Herein, our results revealed that the transportation duration did not affect WBC count ($P \geq 0.05$), (Table 6). These findings concluded that a stress factor may stimulate the adrenal gland to produce glucocorticoid hormones, this hormone is responsible on formation of heterophils (Niken *et al.*, 2018). Lymphocytes act as a specific immune response (formation antibody or cellular response) (Song *et al.*, 2021). Another study found that transportation for 6 or 12 hours decreased the lymphocyte and increased corticosteroid hormone (Jain, 1993). However the our study showed increasing heterophil to lymphocytes ratio at transportation duration of 6 to 12 hr. The long transportation period can induce the corticoid hormones that is responsible increasing secretion of heterophils and decreasing secretion of lymphocyte to the tissues (Aengwanoch, 2007). This result was inconsistent with the findings from studies by Guyton *et al.* (2012) and Niken *et al.* (2018) indicating that early life stress increased the leukocyte production. In addition, Lymphocyte in transported broiler was increased during 1, 5, and 3 h post-hatch. These findings concluded that a stress factor may stimulate the adrenal gland to produce glucocorticoid hormones, this hormone is responsible on formation of heterophils (Niken *et al.*, 2018). Lymphocytes act as a specific immune

response (formation antibody or cellular response) (Song *et al.*, 2021). Another study found that transportation for 6 or 12 hours decreased the lymphocyte and increased corticoid hormone (Jain, 1993). However the our study showed increasing heterophil to lymphocytes ratio at transportation duration of 6 to 12 hr. The long transported duration can induce the corticosteroid hormones that is responsible increasing secretion of heterophil and decreasing secretion of lymphocyte to the tissues (Aengwanoch, 2007). When the birds were exposed to transportation and delay access to feed as both stress at same time, the impact of these was detrimental of the all body organs.

Table 7: Influence of transportation periods after hatching on bacterial count of ileum in Ross 308 Broilers.

Transporta- tion periods (hour)	Total bacte- rial Count ($\times 10^7$)	E.coli ($\times 10^7$)	Colestridia ($\times 10^8$)	Bifido- bacteria ($\times 10^8$)	Lacto- bacilli ($\times 10^8$)
12	11.60 ^a	13.1 ^a	6.84 ^a	3.20 ^{ab}	3.81 ^a
6	6.97 ^{ab}	3.87 ^b	2.29 ^b	1.91 ^b	0.50 ^b
3	2.23 ^b	1.06 ^b	1.33 ^b	5.83 ^a	1.17 ^{ab}
1.5	1.57 ^b	1.98 ^b	0.75 ^b	3.11 ^{ab}	2.65 ^{ab}
Pooled sem	1.861	2.64	9.84	1.60	1.01
Effect	*	*	*	*	*

Values: 10 chicks per treatments, ^{a-b} Means within the same column without the same superscript litters mean significant differences; Pooled **sem:** Pooled Standard error of means; *: $P < 0.05$; statistically significant.

GUT BACTERIOLOGY

On the one hand, the pathogenic bacteria index was used to evaluate the gut health. As illustrated in Table 7, E.coli, Clostridium and total bacterial count ($P < 0.05$) were minimized with the short transportation duration for 1.5 or 3 hours. Broiler intestinal microbiota colonization occurred chiefly in the early stages after hatching, with host and environmental factors impacting the makeup and activity of future microbiota (Kers *et al.*, 2018). Moreover, fluctuations in gut microbiota diversity, composition, and general community structure of chickens are caused by differences in feedstuffs, digestion of substances, and eating or post – hatching stress (Tan *et al.*, 2019; Li *et al.*, 2022); Yadav and Jha, 2019). Furthermore, a relatively stable and sophisticated gut microbiota evolved throughout time (Kogut, 2022). In our study, the intestinal pathogenic bacteria was higher in long transportation duration groups (6–12 h) compared with short transportation duration groups (1.5–3 h). These findings suggest that transported durations influence the variety, composition, and overall community structure of the chicken intestinal microbiota. Additionally, the early transportation provides a species-rich micro ecosystem to withstand external stressors and maintain the healthy development of the intestinal tract. Also, the early transportation post-hatch accelerated the beneficial bacterial colo-

nization in small intestine and minimized the pathogenic bacteria colonization (Ravindran *et al.*, 2021). Simon *et al.* (2015) the composition of microbiota of the birds transferred to the farm during early time post hatch was higher than that in birds with delayed entry into the farm. Enberg *et al.* (2013) and Yahya *et al.* (2015) showed that the *E coli* decreased in the ileum of broiler chicks exposed to early feeding during shipping within 24 hours. Potturi *et al.* (2005) revealed that the aerobic bacteria count increased the ileum of poult when the access to feed was delayed. Hence, the early bacterial colonization in gastrointestinal tract have potentially effect on bacterial species present in the gut during all the life (Groloud *et al.*, 1999). Our result in this regard was not in agreement with that from the study by Alhotan (2011) indicating that early stress factor during 24 h post-hatch did not change the microbiota in broiler chicken. According to this study the short transport period reduced the pathogenic bacteria in small intestine. Besides, researchers have discovered that a range of Bacteroidetes members play a favorable role in broiler digestion by improving nutrient digestion and absorption, and that the quantity of Bacteroidetes is positively correlated with broiler growth. This finding was in line with the study result reported by Ao *et al.* (2012) who demonstrated that early transportation to farm or access to feed reduced *Clostridium spp* and *E coli*. The difference in results among researchers may be attributed to adaptive mechanism exhibited by the birds, it is different for individual stressor compare to combined stressors (e.g heat, nutritional..etc). In brief, short transported duration inhibited the growth of harmful bacteria (clostridium and coliform bacteria) and improved a protective barrier of intestine and enhanced the beneficial microbiota throughout the growth phases.

CONCLUSIONS AND RECOMMENDATIONS

Current Conclusion: According to our article, broiler chicks' growth and health are unaffected by transportation period post hatching ranging from 1.5 to 3 hours. whereas the 6 to 12-hour transportation period post hatching impairs immunological response, gut flora, and growth performance.

Suggested Conclusion: Broiler chicks tolerate transportation periods of 1.5 to 3 hours post-hatch without adverse effects on growth, immunity, or gut health. However, longer transportation periods (6 to 12 hours) impair these parameters.

The study's conclusions provide insight into managerial tactics that may be applied to lessen transportation-related adverse effects in the broiler sector.

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NOVELTY STATEMENTS

This study provides new insights into management strategies that can be used to reduce transportation side effects in the broiler industry and the role of shortening the transportation duration after hatching in improving the health of broiler chicken, so this study identifies the best transportation duration of the chicken after hatching

AUTHOR'S CONTRIBUTIONS

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Methodology: Yahya Sabah Abdulameer and Ali Kumait Alawadi.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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