Research Note: Comparative gastrointestinal, tibia, and plasma attributes in 48-day-old fast- and slow-growing broiler chicken strains[1]

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ABSTRACT Emerging market differentiation for broiler meat from strains exhibiting a range of growth rates is necessitating comparative research on various physiological and production aspects of these strains. The objective of the present study was to compare select gastrointestinal, tibial, and plasma attributes in a sample of 48-day-old (50 male and 50 female) broilers obtained from fast-and slow-growing flocks maintained under similar feed and management regimens. Eight birds were randomly selected from a fast (B; representative of modern commercial strains) and each of the 4 slow-growing strains (**SG**; D, H, M, and E). The strains differed by estimated time to reach 2.2 kg bodyweight corresponding to 36, 50, 42, 44, and 50 D for B, D, H, M, and E, respectively. Blood samples were collected to determine plasma metabolites, and birds were subsequently euthanized, weighed, and necropsied for gizzard and small intestine weight, jejunal tissue for histomorphology, ceca digesta samples for concentration of short-chain fatty acids (**SCFA**) and left tibia for ash content. Gizzard was heavier ($P < 0.01$) for D, H, and M than that for B and E, whereas the small intestine was lighter ($P < 0.01$) for B, D, and H than for M and E. There were no ($P > 0.05$) strain differences on SCFA, jejunal villus height and crypt depth, plasma proteins, and electrolytes. Strains D, H, and M exhibited higher ($P = 0.01$) tibia ash concentration than B; E was intermediate and not different ($P > 0.05$) from any strain. Specifically, the tibia ash for B, D, H, SG 3, and E were 1.24, 1.44, 1.43, 1.49, and 1.39 g/kg BW, respectively. The B birds showed higher ($P < 0.01$) plasma concentrations of aspartate transaminase, creatine kinase, lactate dehydrogenase, and creatinine than SG strains. In conclusion, although B and some SG strains had lighter gastrointestinal tract indicative of energy efficiency, higher circulating plasma enzymes in B birds suggested impaired hepatic function. Moreover, lower tibia ash in B suggested disproportionate body mass relative to skeletal support.

Key words: fast- and slow-growing broiler chickens, gastrointestinal weight, digesta short chain fatty acid, plasma metabolites

INTRODUCTION

Genetic selection has been used to maximize growth rate and feed efficiency in broiler chickens at an unprecedented rate (Tixier-Boichard et al., 2012; Siegel, 2014; Zuidhof et al., 2014; Sakkas et al., 2018). On the other hand, emphasis on production traits negatively affects the ability of broilers to cope with metabolic and skeletal demands (Dawkins and Layton, 2012). Increasing consumer concerns for animal welfare and product quality have stimulated development of slow-growing broiler chickens (Fanatico et al., 2008; Mattioli et al., 2017). The slow-growing genotypes have been suggested as an alternative to address specific concerns on incidences of skeletal disorders and livability (Fanatico et al., 2008).

Growth rate affects metabolic efficiency linked to many underlying biological traits including behavior, appetite, digestive efficiency, protein and lipid accretion, and metabolic activity (Emmerson, 1997). The growth patterns for some of the fast- and slow-growing strains fed industry-standard feed were described (Fanatico et al., 2005); however, the data on comparison of gastrointestinal tract (**GIT**) development and skeletal and blood parameters in fast- and slow-growing broiler

chickens are very limited to date. Therefore, the objective of this experiment was to compare gastrointestinal, tibial, and plasma attributes in fast- and slow-growing broiler strains subjected to similar management.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Animal Care Committee of the University of Guelph (AUP 3746), and birds were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC, 2009) and National Farm Animal Care Council (2016).

Birds, Diets, and Management

Five different strains of broiler chickens including 1 fast-growing strain (B; modern commercial strain) and 4 slow-growing strains (D, H, M, and E) were used in this trial. The eggs were set at Arkell Poultry Research Station hatchery under similar conditions, and all the hatched chicks were vaccinated at day 0. At placement, the chicks were housed in 20 floor pens (238 $\times$ 160 cm) in an environmentally controlled room ($32°C$ to $24°C$; 60% relative humidity). Each pen had 44 birds (22 males and 22 females; 4 pens per strain), and the pens were thinned to 42 birds at 34 D to maintain stocking density. Pens were equipped with a round pan feeder, 5 nipple drinkers, a 25° ramp to a raised platform that was 30 cm above litter, a hanging round scale, a PECKStone mineral block (Protekta Inc., Lucknow, Ontario, Canada), and hanging nylon ropes. The stocking density was $30 \ kg/m^2$. The lighting program was 23L:1D (20+ lux) from 1 to 4 D and subsequently 16L:8D. In accordance with the management guidelines of the breeders, the anticipated age for reaching 2.2 kg of BW for B, D, H, M, and E was 36, 50, 42, 44, and 50 D, respectively. The all-vegetable and antibiotic- and coccidiostats-free corn–soybean meal–based diets were formulated to meet the Global Animal Partnership (**GAP**) nutrient recommendations (Table 1). All the strains were fed with the equal amount of feed in each phase.

Sampling

On day 48, 8 birds (4 males and 4 females) were randomly selected per strain and labeled individually. Blood samples were collected via the brachial vein, into heparinized blood tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Immediately, birds were euthanized by cervical dislocation and weighed. The blood samples were stored on ice and transported to

Table 1. Composition of the basal diets, on a fed basis.

Item	Starter (day 0–14)	Grower (day 15–28)	Finisher (day 28–48)
Ingredients			
Corn, grain	50.40	54.11	57.85
Soybean meal, 46%	28.04	25.98	21.07
Wheat	7.13	7.44	8.77
Corn gluten meal, 60%	4.49	2.70	3.00
Soybean oil	3.90	4.27	4.10
Mono calcium phosphate	1.83	1.61	1.45
Limestone	1.65	1.48	1.36
NaCl	0.36	0.37	0.37
$NaHCO_3$	0.29	0.20	0.21
DL-methionine	0.27	0.26	0.22
L-lysine HCl, 78%	0.33	0.28	0.31
L-threonine, 98%	0.09	0.08	0.10
Choline chloride, 60%	0.22	0.22	0.20
VT premix[1]	1.00	1.00	1.00
Calculated nutrient contents			
AME, mcal/kg	3.04	3.09	3.13
Crude protein, %	21.5	19.71	18.00
SID Lys, %	1.15	1.05	0.95
SID Met + Cys, %	0.86	0.80	0.48
SID Thr, %	0.75	0.69	0.64
SID Trp, %	0.22	0.21	0.19
Ca, %	0.96	0.86	0.78
Available P, %	0.48	0.43	0.39
Ca:aP ratio	2.00	2.00	2.00
Na, %	0.22	0.20	0.20
Cl, %	0.28	0.28	0.28
Analyzed nutrient contents			
Crude protein, %	21.6	19.88	19.99
Ca, %	0.99	0.95	0.82
Total P, %	0.75	0.74	0.64
Na, %	0.22	0.22	0.20
Starch, %	36.29	36.17	42.00

[1]Provided the following per kilogram of diet: vitamin A, 8,800.0 IU; vitamin D_3, 3,300.0 IU; vitamin E, 40.0 IU; vitamin B_{12}, 12.0 mg; vitamin K_3, 3.3 mg; niacin, 50.0 mg; choline, 1,200.0 mg; folic acid, 1.0 mg; biotin, 0.22 mg; pyridoxine, 3.3 mg; thiamine, 4.0 mg; calcium pantothenic acid, 15.0 mg; riboflavin, 8.0 mg; manganese, 70.0 mg; zinc, 70.0 mg; iron, 60.0 mg; iodine, 1.0 mg; copper, 10 mg; and selenium, 0.3 mg.

the laboratory for further analyses. The weights of the empty gizzard, small intestine, and fresh tibia were recorded, and samples of the jejunal tissue and ceca content, collected for histomorphology and short-chain fatty acid (**SCFA**) concentration, respectively. Jejunal tissue samples ($\sim$3 cm) were placed in buffered formalin for fixing. Ceca content samples were placed on ice and transported to the laboratory immediately upon collection and stored at $-20°C$ until required for analyses. The left tibia was defleshed and stored at $-20°C$ until further analyses.

Sample Processing and Analyses

The blood samples were centrifuged at 2,500 $\times$ g for 15 min at 4°C to recover plasma. The plasma samples were further analyzed for avian plasma biochemical profile by photometric method at the Animal Health Laboratory (University of Guelph, Guelph, ON; Greenacre et al., 2008; Robinson and Kiarie, 2019). Specific analytes included total protein, enzymes, metabolites, and minerals. Fixed jejunal tissues were embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin for morphological examinations at the Animal Health Laboratory (University of Guelph, Guelph, ON). In each cross-sectioned tissue, at least 4 to 5 complete villus–crypt structures were examined under a Leica DMR microscope (Leica Microsystems, Wetzlay, Germany) and villus height (**VH**) and crypt depth (**CD**) measured using a calibrated micrometer, and the ratio of VH to CD was calculated (Mohammadigheisar et al., 2019). The concentration of SCFA was analyzed as described by Mohammadigheisar et al. (2019). The tibia samples were subsequently dried at 105°C for 24 h, weighed, and ashed at 600°C for 12 h as described by Akbari Moghaddam Kakhki et al. 2018.

Calculations and Statistical Analysis

The organ weight (gizzard and small intestine) data are reported as g/kg of BW. The ash content of tibia is expressed as g/g of fresh tibia weight and g/kg of BW. The birds were allocated to pens in a randomized complete block design to eliminate any possible environmental effects between pens in the analysis. Two birds (1 male and 1 female) were selected from each pen randomly, and each bird was considered as an experimental unit. The data were subjected to the 2-way ANOVA with no interaction and analyzed using the GLM procedures of SAS (SAS 9.4; SAS Institute Inc., Cary, NY), considering each bird as an experimental unit, and the LS mean values were separated by Tukey's test. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

The fast-growing birds were significantly heavier ($P < 0.01$) than slow-growing strains on day 48 (Table 2). Specifically, B birds were 34.6, 30.2, 19.0, and 25.4% heavier than D, H, M, and E birds, respectively. These results were expected as modern commercial strains are known to attain higher BW relative to slow-growing strains at similar age (Katanbaf et al., 1988; Havenstein et al., 2003). The gizzard weight (g/kg of BW) of B birds was lower ($P < 0.01$) by 63.0, 69.7, 65.9, and 8.6% for D, H, M, and E birds, respectively (Table 2). Moreover, although small intestine weight of B birds was comparable ($P = 0.05$) with that of D and H birds, B and H birds exhibited lighter ($P < 0.05$) small intestines relative to E and M birds. These results agree with those of a previous study that showed that weight of visceral organs of broiler chickens selected for high and low 56-D BW differed at a common age (Katanbaf et al., 1988). Mussini (2012) demonstrated that the gizzard was significantly smaller

Table 2. Relative organ weights, short-chain fatty acids, jejunal histomorphology, and tibia mineral content of different strains of broiler chickens.

Items	B	D	H	M	E	SE	P-value
Days to 2.2 kg BW	36	50	42	44	50		
Bodyweight, kg d 48	3.15[a]	2.06[c]	2.20[b,c]	2.55[b]	2.35[b,c]	0.11	<0.01
Organ weight							
Gizzard, g/kg of BW	9.47[c]	15.44[a]	16.07[a]	15.71[a]	10.28[b,c]	0.84	<0.01
Small intestine, g/kg of BW	30.48[b]	34.54[a,b]	30.44[b]	36.61[a]	37.88[a]	1.33	<0.01
Short-chain fatty acids, μmol/g							
Lactic acid	4.04	5.30	3.22	5.70	4.38	0.8	0.22
Acetic acid	63.52	57.64	65.67	65.99	66.4	3.75	0.47
Propionic acid	3.30	3.51	2.75	3.53	4.07	0.44	0.34
Valeric acid	3.60	3.97	3.26	3.09	4.38	0.40	0.17
Butyric acid	11.84	10.87	10.79	11.72	11.38	1.50	0.98
Total SCFAs	86.29	81.29	85.67	90.03	90.25	50.27	0.75
Histomorphology							
Villus height (VH), μm	2,943.75	2,824.63	2,615.47	2,870.49	2,309.93	162.43	0.06
Crypt depth (CD), μm	412.02	375.62	457.54	456.04	454.35	34.60	0.40
VH:CD ratio	6.95[a,b]	7.94[a]	5.91[a,b]	6.53[a,b]	5.08[b]	0.64	0.05
Tibia mineral content							
Tibia ash, g/kg of BW	1.24[b]	1.44[a]	1.43[a]	1.49[a]	1.39[a,b]	0.05	0.01
Tibia ash, g/g tibia weight	0.24	0.26	0.27	0.26	0.26	0.01	0.06

[a–c]Means in the same row with different superscripts differ ($P < 0.05$).
n = 8.
Abbreviations: B, conventional broiler chicken; D, H, M, and E, slow-growing broiler chickens.

in modern commercial strains relative to that in unselected strains fed the same diet. Selection for higher growth rate has been associated with a reduction of the visceral organ weight relative to BW in modern broilers at a comparable age of old-type strains (Havenstein et al., 2003). Generally, a reduction in visceral organ weights relative to BW is associated with a reduction in maintenance energy and therefore increased overall energy utilization and efficiency (Mitchell and Smith, 1991; Tallentire et al., 2016). For example, birds with the highest growth rate had the smallest relative amount of mucosa in the small intestine and thus a slower rate of cell turnover (Mitchell and Smith 1991). As all strains in the present study were managed in the same way (including being housed in the same room and fed the same diet), we assume that improved efficiency may be the reason B birds had higher BW at the same age.

There were no differences ($P > 0.05$) between strains in the concentration of lactic acid, acetic acid, propionic acid, valeric acid, butyric acid, and total SCFA concentrations (Table 2). The results indicated that cecal microbial activity and consequently SCFA production are not dependent on the strain. Differences in nutrient utilization between poultry strains have been associated with differences in the structure and function of the GIT (Tallentire et al., 2016). Although villus height was not ($P > 0.05$) influenced by strains in the present study, the villus height of SG birds was 4, 11, 2, and 22% lower than for B birds (Table 2). The absolute number of villi is expected to decrease concomitant with the reduction in intestinal mass; however, fast-growing birds have been shown to have higher digestive surface area due to longer intestinal villi (Katanbaf et al., 1988; Mitchell and Smith, 1991). Moreover, embryos from heavy strains had more intestinal membrane transport proteins per unit area than embryos from light strains (Mott et al., 2008). There was a significant difference ($P = 0.05$) in the villus height-to-crypt depth ratio among the various strains. In B birds, this ratio was 14.2% lower than that in D birds but 15.0, 6.0, and 26.9% higher than in H, M, and E birds, respectively. However, Sakkas et al. (2018) reported that strain had no effect on jejunal histomorphology or histomorphological characteristics.

A significant difference ($P = 0.01$) was observed in g/kg BW of tibia ash content among the different strains (Table 2). The tibia ash content of B birds was 16.1, 15.3, 20.2, and 12.1% lower than that of D, H, M, and E birds, respectively. The results showed that tibia ash weight relative to bone weight (g/g) tended ($P = 0.06$) to be different between strains. A comparison of bone ash weight relative to BW (g/kg) among the broiler strains indicated that the emphasis on productive characteristics and improvements in carcass yield have compromised the ability of commercial broilers to cope with skeletal disorders (Williams et al., 2004; Dawkins and Layton, 2012). It has been suggested that improved mineralization is achieved at lower growth rates owing to the increased capacity of the skeleton to adapt to the increasing body mass (Williams et al., 2004;

Brickett et al., 2007; Sakkas et al., 2018). Shim et al., (2012) reported that almost all bone mineralization measurements were greater in slow-growing broiler chickens when expressed per unit of BW at dissection at 6 wk of age than in fast-growing birds.

The plasma biochemical profile is an index to evaluate health and metabolic status through specific grouping of analytes (proteins, enzymes, metabolites, and electrolytes; Greenacre et al., 2008). The plasma biochemical profile of different strains of broiler chickens is summarized in Table 3. The alkaline phosphatase and lipase level did not differ in the present study between strains ($P > 0.05$). The total protein, albumin, globulin, and albumin-to-globulin ratio of plasma did not differ between the strains ($P > 0.05$). Blood proteins are mainly synthesized in the liver. Melillo (2013) listed some of the functions of them as maintaining blood volume through the colloidal osmotic effect, buffering blood pH, transporting hormones and drugs, participating in cell coagulation, catalyzing chemical reactions (enzymes), regulating the metabolism (hormones), and participating in the body immune system against pathogens. It has been reported that decreased total protein can be an indicator of liver failure. On the other hand, the increased level of total protein might be an indicator of inflammatory diseases (Harr 2005). The plasma level of amylase in B chickens was significantly lower ($P < 0.01$) than in D birds, but there were no remarkable differences between B, H, M, and E birds. Harr (2005) reported that increased level of amylase can be the result of pancreatic (e.g., inflammation, infection, neoplasia, necrosis, etc.) or renal diseases. The concentration of plasma aspartate transaminase (**AST**) in B birds was 65.5, 56.2, 58.6, and 52.8% higher ($P < 0.01$) than in D, H, M, and E birds, respectively. Increased level of AST can be an indicator of muscle damage (e.g., seizures, trauma, capture myopathy) or hepatic damage (hemochromatosis, endocrine disease, lipidosis, inflammation, infection, etc.; Harr 2005). The D and E birds had higher ($P = 0.01$) levels of plasma gamma-glutamyl transferase (**GGT**). Some studies have reported that impaired liver function can lead to an accumulation of ammonia in the blood and an increase in plasma hepatic markers including AST and GGT (Shawcross et al., 2010; Shini 2014; Bona et al., 2018). The level of plasma creatine kinase (**CK**) of B birds was significantly higher ($P < 0.01$) than that of D (88.0%), H (69.3%), M (76.2%), and E (57.4%) birds. Harr (2005) defined CK as a magnesium-dependent dimeric enzyme that catalyzes the reaction of adenosine diphosphate (**ADP**) and creatine phosphate in the production of adenosine triphosphate (**ATP**) and creatine in skeletal, cardiac and smooth muscle, as well as brain. Muscle damage (e.g., seizures, capture myopathy, myositis, hyperthermia, hypothermia, vitamin E/Se deficiency, etc.) can lead to an increase in the level of plasma CK. The concentration of glutamate dehydrogenase (**GDH**) of B birds was 42.9, 24.9, 24.9, and 32.0% higher ($P = 0.04$) than that of D, H, M, and E birds, respectively. Glutamate dehydrogenase catalyzes the conversion of ammonium ion and 2-oxoglutarate to glutamate and water. The level of plasma lactate dehydrogenase (**LDH**) did not differ

Table 3. Plasma biochemical profile of the different strains of broiler chickens at 48 d of age.

Items	B	D	H	M	E	SE	P-value
Proteins, g/L							
Total protein,	30.00	33.00	32.00	32.00	32.13	0.83	0.16
Albumin,	11.25	12.87	13.13	12.63	12.25	0.55	0.16
Globulin	18.75	20.12	18.88	19.38	19.88	0.59	0.39
Ratio of albumin to globulin	0.60	0.64	0.70	0.66	0.62	0.03	0.33
Enzymes, U/L							
Alkaline phosphatase	2,027.1	3,564.8	1,870.6	1,506.7	2,537.0	489.28	0.09
Amylase	318.0[b]	594.9[a]	431.1[a,b]	382.5[b]	414.6[a,b]	45.46	<0.01
Aspartate transaminase	582.5[a]	200.9[b]	255.4[b]	241.0[b]	275.1[b]	38.33	<0.01
Creatine kinase	42,287.5[a]	5,088.6[c]	12,963.1[c,b]	10,043.3[c,b]	18,009.0[b]	3407.84	<0.01
Gamma-glutamyl transferase	15.25[b]	21.88[a]	21.00[a,b]	18.25[a,b]	21.88[a]	1.45	0.01
Glutamate dehydrogenase	3.50[a]	2.00[b]	2.63[a,b]	2.63[a,b]	2.38[a,b]	0.33	0.04
Lactate dehydrogenase	1,688.0[a]	686.3[b]	895.0[b]	794.6[b]	1,134.3[b]	249.15	<0.01
Lipase	5.38	5.13	4.75	5.25	5.25	0.30	0.64
Metabolites, μmol/L							
Bile acid	17.63	15.38	14.5	14.4	13.75	2.84	0.89
Cholesterol	3.21	3.19	3.15	3.06	3.25	0.11	0.79
Creatinine	33.50[a]	23.63[b]	22.50[b]	20.50[b]	21.25[b]	2.20	<0.01
Glucose	13.51	13.58	14.10	13.91	13.53	0.32	0.61
Uric acid	297.13	341.50	265.13	282.75	324.13	36.84	0.60
CO_2	23.13	21.50	20.50	22.25	22.50	0.76	0.16
Electrolytes and minerals, mmol/L							
Sodium	151.63	149.25	150.38	151.00	150.13	1.14	0.65
Potassium	6.73	6.95	7.18	6.81	7.01	0.19	0.49
Chloride	112.25	110.50	111.13	112.00	110.63	1.04	0.68
Calcium,	2.46	2.54	2.54	2.59	2.55	0.06	0.59
Phosphorus	1.80	1.98	1.96	1.97	1.87	0.05	0.07

[a-c]Means in the same row with different superscripts differ ($P < 0.05$).

n = 8.

Abbreviations: B, conventional broiler chicken; D, H, M, and E, slow-growing broiler chickens.

between slow-growing birds, but B birds had 59.3, 47.0, 52.9, and 32.8% higher ($P < 0.01$) levels of plasma LH than D, H, M, and E birds, respectively. The lipase level did not differ significantly between strains ($P > 0.05$). Generally, the difference in the enzymatic activity of conventional and slow-growing broiler chickens might have various reasons. Bona et al. (2018) suggested that enzymes such as AST and GGT are associated with liver or muscle damage. Kuttappan et al. (2013) showed that heavier meat–type chickens were more likely to display muscle damage resulting in release of various enzymes.

In the context of plasma metabolites, B birds had significantly higher ($P < 0.01$) level of creatinine than D (29.5%), H (32.8%), M (38.8%), and E (36.6%) birds. Although the creatinine level in B birds was significantly ($P < 0.05$) higher than in slow-growing birds, it was not higher than the normal level of 15-37 mmol/L suggested by Brugere-Picoux et al. (2015). The levels of plasma bile acid, cholesterol, glucose, uric acid, CO_2, sodium, potassium, chloride, calcium, and phosphorus were not significantly different between the strains ($P > 0.05$). Sarica et al. (2014) compared commercial fast-growing strains with slow-growing strains and reported significant differences between the plasma protein, albumin, and globulin level of different strains. They also reported that the plasma levels of cholesterol, Ca, and P were significantly different between strains, which is in contrary with the results of the present study. Manzoor et al. (2003) suggested various factors such as genotype, sex, feed, management, and stress affecting the blood biochemical profile in broiler chickens. However, as, in this study,

the nutritional and environmental conditions were standardized, the observed results of blood parameters must be attributed to differences in strains.

The breed names of the strains tested in the present study are proprietary to several genetics companies, and as such, we cannot reveal their identity. However, we feel that this study has scientific merit without this information. Indeed, there is precedent of publications without breed names (López et al., 2011; Oviedo-Rondón et al., 2017; Olanrewaju et al. 2019. The emphasis on production characteristics has compromised the ability of modern conventional broilers to cope with metabolic and skeletal disorders. This comparison of conventional and slow-growing broilers indicated that genetic selection has improved the gut efficiency in conventional broilers which is one of the influential factors on increased growth rate. However, the relative tibia ash content of conventional birds was lower than in slow-growing broiler chickens which might be an index for skeletal disorders in conventional broiler chickens.

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