Iranian Journal of Applied Animal Science

Effects of a Fat Soluble Vitamin Premix on Performance Indices and Hematological Parameters in Slow- and Fast- Growing Broiler Chicks within a Flock

Research Article

T. Pakzad[1], H. Khosravinia[1*], B. Masourei[1] and B. Parizadian Kavan[1]

[1] Department of Animal Science, Faculty of Agriculture, Lorestan University, Khorramabad, Iran

Received on: 25 Apr 2022
Revised on: 3 Jun 2022
Accepted on: 10 Jun 2022
Online Published on: Dec 2022

*Correspondence E-mail: khosravinia.h@lu.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

A 2 × 7 factorial experiment was conducted to examine the effects of a fat soluble vitamin premix (FSVP, 0 and 1.2 mL/bird/injection) on performance indices and hematological variables in broiler chicken with different growth rate ($\bar{x}$-3SD, $\bar{x}$-2SD, $\bar{x}$-1SD, $\bar{x}$, $\bar{x}$+1SD, $\bar{x}$+2SD and $\bar{x}$+3SD) using 364 male Ross 308 broiler chicks. Body weight (BW) of the birds at marketing age (42 d) was not follow the same pattern as their BW at age of 21 d. The birds receiving a FSVP achieved greater body weight, ate more feed and converted the feed with a greater efficiency than the untreated birds. Heart percentage was significantly lesser in the birds with initial body weight close to the population mean compared with the birds having a lesser or a greater BW than the population mean. Injection of FSVP increased heart weight by 20.48 percent and decreased plateletcrit (PCT) by 33.3 percent. Step-wise multivariate regression analysis showed that heart percentage explained 38.37, 39.07 and 8.57 percent of the variation in BW (42 d), feed intake and feed conversion ratio (21 to 42 d), respectively. Hemoglobin concentration and red cell distribution width (RDW) were the next two variables that collectively explained about 10 percent of the final BW (42 d) variance. It was concluded that birds showing a body weight of $\bar{x}$-3SD at early ages cannot exhibit greater growth rate in later ages probably due to intrinsic limitations. No hematological parameter, excluding heart percentage seems to have a significant influence on intra-flock variance in performance indices in broiler chickens.

KEY WORDS broiler chicken, hematological parameters, intra-flock variation, marketing age, multivariate analysis, performance.

INTRODUCTION

In broiler chicken production, flock uniformity in body weight at a given age, in particular at marketing age is a major determinant of profitability (Szollosi *et al.* 2014; Peak *et al.* 2000; Feddes *et al.* 2002). Indeed, the level of uniformity dictates the final profit where a flock with a poor uniformity inevitably suffering with delayed growth and increased feed conversion ratio (FCR) (Gous, 2018; Vasdal *et al.* 2019). Moreover, lack of uniformity creates many problems in processing plant bringing about greater percent of carcass rejects and an increased rate of carcass defects causing dissatisfaction of the modern distribution network as well as the today's greatly alert consumers (Jacobs, 2016). Therefore, intra-flock uniformity considers as a reliable indicator of the technical or health problems in production chain from the day old breeder up to the broiler delivered to the processing plant (De Jong and Van Riel, 2020; Toudic, 2007).

It is practically accepted that a uniform flock defines as a flock that 90 percent of the bird's live BW falls within a ±10% of the mean of the average flock weight (Boersma *et al.* 2003; Renema *et al.* 2007), but scientifically it claimed that such calculation is not reliable (Gous, 2018; Vasdal *et al.* 2019). Therefore, the topic of intra-flock uniformity in broiler research is still open and warranted further research.

Factually, the basic causes of a diverged body weight (BW), feed intake (FI) and FCR within a flock are still unknown and received little research. There are, however, few reports given some addresses regarding breeder's management, incubation conditions and illegal mixing of chicks from various strains and flocks, early brooding management and sanitary problems as the leading sources of intra flock variation in BW at a specified age (Zuidhof *et al*. 2015; Willson, 2017).

Moreover, a little number of reports has engrossed in the possible adverse effects of intensive genetic selection strategies employed in the line stocks of the commercial broilers with respect to the probable interrelated dysfunctions in certain systems or metabolic pathways (Crespo and Shivaprasad, 2013; Hartcher and Lum, 2020). In other words, certain aspects of BW differences of the birds at a given age in an identical broiler house may originate intrinsically (Parkhurst and Mountney, 2012; Muir and Cheng, 2014). These aspects may eventually realize based on internal factors or their interrelation with the commercial conditions which are approximately practiced alike globally (Butterworth, 2009; Clark *et al*. 2016).

Based on these facts, we hypothesized that flock uniformity may to some degree be connected with the function of certain body systems, such as cardiovascular system, which are playing important roles in the fast growing commercial birds (Azadinia *et al*. 2022). Therefore, the current study aimed at evaluating the effects of a fat soluble vitamin premix on performance indices and hematological variables in a broiler flock with different growth rates and then partitioning the variance in the same performance indices to define the fraction explained by a wide range of the hematological parameters.

MATERIALS AND METHODS

Animal management

A total number of 364 eighteen-day-old broiler chicks were used in this experiment. Birds were selected from a commercial male Ross 308 broiler flock raising in a power ventilated shed where they fed on a starter (0 to 10 days), grower 1 (11 to 20 days), grower 2 (21 to 30 days), finisher 1 (31 to 40 days), and finisher 2 (40 to slaughter age) diet in a pelleted form. The compositions of the diets are described in Table 1. At the end of day 18, based on flock's mean and standard deviation (SD) for body weight (620 and 56 g, respectively) seven experimental treatments were constructed as $\bar{x}$-3SD, $\bar{x}$-2SD, $\bar{x}$-1SD, $\bar{x}$, $\bar{x}$+1SD, $\bar{x}$+2SD and $\bar{x}$+3SD then 52 birds were selected for each body weight group (±5 g) and identified via plastic wing bands.

At the commencement of day 19, the chosen birds were trasfered to 364 battery cages arranged in 24 rows of 14 cage each where they spent three days for adaptation. At the initiation of day 21, birds in each body weight group were randomly shared between two subgroups of 26 birds and one subgroup treated with a fat-soluble multivitamin premix (FSVP) through injection under the skin at the back of the neck. The remainder of the birds continued unhandled. Vitamin injection repeated on days 28 and 35 of age. Each dose contained 63000; 28000; 385 IU of vitamin A, D, and E, respectively as well as 15 mg of vitamin K providing the dietary requirements birds for fat-soluble vitamins in seven days based of catalog released by Ross Company (2022). The experimental design was a randomized complete block design involving 14 treatments with 26 replicates of an individual bird each. The treatments in a 2 × 7 factorial arrangement consisted of seven early growth capability groups with live body weight of $\bar{x}$- 3SD, $\bar{x}$-2SD, $\bar{x}$- 1SD, $\bar{x}$, $\bar{x}$+1SD, $\bar{x}$+ 2SD and $\bar{x}$+ 3SD at day 18 of age with and without a weekly administartion of a fat-soluble multivitamin premix.

This study complied with the animal care and use committee, Lorestan University legislation regarding the use of animals for experimental and other scientific purposes.

Performance data

Individual BW and FI were recorded in days 21 and 42 of age, and the data were used to calculate weight gain (WG), FI and FCR for the same period. Mortality was recorded upon occurrence.

Hematological parameters

At day 42 of age, all the experimental birds were killed by puncturing jugular vein and carotid arteries, scalded, defeathered mechanically, eviscerated manually and evaluated for heart percentage. Before slaughtering, two blood samples of 2 to 3 mL were collected in 5 ml vials with ethylene diamine tetra acetic acid (EDTA anti-coagulant). Blood samples were marked according to wing number of the birds and promptly taken to the laboratory. Within 6 hours, samples were subjected to analyze for cell blood count and automatic hematology analysis (SELECTER E, Netherlands Vital).

Hematological parameters concerned included red blood cell count (Coulter JT, 1988 by Coulter Electronics, America) (RBC, 10^6/mm), hemoglobin (Hb, mg/dL), hematocrit (HCT, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, %), red cell distribution width (RDW, %), platelet count (PLT, per mL), plateletcrit (PCT, ng/mL), mean platelet volume (MPV, fL) and platelet distribution width (PDW, %).

Table 1 Composition of the diets and their approximate analysis

Ingredients (%)	Starter (0-10 day)	Grower 1 (11-20 day)	Grower 2 (21-30 day)	Finisher 1 (31-40 day)	Finisher 2 (41-42 day)
Corn	60.73	60.40	63.59	65.33	69.10
Soybean meal (44 %)	33.94	34.90	31.63	28.94	25.85
Carbonate	1.33	1.3	1.3	0.78	1.37
Dical. phos[1].	1.3	1.3	1.4	1.6	1.6
Soybean oil	1	1.5	1.5	1.8	1.5
Vitamin premix[2]	0.5	0.05	0.05	0.05	0.05
Mineral premix[2]	0.5	0.05	0.05	0.05	0.05
Common salt	0.4	0.2	0.2	0.2	0.2
DL-methionine	0.27	0.11	0.1	0.1	0.08
L-lysine	0.02	0.02	0.04	0.04	0.07
Nutrients					
Metabolism energy (kcal/kg)	2942	3000	3024	3063	3061
Protein (%)	20.43	20.84	19.64	19.15	17.50
Calcium (%)	0.85	0.87	0.88	0.94	0.92
Available phosphate (%)	0.42	0.43	0.43	0.47	0.46
Sodium (%)	0.2	0.12	0.12	0.13	0.13
CL (%)	0.04	0.16	0.16	0.14	0.17
K (%)	1.09	089	0.83	0.79	0.74
Fat (%)	2.47	2.47	2.57	2.63	2.75
Met (%)	0.60	0.44	0.42	0.30	0.37
Met + Cys (%)	0.59	0.61	0.57	0.76	0.51
L-lysine (%)	1.09	1.11	1.05	1.01	0.92
Tryptophan (%)	0.22	0.22	0.21	0.19	0.15
Threonine (%)	0.79	0.81	0.76	0.72	0.68
Arginine	1.29	1.33	1.23	1.15	1.07

[1] Dicalcium phosphate contained: phosphorus: 18% and calcium: 21%.

[2] Vitamin and mineral concentrate supplied per kilogram diet: Retinol: 12000 IU; Cholecalciferol: 5000 IU; Tocopheryl acetate: 75 mg; Menadione: 3 mg; Thiamine:3 mg; Riboflavin: 8 mg; Niacin: 55 mg; Pantothenate: 13 mg; Pyridoxine: 5 mg; Folate: 2 mg; Cyanocobalamin: 16 mg; Biotin: 200 mg; Cereal-based carrier: 149 mg; Mineraloil: 2.5 mg; Cu (sulfate): 16 mg; Fe (sulfate): 40 mg; I (iodide): 1.25 mg; Se (selenate): 0.3 mg; Mn (sulfate and oxide): 120 mg and Zn (sulfate and oxide): 100 mg.

A fresh drop of venous blood from the jugular veins was used to prepare a peripheral blood smear for differential cell count at the slaughtering site. Complete white blood cell counts and differential leukocyte counts were performed through counting at least 200 cell in 20 different high power fields using light microscope to verify changes in absolute numbers of leukocytes, lymphocytes, heterophile, monocytes, eosinophils and basophils (Fair *et al.* 1999). Data were also converted to percentage and used for calculation of heterophile to lymphocytes ratio (H/L ratio) (Gross and Siegel, 1983).

Statistical analysis

The collected data were subjected to analysis of variance using Mixed model procedure in SAS (2002) statistical software. Tukey-Kramer test was used to compare means. For all experiments, the maximum probability of the first type of error was five percent (P>0.05). Pearson's correlation coefficients and tolerance values were calculated using the Correlation and Regression procedures, respectively, in the same software.

Multivariate stepwise liner regression analysis in the same software was also used to extract the share of hematological parameters as a source of the intra-flock variance in final body weight at day 42 and FI as well FCR during age of 21 to 42 days (Mendes, 2011; Adedibu *et al.* 2014).

RESULTS AND DISCUSSION

Weight gain during the days 21 to 42 and mean BW at day 42 of age was differed among the birds but not in the same pattern as the BW at day 21. The birds with an initial BW (21 d) lesser than the population mean grew faster (proportional to their initial weight) than those having a BW greater than the population mean in day 21 of age. Birds with -3, -2 and -1 standard deviation (SD) below the population mean achieved 3.71, 3.41 and 3.52 fold greater weight gain compared with their own initial BW (21 d), respectively, while those with +1, +2 and +3 standard error above the population mean gained 3.13, 2.91 and 2.92 fold, respectively (P<0.05; Table 1). Feed intake during days 21 to 42 of age differed among the birds in the same trend as the initial differences in BW (21 d) from the population mean.

Feed conversion ratio during days 21 to 42 of age was also influenced by the initial body weight at 21d of age but not in the same trend as the early BW at day 21 ($P<0.05$; Table 2). The birds receiving the FSVP gained greater BW, consumed more feed and converted the feed with a greater efficiency than those getting no vitamin treatment during the experimental period ($P<0.05$; Table 2).

Mean heart weight was significantly lesser in the birds with initial BW close to the mean population BW compared with those with an early BW lesser and greater than the population mean ($P<0.05$; Table 3). The same trend was also observed for RBC count, Hb, HCT and MCV but the differences were not significant ($P>0.05$). In other words, the birds with 1, 2 and 3 SD below or above the initial population mean exhibited greater values for all of the above mentioned parameters than those with a BW equal to the population mean at day 21 of age. In the contrary, the birds with a BW close to the mean population BW at day 21 of age, demonstrated greater mean platelet volume (MPV) and platelet distribution width (PDW) in day 42 of age compared with those showing an initial BW of 1, 2 or 3 SD below or above the population mean (Table 3).

Early growth rate in terms of BW at day 21 of age did not influence MCH, MCHC, RDW, PLT, PCT in broiler chicken at day 42 of age ($P>0.05$). Weekly injection of FSVP increased heart weight by 20.48 percent and decreased MCHC and PCT by 3.21 and 33.3 percent, respectively, than the birds did not receive the same treatment ($P>0.05$). Mean MCV was greater by 3.3 percent in the FSVP-treated birds than the untreated birds ($P<0.05$; Table 3).

The broiler chickens with an initial BW equal to the population mean showed a greater heterophile percentage than almost all other birds with an initial BW of 1, 2 or 3 SD below or above the population mean. Heterophile count was significantly increased in the birds receiving the FSVP by 2.5 fold ($P<0.05$; Table 4). No hematological parameter, with the single exception of heterophile count, affected by the IBW × FSVP interaction effect at day 42 of age ($P>0.05$; Table 4). Heterophile count decreased in the birds with an initial body weight of 3 and 2 SD below the population mean while increased in those with an initial body weight close to or above the population mean ($P<0.05$; Table 5).

Body weight at marketing age (42 d) showed, among all hematological variables, a negative moderate correlation with RDW while WG during days 21 to 42, was weakly associated with Hb, HCT, MCV, RDW and MPV showing the correlation coefficients ranging from -0.149 to -0.199. Feed intake showed no correlation with hematological variables, with the exception of RDW and PDW where demonstrated the coefficients of -0.248 and 0.168, respectively (Table 6). Feed conversion ratio, among all performance indices, showed greater and mainly positive associations with either RBC- or Platelet- related variables. However, moderate correlation coefficients were estimated for FCR and Hb, HCT, MCV, PLT, PCT and MPV (Table 6). No association was found between heart weight and hematological parameters excluding MCV and MPV which showed correlation coefficients of 0.228 and -0.252, respectively (Table 6).

Hematological parameters showed also no significant correlation with WBC differential count excepting MCHC which it found to be moderately correlated with basophil count ($r=-0.232$) With the exception of heterophile and basophil count, WBC count showed a diverged coefficients of correlations with WBC-related variables ranging from -0.211 to 0.424 and from –0.254 to 0.353, respectively (Table 7).

Red blood cell count, Hb and HCT were highly positively correlated but other RBC related hematological variables exhibited mainly positive and low to moderate coefficients, with the exception of MCV showing moderate to high correlation coefficients of 0.577, -0.604 and 0.40 with MCH, MCHC and RDW, respectively (Table 7). In general, platelet related variables (PCT, MPV and PDW) showed no correlation with RBC related variables while among themselves they were found to be highly positively correlated with coefficients ranging from 0.533 to 0.931 (Table 7).

Tolerance statistic as the main multicollinearity diagnostic was lesser than 0.1 for all performance indices considered (Table 8). Among hematological parameters, the lowest tolerance values were found for RBC-related parameters, with the exception of RDW ($T=0.555$), while platelet-related variables exhibited greater tolerance values ranging from 0.03 to 0.09 (Table 8). Step-wise multivariate regression analysis, including all hematological parameters concerned provided poor models for partitioning of final BW, FI and in particular, FCR variance (Table 8). As the key predictor, heart weight alone explained 38.73, 39.07 and 8.57 percent of the variation in body weight (42 d), feed intake and FCR during days 21 to 42 days of age, respectively (Table 8). Hemoglobin concentration, RDW, MCV and eosinophil percent were the four succeeding variables which collectively explained about 18 percent of the final BW variance. Red blood cell count was the second predicting variable for FI and FCR prediction accounting for 6.77 and 4.83 percent of the variability in these traits, respectively (Table 9).

The uniqueness of the present study lies in exploring the role of hematological parameters in intra-flock variance of performance indices through multivariate analysis, however, some results on the effects of early growth rate and FSVP on parameters concerned were also interesting.

Table 2 Mean body weight at day 21 and 42 of age (BW; g), weight gain (WG; g), feed intake (FI; g) and feed conversion ratio (FCR) during days 21 to 42 of age in broiler chickens differed in initial body weight at 21d of age and received weekly injection of a fat soluble vitamins premix[1] during days 21 to 42 of age

Factor/level	BW, 21 d	BW, 42 d	WG, 21-42 d	FI, 21-42 d	FCR, 21-42 d
Initial body weight (IBW; g)					
$\bar{X}$-3σ	396.96^{g}	1473.30^{f}	1078.17^{c}	1895.64^{e}	2.071^{a}
$\bar{X}$-2σ	456.40^{f}	1556.09^{ef}	1096.00^{c}	2168.50^{d}	2.047^{ab}
$\bar{X}$-1σ	555.54^{e}	1955.15^{e}	1396.46^{b}	2443.14^{d}	1.929^{c}
$\bar{X}$	646.61^{d}	2048.75^{d}	1402.14^{b}	2684.25^{c}	1.973^{bc}
$\bar{X}$+1 σ	713.56^{c}	2234.73^{c}	1521.60^{ab}	2915.06^{b}	2.045^{ab}
$\bar{X}$+2 σ	794.56^{b}	2311.64^{b}	1517.36^{ab}	2939.87^{b}	2.075^{a}
$\bar{X}$+3 σ	858.72^{a}	2511.43^{a}	1651.14^{a}	3115.87^{a}	1.971^{bc}
SEM	11.142	52.278	43.118	66.013	0.045
Fat soluble vitamin premix (FSVC mL/bird/injection)					
0	638.34	1763.08^{b}	1136.64^{b}	2355.23^{b}	2.193^{a}
1.2	642.80	2467.80^{a}	1800.50^{a}	3018.76^{a}	1.728^{b}
SEM	9.408	54.895	47.467	96.201	0.034
ANOVA results					
IBW	0.0001	0.0001	0.0014	0.0001	0.0432
FSVP	0.0072	0.0001	0.0001	0.0001	0.0001
IBW × FSVP	0.7422	0.1565	0.1241	0.0897	0.8764

[1] Each injection (repeated at days 21, 28 and 35 of age) contained 63000 units of vitamin A, 28000 units of vitamin D, 385 units of vitamin E and 15 mg of vitamin K accounting for the bird's requirements for all fat-soluble vitamin for 7 days.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Mean heart weight and hematology parameters[1] in broiler chickens differed in initial body weight at 21 d of age and received weekly injection of a fat soluble vitamins premix during days 21 to 42 of age

Factor/level	HW (g)	RBC (10^6/mm)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (10^3/mm)	PCT (%)	MPV (fL)	PDW (%)
Initial body weight (IBW; g)												
$\bar{X}$-3σ	10.54	2.04	11.77	30.43	149.06	57.77	38.81	11.07	4.92	0.002	3.94	15.36
$\bar{X}$-2σ	10.94	2.18	12.92	33.09	151.00	58.94	39.07	11.66	4.64	0.002	3.93	15.04
$\bar{X}$-1σ	10.99	1.92	11.19	28.61	149.05	58.30	39.16	11.66	6.15	0.003	4.13	16.10
$\bar{X}$	9.99	1.80	10.52	26.32	144.89	58.05	40.12	10.64	5.87	0.002	4.37	16.36
$\bar{X}$+1σ	12.32	2.12	12.08	30.67	145.01	57.11	39.41	10.95	5.54	0.003	3.88	14.85
$\bar{X}$+2σ	11.95	2.06	11.82	29.56	143.69	57.59	40.16	11.01	3.58	0.002	3.96	14.66
$\bar{X}$+3σ	12.47	2.06	12.03	30.31	146.76	58.33	39.79	11.04	8.69	0.003	3.78	14.49
SEM	0.241	0.041	0.245	0.643	0.751	0.247	0.176	0.124	0.625	0.000	0.074	4.250
Fat soluble vitamin premix (FSVP; mL/bird/ injection)												
0	10.64^{b}	2.03	11.79	29.61	145.54^{b}	58.00	39.90^{a}	11.23	6.17	0.003^{a}	4.07	15.52
1.2	12.82^{a}	1.98	11.50	29.92	150.35^{a}	58.01	38.62^{b}	10.87	4.50	0.002^{b}	3.88	29.12
SEM	0.327	0.062	0.373	1.012	1.005	0.406	0.274	0.175	0.844	0.000	0.107	7.263
ANOVA results												
IBW	0.0366	0.5121	0.4992	0.4421	0.2417	0.5126	0.5513	0.7340	0.4259	0.7939	0.4923	0.0686
FSVP	0.0001	0.7521	0.6737	0.7487	0.0056	0.9461	0.0023	0.1864	0.0584	0.0165	0.3497	0.1792
IBW × FSVP	0.6063	0.0867	0.0696	0.1012	0.7069	0.2427	0.7187	0.9528	0.6010	0.9229	0.9526	0.0574

HW: heart weight; RBC: red blood cell; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; PLT: platelet; PCT: plateletcrit; MPV: mean platelet volume and PDW: platelet distribution width.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Percentage of white blood cells in broiler chickens differed in initial body weight at 21 d of age and received weekly injection of a fat soluble vitamins premix during days 21 to 42 of age

Factor/level	Lymphocyte	Monocyte	Heterophil	Basophil	Eosinophil
Initial body weight, (IBW, g)					
$\bar{X}$-3σ	57.75	30.76	2.19^{d}	5.58	5.12
$\bar{X}$-2σ	58.88	27.76	2.57^{d}	7.09	4.48
$\bar{X}$-1σ	57.80	23.00	11.85^{a}	6.80	5.61
$\bar{X}$	59.24	25.71	12.02^{a}	4.88	4.18
$\bar{X}$+1σ	60.21	25.42	4.97^{c}	6.21	5.02
$\bar{X}$+2σ	53.86	28.21	7.30^{b}	10.09	4.51
$\bar{X}$+3σ	50.71	35.56	6.48b^{c}	9.69	3.28
SEM	1.684	1.530	1.302	0.790	0.382
Fat soluble vitamin premix (FSVP; mL/bird/ injection)[1]					
0	58.12	28.58	4.81^{b}	6.06	4.80
1.2	54.34	26.31	10.16^{a}	9.51	4.15
SEM	2.700	2.467	2.051	1.295	0.529
ANOVA results					
IBW	0.8793	0.4243	0.0029	0.2191	0.7310
FSVP	0.2152	0.4822	0.0036	0.0560	0.8426
IBW × FSVP	0.6120	0.4380	0.0094	0.5482	0.9595

[1] Each injection (repeated at days 21, 28 and 35 of age) contained 63000 units of vitamin A, 28000 units of vitamin D, 385 units of vitamin E and 15 mg of vitamin K accounting for the bird's requirements for all fat-soluble vitamin for 7 days.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Mean heterophile count in day 42 of age for the interaction effect of initial body weight (IBW; g) at 21 d into injection of a fat soluble vitamins premix (FSVP)[1]

IBW; g	FSVP (mL/bird/injection)	Heterophile
$\bar{X}$-3σ	0	3.43^{e}
$\bar{X}$-3σ	1.2	1.94^{f}
$\bar{X}$-2σ	0	2.39^{ef}
$\bar{X}$-2σ	1.2	1.67^{e}
$\bar{X}$-1σ	0	6.91d^{e}
$\bar{X}$-1σ	1.2	10.44^{b}
$\bar{X}$	0	9.48^{bc}
$\bar{X}$	1.2	12.79^{a}
$\bar{X}$+1σ	0	4.52^{e}
$\bar{X}$+1σ	1.2	4.12^{e}
$\bar{X}$+2σ	0	9.49b^{c}
$\bar{X}$+2σ	1.2	10.54^{b}
$\bar{X}$+3σ	0	3.75^{e}
$\bar{X}$+3σ	1.2	6.95^{de}
SEM		2.942
ANOVA results		
IBW × FSVC		0.0094

[1] Each injection (repeated at days 21, 28 and 35 of age) contained 63000 units of vitamin A, 28000 units of vitamin D, 385 units of vitamin E and 15 mg of vitamin K accounting for the bird's requirements for all fat-soluble vitamin for 7 days.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Pearson correlation coefficients among performance indices, heart weight and hematology characteristics in broiler chickens differed in initial body weight at 21 d of age and received weekly injection of a fat soluble vitamins premix during days 21 to 42 of age

Item	BW (g), 42 d	WG (g), 21-42 d	FI (g), 21-42 d	FCR, 21-42 d	Lymphocyte	Monocyte	Hetrophile	Basophil	Eozinophil	HW (g)
BW (g), 42 d	1	0.960**	0.929**	-0.548**	-0.004^{ns}	0.056^{ns}	0.114^{ns}	0.097^{ns}	-0.092^{ns}	0.644**
WG (g), 21-42 d		1	0.887**	-0.677**	-0.014^{ns}	0.034^{ns}	0.089^{ns}	0.054^{ns}	-0.099^{ns}	0.645**
FI (g), 21-42 d			1	-0.329**	-0.005^{ns}	0.017^{ns}	0.075^{ns}	0.054^{ns}	-0.075^{ns}	0.613**
FCR, 21-42 d				1	-0.008^{ns}	-0.038^{ns}	-0.079^{ns}	0.036^{ns}	0.096^{ns}	-0.397**
Lymphocyte					1	0.088^{ns}	0.424**	0.049^{ns}	-0.091^{ns}	-0.052^{ns}
Monocyte						1	-0.211*	- 0.254**	0.153 ^{ns}	-0.036^{ns}
Hetrophile							1	0.353**	-0.024^{ns}	0.007^{ns}
Basophil								1	-0.109^{ns}	0.126^{ns}
Eozinophil									1	-0.033^{ns}
HW (g)										1
RBC (10^6/mm)	- 0.145^{ns}	-0.199**	-0.114^{ns}	0.299**	0.075^{ns}	-0.034^{ns}	0.058^{ns}	0.086^{ns}	-0.085^{ns}	0.004^{ns}
Hb (g/dL)	-0.137^{ns}	-0.179*	-0.105^{ns}	0.261**	0.056^{ns}	-0.034^{ns}	0.029^{ns}	0.030^{ns}	-0.083^{ns}	0.029^{ns}
HCT (%)	-0.122^{ns}	-0.149*	-0.099^{ns}	0.228**	0.045^{ns}	-0.049^{ns}	0.049^{ns}	0.078^{ns}	-0.079^{ns}	0.057^{ns}
MCV (fL)	0.067^{ns}	0.166*	0.027^{ns}	-0.221**	-0.144^{ns}	-0.067^{ns}	-0.008^{ns}	0.036^{ns}	0.008^{ns}	0.228**
MCH (pg)	0.029^{ns}	0.083^{ns}	0.032^{ns}	-0.127^{ns}	-0.129^{ns}	-0.019^{ns}	-0.122^{ns}	-0.193*	-0.007^{ns}	0.122^{ns}
MCHC (g/dL)	-0.049^{ns}	-0.113^{ns}	-0.001^{ns}	0.134^{ns}	0.058^{ns}	0.056^{ns}	-0.104^{ns}	-0.232**	-0.0018^{ns}	-0.144^{ns}
RDW (%)	-0.250**	-0.191*	-0.248**	0.015^{ns}	-0.044^{ns}	-0.084^{ns}	-0.049^{ns}	0.074^{ns}	-0.057^{ns}	0.029^{ns}
PLT (10^3/mm)	-0.026^{ns}	-0.051^{ns}	0.034^{ns}	0.169*	-0.082^{ns}	0.003^{ns}	-0.021^{ns}	-0.024^{ns}	-0.044^{ns}	-0.075^{ns}
PCT (%)	-0.080^{ns}	-0.104^{ns}	-0.013^{ns}	0.242**	-0.050^{ns}	0.042^{ns}	-0.019^{ns}	-0.005^{ns}	-0.020^{ns}	-0.131^{ns}
MPV (fL)	-0.128^{ns}	-0.176*	-0.121^{ns}	0.199*	-0.049^{ns}	0.008^{ns}	0.032^{ns}	0.125^{ns}	-0.043^{ns}	-0.252**
PDW (%)	0.154*	0.148^{ns}	0.168*	-0.036^{ns}	-0.025^{ns}	-0.079^{ns}	-0.041^{ns}	0.017^{ns}	0.076^{ns}	0.026^{ns}

BW: body weight; WG: weight gain; FI: feed intake; FCR: feed conversion ratio; HW: heart weight; RBC: red blood cell; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; PLT: platelet; PCT: plateletcrit; MPV: mean platelet volume and PDW: platelet distribution width, during days 21 to 42.
* ($P<0.05$) and ** ($P<0.01$).
NS: non significant.

Table 7 Pearson correlation coefficients among hematology characteristics in broiler chickens differed in initial body weight at 21 d of age and received weekly injection of a fat soluble vitamins premix during days 21 to 42 of age

Item	RBC (10^6/mm)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (10^3/mm)	PCT (%)	MPV (fL)	PDW (%)
RBC (10^6/mm)	1	0.979**	0.975**	0.159*	0.059^{ns}	-0.134^{ns}	-0.074^{ns}	-0.004^{ns}	-0.013^{ns}	0.005^{ns}	-0.024^{ns}
Hb (g/dL)		1	0.979**	0.262**	0.257**	-0.062^{ns}	-0.038^{ns}	0.006^{ns}	-0.007^{ns}	-0.025^{ns}	-0.047^{ns}
HCT (%)			1	0.369 **	0.183*	-0.256**	0.021^{ns}	0.014^{ns}	0.004^{ns}	-0.021^{ns}	-0.047^{ns}
MCV (fL)				1	0.577**	-0.604**	0.400**	0.099^{ns}	0.097^{ns}	-0.099^{ns}	-0.112^{ns}
MCH (pg)					1	0.301**	0.171*	0.054^{ns}	0.023^{ns}	-0.119^{ns}	-0.104^{ns}
MCHC (g/dL)						1	-0.283**	-0.066^{ns}	-0.092^{ns}	-0.000^{ns}	0.028^{ns}
RDW (%)							1	0.080^{ns}	0.092^{ns}	-0.048^{ns}	-006^{ns}
PLT (10^3/mm)								1	0.919**	0.403**	0.356**
PCT (%)									1	0.533**	0.480**
MPV (fL)										1	0.931**
PDW (%)											1

RBC: red blood cell; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; PLT: platelet; PCT: plateletcrit; MPV: mean platelet volume and PDW: platelet distribution width, during days 21 to 42.
* ($P<0.05$) and ** ($P<0.01$).
NS: non significant.

Firstly, heart weight, RBC count, Hb, HCT and MCV were lesser in the birds having a BW close to the population mean compared to those with a body weight lesser or greater than the population mean. Early growth rate, in terms of "population mean ± a determined SD" reflecting growth potential of a bird and influences by vast number of intrinsic as well as extrinsic factors. In spite of a continuous range in body weight data, these results confirm that greater BW while reflects greater growth potential rate, is not essentially equal to greater health too, an idea which already reported by (Hastings, 2013). It seems that all birds with a BW below or above the population mean are suffering from a certain assortment of stressors forcing them for greater metabolic rate which in turn enforces for a greater heart rate. In the birds with a mean BW above the population mean such distresses may originate form intrinsic sources such as greater metabolic demand for nutrients while for those below the population mean may arise from extrinsic factors such as competition with herdmates. Moreover, we believe that, population mean as a statistical figure, has a distinct biological implication as "greater health and welfare". All birds with a BW below or above the corresponding population mean suffering from certain health problems and distress sources which may be physiological, environmental, or social. Likewise, a bird with a BW below the population mean (in terms of SD) agonizes from certain adverse effects from a complexity of such factors so that those with an initial BW of $\bar{x}$-3SD will never be able to grow such greater to inter the upper class where birds obtaining BW of $\bar{x}$-2SD.

On the other side of the BW distribution, the birds with an initial body weight of $\bar{x}$+3SD are enjoying form a possible combination of the favorite factors providing opportunities for demonestrating a greater growth rate (not necessarily health and welfare). Their superioritiy in BW is so prominent that no bird from the lower BW classess even those with an initial BW of $\bar{x}$+2SD can not demonstrate the same performance in the advanced ages. Therefore, we want to emphasis that growth rate boundaries of $\bar{x}$±nSD reflect biological limitations and convey physiological concepts (Convey, 1987; Nicol, 2015; Grandin and Whiting, 2018). Unfortunately, in poultry production such concepts have received very less concern, while in human medicine they are basis for certain health assements and even diseaes diagnosis and treatment decisions. For instance, infant cases with a birth weight below $\bar{x}$-2SD are considered as abnormal (Rochow *et al.* 2019) and they have to receive medical assessment and follow up. The same concept has been implemented for explanation of many other human health problems (Rosenthal *et al.* 2017).

Our results reveal that the concepts of "bird's health and welfare" and "bird's profitability" may not align with the exception of the mean population point. With increasing demands from animal supporters (Warleigh, 2001), broiler industry may be forced to adopt certain rules against driving the birds for as greater growth rate as possible where physiological distress increasingly confronts their welfare and impair their health. Such regulations may exert promising effects on intra-flock uniformity in performance indices as well as carcass features of the broilers.

Secondly, the well formulated practical commercial diets are anticipated to comprise all vitamins needed to support the growth in the fast broiler chicks. Vitamin premixes are also added to these diets to ensure the vitamin needs coverage. However, in the present study weekly parenteral injection of a fat soluble multivitamin preparation dramatically improved BW and influenced confidently almost all other variables in either slow- or fast- growing birds. These finding create thoughtful doubts against vitamin contents of the feeds and premixes or the current vitamin recommendations for commercial broiler chicks. They also generate uncertainties against the current information on vitamin uptake in bird's gut under commercial conditions. It was shown that diet ingredients may exhaust form vitamin D (Mattila *et al.* 2011) and probably other fat soluble vitamins during preservation, in particular, in hot climates and under inappropriate storage conditions (McDowell and Ward, 2008). Vitamin premixes may also undergo the same deleterious effects during storage (Zhuge and Klopfenstein, 1986).

On the other hand, research results frequently emphasis that absorption of a fat soluble vitamin may affect by many factors including feed composition (Polycarpo *et al.* 2016), bird's health (Yuan *et al.* 2014), gut microbial flora (Goncalves *et al.* 2015), among many others which they impose different impacts on the individual birds within a single flock. Therefore, fat soluble vitamins bioavailability may receive concern as a source of intra-flock diversity in productive performance indices in commercial broilers.

Thirdly, we evaluated the association among production indices and hematological parameters in different ways. In the first step, bivariate product-moment correlation coefficient as a normalized measurement of the covariance (Aubinet *et al.* 2012) were calculated. Results showed that no performance index is strongly correlated with a hematological parameter excluding heart weight which found to be highly associated with BW, WG and FI but not with FCR. Therefore, based on these correlation values, hematological parameters mostly cannot be considered as a reliable source of information for prediction of performance indices in commercial flocks.

However, as with covariance itself, correlation coefficient value can only reflect a linear association of variables and ignores many other types of relationship or correlation (Yu *et al.* 2019).

Table 8 The tolerance estimation[1] to check the collinearity using certain performance indices[2], hematological parameters[2] in broiler chicken

Variable	Parameter estimate	Standard error	t-value	Pr > |t|	Tolerance	Variance inflation
BW (g), 42 d	1.24935	0.18815	6.64	< .0001	0.02074	48.207
WG (g), 21-42 d	-0.00071	0.10280	-0.01	0.9945	0.03471	28.807
FI (g), 21-42 d	229.41941	120.73847	1.90	0.0623	0.09790	10.214
HW (g)	13.32459	7.52400	1.77	0.0817	0.42792	2.336
RBC (10^6/mm)	1160.74449	809.83110	1.43	0.1570	0.00123	812.892
Hb (g/dL)	-239.40789	232.60437	-1.03	0.3076	0.00040	2472.014
HCT (%)	20.54658	71.82879	0.29	0.7758	0.00064	1539.392
MCV (fL)	-12.16391	55.50170	-0.22	0.8273	0.00086	1154.121
MCH (pg)	48.75260	135.17884	0.36	0.7196	0.00171	583.595
MCHC (g/dL)	-13.30521	175.84700	-0.08	0.9399	0.00234	427.779
RDW (%)	-13.73368	14.00753	-0.98	0.3309	0.55578	1.799
PLT (10^3/mm)	25.31231	9.21637	2.75	0.0080	0.05132	19.486
PCT (%)	-66708	28042	-2.38	0.0206	0.03881	25.763
MPV (fL)	188.18535	70.70280	2.66	0.0100	0.08603	11.624
PDW (%)	-36.30264	24.00934	-1.51	0.1359	0.09754	10.252
Lymphocyte	-7.63121	25.41720	-0.30	0.7651	0.00086	1152.013
Monocyte	-6.47502	25.48786	-0.25	0.8003	0.00094	1057.918
Heterophile	-6.16720	25.30127	-0.24	0.8083	0.00358	279.545
Basophil	-6.02548	25.72164	-0.23	0.8156	0.00462	216.274
Eosinophil	-10.05195	26.02527	-0.39	0.7007	0.01139	87.781

[1] The tolerance test was conducted using a without intercept model where body weight at day 24 of age was considered as independent variable.
[2] BW: body weight; WG: weight gain; FI: feed intake; HW: heart weight; RBC: red blood cell; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; PLT: platelet; PCT: plateletcrit; MPV: mean platelet volume and PDW: platelet distribution width.

Table 9 Multivariate linear regression analysis using a stepwise model for partitioning of body weight at day 42 of age and feed intake as well as feed conversion ratio in broiler chickens during days 21 to 42 of age

Step	Variable entered[1]	Partial R-Square	Model R-Square	C (P)	F-value	Pr > F
Body weight, 42 d						
1	HW (g)	0.3873	0.3873	29.0698	49.30	< 0.0001
2	Hb (g/dL)	0.0615	0.4487	20.5300	8.59	0.0045
3	RDW (%)	0.0401	0.4888	15.6551	5.96	0.0170
4	MCV (fL)	0.0458	0.5346	9.8045	7.38	0.0082
5	Eosinophile	0.0321	0.5667	6.3045	5.48	0.0220
Feed intake, 21 to 42 d						
1	HW (g)	0.3907	0.3907	20.9730	50.01	< 0.0001
2	RBC (10^6/mm)	0.0677	0.4584	12.1972	9.63	0.0027
3	RDW (%)	0.0274	0.4858	9.8311	4.06	0.0476
4	Eosinophile	0.0324	0.5182	6.6775	5.04	0.0277
5	HCT (%)	0.0205	0.5387	5.4216	3.28	0.0741
6	RDW (%)	0.0132	0.5519	5.3191	2.15	0.1467
Feed conversion ratio, 21 to 42 d						
1	HW (g)	0.0857	0.0857	7.8340	7.32	0.0084
2	RBC (10^6/mm)	0.0483	0.1340	5.4076	4.29	0.0416
3	MCV (fL)	0.0607	0.1947	1.8430	5.73	0.0192
4	HCT (%)	0.0424	0.2372	-0.0495	4.17	0.0446
5	Monocyte	0.0246	0.2617	-0.3037	2.46	0.1207

HW: heart weight; Hb: hemoglobin; RDW: red cell distribution width; MCV: mean corpuscular volume; RBC: red blood cell; HCT: hematocrit and PDW: platelet distribution width.

In the second step, we extracted the linear dependency among the independent variables, a phenomenon known as multicollinearity (Mela and Kopalle, 2002). There are many methods to estimate the collinearity between the predictors in a multivariate regression model (Jensen and Ramirez, 2013).

In the current study tolerance statistic was used for the same purpose and results attain the independency condition indicated by no parameter exhibiting a tolerance value greater than 1, which considers one of the conditions to make a reliable regression model (Osborne and Waters, 2002).

Finally, step-wise multivariate regression analysis was employed to describe the relationship between the hematological parameters as dependent variables and performance indices as independent variables. In general, no strong model for prediction of either BW or FI and FCR was provided using the hematological variables. Results, however, indicated that heart weight, Hb concentration and RBC count can explain a significant proportion of the intra-flock variation in BW at marketing age, FI and FCR during ages of 21 to 42 days in broiler chicken. As expected, hematocrit percent, and platelet related parameters were not well correlated with body weight at day 42. Step-wise regression analysis also included MCV in the models to predict BW (42 d) and FCR, but they only explained a minor fraction of their variance. Red blood cell distribution width was selected as the third predictor in both BW and FI models, where it accounted for approximately 4 and 3 percent of the intra-flock variability in these indices, respectively. It was shown that RDW is a decisive biomarker associated with certain pathological conditions in human cases (Ycas, 2017) but corresponding data on reliability of such association in chicken are not found.

As mentioned above, the results provide convinced clues for possible involvement of certain circulatory-related variables such as heart weight in final outcome of the growth-related gens. Nevertheless, no evidence based practical recommendations is available for improving heart weight through dietary manipulations or environmental practices aimed at a more uniform broiler flock. Many research results revealed no significant effect of dietary treatments and environmental alterations on a wide range of the hematological parameters (Hernawan, 2014; Mohammadalipour *et al.* 2017; El-Kholy *et al.* 2018), in particular hemoglobin, packed cell volume (PCV), differential leukocyte count (Maroufyan *et al.* 2010). Therefore, a considerable fraction of the defined share of hematological variables in BW, FI and FCR variation may arise inherently. This fraction is tightly allied with the genetic contextual of the birds which has already been strictly congregated for as much growth rate as possible in previous generations. However, it is nowadays possible to precisely evaluate heart dimensions and function and accurately predict the heart weight of the birds via noninvasive imaging modalities. Therefore, such data as selection criteria can feasibly be included in formulation of the genetic programs in parental line flocks. Subsequent studies to further elucidate relationship between the hematological parameters and the intra-flock dissimilarities in the common performance indices for broiler chicken are warranted. Such research may reveal further the adverse effects of the intensified genetic selection strategies for performance indices on other aspects of the bird's life in parental flocks.

CONCLUSION

Broiler chicks with an early BW of $\bar{x}$-3SD were mostly not able to exhibit a greater growth rate for being admitted in the next BW category ($\bar{x}$-2SD) in later ages probably due to a range of intrinsic limitations. Weekly injection of an extra nutritional dosage of a fat soluble vitamin premix dramatically improved performance in the broilers fed on a well formulated diet. This outcome signifies necessity for re-evaluation of the fat soluble vitamins in poultry feeds and diet ingredients. The routine hematological parameters including directly or indirectly assessed RBC, WBC and platelet-related parameters were not able to explain a considerable fraction of the intra-flock variance exhibited in BW at marketing age and FI as well as FCR during days 21 to 42 of age in broiler chicken. However, the role of heart weight, Hb, and RBC count cannot be ignored since they were selected in the multivariate regression models and explained a significant fraction of the variation in these performance indices.

ACKNOWLEDGEMENT

Special thanks to Mr. S. Valikhanei, director, Pardis Badeih farm, for generous assistance in providing all facilities needed in the pre-experimental phase and Dr. E. Rafiei Alavei, Central pathobiology, Khorramabad, Lorestan, Iran, for his assistance in hematological analysis.

REFERENCES

Adedibu I.I., Ayorinde K. and Musa A.A. (2014). Multifactorial analyses of morphological traits of extensively reared helmeted guinea fowls (*Numidia meleagris*) in kaduna and katsina states of Nigeria. *British J. Appl. Sci. Technol*. **4,** 3644-3652.

Aubinet M., Vesala T. and Papale D. (2012). Eddy Covariance: A Practical Guide to Measurement and Data Analysis. Springer, Germany.

Azadinia B., Khosravinia H., Masourei B. and Parizadian Kavan B. (2022). Effects of early growth rate and fat-soluble vitamins on glucose tolerance, feed transit time, certain liver and pancreas related parameters and their share in intra-flock variation in performance indices in broiler chicken. *Poult. Sci.* **101(5),** 101783-101791.

Boersma S.I., Robinson F.E., Renema R.A. and Fasenko G.M. (2003). Administering oasis hatching supplement prior to chick placement increases initial growth with no effect on body weight uniformity of female broiler breeders after three weeks of age. *J. Appl. Poult. Res.* **12,** 428-434.

Butterworth A. (2009). Animal welfare indicators and their use in society. Pp. 371-389 in Welfare of Production Animals: Assessment and Management of Risks. F.J.M. Smulders, Ed., Food Safety Assurance and Veterinary Public Health Series, Sumas, Washington, USA.

Clark B., Stewart G.B., Panzone L.A., Kyriazakis I. and Frewer L.J. (2016). A systematic review of public attitudes, perceptions and behaviors towards production diseases associated with farm animal welfare. *J. Agric. Environ. Ethics.* **29,** 455-478.

Convey E.M. (1987). Growth research: Challenges and opportunities. *Anim. Sci. J.* **65,** 128-139.

Crespo R. and Shivaprasad H.L. (2013). Developmental, metabolic, and other noninfectious disorders. *Dis. Poult.* **4,** 1233-1270.

De Jong I.C. and Van Riel J.W. (2020). Relative contribution of production chain phases to health and performance of broiler chickens: A field study. *Poult. Sci. J.* **99,** 179-188.

El-Kholy M.S., El-Hindawy M.M., Alagawany M., Abd El-Hack M.E. and El-Sayed S.A. (2018). Use of acetylsalicylic acid as an allostatic modulator in the diets of growing Japanese quails exposed to heat stress. *J. Therm. Biol.* **74,** 6-13.

Fair J.M., Hansen E.S. and Ricklefs R.E. (1999). Growth, developmental stability and immune response in juvenile Japanese quails. *(Coturnix coturnix japonica*). *Proc. Biol. Sci.* **266,** 1735-1742.

Feddes J.J., Emmanuel E.J. and Zuidhoft M.J. (2002). Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities. *Poult. Sci. J.* **81,** 774-779.

Goncalves A., Roi S., Nowicki M., Dhaussy A., Huertas A., Amiot M.J. and Reboul E. (2015). Fat-soluble vitamin intestinal absorption: absorption sites in the intestine and interactions for absorption. *Food Chem.* **172,** 155-160.

Gous R.M. (2018). Nutritional and environmental effects on broiler uniformity. *World's Poult. Sci. J.* **74,** 21-34.

Grandin T. and Whiting M. (2018). Are We Pushing Animals to their Biological Limits? Welfare and Ethical Implication. CABI, USA.

Gross W.B. and Siegel H.S. (1983). Evaluation of the heterophile / lymphocyte ratio as a measure of stress in chickens. *Avian Dis.* **27,** 972-979.

Hartcher K.M. and Lum H.K. (2020). Genetic selection of broilers and welfare consequences: a review. *World's Poult. Sci. J.* **76,** 154-167.

Hastings A. (2013). Population Biology: Concepts and Models. Springer, Germany.

Hernawan E. (2014). Effect of banana peel application in ration on hematological level, nitrogen retention and body weight gain of heat exposed broiler chicken. *Sci. Papers Ser. D. Anim. Sci*. **42,** 101-107.

Jacobs L. (2016). Road to better welfare-Welfare of broiler chickens during transportation. PhD Thesis. Ghent Univ., Ghent, Belgium.

Jensen D.R. and Ramirez D.E. (2013). Revision: Variance inflation in regression. *Adv. Dec. Sci.* **2013,** 1-15.

Maroufyan E., Kasim A., Hashemi S.R., Loh T.C. and Bejo M.H. (2010). Responses of performance and differential leukocyte count to methionine and threonine supplementations on broiler chickens challenged with infectious bursal disease in tropical condition. *Asian J. Biol. Sci*. **3,** 68-76.

Mattila P.H., Valkonen E. and Valaja J. (2011). Effect of different vitamin D supplementations in poultry feed on vitamin D content of eggs and chicken meat. *J. Agric. Food Chem.* **59,** 8298-8303.

McDowell L.R. and Ward N.E. (2008). Optimum vitamin nutrition for poultry. *Int. Poult. Prod.* **16,** 29-36.

Mela C.F. and Kopalle P.K. (2002). The impact of collinearity on regression analysis: the asymmetric effect of negative and positive correlations. *Appl. Econ.* **34,** 667-677.

Mendes M. (2011). Multivariate multiple regression analysis based on principal component scores to study relationship between some pre- and post- slaughter traits of broilers. *Agric. Sci. J.* **17,** 77-83.

Mohammadalipour R., Rahmani H.R., Jahanian R., Riasi A., Mohammadalipour M. and Nili N. (2017). Effect of early feed restriction on physiological responses, performance and ascites incidence in broiler chickens raised in normal or cold environment. *Animal*. **11,** 219-226.

Muir W.M. and Cheng H.W. (2014). Genetic influences on the behavior of chickens associated with welfare and productivity. Pp. 317-359 in Genetics and the Behavior of Domestic Animals. T. Grandin and M.J. Deesing, Academic Press, Cambridge, Massachusetts, USA.

Nicol C.J. (2015). The Behavioral Biology of Chickens. CABI, USA.

Osborne J.W. and Waters E. (2002). Four assumptions of multiple regression that researchers should always test. *Pract. Assess. Res. Eval*. **8,** 2-12.

Parkhurst C. and Mountney G.J. (2012). Poultry Meat and Egg Production. Springer, Germany.

Peak S.D., Walsh T.J., Benton C.E., Brake J. and Van Horne P.L.M. (2000). Effects of two planes of nutrition on performance and uniformity of four strains of broiler chicks. *J. Appl. Poult. Res*. **9,** 185-194.

Polycarpo G.V., Burbarelli M.F.C., Carao A.C.P., Merseguel C.E.B., Dadalt J.C., Maganha S.R.L. and Albuquerque R. (2016). Effects of lipid sources, lysophospholipids and organic acids in maize-based broiler diets on nutrient balance, liver concentration of fat-soluble vitamins, jejunal microbiota and performance. *British Poult. Sci. J.* **57,** 788-798.

Renema R.A., Robinson F.E., Beliveau R.M., Davis H.C. and Lindquist E.A. (2007). Relationships of body weight, feathering, and footpad condition with reproductive and carcass morphology of end-of-season commercial broiler breeder hens. *Appl. Poult. Res. J.* **16,** 27-38.

Rochow N., Landau-Crangle E., So H.Y., Pelc A., Fusch G., Däbritz J., Göpel W. and Fusch C. (2019). Z-score differences based on cross-sectional growth charts do not reflect the growth rate of very low birth weight infants. *PLoS One*. **14(5),** e0216048.

Rosenthal R.J., Morton J., Brethauer S., Mattar S., De Maria E., Benz J.K. and Sterrett D. (2017). Obesity in America. *Surg. Obes. Rel. Dis*. **13,** 1643-1650.

SAS Institute. (2002). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Szollosi L., Szucs I. and Nabradi A. (2014). Economic issues of broiler production length. *Econ. Agric. J.* **61,** 633-646.

Toudic C. (2007). Evaluating uniformity in broilers-factors affecting variation. WebMD. https://thepoultrysite.com/articles/evaluatinguniformity-in-broilers-factors-affectingvariation, 2007.

Vasdal G., Granquist E.G., Skjerve E., De Jong I.C., Berg C., Michel V. and Moe R.O. (2019). Associations between carcass weight uniformity and production measures on farm and at slaughter in commercial broiler flocks. *Poult. Sci. J.* **98,** 4261-4268.

Warleigh A. (2001). 'Europeanizing'civil society: NGOs as agents of political socialization. *J. Common Market Stud*. **39,** 619-639.

Willson N.L. (2017). Identification of biological factors that can be consistently linked to performance variation in modern commercial broiler flocks. PhD Thesis. University of Adelaide, Adelaide, Australia.

Yčas J.W. (2017). Toward a blood-borne biomarker of chronic hypoxemia: red cell distribution width and respiratory diseases and advanced clinical chemistry. *Adv. Clin. Chem*. **82,** 105-197.

Yu W.H., Sing S.L., Chua C.K., Kuo C.N. and Tian X.L. (2019). Particle-reinforced metal matrix nanocomposites fabricated by selective laser melting: A state of the art review. *Prog. Mater Sci*. **104,** 330-379.

Yuan J., Roshdy A.R., Guo Y., Wang Y. and Guo S. (2014). Effect of dietary vitamin A on reproductive performance and immune response of broiler breeders. *PLoS One*. **9,** e105677.

Zhuge Q. and Klopfenstein C.F. (1986). Factors affecting storage stability of vitamin A, riboflavin, and niacin in a broiler diet premix. *Poult. Sci. J.* **65,** 987-994.

Zuidhof M.J., Holm D.E., Renema R.A., Jalal M.A. and Robinson F.E. (2015). Effects of broiler breeder management on pullet body weight and carcass uniformity. *Poult. Sci. J.* **94,** 1389-1397.