

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

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ABSTRACT Three hundred fifty 18-day-old Ross 308 male chicks were used to examine the effects of early growth rate (\bar{x} -3SD, \bar{x} -2SD, \bar{x} -SD, \bar{x} , \bar{x} +SD, \bar{x} +2SD and \bar{x} +3SD) and a fat soluble vitamin (**FSV**) cocktail on glucose tolerance, whole tract feed transit time (**FFT**), certain liver, and pancreas related traits as well as their share in intra-flock variance of body weight (**BW**) at d 42 and feed intake (**FI**) and feed conversion ratio (**FCR**) in d 21 to 42 of age. Birds with a greater initial BW (21 d) showed greater FI during d 21 to 42 of age and gained a higher final BW at d 42 of age. The broilers injected with a FSV cocktail consumed more feed with an improved FCR and achieved a noticeable greater BW at d 42 of age compared with the untreated birds ($P < 0.05$). Blood glucose at 15 min after oral gavage of a glucose solution was elevated in all birds faster than those

with a body weight close to the mean population BW. Lipase activity increased by 9.75% and amylase activity decreased by 14.9% in the birds treated with FSV injections compared with those received no vitamin. Multivariate step-wise regression analysis showed liver percentage as the leading variable accounting for about 75 and 62.77% of BW and FI variance, respectively. Serum cholesterol concentration was the major predictor in a poor model ($R^2 = 52.07$) generated for FCR, explaining 29.3 of the FCR variability ($P < 0.150$). It was concluded that the slow and fast growing birds within a flock showed the same efficiency in dietary glucose absorbing and blood glucose clearing. The faster-growing birds demonstrated slower FTT. Liver percentage was the major parameter explaining a significant fraction of the intra-flock variance in BW at marketing age and FI during days 21 to 42 d.

Key words: broiler chicken, glucose tolerance, feed transit time, intra-flock variability, performance

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INTRODUCTION

Today's modern strains of broiler chickens are frequently reported as the most genetically uniform flocks in animal industry (Chang et al., 2016). It is well known that commercial broiler chicks originate from nucleus breeding flocks that are bred by a few numbers of international poultry breeding companies adopting vastly specialized and resource-intensive methods (Warren, 1996; Notter, 1999; Zuidhof et al., 2014). Specialist breeding companies are continuously improving their breeding lines to select for a few important economic traits such as live body weight, meat yield, and the efficiency with which birds convert feed to meat

(Pollock, 1999; Decuyper et al., 2003). Therefore, one anticipate all chicks in a grow-out house demonstrate an uniform growth rate, feed intake, and feed efficiency, where they expose to the same environmental conditions, receive the same feed for ad libitum consumption and take the benefits of all attempts of the producer in providing an optimum circumstance for their maximum growth and profitability.

However, broiler producers almost always complain about lack of uniformity, in particular, with respect to body weight at marketing age (Gous and Berha, 2006; Madsen and Pedersen, 2010; Gous, 2018). While there is little scientific data available, a broiler flock with 10 to 12% coefficient of variation (**CV**) at 40 to 42 d is considered uniform (Corzo et al., 2004; Griffin et al., 2005). Links among high flock CV and increased weekly mortality, total mortality, and abattoir rejection, as well as poor FCR and growth rates, have been reported in multiple global research works (Heier et al., 2002; Vasdal et al., 2019). A major question is what are the

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causes of lack of uniformity in performance indices? Several recent reports confirmed that such question has not been yet answered in detail by poultry researchers (Madsen and Pedersen, 2010; Hughes et al., 2017).

Evidently, plant seeds comprise the major feed ingredients in broiler diets and starch consists more than 50% of the whole diet (Carre, 2004; Singh et al., 2010; Svihus, 2014). Therefore, it seems that gut phenomena responsible for digestion and absorption of glucose may elucidate a noticeable fraction of the differences in growth rate and feed efficiency among the birds within a flock. On the other hand, feed transit time through gut is a key factor in a healthy digestive system (Svihus and Itani, 2019). It was shown than in human cases, the longer food takes to pass through the colon, the more harmful bacterial degradation products are produced. Conversely, when the transit time is shorter, there are a higher amount of the substances that are generated when the colon renews its inner surface, which may be a sign of a healthier intestinal wall (Henrik et al., 2016).

Taking into consideration the prominent role of glucose absorption, feed passage, liver, and pancreas functions in bird's metabolism, we hypothesized that they may explain a remarkable fraction of the intra-flock variance of the performance indices in a boiler flock. Therefore, this study intended to evaluate the effects of early growth rate and a fat soluble vitamin cocktail on glucose tolerance test parameters, feed transit time, liver and pancreas parameters, their associations, as well as their share in intra-flock variation in final body weight, feed intake and feed conversion ratio during d 21 to 42 of age in broiler chicken.

MATERIALS AND METHODS

All procedures carried out in this experiment were reviewed and approved by the Animal Care and Use Committee of Lorestan University, Khorramabad, Iran.

Pre-experimental Phase

A total number of 20,000 male Ross 308 broiler chicks provided from a local hatchery (Mahan hatchery, Zanjan, Iran) and raised in a force ventilated growout house (Pardis Badieah farm, Khorramabad, Lorestan, Iran). Birds were fed on a starter (1–10 d), grower 1 (11–20 d), grower 2 (21–30 d), finisher 1 (31–40 d), and finisher 2 (40 to slaughter age) diet for ad libitum consumption (Table 1). All diets were provided in crumble/pelleted form. The brooding temperature was held at 32 to 33°C for the first 3 d and then progressively lowered to 23 to 25°C by the end of the raising period. Photoperiods were maintained at 24 h during the first day and decreased to 20: 4 h lightness: darkness schedule for the rest of the raising period. Weekly weighing (at 1 g precision) was performed on a random sample of the birds consisting at least 5% of the flock. At the close of d 18, mean and standard (SD) of body weight (620 and 56 g, respectively) for the entire of the flock was calculated using a random

sample including 10% of the birds. Based on live weight differences with population mean, 7 experimental treatments were constructed as \bar{x} -3SD, \bar{x} -2SD, \bar{x} -SD, \bar{x} , \bar{x} +SD, \bar{x} +2SD and \bar{x} +3SD, then 52 birds were selected for each body weight group (± 5 g), identified via a plastic wing band and transferred to the research site (Faculty of Agriculture, Lorestan University, Khorramabad, Iran).

Experimental Phase

In d 18, the selected birds were distributed into 364 wire cages (arranged in 26 rows of 14 cage each) where they spent 3 d for adaptation under the same circumstances as the pre-experimentation period. At d 21, birds belonging to each body weight group were divided into 2 subgroups of 26 birds and one subgroup administrated with a fat soluble vitamin (FSV) cocktail through SC injection in the back of the neck. The reminder of the birds remained unhandled. Vitamin cocktail injection (1.2 mL/bird) repeated on d 28 and 35 of age when the treatment dosage was adjusted in relation to dietary requirements for a given age. Each injection dosage contained 63,000 units of vitamin A, 28,000 units of vitamin D, 385 units of vitamin E and 15 mg of vitamin K accounting for the birds need for all fat-soluble vitamin for 7 d. The experimental design was a randomized complete block design comprising of 14 treatments with 26 replicates each. The treatments in a 7 \times 2 factorial fashion consisted of seven initial growth rate groups with live BW of \bar{x} +3SD, \bar{x} +2SD, \bar{x} +SD, \bar{x} , \bar{x} -SD, \bar{x} -2SD and \bar{x} -3SD at d 21 of age with and without a weekly FSVs injection.

Performance and Organ Data

Individual BW and FI were recorded in days 21 and 42 of age, and the collected data were used to generate WG, FI, and FCR for the same period. Mortality was recorded upon occurrence. At d 42 of age, all the birds were killed by puncturing jugular vein and carotid arteries, then scalded, defeathered mechanically, eviscerated manually, and evaluated for liver and pancreas percentage.

Enzymes Activity

At d 42 of age, prior to the killing of each bird, a 3 to 4 mL blood sample was collected in a 5-mL tube with no anticoagulant. Blood samples were kept on ice slashes and quickly transferred to laboratory where sera samples were collected (3,000 \times g for 10 min) and preserved in -20°C pending further analysis. Serum concentrations of amylase and lipase were assayed using an autoanalyzer (Clima Ral. Co, Barcelona, Spain). The analyzer employed enzymatic procedures based on the methodology introduced by Elliott (1984) using SEPPIM Diagnostic Kits (SEPPIM S.A.S., Sees, France).

Table 1. Composition of the diets and their approximate analysis.

Ingredients (%)	Starter (0–10 d)	Grower 1 (11–20 d)	Grower 2 (21–30 d)	Finisher 1 (31–40 d)	Finisher 2 (41–42 d)
Corn	60.73	60.40	63.59	65.33	69.10
Soybean meal (48%)	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
Mineral premix ²	0.5	0.05	0.05	0.05	0.05
Vitamin 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)	2,942	3,000	3,024	3,063	3,061
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	0.89	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

¹Dicalcium phosphate contained: phosphorus, 18%; calcium, 21%.

²Vitamin and mineral concentrate supplied per kilogram diet: retinol, 12,000 IU; cholecalciferol, 5,000 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; Zn (sulfate and oxide), 100 mg.

Gut Feed Transit Time

Commencing at d 35, 6 a.m., all birds were fasted for four hours followed by oral administration of a gelatin capsule containing ferric oxide (Fe_2O_3 , 200 mg/kg BW) as described by Hughes et al. (2008). The birds were closely monitored in at least 5-min interval sequence for red signs of ferric oxide in excreta. Whole tract feed transit time for each birds was considered as the time elapsed from the moment of oral administration of the Fe_2O_3 -containing capsule to time of the first observation of the distinctive red coloration in droppings in term of minutes.

Glucose Tolerance Test

The oral glucose tolerance test was performed after a 16 h (overnight) fast (Gueritault et al., 1990). The birds in each growth rate group were inoculated with a dosage of 5 mL/kg BW glucose (50% weight by volume glucose solution, Dextrose monohydrate powder, Iran dextrose Co., Tehran, Iran) into the crop (Muellenbach et al., 2009). Fasting levels of blood glucose were measured prior to the administration of the glucose load using a portable glucometer (eBchek, eB0D0G1I50598, Taiwan). A venous blood sample of approximately 50 microliters from the brachial vein was used for serial glucose assessments. Before the venipuncture using a blood Lancet (Chongqing new world trading Co., LTD, China), the area surrounding the wing vein was clipped of its feathers and sterilized with alcohol soaked gauze swabs. Similarly, consecutive blood glucose assessment were carried out at fixed time intervals of 15, 45, 75,

105 min after the administration of the oral glucose load (Loxham et al., 2007; Muellenbach et al., 2009).

Statistical Analysis

The collected data were subjected to analysis of variance in a randomized complete block design using Mixed model procedure in SAS (2003) statistical software. Tukey-Kramer test was used to compare means. For all experiments, the maximum probability of the first type of error was 5% ($P > 0.05$). Multivariate liner regression analysis using a stepwise model in the same software was used to extract the share of hematological parameters as a source of the intra-flock variance in final BW at d 42 and FI as well FCR during 21 to 42 d of age (Mendes, 2011; Adedibu et al., 2014).

RESULTS

Birds differing in initial growth rate at d 21 of age exhibited significant differences in live BW at age of 42 d, WG and FI during d 21 to 42 of age but no difference was observed in FCR in the same period ($P < 0.05$; Table 2). Body weight in 42 d of age, WG as well as FI during d 21 to 42 of age differed in an approximately similar pattern as the BW at d 21 of age ($P < 0.05$; Table 2). In general, birds with a greater initial BW demonstrated greater WG and FI during d 21 to 42 of age and showed a greater final BW at d 42 while exhibited almost higher FCR than the birds with lesser initial BW in terms of SD units below or above the population mean. The broilers providing with a FSV, cocktail

Table 2. Effects of early growth rate and fat soluble vitamins¹ on body weight (BW, g), weight gain (WG, g), feed intake (FI, g), feed conversion ratio (FCR, g; g), liver weight, pancreas weight, and feed transit time (FTT; min) in broiler chickens.

Factor/level	BW, 18d	BW, 42 d	WG, 21–42 d	FI, 21–42 d	FCR, 21-42 d	Liver, %	Pancreas %	FTT, min
Early growth rate (EGR, g)								
$\bar{X}-3\sigma$	455 ^g	1,502.25 ^f	1,047.75 ^d	1,924.75 ^e	1.84	2.55 ^a	0.27	295.70 ^{cd}
$\bar{X}-2\sigma$	510 ^f	1,540.76 ^f	1,030.15 ^d	2,037.40 ^f	1.96	2.50 ^a	0.26	292.74 ^d
$\bar{X}-1\sigma$	564 ^e	1,927.24 ^e	1,363.80 ^c	2,336.00 ^e	1.71	2.45 ^b	0.24	308.00 ^b
\bar{X}	620 ^d	2,012.43 ^d	1,391.94 ^c	2,695.22 ^d	1.94	2.46 ^b	0.23	307.11 ^b
$\bar{X}+1\sigma$	676 ^c	2,218.83 ^{bc}	1,542.31 ^b	2,822.61 ^c	1.83	2.23 ^c	0.23	301.96 ^{bc}
$\bar{X}+2\sigma$	733 ^b	2,259.00 ^b	1,526.93 ^b	2,728.90 ^b	1.78	2.26 ^c	0.20	303.12 ^b
$\bar{X}+3\sigma$	790 ^a	2,481.07 ^a	1,691.07 ^a	3,096.52 ^a	1.83	2.23 ^c	0.20	323.35 ^a
SEM	23.163	38.747	31.950	48.431	0.030	0.055	0.008	4.200
Fat soluble vitamins (FSV; mL/ injection)								
0	627	1,782.67 ^b	1,153.72 ^b	2,323.84 ^b	2.17 ^a	2.25 ^b	0.24	309.68 ^a
1.2	624	2,444.40 ^a	1,790.25 ^a	3,005.00 ^a	1.72 ^b	2.31 ^a	0.22	295.48 ^b
SEM	3.461	47.925	35.451	66.536	0.029	0.031	0.006	5.121
ANOVA results								
EGR	0.0001	0.0001	0.0001	0.0001	0.8869	0.0001	0.5270	0.0160
FSV	0.6792	0.0001	0.0001	0.0001	0.0001	0.0001	0.4569	0.0487
EGR×FSV	0.4584	0.0118	0.0073	0.1517	0.4962	0.0715	0.5174	0.0328

¹Each injection (repeated at d 21, 28, and 35) contained 63,000 units of vitamin A, 28,000 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 d.

^{a–g}Means in the same column without the same superscript differ significantly ($P < 0.05$). SEM: standard error of mean.

consumed more feed with an improved conversion ratio, and finally achieved a noticeable greater BW at d 42 of age compared with the birds receiving no FSV during d 21 to 42 of age ($P < 0.05$; Table 2).

Pancreas percentage was not affected by early growth rate but liver percentage was greater in the birds having a BW lesser than the population mean (Table 2; $P < 0.05$). Weekly injection of a FSV cocktail did not influence pancreas percentage but increased liver percentage by 2.6% compared with the untreated birds (Table 2; $P < 0.05$).

Whole gut feed transit time influenced by early growth rate where it was increased in the birds with greater initial body weight than the birds with a lesser initial weight. Feed transit time mainly decreased in response to FSV injection (Table 2; $P > 0.05$).

Lipase as well as amylase activity was not varied among the birds with different initial growth rate. Lipase activity increased by 9.75% and amylase activity decreased by 14.9% in the birds offering with a weekly injection FSV compared with those received no vitamin treatment during d 21 to 42 of age.

Fasting blood glucose (FBG) was significantly differed among the broilers with different early growth rate where birds with a BW close to the mean population weight showed greater FBG and those far from the mean (in term of SD) exhibited lesser values (Table 3; $P < 0.05$). In response to receiving a glucose solution through oral gavage, blood glucose was elevated in all birds faster than those with mean population body weight after 15 min. However, no pattern of change in blood glucose was found in 45, 75, and 105 min after glucose gavage so that the components of the nonlinear equations fitted for post administration blood glucose changes (a, b, and c) were not differ among the birds exhibiting different initial growth rate (Table 3; $P > 0.05$). Administration of a FSV cocktail in a weekly schedule in d 21, 28, and 35 of age showed no effect on

fasting as well as post-gavage blood glucose in the birds differing in initial BW and growth rate (Table 3; $P > 0.05$).

Body weight at marketing age (42 d) and WG during d 21 to 42 of age were affected by IBW × FSV interaction where vitamin injection increased growth performance inequitably in all groups and improvement was lesser in the birds with BW closer to the population mean compared with the birds in 2 extreme tails BW distribution (Table 4). Feed transit time was also influenced by IBW × FSV interaction where FSV was often decreased FTT and the reduction did not exhibited in an obvious pattern (Table 4).

Body weight at d 42 and weight gain as well as FI in d 21 to 42 were highly positively correlated while they showed lesser and negative association with FCR during d 21 to 42 of age. No significant correlation was found between FTT and all variables concerned with the exception of blood sugar where they showed a positive weak correlation ($r = 0.171$). Interestingly, no performance index exhibited a significant correlation with either fasting blood sugar or a post-gavage concentration of blood sugar. Performance indices were found to be weakly correlated with serum lipase and amylase activity. Pre- as well as post-gavage concentration of blood glucose were mainly correlated and in general showed inconsistent low-to-moderate positive associations. No correlation was realized among a blood sugar concentration and lipase as well as amylase activity (Table 5).

Tolerance statistic for multicollinearity diagnosis was lesser than 1 ranging from 0.0001 to 0.831, for all variables considered (Table 6). In general, lower tolerance values were found for pancreas weight, while FTT, blood lipase, and amylase activity exhibited greater tolerance values (Table 6).

Multivariate step-wise regression analysis showed that liver weight alone explained 74.57% of the variation

Table 3. Effects of early growth rate and fat soluble vitamins¹ on blood glucose concentration at 0, 15, 45, 75, 105 min post-gavageing, equations parameter (a, b, and c), and serum enzymes activity in broiler chickens.

Factor/level	Post-gavageing blood glucose time, min					Equation parameter			Serum enzyme activity(U/L)	
	0	15	45	75	105	a	b	c	Lipase	Amylase
Early growth rate (EGR, g)										
\bar{X} -3 σ	225.6 ^{a,b}	346.73	318.00	257.47	232.47	136.86	131.31	-23.15	39.82	735.82
\bar{X} -2 σ	219.14 ^b	324.07	318.93	245.36	227.07	128.34	128.65	-22.49	39.50	766.38
\bar{X} -1 σ	212.93 ^c	325.79	316.86	247.07	215.21	110.67	142.71	-25.02	42.48	847.83
\bar{X}	233.00 ^a	304.36	333.79	252.21	231.00	139.64	121.30	-21.15	40.18	599.58
\bar{X} +1 σ	230.50 ^b	353.21	338.29	256.71	224.36	124.86	150.60	-26.91	39.52	679.58
\bar{X} +2 σ	217.93 ^{bc}	344.93	328.64	258.93	224.93	108.96	153.70	-26.76	41.31	661.85
\bar{X} +3 σ	210.57 ^c	336.50	344.71	259.50	217.93	78.31	177.38	-30.60	39.50	666.19
SEM	2.528	6.701	6.922	3.973	3.933	7.173	6.852	1.156	0.591	30.527
Fat soluble vitamins (FSV; mL/injection/bird)										
0	221.88	340.12	332.22	257.68	227.04	115.29	148.94	-26.01	38.97 ^b	751.71 ^a
1.2	220.94	327.06	324.25	249.96	222.39	121.75	137.80	-24.20	42.77 ^a	609.43 ^b
SEM	3.502	9.321	9.633	5.401	5.359	10.052	9.573	1.615	0.808	35.573
ANOVA results										
EGR	0.0315	0.2456	0.8974	0.9397	0.9157	0.2801	0.3608	0.3455	0.6068	0.6539
FSV	0.9125	0.4471	0.5254	0.3715	0.7218	0.6887	0.3828	0.3937	0.0017	0.0230
EGR × FSV	0.0991	0.8961	0.8350	0.5478	0.5255	0.8675	0.7699	0.6363	0.2588	0.7135

¹Each injection (repeated at d 21, 28 and 35) contained 63,000 units of vitamin A, 28,000 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 d.

^{a,b}Means in the same column without the same superscript differ significantly ($P < 0.05$).

in body weight at marketing age (42 d) followed by pancreas weight and post-gavageing blood glucose (75 min), explaining 1.98 and 1.71 percent of the final live weight variance, respectively (Table 7). Inclusion of blood glucose at 15 min post-gavageing (BS15), fasting BS and serum amylase activity totally increased the accuracy of the model by approximately 2 units (Table 7). For FI prediction, liver weight, pancreas weight, post-gavageing glucose levels at 75 and 15 min were the fore leading variables accounting for about 70% of the variability (Table 7; $P < 0.150$). A poor model using only liver weight and post-gavageing blood glucose at 45 min was achieved for FCR ($R^2 = 29.87$; Table 7).

Table 4. Interaction effects of early growth rate (EGR) and fat soluble vitamins¹ (FSV) on body weight (BW, g), weight gain (WG, g), and feed transit time (FTT; min) in broiler chicken.

EGR	FSV	BW, 42 d	WG, 21-42 d	FTT, min
\bar{X} -3 σ	0	1,274.66 ⁱ	897.73 ^g	300.47 ^{bc}
\bar{X} -3 σ	1.2	1,881.56 ^{figh}	1,465.78 ^{bc}	287.78 ^{def}
\bar{X} -2 σ	0	1,453.89 ⁱ	995.89 ^{fg}	302.45 ^{bc}
\bar{X} -2 σ	1.2	1,776.57 ^{gh}	1,301.43 ^{cd}	265.00 ^f
\bar{X} -1 σ	0	1,605.00 ^{hi}	1,044.60 ^{fg}	326.81 ^a
\bar{X} -1 σ	1.2	2,410.60 ^{cd}	1,857.60 ^b	277.90 ^{ef}
\bar{X}	0	1,866.91 ^{figh}	1,227.73 ^{def}	292.96 ^{cd}
\bar{X}	1.2	2,332.60 ^{cde}	1,689.20 ^{bc}	334.00 ^a
\bar{X} +1 σ	0	1,957.26 ^{fg}	1,257.11 ^{def}	315.83 ^{ab}
\bar{X} +1 σ	1.2	2,715.80 ^b	1,979.80 ^{ab}	277.00 ^{ef}
\bar{X} +2 σ	0	1,970.61 ^{fg}	1,192.94 ^{ef}	293.47 ^{cd}
\bar{X} +2 σ	1.2	2,835.78 ^{ab}	2,032.89 ^a	306.12 ^{ab}
\bar{X} +3 σ	0	2,225.89 ^{de}	1,385.78 ^{cd}	329.78 ^a
\bar{X} +3 σ	1.2	2,940.40 ^a	2,050.20 ^a	311.80 ^{ab}
SEM		76.445	72.285	14.340
ANOVA results				
EGR × FSV	0.0118	0.0073	0.0328	

¹Each injection (repeated at d 21, 28, and 35) contained 63,000 units of vitamin A, 28,000 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 d.

^{a-i}Means in the same column without the same superscript differ significantly ($P < 0.05$).

DISCUSSION

The unique feature of the present study is quantifying the possible share of whole gut tract feed passage time and glucose tolerance in the starved broilers. However, results of the present study were interesting in certain aspects.

At first, we found that categorization of the birds in terms of BW± standard deviations (SD) from the population mean may convey certain biological concepts for each category. In a more clear language, a bird with a BW, 1, 2, and 3 SD below or above population mean at early ages may never receive an opportunity for shifting to the upper class. Early growth rate, in terms of “population mean ± a determined SD” reflecting growth potential of a bird and affected by vast number of intrinsic as well as extrinsic

Table 5. Pearson correlation coefficients among performance indices, fasting and post-gavageing blood sugar test, pancreatic amylase, and lipase enzymes and liver percentage in broiler chickens.

	BW, 42 d	FI, 21-42 d	FCR, 21-42 d
BW, 42 d	1	0.929 ^{**}	-0.547 ^{**}
WG, 21-42 d	0.960 ^{**}	1	-0.676 ^{**}
FI, 21-42 d	0.929 ^{**}	1	-0.329 ^{**}
FCR, 21-42 d	-0.547 ^{**}	-0.329 ^{**}	1
FTT	-0.025 ^{ns}	-0.027 ^{ns}	-0.013 ^{ns}
BS, 0 s	0.021 ^{ns}	0.021 ^{ns}	-0.143 ^{ns}
BS, 15 min	0.077 ^{ns}	0.044 ^{ns}	-0.091 ^{ns}
BS, 45 min	0.083 ^{ns}	0.030 ^{ns}	0.097 ^{ns}
BS, 75 min	0.079 ^{ns}	-0.059 ^{ns}	0.128 ^{ns}
BS, 105 min	0.034 ^{ns}	-0.123 ^{ns}	0.046 ^{ns}
Lipase, u/ L	0.187 [*]	0.164 [*]	-0.192 ^{**}
Amylase, u/ L	-0.158 [*]	-0.152 [*]	0.021 ^{ns}
Liver, %	0.804 ^{**}	0.725 ^{**}	-0.504 ^{**}

¹Body weight (BW, 42 d, g), weight gain (WG, 21-42 d; g), feed intake (FI, 21-42 d; g), and feed conversion ratio (FCR, 21-42 d; g:g) during days 21 to 42. Blood sugar concentration for 0, 15, 45, 75, 105 min post-gavageing (BS0, BS15, BS45, BS75, BS105; mg/dL), lipase and Amylase activity (u/L).

^{*} $P < 0.05$.

^{**} $P < 0.01$.

^{ns}Nonsignificant.

Table 6. The Tolerance estimation¹ to check the collinearity using production indices, blood sugar test, liver characteristics, pancreatic weights, serum lipase, and amylase activity in broiler chicken.

Variable ²	Parameter estimate	S.E.	T-value	Pr > t	Tolerance	Variance inflation
FI, 21–42 d	0.297	0.072	4.09	0.01	0.076	13.04
WG, 21–42 d	0.793	0.128	6.16	0.01	0.044	22.25
FCR, 21–42 d	-18.788	60.471	-0.15	0.88	0.187	5.33
FTT	0.026	0.245	0.11	0.91	0.912	1.10
BS, 0	-1.081	0.652	-1.66	0.10	0.714	1.39
BS, 15	0.176	0.230	0.76	0.44	0.816	1.22
BG, 45	-0.01	0.228	0.52	0.60	0.846	1.18
BS, 75	0.822	0.625	1.31	0.19	0.440	1.28
BS, 105	0.048	0.691	0.07	0.94	0.441	2.26
Lipase (U/L)	-1.539	1.894	-0.81	0.41	0.856	1.68
Amylase (U/L)	-0.053	0.043	-1.23	0.22	0.849	1.17
Pancreas, %	-8.523	17.161	-0.50	0.62	0.426	2.34
Liver, %	4.087	2.163	1.89	0.06	0.213	4.694

¹The tolerance test was conducted using a without intercept model where body weight at d 42 of age was considered as independent variable.

²Feed intake (FI 21–42 d; g), weight gain (WG, 21–42 d; g), feed conversion ratio (FCR 21–42 d; g:g), feed transit time (FTT; min), blood sugar concentration at 0, 15, 45, 75, 105 min post-gavageing (BS0, BS15, BS45, BS75, BS105; mg/dL).

Table 7. Multivariate liner regression analysis using a stepwise model for partitioning of feed intake in broiler chickens during d 21 to 42 of age.

Step ¹	Variable entered	Partial R-square	Model R-square	C(p)	F value	Pr > F
Body weight, 42 d						
1	LIVW	0.7457	0.7457	19.330	208.17	<0.0001
2	PANWET	0.0198	0.7655	14.453	5.91	0.0176
3	BS75	0.0171	0.7826	10.501	5.44	0.0226
4	BS15	0.0133	0.7959	7.871	4.44	0.0388
5	BS0	0.0097	0.8057	6.491	3.36	0.0714
6	Amylase	0.0111	0.8167	4.630	4.01	0.0495
Feed intake, 21 to 42 d						
1	LIVW	0.6277	0.6277	16.00	117.16	<0.0001
2	PANWET	0.0312	0.6539	10.96	6.31	0.0143
3	BS75	0.0285	0.6823	6.55	6.18	0.0153
4	BS15	0.0176	0.6990	4.80	3.76	0.0566
5	BS45	0.0094	0.7084	4.68	2.16	0.1460
Feed conversion ratio, 21 to 42 d						
1	LIVW	0.2608	0.2608	2.48	25.06	0.0001
2	BS45	0.0379	0.2987	0.81	3.78	0.0558

¹Liver weight (LIVW; %), Pancreas wet weight (PANWET; %), Blood sugar concentration at 0, 15, 45, 75, 105 min post-gavageing (BS0, BS15, BS45, BS75, BS105; mg/dL).

factors. An individual bird with a body weight below or above the population mean ($\pm nSD$) is organized with a specific package of genetic materials which in interrelation with environmental factors which are almost identical to every birds within a flock, provide a change to express a predetermined body weight. A bird with a body weight below the population mean (in terms of SD) agonizes from certain adverse effects from a complexity of such factors so that those with initial body weight of $\bar{x} - 3SD$ are never able to grow such faster to inter the upper class where birds obtaining body weight of $\bar{x} - 2SD$. Therefore, we want to emphasis that growth rate boundaries of $\bar{x} \pm nSD$ refelect biological limitations and convey physiological concepts (Convey,1987; Nicol, 2015; Grandin and Whiting, 2018). Unfortunately, in poultry production such concepts have received very less attention, while in human medicin they are considered as decisive ceriteria for convinced health assemments and even diseases diagnosis and treatment. For instance, human cases with blood pressure above $\bar{x} + 2SD$ are considered as hypertention (Hermida et al., 2013) and they have to receive medical care and treatment. The same concepts has been used for difination of diabet (Forouhi and Wareham, 2014), anemia (Green and Dwyre, 2015), tachycardia (Low and

Sandroni, 2012), tachypnea (Deterding et al., 2005), and many other health problems (Rosenthal et al., 2017).

Second, the common commercial diets are anticipated to encompass all nutrient requirements of the fast growing broiler chicks. Vitamin premixes are also added to the same diets to ensure the vitamin needs coverage. In the current study, weekly SC injection of a FSV preparation dramatically improved BW and influenced remarkably almost all other variables in later ages. These finding create many doubts against vitamin contents of feed ingredients and premixes or vitamin recommendations for commercial broiler chicks. They also urge for reassessment of the current information on vitamin uptake in bird's gut under commercial conditions. It was shown that poultry feedstuffs may depleted form vitamin D (Mattila et al., 2011) and perhaps other fat soluble vitamins during storage, in particular, in hot climates and 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, birds health (Yuan et al., 2014), gut

bacterial (Goncalves et al., 2015), and the like, which they are not uniformly realize in the birds in a flock. Therefore, fat soluble vitamins bioavailability may receive concern as a source of intra-flock diversity in the productive performance indices in commercial broilers.

Third, starch is one of the most important energy sources in a broiler diet. Up to 65% of the metabolizable energy in a broiler ration is attributed to carbohydrates (Aderibigbe et al., 2020). Both starch and sugars are digested to glucose, which is readily absorbed as energy source. Therefore, the ability of the boilers to dispose of a blood glucose load, known as glucose tolerance (Hu et al., 2018), has received high, but inadequate concern, in broiler breeding. It is very reasonable to correspond the body weight variation in a broiler flock, in part, to individual capacity of each bird for glucose absorption, and glucose tolerance. For the same purpose, we calculated Pearson's correlation coefficients among the production indices and post-gavageing blood glucose in different times. No performance index exhibited a significant correlation with either fasting blood sugar or post-gavageing blood glucose concentration. In the present study we also considered whole gut feed transit time (FTT) as a potential variable explaining significant part of BW variation at a given age. Again, no significant correlation was found between FTT and all variables concerned with the exception of blood sugar where they showed a positive weak correlation ($r = 0.171$). In the next step, multicollinearity diagnosis was done through tolerance test and tolerance statistic was found to be lesser than 1, ranging from 0.0001 to 0.831, confirming the reliability of all the variables concerned for inclusion in multivariate regression models (Vatcheva et al., 2016).

Finally, multivariate step-wise regression analysis demonstrated liver weight as important variable influencing intra-flock variability in BW evidenced by its significant share of approximately 75% in the variance of BW as well as FI.

Several researches explaining the vital roles of liver in broiler metabolism (Nguyen et al., 2008; Zaefarian et al., 2019) confirmed vital role of liver health in improved productivity (Zaefarian et al., 2019; Kalra et al., 2021). Most of the studies intended to improve broiler health or production are in fact targeting liver function through dietary feed additives such as antioxidants (Surai et al., 2019; Emami et al., 2020), immunoinhncers (Parvar et al., 2013; Yang et al., 2018), and phytogetic remedies (VanHieu et al., 2020; Irawan et al., 2021).

In the current study, interestingly, fasting blood sugar as well as blood glucose levels in no post-gavageing time was able to explain a remarkable part of intra-flock variability in performance indices. These findings are not in the line with the results of Sumners et al. (2014) and Zhang et al. (2015) who reported significant differences in insulin sensitivity and glucose clearance rate between the hypophagic low weight and hyperphagic high weight lines of chicken. In the present study no difference was found among slow- and fast-growing birds within a single flock in dietary glucose absorbing and blood glucose clearing.

In this study, the trend of changes in post-gavageing serum glucose results was also inconsistent with the results of the previous studies. We found that slow- and fast-growing birds in the same flock were almost similar in post gavageing blood glucose peak since all the birds quickly absorbed the glucose, cleaned the blood glucose and reached the normal blood levels within 60 min post-gavageing. In our study, pancreas weight was the second variable appeared in the predictive models for intra-flock variance of BW and FI, however, it was able to explain a small fraction of the variance in both variables. This results are in part consistent with the findings of Sumners et al. (2014) who described pancreas, as a key endocrine organ of insulin and showed that its relative weight (ratio of absolute pancreas weight to body weight) was heavier in low weight selected chickens than in high weight selected lines on both days 65 and 56 of age. In the current study no difference was found among the fast- and slow-growing birds therefore no significant share in performance variation can be attributed to the pancreas percentage.

CONCLUSIONS

Within flock growth rate showed no effect on oral glucose tolerance. The slower growing birds within a flock showed the same efficiency in dietary glucose absorbing and blood glucose clearing as the faster growing ones. The exclusive feature of the faster growing birds was a slower feed transit time.

DISCLOSURES

The authors have not declared any conflict of interests in the subject matter discussed in this manuscript.

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