Growth performance, organ attributes, nutrient and caloric utilization in broiler chickens differing in growth rates when fed a corn-soybean meal diet with multienzyme supplement containing phytase, protease and fiber degrading enzymes[1]

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ABSTRACT Growth performance, organ weight, ceca digesta short chain fatty acids (**SCFA**), jejunal histomorphometry, tibia ash, apparent retention (**AR**) of components and caloric efficiency were investigated in broiler chicken strains differing in growth rate fed diets with multienzyme supplement (**MES**). The strains differed in estimated time to reach 2.1 kg BW: 37, 43, 47, and 50 d and were designated C, F, J, and N, respectively. A corn-soybean meal diet was formulated for 2-phase program (starter and grower) and fed without or with MES containing phytase, protease and fiber-degrading enzymes. A total of 640-day-old chicks (42.3 + 0.01 g/bird) were housed in cages (5 cockerels and 5 pullets/cage) and allocated to give 8 replicates/strain and diet combination. Equal amount of feed was fed based on observed ad-libitum intake of C strain in the starter (d 0−14) and grower (d 15−28). Body weight was monitored, grab excreta samples taken and at completion of allocated feed one bird per cage necropsied for samples. With exception of P, apparent metabolizable energy corrected for nitrogen (**AMEn**) and ceca digesta acetic acid, there was no ($P > 0.05$) interaction between strain and MES on examined responses. Strains differed ($P < 0.01$) on growth, FCR, gizzard weight, tibia ash, breast weight, ceca digesta concentration of lactic, propionic, and isobutyric acid and caloric efficiency. The final body weight (**BW**) was 1,344, 1,134, 959, and 916 g/bird for C, F, J, and N, respectively. Corresponding caloric efficiency was 4,930, 5,807, 6,680 and 7,199 kcal/kg BW gain, respectively. Birds fed MES had higher BW gain ($P < 0.05$) in grower phase, larger gizzard, higher AR of CP, crude fat, neutral detergent fiber, and Ca than non-MES birds. In conclusion, growth rate influenced organ attributes, nutrient, and caloric utilization. Enzyme supplementation improved growth in grower phase and nutrient utilization independent of strain, suggesting that effects of feed enzymes are not influenced by inherent growth rate.

Key words: broiler, nutrient utilization, growth rate, feed enzymes, organ attributes

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INTRODUCTION

The growing demand for chicken meat as a healthier, and relatively cheaper animal protein compared to red meats has attracted significant investments in research and development in broiler meat value chain (Mottet and Tempio, 2017). In this context, genetic, nutrition, and management strategies have been implemented to optimize growth rate and feed efficiency to unprecedented levels (Tixier-Boichard et al., 2012; Siegel, 2014; Zuidhof et al., 2014; Sakkas et al., 2018). Despite all the improvements reported in broiler production, issues such as lameness, sudden death, enteric diseases, and myopathies are prevalent in modern strains of broiler chickens (Julian, 2005; Bessei, 2006; Blagojevic et al., 2009; Dawkins and Layton, 2012; Torrey et al., 2021). Because some of these issues have been associated with accelerated growth rate, selection for slower-growing strains has been suggested as a mitigation strategy (Bessei, 2006; Fanatico et al., 2008; Dawkins and Layton, 2012; Mattioli et al., 2017).

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The growth patterns for some of the fast, medium, and slow-growing strains fed industry-standard feed has been described (Fanatico et al., 2005; Torrey et al., 2021; Singh et al., 2021). However, data on the comparison of gastrointestinal tract development, digestibility, and skeletal parameters in broiler chickens differing in growth rate are limited. We previously showed that tibia ash content of fast-growing broiler chickens was lower than for slow-growing broiler chickens (Mohammadigheisar et al., 2020). Fast-growing birds were shown to have lower femur and tibia mineral density compared with medium-growing birds (Damaziak et al., 2019). Suggesting linkage between growth rate and skeletal integrity in broiler chickens. Slow-growing genotypes have fewer metabolic disorders and leg problems, lower mortality, and less processing downgrades than do fast-growing birds (Julian, 1998; Fanatico et al., 2008). On the other hand, the increased number of grow-out days, poor feed efficiency, and lower yields are linked with increased feed and water use, manure output, and cost of production; raising concerns over sustainability (Fisher, 2016, Fisher 2017; Tallentire et al., 2018).

A recent comparison of slow- and fast-growing broiler chickens showed that fast-growing birds digested more energy than slow-growing birds when fed the same diet (Singh et al., 2021). Further research has shown differences in expression of intestinal brush border nutrient transporters in broiler chickens differing in growth rate (Miska and Fetterer, 2019). Exogenous feed enzymes are widely used by the poultry industry to improve growth performance, nutrient utilization, and indices of gastrointestinal health and skeletal integrity (Bedford and Schulze, 1998; Slominski, 2011; Kiarie et al., 2013). However, there is paucity of data on response of supplementation of exogenous feed enzymes in broiler chickens exhibiting differences in growth rates. The present study evaluated growth performance, organ weight, ceca digesta short chain fatty acids (**SCFA**), jejunal histomorphometry, tibia ash content, apparent retention (**AR**) of components and caloric efficiency in broiler chicken strains differing in growth rates when fed corn-soybean meal diet supplemented with multienzyme supplement (**MES**) containing phytase, protease, and fiber degrading enzymes.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by 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 the Canadian Codes of Practice (National Farm Animal Care Council, 2016).

Enzyme and Diets

The corn-soybean meal-based diets were formulated to meet the specifications of a moderate slower growing broiler chicken (Mohammadigheisar et al., 2020; Torrey et al., 2021) in a 2-phase feeding program: starter and grower (Table 1). The MES had target main activities of phytase, protease, β-glucanase, and cellulase at 1,500 FYT, 8,000 PROT, 70 U, and 80 U per kg of complete starter feed, respectively (Victus, DSM Nutritional products Inc., Parsippany, NJ). The main target activities for the grower were similar to that of starter with the exception of protease at 3,750 PROT/kg of feed. The MES was also rich in a wide range of side activities such as arabinofuranosidase, exoarabinase, pectin lyase, and pectin methyl esterase (Sluis et al, 2017; Kiarie et al., 2020; Ward, 2020) considered important in facilitating accessibility of complex substrates to the main activities (Mohnen, 2008; Rytioja et al., 2014; Ward, 2021). Inclusion of MES accounted for the recommended nutrient contributions. For starter phase: P and available P, 454%; Ca, 400%; Na, 54%; metabolizable energy, 205 mcal/kg; standardized ileal digestible (**SID**) Lys, Met, Met + Cys, Thr, Ile, and Val were 80, 27, 27, 80, 27, and 27%, respectively. For grower phase:

Table 1. Composition of the experimental diets, as fed basis.

	Starter		Grower	
Item	Control	MES[1]	Control	MES
Ingredients, %				
Corn	50.0	51.1	53.5	55.6
Soybean meal-46%	28.1	27.1	26.1	23.7
Wheat	7.48	10.0	7.66	10.0
Corn gluten meal-60%	4.42	4.73	2.67	3.83
Soybean oil	3.94	2.07	4.40	2.08
Limestone	1.65	1.58	1.48	1.42
Mono calcium phosphate	1.82	1.01	1.61	0.80
Vitamin and trace premix[2]	1.00	1.00	1.00	1.00
Salt	0.36	0.36	0.37	0.36
Sodium carbonate	0.29	0.21	0.20	0.14
L-Lysine HCl; 78%	0.33	0.32	0.28	0.31
DL-Methionine	0.27	0.25	0.26	0.24
Choline chloride, 60%	0.22	0.22	0.20	0.20
L-Threonine-98%	0.09	0.06	0.08	0.06
Titanium dioxide	-	-	0.20	0.20
MES[1]	-	0.04	-	0.04
Calculated provisions				
AME, mcal/kg	3.04	3.04	3.09	3.09
Crude protein, %	21.5	21.5	19.7	19.7
SID Lys, %	1.15	1.15	1.05	1.05
SID Met, %	0.58	0.57	0.54	0.53
SID Met + Cys, %	0.86	0.86	0.80	0.80
SID Thr, %	0.75	0.75	0.69	0.69
SID Trp, %	0.22	0.22	0.21	0.21
Ca, %	0.96	0.96	0.86	0.86
Available P, %	0.48	0.48	0.43	0.43
Na, %	0.22	0.22	0.20	0.20
Cl, %	0.28	0.28	0.28	0.28

[1]Multienzyme supplement a blend of phytase, protease, β-glucanase and cellulase targeting 1,500 FYT, up to 8,000 PROT, 70 U, and 80 U per kg of complete feed, respectively (Victus^{TM}, DSM nutritional products, Ayr, Ontario, Canada). Formulation of MES diets considered supplier recommended nutrients contribution diets. For starter phase: P, 454%; C, 400%; metabolizable energy, 205 mcal/kg; SID Lys,80%; SID total sulphur amino acids, 27%; SID Thr, 80%, SID Ile 27%, SID Val 27% and Na, 54%. For grower phase: nutrient contributions for grower were similar to starter phase with exception of AME, 235 mcal/kg and SID Lys, 53%.

[2]Provided 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.

nutrients contribution was similar to starter phase with exception of AME, 235 mcal/kg and SID Lys, 53%. The grower diets contained 0.2% TiO_2 as an indigestible marker and diets were prepared in crumble form. During the pelleting process the temperature of the processing conditioner was 65 to 70°C with a steam pressure of 30 psi.

Birds and Management

Four different strains of broiler chickens included: (**C**; modern fast-growing strain) and 3 medium- to slow-growing (**F, J**, and **N**) strains. The breed names of the strains tested in the present study are proprietary to several genetic companies and the brand names could not be revealed in alignment with our previous publications (Mohammadigheisar et al., 2020; Torrey et al., 2021). The anticipated age for reaching 2.1 kg of BW for C, F, J, and N was 37, 43, 47, and 50 d, respectively. Fertile eggs were procured at the same time and set at Arkell Poultry Research Station hatchery under similar conditions, and all the hatched chicks were sexed, vaccinated, and weighed. All the chicks were weighed on day 0 and based on initial BW placed in wire floored metabolism cages (10 birds/cage: 5 males and 5 females), 8 cages per strain. The floors were lined with chick paper in the first week. The cages (each measuring 70 × 76 cm, Ford Dickison Inc., Mitchell, ON, Canada) were in an environmentally controlled room. There was a total of 64 cages in the room, installed in 2 rows separated by a 91 cm walkway and cages in a row stacked in two tiers of 16 cages each. The room temperature was set at 32°C on d 0 and gradually reduced to 24°C by d 13. The lighting program was 23 h of light (>20 LUX) from d 0 to 3 followed by 20 h of light (10−15 LUX) from d 4 onward. The cages were equipped with a side trough feeder and an adjustable water line with 2 nipple drinkers which was located inside the cages to allow the bird's free access to feed and water throughout the experiment.

Experimental Procedures and Sampling

The diets with or without MES were randomly assigned within strain. Differences in growth rates of broiler chickens is highly correlated with feed intake as we recently demonstrated in 16 strains of broiler chickens with fast-growing birds showing higher feed intake than medium and slow-growing birds (Torrey et al., 2021). However, feed intake differences have been shown to influence nutrient digestibility and metabolic response through mechanisms such as digesta passage rate (Baker, 1984; Svihus, 2011a; Massuquetto et al., 2020). Therefore, in the present study, an attempt was made to provide equal amount of feed to all birds. In this context, all birds were fed equal amounts of feed based on observed ad-libitum feed intake of C strain in the starter (d 0−14) and grower (d 15−28) phases. In the last week of the trial, excreta collection trays were installed under the cages and fresh grab excreta samples collected for 3 consecutive days and placed on ice for transportation the laboratory for storage at −20°C until further analyses. All the birds (cage basis) were weighed upon the consumption of their allocated feed to calculate body weight gain (**BWG**) and feed conversion ratio (**FCR**). One bird per cage (4 males and 4 females per treatment) was selected randomly, individually weighed and euthanized by cervical dislocation. Jejunum was immediately located and excised at duodenal loop and 2 cm anterior to Meckel's diverticulum. Segments (~3 cm) of mid-jejunum were excised and placed in buffered formalin for histomorphometry analysis. The breast, gizzard, rest of the small intestine, and ceca were removed, emptied, and weighed. Ceca digesta samples were placed on ice and transported to the laboratory immediately upon collection and stored at −20°C until required for analyses. The left leg was excised, tibia separated, defleshed, fresh weight recorded, and stored at −20°C until further analyses.

Sample Processing and Analyses

The pooled excreta samples were air dried in an oven at 60°C for 48 h. The feed samples and air-dried excreta samples were finely ground using a coffee grinder (KitchenAid, Mississauga, ON, Canada). All the samples were analyzed for dry matter (**DM**), gross energy (**GE**), nitrogen (**N**), crude fat, neutral detergent fiber (**NDF**), calcium (**Ca**), phosphorus (**P**), and titanium (**Ti**). The DM content was determined according to the standard procedures (AOAC International, 2005; method 930.15). The NDF content was determined according to Van Soest et al. (1991) using Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Crude fat content was determined using ANKOM XT 20 Extractor (Ankom Technology, Fairport, NY). Gross energy was measured using IKA bomb calorimeter (C5000; IKA Works, Wilmington, NC). Determination of N was carried out by using Leco N analyzer (FP-528; Leco, Saint Joseph, MI) and crude protein calculated by multiplying N values by 6.25. For measuring the mineral content, the samples were dry ashed, followed by acid digestion with HCl. Minerals were then analyzed by inductively coupled plasma after appropriate dilution (AOAC, method 985.01). The content of Ti was measured on a UV spectrophotometer according to the method described by Myers et al. (2004). The content of starch in the diets was measured in a commercial laboratory (SGS Canada Inc, Guelph, ON, Canada). Fixed jejunal tissues were embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin at 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 the ratio of VH: CD was calculated (Mohammadigheisar et al., 2019). The concentration of SCFA was analyzed as described

by Mohammadigheisar et al. (2019). The tibia samples were weighed and defatted by soaking in hexane for 48 h and 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. (2019). The enzyme recovery (phytase, protease, xylanase, and β-glucanase) was analyzed at DSM Nutritional Products laboratories (Belvidere, NJ). One unit of xylanase was defined as the quantity of the enzyme that liberated 1 μmoL of xylose equivalent per min. One unit of β-glucanase was defined as the amount of enzyme that produces 1 micromole of glucose reducing per min. Protease is measured in PROT units, with 1 unit defined as the amount of enzyme that released 1 μmol of p-nitroaniline from 1 μM of substrate (Suc-Ala-Ala-Pro-Phe-p-nitroaniline) per minute. For phytase, one FYT was defined as the quantity of enzyme that liberates 1 μmol of inorganic phosphate per minute from 5.0 μmol/L sodium phytate.

Table 2. Analyzed chemical composition of experimental diets.

	Starter		Grower	
Item	Control	MES[1]	Control	MES[1]
Dry matter, %	88.1	87.3	88.5	87.5
Gross energy, mcal/kg	4.07	3.99	4.05	397
Crude protein, %	21.6	21.1	19.6	19.0
Crude fat, %	5.38	3.84	5.84	4.15
Starch, %	37.7	39.7	40.5	42.2
Neutral detergent fiber, %	6.63	6.88	6.20	6.61
Ash, %	6.27	4.90	5.60	5.25
Calcium, %	1.14	0.98	0.85	0.80
Phosphorous, %	0.86	0.84	0.66	0.64
Potassium, %	0.84	0.88	0.79	0.75
Magnesium, %	0.18	0.17	0.16	0.15
Sodium, %	0.23	0.22	0.22	0.21
Phytase, FYT/kg	<100	686	<100	514
Protease, PRT/kg	0	8,738	0	3,978
β-glucanase, IU/kg	<15	69	<15	65
Cellulase, IU/kg	51	128	68	121

[1]Multienzyme supplement a blend of phytase, protease, β-glucanase and cellulase targeting 1,500 FYT, up to 8,000 PROT, 70 U, and 80 U per kg of complete feed, respectively.

Calculations and Statistical Analyses

The AR of components was calculated according to Kiarie et al. (2014) as follows:

$$\text{AR, \%} = \left[\left((NT/Ti)_{\text{diet}} \times (NT/Ti)_{\text{excreta}}\right)/(NT/Ti)_{\text{diet}}\right] \times 100,$$

where $(NT/Ti)_{diet}$ is ratio of component and Ti in the diet, and $(NT/Ti)_{excreta}$ = ratio of component and Ti in excreta. Component can be DM, CP, crude fat, Ca, P, or NDF. The apparent metabolizable energy corrected for nitrogen (**AMEn**) was calculated as previously described by Mwaniki and Kiarie (2019). The caloric efficiency (**CE**) was calculated as: (AMEn × FI)/BWG (Leung et al., 2019). The organ weight (breast, gizzard, small intestine, and ceca) data were reported as g/kg of BW. The tibia ash content is expressed as g/g of dry tibia weight and g/kg of BW. The data were subjected to the 2-way ANOVA with fixed effects of MES, strains and associated interactions using the GLM procedures of SAS (SAS 9.4; SAS Institute Inc., Cary, NY) with cage as an experimental unit (n = 8). The LS means were separated by Tukey's test and the *P*-values less than 0.05 were considered significant.

RESULTS

Analyzed chemical composition of experimental diets is shown in Table 2. The concentration of GE, crude fat, Ca, and total P was slightly higher in the control than MES. In contrast, the concentration of starch and NDF was slightly higher in MES than control diets in both phases. With exception of phytase, the recovery of enzyme activities in starter and grower diets was more than 93% (Table 2).

Strains J and N took 3 d longer than strains C and F to finish allocated starter and grower feed (Table 3). There was no interaction ($P > 0.10$) between MES and strain on BW, BWG, and FCR throughout the experiment (Table 3). Strains differed ($P < 0.01$) in BW, BWG, and FCR throughout the experiment (Table 3). In the starter phase, BWG and FCR differed ($P < 0.01$) among the strains with C and F having higher BWG and better (lower) FCR than strains J and N birds, however, strain J had higher BWG and better (lower) FCR than strain N. At the end of starter phase strains C and F were heavier ($P < 0.01$) than strains J and N whereas strain J was heavier than strain N. In the grower phase, C birds were heavier ($P < 0.01$) than slow-growing strains and gained 21.7, 34.1, and 35.4% more BW than F, J, and N birds, respectively. The FCR in C birds was 26.0, 42.9, and 48.1% lower than that of F, J, and N strains, respectively. Over the entire experimental period, BWG and FCR were different ($P < 0.01$) among strains and the overall BWG of C birds was 16.2, 29.6, and 32.9% higher than F, J, and N strains, respectively. Strain F exhibited higher ($P < 0.01$) final BW, BWG and lower FCR than J and N over the entire experimental period. However, strain J had lower ($P < 0.01$) overall FCR than strain N. There was no effect ($P > 0.05$) of MES on BW and BWG in starter phase; however, birds fed MES had higher (713 vs. 684 g/bird, $P = 0.03$) BWG in the grower phase resulting in a tendency (1,062 vs. 1,030 g/bird, $P = 0.08$) for higher total BWG (Table 3). Birds fed the diets supplemented with MES tended (1,104 vs. 1,072 g/bird, $P = 0.08$) to have higher final BW compared to the birds fed with the control diet. The MES had no ($P > 0.10$) effect on FCR throughout the experiment.

There was no interaction between MES and strain on gastrointestinal weight and jejunal histomorphometry (Table 4). Strains C and N differed in gizzard weight ($P = 0.02$), but these 2 strains were not different from strains F or J. Strain C had lighter ($P < 0.01$) ceca than strains J and N whereas strain F had lighter ceca than strain J but similar to strains C and N. Supplemental MES increased gizzard weight ($P = 0.02$) but had no effects on ceca weight. There were no ($P > 0.10$) strain

Table 3. Growth performance in broiler chicken strains differing in growth rates and fed diet without or with multienzyme supplement[1].

		Starter[3]						Grower					Overall		
Strain	MES[2]	Duration, d	iBW, g/bird	BW, g/bird	BWG, g/bird	FI, g/bird	FCR, g/g	Duration, d	BW, g/bird	BWG, g/bird	FI, g/bird	FCR, g/g	BWG, g/bird	FI, g/bird	FCR, g/g
C	–	14	42.3	441	399	490	1.23	14	1,333	892	1,386	1.54	1,291	1,876	1.45
F	–	14	42.3	425	383	486	1.26	14	1,113	687	1,345	1.97	1,070	1,831	1.71
J	–	15	42.3	365	322	451	1.39	16	942	578	1,305	2.21	900	1,756	1.94
N	–	15	42.3	323	280	471	1.57	16	901	578	1,336	2.32	859	1,807	2.11
C	+	14	42.3	438	396	487	1.21	14	1,356	918	1,402	1.53	1,313	1,889	1.44
F	+	14	42.3	426	384	487	1.25	14	1,155	729	1,393	1.90	1,113	1,880	1.69
J	+	15	42.3	360	318	455	1.43	16	976	616	1,339	2.18	934	1,794	1.91
N	+	15	42.3	340	297	479	1.50	16	931	591	1,350	2.24	888	1,829	2.06
SEM				11.3	11.3	14.0	0.04		28.1	21.2	41.8	0.06	28.1	49.2	0.04
Main effects of strain															
C				440[a]	397[a]	489	1.22[c]		1,344[a]	905[a]	1,394	1.54[c]	1,302[a]	1,883	1.45[d]
F				426[a]	383[a]	487	1.25[c]		1,134[b]	708[b]	1,369	1.94[b]	1,092[b]	1,856	1.70[c]
J				363[b]	320[b]	453	1.41[b]		959[c]	597[c]	1,322	2.20[a]	917[c]	1,775	1.93[b]
N				331[c]	289[c]	475	1.53[a]		916[c]	585[c]	1,342	2.28[a]	874[c]	1,818	2.08[a]
SEM				8.87	8.68	10.7	0.03		21.5	16.3	32.0	0.04	21.527	37.7	0.03
Main effects of MES															
-				388	346	475	1.36		1,072	684[b]	1,343	2.01	1,030	1,818	1.80
+				391	349	477	1.35		1,104	713[a]	1,371	1.96	1,062	1,848	1.78
SEM				5.12	5.12	6.30	0.02		12.7	9.59	18.9	0.03	12.7	22.2	0.02
P-value															
Strain				<0.001	<0.001	0.310	<0.001		<0.001	<0.001	0.655	<0.001	<0.001	0.499	<0.001
MES				0.721	0.721	0.797	0.512		0.081	0.035	0.299	0.204	0.081	0.339	0.350
Strain × MES				0.712	0.712	0.977	0.316		0.984	0.876	0.967	0.921	0.984	0.978	0.984

[1]The anticipated age for reaching 2.1 kg BW for C, F, J, and N was 37, 43, 47, and 50 d, respectively.
[2]Multienzyme supplement a blend of phytase, protease, β-glucanase and cellulase targeting 1,500 FYT, up to 8,000 PROT, 70 U, and 80 U per kg of complete feed, respectively.
[3]iBW, initial body weight; BW, body weigh; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.
[a-c]Means assigned different letters within a factor of analysis (strain, MES, and their interactions) are significantly different, $P < 0.05$.

Table 4. Gastrointestinal weight and jejunal histomorphometry in broiler chicken strains differing in growth rates and fed diet without or with multienzyme supplement[1].

		Weight (g/kg of BW)			Histomorphometry, μm		
Strain	MES[2]	Gizzard	Small intestine	Ceca	Villus height (VH)	Crypt depth (CD)	VH: CD ratio
C	–	15.8	55.4	2.19	1,507	265	6.12
F	–	16.0	54.8	3.49	1,301	206	5.43
J	–	17.8	59.9	4.68	1,155	172	7.03
N	–	17.5	54.5	3.98	1,199	201	4.38
C	+	16.8	54.4	2.86	1,175	242	7.50
F	+	17.5	52.3	3.66	1,330	300	5.44
J	+	18.8	55.2	4.21	1,225	200	6.45
N	+	19.9	52.6	3.86	1,176	195	6.08
SEM		0.87	2.20	0.31	102.5	35.47	0.88
Main effects of strain							
C		16.3[b]	54.9	2.89[c]	1,341	253	6.81
F		16.7[ab]	53.6	3.58[bc]	1,316	253	5.43
J		18.3[ab]	57.5	4.44[a]	1,190	186	6.74
N		18.7[a]	53.5	3.92[ab]	1,188	198	5.23
SEM		0.62	1.55	0.22	72.5	25.1	0.62
Main effects of MES							
-		16.8[b]	56.1	3.76	1,291	211	5.74
+		18.3[a]	53.6	3.65	1,227	234	6.37
SEM		0.44	1.10	0.16	51.3	17.7	0.44
P-value							
Strain		0.020	0.233	<0.001	0.295	0.122	0.154
MES		0.020	0.110	0.616	0.382	0.360	0.319
Strain × MES		0.834	0.858	0.787	0.199	0.379	0.511

[1]The anticipated age for reaching 2.1 kg BW for C, F, J, and N was 37, 43, 47, and 50 d, respectively.
[2]Multienzyme supplement a blend of phytase, protease, β-glucanase and cellulase targeting 1,500 FYT, up to 8,000 PROT, 70 U, and 80 U per kg of complete feed, respectively.
[a-c]Means assigned different letters within a factor of analysis (strain, MES, and their interactions) are significantly different, $P < 0.05$.

and MES effects on small intestine weight and jejunal histomorphometry. There were no interactions between strain and MES ($P > 0.05$) on breast and tibia attributes (Table 5). Tibia ash as a function of tibia weight (g/g of bone weight) and percentage (%) was not ($P > 0.10$) influenced by MES, strain or their interactions. However, there was tendency ($P = 0.07$) for interaction between strain and MES on these parameters. In this context, in strain N, tibia weight, and ash percentage in birds fed MES were 7.7 and 6.7% higher than for birds fed the control diets. When tibia ash was expressed as function of BW (g/kg of BW), strain J exhibited higher

Table 5. Tibia attributes and breast meat yield in broiler chicken strains differing in growth rates and fed diet without or with multienzyme supplement[1].

Strain	MES[2]	Tibia ash (g/g of bone wt)	Tibia ash (%)	Tibia ash (g/kg of BW)	Breast yield (g/kg of BW)
C	–	0.41	41.0	1.12	208
F	–	0.40	40.3	1.20	203
J	–	0.41	40.8	1.27	159
N	–	0.39	39.1	1.18	161
C	+	0.40	39.6	1.11	206
F	+	0.40	40.4	1.17	209
J	+	0.42	41.7	1.29	163
N	+	0.42	41.7	1.29	165
SEM		0.01	0.73	0.04	4.96
Main effects of strain					
C		0.40	40.3	1.11[b]	207[a]
F		0.40	40.4	1.18[b]	206[a]
J		0.41	41.2	1.28[a]	161[b]
N		0.40	40.4	1.24[ab]	163[b]
SEM		0.01	0.52	0.03	3.51
Main effects of MES					
–		0.40	40.3	1.19	183
+		0.41	40.9	1.21	186
SEM		0.00	0.37	0.02	2.48
P-value					
Strain		0.558	0.549	<0.001	<0.001
MES		0.283	0.272	0.373	0.371
Strain × MES		0.068	0.068	0.243	0.826

[1]The anticipated age for reaching 2.1 kg BW for C, F, J, and N was 37, 43, 47, and 50 d, respectively.
[2]Multienzyme supplement a blend of phytase, protease, β-glucanase and cellulase targeting 1,500 FYT, up to 8,000 PROT, 70 U, and 80 U per kg of complete feed, respectively.
[a-c]Means assigned different letters within a factor of analysis (Strain, MES, and their interactions) are significantly different, $P < 0.05$.

Table 6. Ceca digesta concentration (μmol/g) of short chain fatty acids in broiler chicken strains differing in growth rates and fed diet without or with multienzyme supplement[1].

Strain	MES[2]	Lactic	Acetic	Propionic	Isobutyric	Butyric
C	–	20.7	58.8[ab]	5.88	4.91	11.7
F	–	15.0	61.1[ab]	6.79	5.98	15.9
J	–	11.5	61.0[ab]	4.51	4.76	14.2
N	–	17.2	75.1[a]	6.35	4.94	15.8
C	+	15.6	68.7[ab]	5.74	4.73	12.8
F	+	13.2	65.3[ab]	6.83	5.62	13.1
J	+	13.2	52.3[b]	5.50	5.08	10.2
N	+	16.5	52.8[b]	5.40	6.00	16.7
SEM		2.09	4.06	0.51	0.38	1.90
Main effects of strain						
C		18.2[a]	63.8	5.81[ab]	4.82[b]	12.3
F		14.1[ab]	63.2	6.81[a]	5.80[a]	14.5
J		12.3[b]	56.6	5.00[b]	4.92[ab]	12.2
N		16.8[ab]	64.0	5.87[ab]	5.47[ab]	16.3
SEM		1.48	2.87	0.36	0.27	1.35
Main effects of MES						
-		16.1	64.0	5.88	5.15	14.4
+		14.6	59.8	5.86	5.36	13.2
SEM		1.05	2.03	0.25	0.19	0.95
P-value						
Strain		0.034	0.228	0.012	0.036	0.108
MES		0.310	0.146	0.965	0.429	0.373
Strain × MES		0.431	0.001	0.299	0.259	0.438

[1]The anticipated age for reaching 2.1 kg BW for C, F, J, and N was 37, 43, 47, and 50 d, respectively.

[2]Multienzyme supplement a blend of phytase, protease, β-glucanase and cellulase targeting 1,500 FYT, up to 8,000 PROT, 70 U, and 80 U per kg of complete feed, respectively.

[a-b]Means assigned different letters within a factor of analysis (strain, MES, and their interactions) are significantly different, $P < 0.05$.

values ($P < 0.01$) than strains C and F strains but the value for strain N was intermediate and similar ($P > 0.05$) to that of other strains. The breast weight differed ($P < 0.01$) between strains with C and F strains showing heavier (~1.3 fold) breast compared with strains J and N (Table 5).There was an interaction ($P < 0.01$) between strain and MES on the concentration of ceca digesta acetic acid (Table 6). Strain N fed the diet without MES showed a higher ($P < 0.01$) concentration of digesta acetic acid than strains J and N fed diet with MES. Strains differed in ceca digesta concentration of lactic ($P = 0.03$), propionic ($P = 0.01$), and iso-butyric ($P = 0.04$) acids (Table 6). The lactic acid in C strain was higher ($P = 0.03$) than strain J but similar ($P > 0.05$) to that of strains F and N. Whereas the concentration of propionic acid in F birds was higher ($P = 0.01$) than in J birds it was similar to that of C and J birds. The F birds also showed higher ($P = 0.04$) concentration of isobutyric than C strains but similar to J and N birds (Table 6).

An interaction ($P < 0.01$) between MES and strain was observed for AR of P and AMEn (Table 7). Supplementation of MES improved AR of P in strains C and F but reduced the same in strains J and N. Strain J had higher AMEn than strain F in absence of MES and MES improved AMEn in strain F only. The AR of crude fat in F and N birds was lower ($P < 0.01$) than that of C strain but similar ($P > 0.05$) to that of strain J. AR of NDF was not affected by strains. Strain N showed higher ($P < 0.01$) AR of Ca than strain F, however, similar to the AR of Ca in strains C and J. Regardless of strain, the birds fed MES exhibited higher AR of DM (75.5 vs. 72.9%, $P < 0.01$), CP (65.7 vs. 63.4%, $P = 0.01$), crude fat (89.2 vs. 85.8%, $P < 0.01$), NDF (33.7 vs. 27.0%, $P < 0.01$), and Ca (42.0 vs. 29.4%, $P<0.01$ than birds feed the diet without MES. The was no ($P > 0.05$) interaction between strain and MES or MES effect on CE. The main effect ($P < 0.001$) of strain was such that, strains differed in utilization of calories for BWG. The CE was 4,930, 5,807, 6,680, and 7,199 kcal/kg BWG for strain C, F, J. N, respectively.

DISCUSSION

The higher BWG of fast-growing broiler chickens is often attributed to higher FI and thus a higher AME intake, resulting in more energy being available for growth (N'dri et al., 2006; Yamak et al., 2014; Siegel, 2014; Brameld and Parr, 2016; Lee and Aggrey, 2016; Tallentire et al., 2016; Xu et al., 2016; Torrey et al., 2021). Poor feed utilization efficiency resulting in increased feed and water use have raised concerns over sustainability of slow-growing broilers (Tallentire et al., 2018; Fisher, 2016, 2017). Feeding strategies such as supplementation with feed enzymes are known to improve nutrient utilization in poultry (Bedford and Schulze, 1998; Slominski, 2011). Feed intake differences have been shown to influence nutrient digestibility and metabolic response through mechanisms such as digesta passage rate (Baker, 1984; Svihus, 2011a; Massuquetto et al., 2020). Thus, we attempted to allocate the same amount of feed based on observed intake of strain C as a basis of investigating whether feed enzymes may influence nutrient utilization in broilers differing in growth rates. We did not observe interactions between strain and feed enzymes on growth performance and many other response parameters perhaps suggesting that feed interventions may not narrow differences in nutrients utilization in broiler chickens differing in growth rates. The results of the present study showed that even when offered equal amount of feed, growth rate, feed efficiency, and breast yield of fast-growing birds were higher than for slow-growing birds. Improved nutrient utilization efficiency in fast-growing broiler chickens is one of the influential factor associated with superior growth rate (Siegel, 2014; Zuidhof et al., 2014; Tallentire et al., 2016). Wen et al. (2018) suggested that the poor feed efficiency was indeed the major challenge of slow-growing broilers.

Practical application of feed enzyme takes 2 approaches: 1) top dressing in a diet formulated to specifications and 2) accounting for expected nutrient uplift (e.g., metabolizable energy, crude protein/amino acids, and minerals) (Kiarie et al., 2013). The second approach was used in the present study and is linked with observed differences in analyzed diet chemical composition. It is rather hard to explain the lower phytase recovery in the present study, however, in terms of application and efficacy, such variation in

Table 7. Apparent retention of components, metabolizable energy and caloric efficiency (CE) in broiler chicken strains differing in growth rates and fed diet without or with multienzyme supplement[1].

		Apparent retention, %							
Strain	MES[2]	DM	CP	CF	NDF	Ca	P	AMEn, mcal/kg DM	CE, mcal/kg BWG
C	–	73.0	65.5	87.3	27.7	28.0	30.9[b]	3.41[ab]	4.94
F	–	72.7	66.7	85.5	27.6	26.3	28.5[b]	3.34[b]	5.80
J	–	73.4	60.6	87.0	27.3	33.3	51.5[a]	3.47[a]	6.77
N	–	72.5	60.9	83.2	25.5	30.0	51.9[a]	3.41[ab]	7.12
C	+	75.4	68.1	91.7	30.2	41.5	48.2[a]	3.46[a]	4.92
F	+	75.8	68.3	88.2	33.3	38.4	46.1[a]	3.44[a]	5.82
J	+	75.2	62.4	89.1	34.7	41.5	20.3[b]	3.43[a]	6.59
N	+	75.6	64.1	88.0	36.6	46.6	28.1[b]	3.45[a]	7.12
SEM		0.63	1.28	0.94	2.62	2.23	2.48	0.02	0.14
Main effects of strain									
C		74.2	66.8[a]	89.5[a]	29.0	34.7[ab]	39.5	3.44[a]	4.93[d]
F		74.3	67.5[a]	86.8[b]	30.4	32.3[b]	37.3	3.39[b]	5.81[c]
J		74.3	61.5[b]	88.1[ab]	31.0	37.4[ab]	35.9	3.45[a]	6.68[b]
N		74.1	62.5[b]	85.6[b]	31.1	38.3[a]	40.0	3.43[ab]	7.12[a]
SEM		0.45	0.91	0.67	1.85	1.58	1.75	0.01	0.10
Main effects of MES									
–		72.9[b]	63.4[b]	85.8[b]	27.0[b]	29.4[b]	40.7[a]	3.41[b]	6.16
+		75.5[a]	65.7[a]	89.2[a]	33.7[a]	42.0[a]	35.7[b]	3.44[a]	6.11
SEM		0.32	0.64	0.47	1.31	1.12	1.24	0.01	0.07
P-value									
Strain		0.977	<0.001	0.001	0.834	0.044	0.301	0.004	<0.001
MES		<0.001	0.014	<0.001	<0.001	<0.001	0.006	0.001	0.655
Strain × MES		0.728	0.913	0.437	0.428	0.335	<0.001	0.001	0.892

Abbreviations: AMEn, apparent metabolizable energy corrected for nitrogen; Ca, calcium; CP, crude protein; CF, crude fat; DM, dry matter; NDF, neutral detergent fiber; P, phosphorus.

[1]The anticipated age for reaching 2.1 kg BW for C, F, J, and N was 37, 43, 47, and 50 d, respectively.

[2]Multi-enzyme supplement a blend of phytase, protease, β-glucanase and cellulase targeting 1,500 FYT, up to 8,000 PROT, 70 U, and 80 U per kg of complete feed, respectively.

[a-b]Means assigned different letters within a factor of analysis (strain, MES, and their interactions) are significantly different, $P < 0.05$.

target and recovered activities is acceptable by industry standards (Bedford, 2018). We observed an improved BWG in the grower phase and a tendency in improving overall BWG with inclusion of the dietary enzymes. The improvement in the growth of the broiler chickens fed with the diet supplemented with MES, independent of growth rate, might be partly linked to improved digestibility of the nutrients and energy. Cowieson (2010) estimated that there is about 400 to 450 kcal of energy per kg of diet not being digested when birds are fed a typical corn-soybean ration. Cowieson (2010) noted that a combination of undigested components including fat, protein, and starch contributes to this energy loss, and supplementing the diets with feed enzymes can make this energy available to birds. However, it was rather surprising enzyme effects on growth was not observed in starter phase. Given the nutrient contribution of enzymes were accounted for in the diet formulation, it also plausible the BWG response in grower phase could have been due to functional enzyme effects such as in gut health and function (Kiarie et al., 2013).

The broiler chicken growth rates have also been associated with gastrointestinal development and function (Miska and Fetterer, 2019; Singh et al., 2021). Gizzard has several important functions, such as aiding digestion by particle size reduction, chemical degradation of nutrients and regulation of feed flow (Svihus, 2011b). Strain N had lower relative gizzard weight compared to strain C which could be attributed to lighter BW. The effect of MES on gizzard weight was difficulty to explain as only the structural feed components have been linked to increased gizzard weight (Svihus, 2011b; Kiarie and Mills, 2019). There is a correlation between visceral organs and maintenance energy requirements (Mitchell and Smith, 1991; Havenstein et al., 2003; Fanatico et al., 2005; Tallentire et al., 2016). Mitchell and Smith (1991) noted that the relative amount of mucosa in the small intestine and, therefore, the rate of cell turnover had a negative correlation with the growth rate of the birds. 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). The relative weight of small intestine, as the major site of the absorption of nutrients, and VH and CD did not differ between strains in the present study. Previous studies have found that the fast-growing birds have higher digestive surface area and more intestinal membrane transport proteins per unit area (Mitchell and Smith, 1991; Katanbaf et al., 1988; Mott et al., 2008; Miska and Fetterer, 2019). Strain effect was noted for the ceca weight with J strain showing higher ceca weight than strains C and F but similar to strain N. The quantitative significance of ceca in high-yielding broiler chickens remains to be elucidated (Svihus et al., 2013). However, increased ceca weight in broiler chickens was linked to increased flow of undigested from the small intestine (Huang et al., 2006). Such effects could lead to changes in microbial growth and activity in the ceca. However, although strains

showed differences in the concentration of ceca digesta SCFA this could not be associated with observed ceca weight.

Bone ash is an indicator of bone mineralization (Shim et al., 2012). We previously reported that the tibia ash content (g/kg BW and g/g of tibia weight) of fast-growing broiler chickens was lower than slow-growing broiler chickens raised under similar management regimen (Mohammadigheisar et al., 2020). Similarly, the strains exhibiting slower growth rate had higher tibia ash (g/kg BW) in the present study. The effect of strain may be primarily due to the differences in BW, but it may also serve as an index of the higher susceptibility of fast-growing broiler skeletal disorders. The effects of feed enzyme on bone mineralization are linked with increased retention of minerals particularly Ca and P (Kiarie et al., 2015). However, although birds fed MES showed higher Ca retention this did not translate to improved tibia attributes.

Undigested starch and protein, as well as fiber that bypass the small and enter the ceca for fermentation to produce SCFA and onward voiding with excreta. The anaerobic fermentation of material entering the ceca produces mainly SCFA in largely conservative molar proportions of acetic acid > butyric acid > propionic acid (Svihus et al., 2013). Contrary to the findings of our previous study (Mohammadigheisar et al., 2020), the concentration of lactic, propionic, and iso-butyric acids was affected by strains in the present study. Although not investigated in the present study, differences between strains in ceca fermentation metabolites may be linked to differences in microbial composition. Several studies have reported differences in microbial diversity and composition in broiler chicken lines differing in growth efficiency (as reviewed, Kers et al., 2018). Thus, strain N birds had lower acetic acid concentration when fed diet supplemented with MES. It is thought that supplemental feed enzymes reduce the quantity of undigested nutrients flowing in the ceca for fermentation (Kiarie et al., 2013).

The disappearance of components in the gut can be an index of absorption by host animal and/or microbial digestion at the hindgut. Microbial digestion or fermentation accounts for 8 to 16% of the organic matter disappearing from the gastrointestinal tract (McDonald et al., 1995) which plays a crucial role in increasing the retention of components. Fast-growing broiler chickens digested more energy than slow growing birds when fed the same diet on ad libitum basis (Singh et al., 2021). Free access to feed may not allow separation of a feed intake response from a metabolic efficiency response (Baker, 1984). However, equalized (controlled) feed intake allows separation of these responses. Although the strains used in the present study showed differences in growth rate, the patterns of CP, crude fat, and NDF retention were not associated with growth rates. It is therefore plausible that broiler chicken growth rate does not influence digestibility/metabolizability of energy and energy yielding nutrients. Independent of strain, MES supplementation improved the AR of DM, CP, crude fat, NDF, and Ca. Thus, the improvement in the growth performance of the broiler chickens fed with the diet supplemented with MES regardless of growth rate was due to increased digestibility of the nutrients.

Previous researchers reported that the fiber degrading enzymes can enhance the access of endogenous enzymes to nutrients trapped in complex cell wall molecules (Yu and Chung, 2004; Leslie et al., 2007; Kiarie et al., 2014). On the other hand, several studies have shown that addition of exogenous enzyme products in corn-soybean meal based diets can also increase digestibility of CP (Zanella et al., 1999; D'Alfonso, 2005; Cowieson and Ravindran, 2008). The indigestible feed components are exceedingly complex and it has been suggested enzyme composites beyond the simple core mixes of xylanases, cellulases, and β-glucanases can exploit synergistic benefits generated by this class of enzymes (Kiarie et al., 2020; Ward, 2021). Thus, effects of MES on components retention may be linked to the side activities. Considering the fact that over 90% of Ca and 80% of P content in the body of the birds is found in the bones, availability of these minerals are essential for skeletal development (Shim et al., 2012). Calcium and P retention are closely related to skeletal development. It was rather surprising that strain J had the highest tibia ash (g/kg BW) yet had the similar AR of Ca compared to other strains. Strain N had the highest AR of Ca and similar tibia ash (g/kg BW). The interaction between strain and MES on AR of P was such that, MES improved P retention in strains C and F but reduced it in strains J and N. These patterns of MES responses on AR of P are difficult to interpret but they were associated with growth rate and tibia as (g/kg BW) in the present study.

The present study extends observations reported elsewhere (e.g., Torrey et al., 2021) that growth rate and feed utilization efficiency are closely linked in broiler chickens. There was no interaction between strains and supplemental feed enzymes on feed efficiency suggesting improving digestibility *per se* may not close the gap in feed efficiency in birds exhibiting different growth rates. Enzyme supplementation improved growth and nutrient retention independent of strain, suggesting feed enzyme responses are not influenced by growth rates in broiler chickens. It is also noteworthy that formulation of MES accounted for targeted nutrient release by the core main activities implying that enhanced retention was associated with side activities.

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DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Nelson E. Ward is an employee of DSM nutritional products.

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