The interactive influence of barley particle size and enzyme supplementation on growth performance, nutrient utilization, and intestinal morphometry of broiler starters

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ABSTRACT The influence of barley particle size and enzyme supplementation on performance, nutrient and energy utilization, and intestinal morphometry of broiler starters (day 1–21) fed pelleted barley-based diets was evaluated. Two barley particle sizes (fine and coarse) and 4 enzyme treatments (non-supplemented [control], carbohydrase [0.15 g/kg of feed; Carb], phytase [0.10 g/kg; Phy] and combination of carbohydrase and phytase [0.15 and 0.10 g/kg, respectively; Carb + Phy]) were evaluated in a 2 × 4 factorial arrangement. Fine and coarse barley particles were achieved by grinding whole barley in a hammer mill to pass through 2.0 and 8.0 mm screens, respectively. A total of 384, 1-day-old male broilers (8 birds/cage; 6 cages/treatment) were used. Supplemental enzymes tended ($P = 0.056$) to increase the weight gain of birds with a synergetic effect from Carb + Phy. The response of feed intake to supplemental enzymes interacted ($P < 0.05$) with barley particle size, as Phy increased feed intake only in fine barley diets. Both coarse particles and supplemental Carb, either individually or in combination with Phy, reduced feed per gain ($P < 0.001$). Digestibility of DM, nitrogen, and fat was greater in birds fed coarse barley diets ($P < 0.05$). Dry matter, starch, fat, and phosphorus digestibility values were improved by supplemental enzymes ($P < 0.05$). Coarse barley ($P < 0.05$) and Carb ($P < 0.001$), either individually or in combination, increased the AMEn. Coarse barley reduced the gizzard pH ($P < 0.001$). Birds fed diets with supplemental enzymes had shorter jejunum ($P < 0.05$). Neither the barley particle size nor supplemental enzymes ($P > 0.05$) affected the jejunal digesta viscosity. In summary, feeding coarse barley particles and supplemental Carb improved the feed efficiency and nutrient and energy utilization. The effects of barley particle size on measured parameters suggest that the particle size effect was preserved even after pelleting. The combination of Carb and Phy tended to improve the weight gain but caused no further improvements in nutrient utilization.

Key words: barley, broilers, carbohydrase, phytase, particle size

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INTRODUCTION

Owing to possible impairment of pellet physical quality associated with coarser grain particles, fine grinding of ingredients followed by pelleting has become the standard practice in feed manufacture. However, the lack of structural components in highly processed poultry diets masks the benefits offered by superior pellet quality and results in suboptimal functionality of the foregut followed by feed overconsumption, poor nutrient digestibility, and increased consumption of litter leading to poor intestinal health (Hetland et al., 2004; Svihus, 2011a; Rodrigues and Choct, 2018). This concern has increased the interest on methods to restore the structure of the diet. Inclusion of insoluble fiber sources (Hetland et al., 2004), coarse cereal particles (Amerah et al., 2007a; Abdollahi et al., 2019), or whole grains (Singh et al., 2014) in broiler diets has been practised to improve the physical microstructure of feed. However, insoluble fiber and whole grains can only be incorporated up to a certain level because of possible nutrient dilution, feed intake (**FI**) depression, and increased segregation (Singh et al., 2014; Rodrigues and Choct, 2018). Manipulation of grain particle size therefore provides a promising solution because of easier adaptation into normal feed processing practice.

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Cereal grains are ground to reduce the particle size with the aim of modifying their physical characteristics. Grinding facilitates handling, mixing, and further processing (extrusion and pelleting) and increases the exposure of nutrients in the endosperm to digestive enzymes (Amerah et al., 2011). Fine grinding results in greater surface area, and consequently greater substrate availability for enzymatic digestion, and decreases segregation ensuring the homogeneity of mixed feed. Coarse grinding, on the other hand, stimulates gizzard development and functionality, facilitating digestion of nutrients through enhanced grinding activity and gut motility (Amerah et al., 2007a). A key benefit of feeding coarse particles is stronger reverse peristaltic contractions between the gizzard and proventriculus resulting in increased secretion of hydrochloric acid and proteolysis by pepsin (Svihus, 2011a). Accordingly, the use of coarse particles in pelleted diets may optimize intestinal development and function (Abdollahi et al., 2019).

However, the pelleting process may further reduce the size of feed particles, especially of coarser particles, and equalize the differences in particle size distribution (Svihus et al., 2004; Amerah et al., 2007b; Abdollahi et al., 2013), suggesting that the particle size impact is more pronounced in mash diets than in pelleted or crumbled diets (Zaefarian et al., 2016). However, some reports indicate that the effects of feed particle size on bird performance exist even after pelleting (Nir et al., 1995; Naderinejad et al., 2016). Moreover, recommendations regarding the optimum particle size are contradictory due to the confounding effects from several factors including grain type, feed form, complexity of the diet, endosperm hardness, grinding method, particle size distribution, and pellet quality (Amerah et al., 2007a; Abdollahi et al., 2018). The influence of grain particle size on growth performance and nutrient utilization of broilers fed corn-based (Amerah and Ravindran, 2009; Naderinejad et al., 2016) and wheat-based (Lentle et al., 2006; Amerah et al., 2007b; Abdollahi et al., 2019) diets has been examined, but corresponding studies with barley are lacking.

In addition to carbohydrases (**Carb**) that target nonstarch polysaccharides (**NSP**) present in viscous grains such as wheat and barley, phytases (**Phy**) are routinely added to cereal-based diets to facilitate the release of phytate-bound phosphorus (**P**) and reduce the P effluent from intensive poultry production (Ravindran et al., 1995). Several researchers have evaluated the individual and combined supplementation of Carb and Phy to corn-based (Juanpere et al., 2005), wheat-based (Ravindran et al., 1999; Wu et al., 2004a,b; Juanpere et al., 2005; Abdollahi et al., 2016) and barley-based (Ravindran et al., 1999; Wu et al., 2004a; Juanpere et al., 2005) diets. The combination of Carb and Phy is believed to facilitate each other's substrate access; however, the effects seem to be inconsistent (Selle et al., 2003) and require further elucidation.

With the aim of maximizing the benefit from supplemental enzymes, only a limited number of studies has focused on determining the optimum dietary conditions for enzyme action. Along with many other factors, particle size was recognized to cause variability in responses to supplemental enzymes (Ravindran, 2013), and their effectiveness could be improved by optimizing the particle size in diet formulations (Amerah et al., 2008b). Consequently, there has been some interest in the interaction between particle size and supplemental enzymes (Amerah et al., 2011). Findings from limited studies that evaluated the interaction between particle size of corn (Kasim and Edwards, 2000; Amerah and Ravindran, 2009) and wheat (Amerah et al., 2008b) and supplemental enzymes are contradictory, and to the authors' knowledge, no corresponding studies are available with barley. Moreover, the interaction of particle size and supplemental enzymes can be influenced by the feed form due to pelleting-induced particle size reduction. Accordingly, the present study was conducted to assess the potential interactive influence of barley particle size and Carb and Phy addition, individually or in combination, on growth performance, nutrient digestibility, and intestinal morphometry of broiler starters fed pelleted diets.

MATERIALS AND METHODS

Enzymes

A multicomponent NSP-degrading enzyme, Ronozyme Multigrain (produced by *Trichoderma reesei,* also known as *Trichoderma longiabrachiatum*) and Ronozyme HiPhos were obtained from DSM Nutritional Products, East Wagga Wagga, Australia. The activities of endo-1,4-β- glucanase, endo-1,3 (4)-β-glucanase and endo-1,4-β-xylanase in Ronozyme Multigrain were 800 β-glucanase activity (**BGU**)/g, 700 BGU/g, and 2,700 XU/g, respectively. One unit of xylanase activity (**XU**) is defined as the quantity of enzyme that releases 1.0 μmol of reducing moieties from 1.5% arabinoxylan per minute at pH 5.0 and incubation temperature of 40°C for 20 min. One unit of BGU is defined as the quantity of enzyme that releases 1.0 μmol of reducing moieties from 1.5% β-glucan per minute at pH 5.0 at incubation temperature of 40°C for 20 min. Ronozyme HiPhos is a granular 6-Phy preparation expressed by submerged fermentation of *Aspergillus oryzae* and contains >10,000 Phy units/g (**FYT**). One FYT is defined as the activity of enzyme that releases 1.0 μmol of inorganic P/minute from 5.0 mmol sodium phytate at pH 5.5 at 37°C (DSM Nutritional Products Ltd., 2013). The activities of Phy, endo-1,3 (4)-β-glucanase, and endo-1,4-β-xylanase in samples of pelleted diets were measured at Biopract GmbH, Berlin, Germany. The enzyme recovery was calculated as the percentage of measured enzyme activity in the diet to the expected enzyme activity estimated from the amount and minimum activity (DSM Nutritional Products Ltd., 2013) of enzymes added to the diets.

Diets

Normal-starch hulled barley (cultivar, Fortitude), obtained from a seed company (Luisetti Seeds Ltd., Rangiora, New Zealand), was ground in a hammer mill to pass through 2.0 and 8.0 mm screens to achieve fine and coarse barley particles, respectively. Nutrient composition, AMEn, and standardized digestible amino acid contents of barley, determined in a previous study (Perera et al., 2019a), were used to formulate a basal diet to meet the Ross 308 strain recommendations for major nutrients for broiler starters (Ross, 2019; Table 1). The basal diet contained 4.8 g/kg nonphytate P. Two diets, mixed using fine or coarse barley, were developed into 8 dietary treatments using 4 methods of enzyme supplementation: nonsupplemented (control), carbohydrase (0.15 g/kg of feed; Carb), phytase (0.10 g/kg; Phy), and combination of carbohydrase and phytase (0.15 and 0.10 g/kg, respectively; Carb + Phy). The diets contained 5.0 g/kg of titanium dioxide (TiO_2, Merck KGaA, Darmstadt, Germany) as an indigestible marker to determine ileal nutrient digestibility. Diets were mixed in a single-screw paddle mixer. Following mixing, all diets were steam-conditioned to 70°C for 30 s and pelleted using a pellet mill (Model Orbit 15; Richard Sizer, Kingston-upon-Hull, UK) with capacity of manufacturing 180 kg of feed/hour and equipped with a die ring with 3.0 mm holes and 35 mm thickness. Representative diet samples were collected after pelleting for chemical analysis and determination of particle size distribution and pellet durability.

Table 1. Composition, calculated analysis, and analyzed values (g/kg, as fed) and pellet durability index (%) of the basal diet.

	Inclusion (g/kg)
Item	
Normal starch hulled barley	550
Soybean meal	318.4
Corn gluten meal	50.0
Soybean oil	33.8
Di-calcium phosphate	20.4
Limestone	6.0
L-Lysine HCl	3.1
DL-Methionine	2.4
L-Threonine	1.2
Sodium chloride	1.9
Sodium bicarbonate	3.8
Vitamin premix[1]	1.0
Mineral premix[1]	1.0
Titanium dioxide[2]	5.0
Pellet binder[3]	2.0
Calculated analysis	
AMEn, kcal/kg	2,850
Total protein	238
Digestible protein	196
Digestible methionine	5.8
Digestible methionine + cysteine	9.0
Digestible lysine	12.2
Digestible threonine	8.2
Digestible arginine	13.1
Digestible valine	9.5
Crude fat	46.0
Crude fiber	43.9
Calcium	9.6
Nonphytate phosphorus	4.8
Sodium	2.0
Chloride	2.0
Potassium	8.4
Analyzed values	
DM	900
Gross energy, kcal/kg	4,135
Crude protein (Nitrogen × 6.25)	248
Starch	315
Fat	49.5
Calcium	8.5
Total phosphorus	7.6
Pellet durability index (%)[4]	
Finely ground diet	82.5[a]
Coarsely ground diet	79.0[b]

[1]Supplied per kg of diet: antioxidant, 125 mg; biotin, 0.2 mg; calcium pantothenate, 20 mg; cholecalciferol, 5000 IU; cyanocobalamin, 0.02 mg; folic acid, 2.0 mg; menadione, 4 mg; niacin, 80 mg; pyridoxine, 5.0 mg; trans-retinol, 15,000 IU; riboflavin, 9.0 mg; thiamine, 4.0 mg; dl-α-tocopheryl acetate, 80 IU; choline, 0.45 mg; ascorbic acid, 100 mg; Co, 1.0 mg; Cu, 20 mg; Fe, 40 mg; I, 2.0 mg; Mn, 100 mg; Mo, 1.0 mg; Se, 0.15 mg; Zn, 100 mg. [1]Image Holdings Ltd., Auckland, New Zealand.

[2]Merck KGaA, Darmstadt, Germany.

[3]KEMBIND (Kemin Industries [Asia] Pte Ltd.) pellet binder, which contained modified lignosulphonate, guar gum, edible fatty acids, and mineral oil.

[4]Each value represents the mean of 5 replicate samples. Means not sharing common letters (a,b) are different ($P < 0.05$).

Determination of Particle Size Distribution

Particle size distribution of ground barley samples was determined using a dry sieving method as described by Baker and Herrman (2002). Briefly, ground barley samples (100 g; 4 replicates per particle size) were passed through a sieve stack with a set of 6 sieves (2.0, 1.0, 0.5, 0.25, 0.125, and 0.063 mm) on shakers for 5 min. The amount of sample retained on each sieve was determined, and the geometric mean diameter (**GMD**) and geometric standard deviation (**GSD**) was calculated for each sample. These calculations assumed that weight distribution of the sample was logarithmically normal. The following equations were used to calculate the GMD and GSD.

$$di = (du \times do)\ \hat{}0.5$$

$$GMD = \log^{-1}\left\{\sum(Wi \log di) \Big/ \sum Wi\right\}$$

$$GSD = \log^{-1}\left\{\sum Wi(\log di - \log GMD)^2 \Big/ \sum Wi\right\}^{0.5}$$

Where.

di = diameter of i^{th} sieve on stack

du = diameter opening through which particles were passed (sieve preceding i^{th})

do = diameter opening through which particles were not passed (i^{th} sieve)

Wi = weight fraction of sample on i^{th} sieve.

Particle size distribution of the 2 basal pelleted diets were determined by wet sieving using the method described by Lentle et al. (2006). Two weighed samples (100 g each; 2 replicates per particle size) of diets were used in the analysis. One sample was dried at 80°C in a forced draft oven for 3 D for the determination of DM. The second sample was soaked in 400 mL water and was left to stand for 2 h before sieving. The same

sieve sizes used in the dry sieving method were used. The contents of each of the sieves were subsequently washed onto dried, preweighed filter papers, dried in a forced draft oven at 80°C for 24 h, and re-weighed. The dry weight of particles retained by each sieve was expressed as proportion of total DM recovered.

Pellet Durability

Pellet durability was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK) using the method described by Abdollahi et al. (2013). Briefly, samples of whole pellets (100 g; 5 replicates per diet) with no fines were rapidly circulated in an air stream around a perforated test chamber for 30 s. Resulting fines were removed continuously through the perforations during the test cycle. After the test cycle, pellets were ejected and weighed manually. The pellet durability index was calculated as the percentage of weight of pellets not passing through the perforations at the end of the test to weight of whole pellets at the start.

Birds and Housing

The experimental procedures were approved by the Massey University Animal Ethics Committee and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes. A total of 384, 1-day-old male broilers (Ross 308), obtained from a commercial hatchery, were individually weighed and allocated to 48 cages containing 8 birds each of similar weight in electrically heated battery brooders so that the average bird weight per cage was similar. Each of the 8 dietary treatments was randomly assigned to 6 cages. The birds were transferred to grower cages on day 12 and continued on the same starter diets until the end of the trial (day 21). The space allocation per bird in brooder and grower cages was 530 and 640 cm^2, respectively. The battery brooders and grower cages were housed in an environmentally controlled room with 20 h of fluorescent illumination per day. The temperature was maintained at 31°C on day 1 and was gradually reduced to 22°C by 21 D of age. The diets were offered *ad libitum* and water was available at all times.

Performance Data

Body weights (**BW**) and FI were recorded on a cage basis at weekly intervals. Mortality was recorded daily. Feed per gain (**F/G**) values were corrected for the BW of any bird that died during the course of the experiment.

Energy and Nutrient Utilization

Nitrogen-corrected apparent metabolizable energy

The AME_n was determined using the classical total excreta collection method. Feed intake and total excreta output of each cage were quantitatively measured from day 17 to 20 posthatch. Daily collections from each cage were pooled, mixed in a blender, and subsampled. Subsamples were lyophilized (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5 mm sieve, and stored in airtight plastic containers at 4°C pending analysis. The diets and excreta samples were analyzed for DM, gross energy (**GE**), and nitrogen (**N**).

Coefficient of apparent ileal digestibility of nutrients

On day 21, six broilers per cage were euthanized by intravenous injection (0.5 mL per kg BW) of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand), and digesta were collected from the lower half of the ileum by gently flushing with distilled water, as described by Ravindran et al. (2005). The ileum was defined as that portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo-cecal junction. The ileum was then divided into 2 halves, and the digesta were collected from the lower half toward the ileo-cecal junction. Digesta from birds within a cage were pooled, frozen immediately after collection, and subsequently lyophilized. Diet and lyophilized digesta samples were ground to pass through a 0.5 mm sieve and stored at 4°C until laboratory analysis. The diets and digesta were analyzed for DM, titanium (**Ti**), N, starch, fat, calcium (**Ca**), and P.

Gizzard pH and Jejunal Digesta Viscosity

Gizzard pH was measured in 2 birds, from each replicate cage, euthanized for ileal collection using a pH meter (pH spear, Oakton Instruments, Vernon Hill, IL). The glass probe was inserted directly through an opening made in the gizzard and placed in the digesta. Three values were taken from the proximal, middle, and distal sections of gizzard, and the average value was considered as the final pH value.

The viscosity of jejunal digesta from 2 birds euthanized for ileal collection from each replicate cage was also measured. The jejunum is defined as portion of small intestine extending from pancreatic loop to the Meckel's diverticulum. The jejunum was divided into 2 halves, and the digesta were collected from the lower half toward the Meckel's diverticulum. Digesta collected from each bird were centrifuged at 3,000 × *g* at 20°C for 15 min. A 0.5 mL aliquot of the supernatant was used in a viscometer (Brookfield digital viscometer, Model DV2TLV, Brookfield Engineering Laboratories Inc., Stoughton, MA) fitted with CP-40 cone spindle with shear rates of 5 to 500/s to measure the viscosity.

Digestive Tract Measurements

On day 22, two additional birds with BW closest to the mean weight of the cage were weighed and euthanized by cervical dislocation. The digestive tract from the proventriculus to ceca was carefully excised, and adherent fat was removed. The length of duodenum (pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to ileocecal junction), and ceca were recorded as described by Amerah et al. (2008b) and reported as

cm/kg of BW. The empty weights of proventriculus, gizzard, duodenum, jejunum, ileum, and ceca were determined and reported as g/kg of BW.

Chemical Analysis

Dry matter was determined using standard procedures (Method 930.15; AOAC, 2016). Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a CNS-200 carbon, N, and sulphur auto-analyzer (LECO Corporation, St. Joseph, MI). An adiabatic bomb calorimeter (Gallenkamp Autobomb, London, UK) standardized with benzoic acid was used for the determination of GE. Starch was measured using a Megazyme kit (Method 996.11; AOAC, 2016) based on thermostable α-amylase and amyloglucosidase (McCleary et al., 1997). Fat was determined using the Soxtec extraction procedure for animal feed, forage, and cereal grains (Method 2003.06; AOAC, 2016). For mineral analysis, the samples were wet digested in a nitric and perchloric acid mixture, and concentrations of P and Ca were determined by inductively coupled plasma-optical emission spectroscopy using a Thermo Jarrell Ash IRIS instrument. Samples were assayed for Ti on a UV spectrophotometer following the method of Short et al. (1996).

Calculations

The AME of diets was calculated using the following formula:

$$\text{AME}_{\text{diet}}\ (\text{kcal}/\text{kg}) = \frac{(\text{FI} \times \text{GE}_{\text{diet}}) - (\text{Excreta output} \times \text{GE}_{\text{excreta}})}{\text{Total FI}}$$

Correction for 0 N retention was made using a factor of 8.73 kcal per g N retained in the body (Hill and Anderson, 1958).

$$\text{AMEn}_{\text{diet}}\left(\text{kcal}/\text{kg}\right) = \frac{\text{AME}_{\text{diet}} - (8.73 \times \text{N retention})}{1000}$$

Apparent ileal digestibility coefficients of nutrients were calculated from the dietary ratio of nutrients to Ti relative to the corresponding ratio in the ileal digesta.

$$\text{CAID of nutrient} = \frac{(\text{Nutrient}/\text{Ti})_{\text{d}} - (\text{Nutrient}/\text{Ti})_{\text{i}}}{(\text{Nutrient}/\text{Ti})_{\text{d}}}$$

where, $(\text{Nutrient}/\text{Ti})_{\text{d}}$ = ratio of nutrient to Ti in diet and $(\text{Nutrient}/\text{Ti})_{\text{i}}$ = ratio of nutrient to Ti in ileal digesta.

Statistical Analysis

The data were analyzed as a 2 × 4 factorial arrangement of treatments using the general linear model procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC.). Cage served as the experimental unit. Significant differences between means were separated by least significant difference test. Significance was declared at $P < 0.05$.

RESULTS

Particle Size Distribution and Pellet Durability

As shown in Table 2, the GMD of barley ground through 2.0 and 8.0 mm screen sizes were 648 and 1,249 μm, respectively, with corresponding GSD values of 2.0 and 1.9 μm. The GMD values of fine and coarse barley-based diets were 215 and 263 μm, respectively, with corresponding GSD values of 3.6 and 4.0 μm.

A significant effect of barley particle size ($P < 0.001$; Table 1) was observed for pellet durability index, with poorer pellet durability in diets made of coarsely ground barley (79.0%) compared with the diets made of finely ground barley (82.5%).

Enzyme Recovery

The average recovery of Phy, endo-1,3 (4)-β-glucanase, and endo-1,4-β-xylanase from enzyme-supplemented diets were 78, 52, and 67%, respectively.

Growth Performance

Mortality during the experiment was insignificant. Only 7 out of the 384 birds died, and the deaths were not related to any dietary treatment.

As summarized in Table 3, supplemental enzymes tended ($P = 0.056$) to improve the weight gain (**WG**) of birds with a synergetic effect from the combined use of enzymes. Regardless of barley particle size and in comparison to the control diet, the combination of enzymes increased the WG by 28 g/bird. The FI response to the supplemental enzymes interacted ($P < 0.05$) with barley particle size, as the individual supplementation of Phy resulted in greater FI only in fine barley diets. Coarse particle size and supplemental Carb, either individually or in combination with Phy, reduced ($P < 0.001$) the F/G.

Table 2. Determined particle size distribution (percentage of retained particles on sieves) and geometric mean diameter ± geometric standard deviation (GMD ± GSD) of ground barley and diets.

	Sieve pore size (μm)							
Particle size	2,000	1,000	500	250	125	63	< 63	GMD ± GSD
Ground barley[1]								
Fine	0.04	28.9	43.1	17.5	7.93	1.83	0.70	648 ± 2.0
Coarse	31.8	44.1	15.0	5.74	2.25	0.94	0.17	1,249 ± 1.9
Pelleted diets[2]								
Fine	0.66	13.1	20.0	18.1	7.55	4.82	35.8	215 ± 3.6
Coarse	5.96	16.9	16.8	16.3	7.60	3.39	33.0	263 ± 4.0

Fine and coarse grade were achieved using screen sizes of 2.0 and 8.0 mm, respectively.
[1]Each value represents the mean of 4 replicates.
[2]Each value represents the mean of 2 replicates. Fine and Coarse refers to particle size of barley used to make pellets.

Nutrient and Energy Utilization

The effects of barley particle size and enzyme supplementation on nutrient and energy utilization are summarized in Table 4. No significant ($P > 0.05$) interaction between particle size and enzyme supplementation was observed for the coefficient of apparent ileal digestibility (**CAID**) of any nutrient or AMEn. Greater ($P < 0.05$) CAID of DM, N, and fat were observed in birds fed coarse barley diets. Feeding coarse barley tended ($P = 0.071$) to increase the CAID of Ca. Regardless of barley particle size, all supplemental enzymes increased ($P < 0.05$) the DM digestibility. Carb addition (Carb and Carb + Phy) improved ($P < 0.05$) starch and fat digestibility. Phosphorus digestibility was positively influenced ($P < 0.01$) by enzyme supplementation, with greater P digestibility in diets with Phy (Phy and Carb + Phy; $P < 0.05$). Coarse grinding of barley ($P < 0.05$) and Carb enzyme improved ($P < 0.001$) the AMEn.

Relative Weight and Length of Intestinal Segments, Gizzard pH and Jejunal Digesta Viscosity

Table 5 shows the influence of barley particle size and enzyme supplementation on the relative weight and length of intestinal segments, gizzard pH, and jejunal digesta viscosity. A significant ($P < 0.01$) barley particle size × enzyme interaction was observed for the relative weight of gizzard, as supplemental Phy in fine and coarse barley diets resulted in the lowest and the highest relative gizzard weights, respectively. No significant

Table 3. The influence of barley particle size and carbohydrase (Carb) and phytase (Phy) supplementation, individually or in combination (Carb + Phy) in pelleted diets on weight gain (WG; g/bird), feed intake (FI; g/bird), and feed per gain (F/G; g feed/g gain) of broiler starters[1] (1–21 D of age).

Particle size	Enzyme	WG	FI	F/G
Fine	Control	1,185	1,477^{b,c}	1.246
	Carb	1,198	1,442^{c}	1.214
	Phy	1,208	1,519^{a}	1.256
	Carb + Phy	1,223	1,501^{a,b}	1.235
Coarse	Control	1,197	1,474^{b,c}	1.235
	Carb	1,204	1,456^{c}	1.209
	Phy	1,199	1,463^{c}	1.220
	Carb + Phy	1,215	1,458^{c}	1.203
SEM[2]		9.8	12.6	0.0074
Main effects				
Particle size				
Fine		1,203	1,485	1.238^{a}
Coarse		1,204	1,463	1.217^{b}
Enzyme				
	Control	1,191	1,475	1.240^{a}
	Carb	1,201	1,449	1.211^{b}
	Phy	1,204	1,491	1.238^{a}
	Carb + Phy	1,219	1,479	1.219^{b}
Probabilities, $P \leq$				
Particle size		0.962	0.018	0.001
Enzyme		0.056	0.014	0.001
Particle size × Enzyme		0.634	0.026	0.107

[a-c]Means in a column not sharing common letters are different ($P < 0.05$).
[1]Each value represents the mean of 6 replicates (8 birds per replicate).
[2]Pooled standard error of mean.

Table 4. The influence of barley particle size and carbohydrase (Carb) and phytase (Phy) supplementation, individually or in combination (Carb + Phy) in pelleted diets on coefficient of apparent ileal digestibility (CAID)[1] of dry matter (DM), nitrogen (N), starch, fat, calcium (Ca), phosphorus (P), and N-corrected apparent metabolisable energy (AMEn; kcal/kg DM)[2] of 21-day-old broiler starters.

		CAID						
Particle size	Enzyme	DM	N	Starch	Fat	Ca	P	AMEn
Fine	Control	0.582	0.725	0.930	0.811	0.311	0.455	3,004
	Carb	0.626	0.757	0.947	0.876	0.381	0.486	3,075
	Phy	0.607	0.751	0.936	0.810	0.343	0.557	2,977
	Carb + Phy	0.621	0.741	0.945	0.852	0.355	0.560	3,065
Coarse	Control	0.608	0.757	0.927	0.850	0.387	0.485	3,011
	Carb	0.631	0.768	0.940	0.864	0.382	0.508	3,080
	Phy	0.633	0.772	0.933	0.873	0.398	0.529	3,031
	Carb + Phy	0.639	0.775	0.948	0.905	0.374	0.554	3,092
SEM[3]		0.0112	0.0101	0.0067	0.0161	0.0287	0.0237	12.6
Main effects								
Particle size								
Fine		0.609[b]	0.744[b]	0.939	0.837[b]	0.347	0.514	3,030[b]
Coarse		0.628[a]	0.768[a]	0.937	0.873[a]	0.385	0.519	3,053[a]
Enzyme								
	Control	0.595[b]	0.741	0.929[b]	0.831[c]	0.349	0.470[c]	3,007[b]
	Carb	0.629[a]	0.763	0.943[a]	0.870[a,b]	0.381	0.497[b,c]	3,078[a]
	Phy	0.620[a]	0.761	0.935[a,b]	0.842[b,c]	0.370	0.543[a,b]	3,004[b]
	Carb + Phy	0.630[a]	0.758	0.946[a]	0.878[a]	0.365	0.557[a]	3,078[a]
Probabilities, $P \leq$								
Particle size		0.022	0.002	0.600	0.003	0.071	0.773	0.012
Enzyme		0.012	0.129	0.044	0.014	0.722	0.002	0.001
Particle size × Enzyme		0.754	0.645	0.877	0.108	0.559	0.607	0.217

[a-c]Means in a column not sharing common letters are different ($P < 0.05$).
[1]Each value represents the mean of 6 replicates (6 birds per replicate).
[2]Each value represents the mean of 6 replicates (8 birds per replicate) measured from day 17 to 20 posthatch.
[3]Pooled standard error of mean.

($P > 0.05$) differences in the weight of other digestive organs and segments were observed in response to either barley particle size or supplemental enzymes.

Barley particle size had no effect ($P > 0.05$) on the relative length of intestinal segments. Supplemental Carb and Carb + Phy tended ($P = 0.055$) to reduce

Table 5. The influence of barley particle size and carbohydrase (Carb) and phytase (Phy) supplementation, individually or in combination (Carb + Phy) in pelleted diets on relative weight (g/kg of body weight) of proventriculus (Prov.), gizzard (Giz.), duodenum (Duo.), jejunum (Jej.), ileum (Ile.) and ceca; relative lengths (cm/kg of body weight) of Duo., Jej., Ile., and ceca; pH of the gizzard; and jejunal digesta viscosity (cP) of 21-day-old broilers.[1]

		Relative empty weight						Relative length					
Particle size	Enzyme	Prov.	Giz.	Duo.	Jej.	Ile.	Ceca	Duo.	Jej.	Ile.	Ceca	Giz. pH	Jej. digesta viscosity
Fine	Control	3.80	9.13[c,d]	4.09	7.53	5.71	2.11	22.2	64.0	64.8	13.8	3.66	2.83
	Carb	4.29	9.90[b,c]	3.81	7.40	5.40	2.18	22.3	62.2	63.3	14.7	3.26	3.09
	Phy	3.59	8.33[d]	4.24	7.64	5.64	1.94	22.1	62.6	62.8	13.6	3.77	2.70
	Carb + Phy	4.17	9.69[c]	3.78	7.42	5.35	1.99	20.8	59.3	61.2	13.3	3.69	2.81
Coarse	Control	3.79	10.3[b,c]	3.64	7.27	5.55	2.04	23.3	69.6	65.6	14.0	2.77	2.93
	Carb	3.70	11.2[a,b]	3.82	7.93	5.46	2.17	20.9	61.0	60.8	13.8	2.81	2.50
	Phy	3.98	12.2[a]	3.63	7.46	5.30	1.97	23.3	61.5	64.6	13.6	2.67	2.91
	Carb + Phy	3.57	10.4[b,c]	3.66	7.87	5.06	1.97	21.9	58.0	61.2	13.5	2.92	2.68
SEM[2]		0.286	0.459	0.331	0.557	0.319	0.134	0.62	2.27	2.31	0.55	0.228	0.159
Main effects													
Particle size													
Fine		3.96	9.26	3.98	7.50	5.52	2.06	21.85	62.0	63.0	13.84	3.60[a]	2.86
Coarse		3.76	11.0	3.69	7.63	5.34	2.04	22.34	62.5	63.0	13.70	2.79[b]	2.75
Enzyme													
	Control	3.79	9.70	3.86	7.40	5.63	2.08	22.74	66.8[a]	65.2	13.9	3.22	2.88
	Carb	4.00	10.53	3.82	7.67	5.43	2.18	21.61	61.6[b]	62.0	14.2	3.03	2.79
	Phy	3.79	10.26	3.93	7.55	5.47	1.95	22.70	62.1[b]	63.7	13.6	3.22	2.80
	Carb + Phy	3.87	10.06	3.72	7.64	5.20	1.98	21.33	58.6[b]	61.2	13.4	3.31	2.75
Probabilities, $P \leq$													
Particle size		0.327	0.001	0.218	0.732	0.423	0.841	0.273	0.756	0.999	0.712	0.001	0.357
Enzyme		0.874	0.338	0.933	0.963	0.605	0.346	0.055	0.010	0.319	0.438	0.674	0.872
Particle size × Enzyme		0.250	0.006	0.768	0.844	0.921	0.986	0.132	0.354	0.819	0.720	0.543	0.071

[a,b]Means in a column not sharing common letters are different ($P < 0.05$).
[1]Each value represents the mean of 6 replicates (2 birds per replicate).
[2]Pooled standard error of mean.

the relative length of duodenum and significantly ($P < 0.01$) reduced the relative length of jejunum. Coarse grinding of barley reduced ($P < 0.001$) the gizzard pH. Neither barley particle size nor supplemental enzymes influenced ($P > 0.05$) jejunal digesta viscosity, but a tendency ($P = 0.071$) for an interaction between barley particle size and enzyme supplementation was observed.

DISCUSSION

Particle size distribution results showed that the relative proportion of particles >1,000 μm increased from 28.9% in the fine barley grind to 75.9% in coarse barley grind, showing the improvement in the diet structure by incorporating coarsely ground barley. Proportion of particles >1,000 μm in the diet were 13.8 and 22.9% for fine and coarse barley diets, respectively. Previous reports on the effect of grain particle size on pellet durability are contradictory. Some authors reported no effect of grain particle size on pellet durability (Reece et al., 1986a; Amerah et al., 2007b; Naderinejad et al., 2016), whereas Reece et al. (1986b) observed superior pellet durability of pellets made from coarsely ground corn particles compared with those made from fine particles. The current study showed a significant impact of barley particle size on pellet durability, which agrees with that of Angulo et al. (1996), supporting the suggestion that coarse grain particles result in more weak points in pellets, leading to pellet breakages and consequent poor pellet durability (Thomas et al., 1998). Although starch gelatinization was not measured in the current study, it may be postulated that larger grain particles were more resistant to gelatinization during processing than fine particles (Svihus et al., 2004) and, thus, resultant pellets were less durable.

Based on the lack of effect from grain particle size in pelleted diets, previous studies hypothesized that pelleting can mask the influence of particle size (Amerah et al., 2007b; Chewning et al., 2012). Amerah et al. (2007b) evaluated the effect of wheat particle size (3.0 vs. 7.0 mm) in mash and pelleted diets and reported improvements in WG and F/G in broilers (day 1–21) fed 7.0 mm wheat in mash diets. In pelleted diets, however, wheat particle size had no influence on growth performance. Chewning et al. (2012) evaluated the effect of feed form (mash vs. pellets) and corn particle size (300 vs. 600 μm) on broiler performance and also reported the lack of particle size effect on performance of broilers (day 1–44) fed pelleted diets. In contrast, the present study showed that the effect of barley particle size on FI was preserved after pelleting and interacted with supplemental enzymes. The response of Phy on FI in the current study was influenced by barley particle size. Amerah and Ravindran (2009), in a study with broilers (day 1–21), evaluated medium and coarse grinds (3.0 and 7.0 mm, respectively) of corn in mash diets, without and with microbial Phy and reported increased FI by supplemental Phy regardless of particle size. Adding Phy to low P diets is expected to result in better FI and WG; however, Phy inclusion in diets with adequate P levels do not necessarily generate greater responses in broilers (Selle and Ravindran, 2007). However, the FI response to Phy addition seems to be dependent on diet particle size, a finding that is not readily explainable.

Lentle et al. (2006) examined the performance of broiler starters fed pelleted diets based on wheat cultivars that were similar in nutrient composition and NSP content but differed in grain hardness. After grinding in a hammer mill to pass through a 4.0 mm screen, the wheat cultivars showed different particle size distributions owing to differences in grain hardness. The diet with a greater proportion of coarse particles resulted in improved feed efficiency. Amerah et al. (2007b) reported that wheat particle size did not influence the performance of birds fed pelleted diets, but in mash diets, F/G improved by 8.8 points in birds fed coarse compared with medium grind. Moreover, Deaton et al. (1995) and Naderinejad et al. (2016) reported that pelleting eliminated any possible effect of particle size on the F/G of birds fed pelleted corn-based diets. In the present study, however, the effects of particle size existed even after pelleting, with birds fed pellets made with coarsely ground barley having F/G improved by 2.1 points. This contradictory evidence from comparisons of pelleted diets with different grain particle sizes on growth performance can be explained, at least in part, by the changes in particle size distribution following pelleting process. It is evident that when particle size differences were preserved after pelleting, diets with coarser particles improved feed efficiency of broilers (Lentle et al., 2006). On the other hand, when pelleting evened out any differences in particle size distribution, no particle size effect on performance was observed (Naderinejad et al. 2016). It is, therefore, reasonable to speculate that grain hardness may have a substantial impact on the resistance of grain particles in the feed to the frictional force inside the pellet die and, hence, the particle size distribution after pelleting.

The presence of Carb in the diet (Carb and Carb + Phy) improved the F/G by an average of 2.5 points. Previous studies (Bedford et al., 1991; Shakouri et al., 2009; Perera et al., 2019b, 2020), consistent with this finding, attributed the improvement in F/G to reduction in digesta viscosity by the action of Carb, but the viscosity effect was not observed in the current study. Amerah et al. (2008b) evaluated coarse and medium ground wheat (7.0 and 3.0 mm, respectively), without and with supplemental xylanases, on performance of broiler starters. They observed a significant particle size × xylanase interaction for F/G as xylanase improved F/G only in the coarse wheat diet. In agreement with the current results with barley, these researchers did not observe any effect of wheat particle size or supplemental enzymes on digesta viscosity.

Surface area per unit volume of grain is increased with the extent of grinding, which can facilitate the in situ gel formation by partial solubilization of NSP in finely ground cereals, leading to poor efficacy of exogenous

enzymes (Amerah et al., 2007a). In coarsely ground grains, on the other hand, in situ gel formation happens to a lesser extent, causing only a minor impact on the efficacy of supplemental enzymes (Amerah et al., 2008b). Accordingly, the improvement in F/G observed only in birds fed coarse wheat diets by Amerah et al. (2008b) was attributed to enzyme action of hydrolyzing the cell wall matrix (Bedford and Schulze, 1998), which can happen more effectively in coarse grain particles because of lower extent of in situ gel barriers. In the current study, however, as indicated by the absence of significant interaction, the action of supplemental enzymes on F/G was not influenced by the barley particle size. Furthermore, owing to the lack of effect of barley particle size on jejunal digesta viscosity, it can be speculated that other mechanisms, as suggested by Amerah et al. (2008b), might have contributed to the 2.5 points improvement in feed efficiency by added Carb.

The improvements of 3.1, 3.2, and 4.3% in the CAID of DM, N, and fat, respectively, in coarsely ground barley diets is contrary to that of Naderinejad et al. (2016) and Abdollahi et al. (2019), who reported no effect of corn and wheat particle size on the digestibility of nutrients. Improved DM, N, and fat digestibility in birds fed coarse-barley diets can be attributed to a greater functionality of the gizzard (Svihus et al., 2011a), which results in greater mechanical breakdown of digesta (Svihus et al., 1997; Hetland et al., 2002) and lower digesta pH, as illustrated by the lower gizzard pH of birds fed coarse barley in the current study. Moreover, coarse grain particles reduce the digesta passage rate through the gizzard (Nir et al., 1994b) and therefore, are retained longer than finer particles in the digestive tract (Amerah et al., 2007a), increasing the exposure time of nutrients to digestive enzymes.

With reference to protein digestion, extended retention and mixing in the gizzard is necessary for better contact between feed, gastric juices, and pepsin, thus facilitating the denaturation and digestion of proteins. Accordingly, the larger gizzards in birds fed coarse barley and the consequent increased gastric reflux between gizzard and proventriculus results in more time for gastric enzyme and protease activities in the foregut, aiding protein digestion. In addition, the lower gizzard pH increases the pepsin activity (Gabriel et al., 2003), which facilitates initial protein hydrolysis. All these modifications might have acted to enhance the CAID of N in birds fed coarsely ground barley in the current study. The improvement in CAID of N in birds fed coarse barley in the current study is, however, contrary to the findings by Naderinejad et al. (2016), who reported no effect of corn particle size on CAID of N in both mash and pelleted diets, despite well-developed gizzards and lower gizzard pH in birds fed coarser corn-based diets. Jacobs et al. (2010) also reported no effect of corn particle size on the apparent total tract digestibility of most amino acids in birds fed corn-based mash diets. According to Mtei et al. (2019), who evaluated the interaction between bird type (broilers and layers) and corn particle size, the CAID of N was not influenced by corn particle size, regardless of bird type. In the studies of Jacobs et al. (2010) and Mtei et al. (2019), despite well-developed gizzards, gizzard pH remained unaffected, suggesting that gizzard pH might be of more importance in enhancing the protein digestibility compared to other mechanisms facilitated by a functional gizzard.

The gizzard has been identified as a key site for regulating the digestibility of starch by preventing starch overload into the lower gut, and a positive correlation between gizzard weight and starch digestibility has been reported (Svihus, 2011b). Despite larger gizzards in birds fed coarse barley diets, no influence of barley particle size on the CAID of starch was observed in the current study. Fine feed structures do not facilitate gizzard development and can result in poor starch utilization because of suboptimal regulation of feed flow (Svihus, 2011a). Naderinejad et al. (2016) reported a greater starch digestibility in pelleted coarse corn diets, whereas Péron et al. (2005) reported improved starch digestibility in birds fed pelleted fine wheat (hard cultivar) diets. The improved starch digestibility in coarse corn-based pelleted diets (Naderinejad et al., 2016) was attributed to higher gizzard weights and reduction in gizzard pH. On the other hand, the poor starch digestibility in coarse wheat-based pelleted diets (Péron et al., 2005) was attributed to a starch accessibility problem because of physical entrapment of starch granules in coarse particles of hard wheat (Carré, 2004) and, hence, the improved digestibility with fine grinding. The inconsistent response of starch digestibility with grain particle size is likely related to a complex array of confounding factors such as grain type (Carré, 2004), hardness (Carré et al., 2002), and feed form (Naderinejad et al., 2016).

The CAID of Ca in birds fed coarse barley diets tended to be greater in the current study (0.347 vs. 0.385, $P = 0.071$), possibly because of a more acidic pH in the gizzard of birds fed coarse particles. Most phytate-mineral complexes are soluble at pH lower than 3.5 and become insoluble at pH values between 4 and 7 (Champagne, 1988; Selle et al., 2000). The low gizzard pH of birds fed coarse barley diets (2.79) fell within the soluble range of pH (< 3.5) and could explain the observed results. However, the lower pH failed to enhance the CAID of P in the current study. Naderinejad et al. (2016) evaluated the effect of different particle sizes of corn on the digestibility of minerals and reported an 18.3% improvement in Ca digestibility (0.429 vs. 0.508) and an improvement in CAID of P by 7.82% (0.467 vs. 0.504) for medium and coarse grinding compared with finely ground corn. Amerah and Ravindran (2009) also reported that coarse corn diets improved the total tract retention of Ca, by 16.4%, but had no effect on P retention.

Increasing coarseness of the barley grind in the current study caused a small, but significant, improvement of AMEn by 23 kcal/kg (from 3,030–3,053 kcal/kg DM). Naderinejad et al. (2016) observed greater AME in birds fed coarse corn-based pelleted diets (3,573 kcal/kg DM)

compared with fine and medium corn-based pelleted diets (3,516 and 3,540 kcal/kg DM, respectively). Highlighting the inconsistent nature of particle size effect on energy utilization, Svihus et al. (2004) and Amerah et al. (2007b) reported that different particle sizes in either mash or pelleted wheat-based diets had no effect on energy utilization. These contradictory results may be explained by confounding factors such as grain type (Amerah et al., 2007b), hardness (Péron et al., 2005), and feed form (Kilburn and Edwards, 2001).

Regardless of the nature of response, previous studies (Péron et al., 2005; Svihus et al., 2011b) observed a strong correlation between starch digestibility and energy utilization. The 4.66% improvement in AMEn (from 2,923–3,059 kcal/kg DM) reported by Péron et al. (2005) was attributed to a 6.18% enhancement in starch digestibility in response to increasing fineness. In contrast, the increase in AMEn with increasing coarseness of barley grind in the present study was not reflected in starch digestibility response. Nevertheless, similar trends in AMEn with CAID of DM, N, and fat responses to barley particle size are reflective of a link between energy utilization and nutrient digestibility.

Irrespective of the barley particle size, the magnitude of response to Carb, Phy, and Carb + Phy on the ileal digestibility of DM were 5.7, 4.2, and 5.9%, respectively. Phytate in wheat and barley is largely located in the aleurone layer (Eeckhout and De Paepe, 1994). Therefore, improvement of CAID of DM in response to supplemental Phy, at least in part, was caused by the action of Phy in disrupting of cell wall and consequent release of encapsulated nutrients in a manner similar to that of Carb (Ravindran et al., 1999).

Nutrients, such as starch and protein, encapsulated within intact endosperm cell walls in barley (Perera et al., 2019a) are released because of the action of supplemental Carb on cell wall integrity (Bedford, 2018), and as a consequence digestibility increases. Similarly, supplemental Phy releases not only phytate P but also phytate-bound protein and proteolytic enzymes, thus enhancing protein digestion (Ravindran et al., 2000; Selle and Ravindran, 2007). The benefits of individual and combined supplementation of Carb and Phy in wheat-based and barley-based diets in terms of protein and amino acid digestibility has been previously reported (Ravindran et al., 1999; Wu et al., 2004a). Wu et al. (2004a) reported that CAID of N enhanced by 13.8, 10.8, and 13.8% in broiler starters fed barley-based diets in response to glucanase, Phy, and glucanase + Phy, respectively. The observations of the current study contrast from these findings by showing no effect from supplemental enzymes on the CAID of N, with only numerical improvements in the CAID of N (3.0, 2.7, 2.2% increments in response to Carb, Phy, and Carb + Phy, respectively) being observed.

Regardless of barley particle size, starch digestibility was enhanced by Carb in both individual and combined supplementation, with magnitude of response of 1.51 and 1.83%, respectively. The effect of supplemental carbohydrase on enhanced starch digestibility in barley is well documented (Bergh et al., 1999; Ravindran et al., 2007; Perera et al., 2019a,b; 2020). Carbohydrase disrupts the endosperm cell wall and releases encapsulated starch granules, thus allowing them to interact unhindered with digestive enzymes. However, reports on improved starch digestibility in diets supplemented with Phy are limited (Camden et al., 2001). Improvements have been attributed to the release of starch granules bound in phytate complexes (Thompson, 1988) and alleviation of the inhibitory action of phytate on α-amylases (Sharma et al., 1978).

Fat digestibility is detrimentally affected by greater digesta viscosity (Edney et al., 1989; Almirall et al., 1995). In this study, however, despite the lack of enzyme effect on digesta viscosity, CAID of fat was increased by 4.7 and 5.7% because of individual use of Carb and combination with Phy, respectively. Carbohydrase is believed to enhance fat digestibility by the release of encapsulated nutrients, while Phy can partially prevent the formation of metallic soaps by prior hydrolysis of phytate in more proximal parts of the gut and thereby increasing fat digestibility (Selle and Ravindran, 2007). This beneficial impact of Phy on fat digestibility, however, was not evident in the current study.

Individual addition of Phy increased the CAID of P by 15.5%, from 0.470 to 0.543. The CAID of P was further improved by 18.5% when Carb and Phy were added together, showing that activity of Phy was facilitated by NSP-degrading enzymes, perhaps by allowing greater access to substrates. Juanpere et al. (2005) evaluated the effect of Carb and a Phy, individually and in combination, in corn-, wheat- and barley-based diets and reported a synergistic effect of Phy + xylanase on P retention of wheat-based diets and Phy + β-glucanase on P and Ca retention of barley-based diets. The improvement of Ca retention by supplemental enzymes, however, was not observed in the current study.

Regardless of barley particle size, 2.4% (71 kcal/kg DM) improvement in AMEn was observed in response to both individual and combined supplementation of Carb. The beneficial effect from individual use of Phy (Selle et al., 2003) on energy utilization, reported in previous studies, was not observed in the current study. The improvement in AMEn in response to addition of Carb was closely associated with enhanced digestibility of starch and fat, the main energy yielding nutrients. In agreement to the current findings and despite the absence of effect on digesta viscosity, Amerah et al. (2008b) reported improved AMEn in response to added Carb in both medium (1.6%) and coarse (5.6%) wheat diets. These improvements were attributed to the action of Carb on the physical barriers of endosperm cell wall and gel barriers on digesta particles formed by partial solubilization of NSP.

Barley particle size influenced the response of gizzard size to supplemental enzymes, as Phy in fine, and coarse barley diets resulted in the lowest and the highest relative gizzard weights, respectively. This finding is hard to explain and highlights the need for evaluating the mechanisms of Phy interactions at different particle sizes.

Increased gizzard weights in birds fed coarse corn (Nir et al., 1994a,b; Parsons et al., 2006) and wheat (Amerah et al., 2007b; Abdollahi et al., 2019) have been observed previously. Amerah et al. (2008a) reported higher gizzard weights in response to increasing grain particle size from 1.0 mm to 7.0 mm in corn-based (34% increase from 9.40 to 12.6 g/kg of BW) and wheat-based pelleted diets (10.7% increase from 9.03 to 10.0 g/kg of BW). Nir and Ptichi (2001) and Svihus et al. (2004) reported that coarse grinding increased gizzard size only when mash diets were fed, whereas this effect was not apparent in pelleted feeds. In the present study, however, the effects of particle size on gizzard size remained even after pelleting with 18.8% increase from 9.26 to 11.0 g/kg of BW.

In agreement with the present results, previous researchers reported no influence of corn (Naderinejad et al., 2016) and wheat (Péron et al., 2005) particle size on the relative weight and length of digestive tract components apart from the gizzard. However, Nir et al. (1994a) reported reduced duodenal weight in coarse wheat fed birds, but in a follow-up study, Nir et al. (1995) observed greater relative weights of jejunum, ileum, and small intestine in birds fed coarse corn particles.

In a study by Wu et al. (2004b), supplementation of xylanase and Phy individually reduced the relative length (16.5 and 14.1%, respectively) and weight (15.5 and 11.4%, respectively) of the small intestine, whereas the combination of enzymes had no further effect. It was suggested that the heavier intestinal weight was caused by greater digesta viscosity (Wu et al., 2004b), reduced passage rate and subsequent rise in pathogenic microbial activity (Brenes et al., 2002) that stimulated intestinal tissue growth. In the current study, individual additions of Carb and Phy significantly shortened the jejunum by 8.38 and 7.54%, respectively, whereas combining the 2 enzymes had a synergetic effect causing 13.9% reduction. As the reduction in the relative length of the jejunum paralleled the improvements in DM, starch, and fat digestibility in response to supplemental enzymes, it is tempting to speculate that the reduced jejunal length may be a consequence of the decreased need for digestive and absorptive capacity resulting from supplemental enzymes.

Birds fed coarse barley diets showed lower gizzard pH that tended ($P = 0.058$) to negatively correlate ($r = -0.276$) with the relative weight of gizzard. A significant negative correlation ($r = -0.451$) between the relative gizzard weight and gizzard pH reported by Liu et al. (2015) lends support to the present observation. Nir et al. (1994b) evaluated coarse, medium, and fine particle sizes of corn, wheat, and sorghum and observed that the pH of the gizzard contents decreased with increasing particle size, irrespective of the grain type. Naderinejad et al. (2016) reported a particle size × feed form interaction for gizzard pH of birds fed different corn particle sizes in mash and pellet diets, as in mash diets, gizzard pH was not influenced by particle size, whereas, in pelleted diets, medium and coarse grinding lowered gizzard pH compared with fine grinding. In addition, secretion of pepsinogen and hydrochloric acid from proventriculus is encouraged when digesta is refluxed into the proventriculus by contraction of a functional gizzard (Duke, 1992). On other hand, smaller gizzards may have resulted in fewer refluxes, which inhibited gastric secretions (Svihus, 2011a) and contributed to elevated pH in birds fed fine barley diets. Contrary to the current findings, Wu et al. (2004b) reported that addition of xylanase or the combination of xylanase plus Phy reduced the viscosity of digesta in all segments of the intestine.

The potential impact of grain hardness on particle size distribution, particularly after pelleting, justifies the need for further evaluation of the optimum particle size for different barley types that vary in grain hardness. Moreover, as a potential approach for restoring the structure in pelleted barley-based diets, whole barley inclusion should be evaluated concerning optimum inclusion and interactions with supplemental enzymes.

CONCLUSIONS

In summary, improving the structure of the diet by increasing coarseness of barley grind enhanced the feed efficiency, and nutrient and energy utilization in broiler starters fed pelleted diets. The fine barley diet was superior only from a pellet quality perspective. Pelleting did not mask the effect of barley particle size. Supplementation of Carb individually or in combination with Phy enhanced the feed efficiency, and starch, fat and energy utilization, whereas addition of Phy individually or in combination enhanced P utilization. Supplementation of either enzyme improved DM digestibility, and the combination of Carb plus Phy tended to improve WG.

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