

The effects of the fiber source and xylanase supplementation on production, egg quality, digestibility, and intestinal morphology in the aged laying hen

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ABSTRACT This study was conducted to evaluate the effects of the fiber source (wheat bran [WB] or sugar beet pulp [SBP]) and xylanase supplementation on production, egg quality, ileal digestibility, intestinal morphology, and gastrointestinal pH in aged laying hens. A total of 540 laying hens (Lohman LSL Lite; 70 wk of lay) were randomized into 10 treatments (6 replicate cages of 9 birds) consisting of a corn soy control supplemented with 0, 3, or 6% WB or SBP with or without xylanase (100 mg of xylanase preparation per kg) for a period of 9 wk in a 5 × 2 factorial arrangement. Hens fed with the diets containing either of the levels of SBP or 6% WB had lower hen-day production, and addition of the enzyme improved hen-day production ($P < 0.05$), but it could not compensate for the lost production due to the higher levels of either of the fiber sources. Supplementation of 6% SBP to the control diet decreased egg mass ($P < 0.05$). All fiber-supplemented diets significantly decreased ADFI, which was restored on

enzyme addition, with the exception of 3% WB diet. Treatments had no effects on egg weight, feed conversion ratio, egg quality, and serum and carcass traits, except for ileum weight, which was greater in hens fed with the 6% SBP diet ($P < 0.05$). Adding 3% SBP increased ileal DM digestibility ($P < 0.05$). Addition of 3% WB improved jejunal villus height, villus height-to-crypt depth ratio, and villus surface area ($P < 0.05$). Villus surface area, DM, organic matter, and protein digestibility increased as a result of enzyme supplementation ($P < 0.05$). Cecal pH was reduced on feeding diets containing 3% WB, containing 3% SBP, and with enzyme supplementation ($P < 0.05$). In conclusion, addition of 3% WB in a corn soy control diet has the potential to improve small intestine morphology in older hens without adverse effects on performance, especially if accompanied by the use of an enzyme, which simultaneously improved morphological traits and nutrient digestibility.

Key words: sugar beet pulp, xylanase, laying hen, viscosity, wheat bran

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INTRODUCTION

In recent years, providing an appropriate fiber source in the diets of nonruminants has gained considerable interest as a means to improve gastrointestinal tract (GIT) health, which is concomitant with balanced microbiota (Mateos et al., 2012). Dietary fiber is derived from the constituent parts of the cell wall structures in plant ingredients. It is composed of 3 forms based on solubility:

soluble high-molecular-weight polysaccharides that are potentially viscous, soluble oligosaccharides and smaller polysaccharides that do not confer viscosity, and insoluble nonviscous fiber. The nutritional behavior and functional properties of these different fiber types in poultry depend on their sugar composition, molecular weight, associations with other components such as lignin, and the maturity of the bird's digestive system and microbiome (Slavin, 2013; Koca et al., 2015). Feeding significant quantities of a fiber source that is rich in highly soluble, high-molecular-weight polysaccharides can cause the formation of viscous digesta. Such an effect can compromise bird performance via a cascade of events including slowing the passage rate of digesta, reducing emulsification and thus efficiency of micelle formation and fat digestibility, and providing

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an environment conducive to microbial growth and thus competition for nutrients with the host (Annison, 1993; Gonzalez-Alvarado et al., 2010). In contrast, insoluble fiber sources tend not to influence viscosity of digesta and can positively influence bird performance if fed at appropriate levels. Again, this is through a cascade of effects including extending the retention time of digesta in the upper segments of the GIT, resulting in enhanced duodenum–gastric reflux and gizzard development, increased secretion of hydrochloric acid, pancreatic enzymes, and bile acids (Rogel et al., 1987; Mosenthin et al., 1999; Hetland et al., 2003, 2004), and modifications of microbial fermentation patterns in the lower parts of the gut, which are of advantage to the host (Williams et al., 2005; Slavin, 2013; Makki et al., 2018). The lack of an adequate source and level of fiber in the diet could result in fermentation of undigested amino acids, proteins, and fats rather than fibrous carbohydrates as the favored substrates, which results in production of harmful as opposed to beneficial end products from the standpoint of the host (Williams et al., 2005, 2017; Kieffer et al., 2016; Koh et al., 2016).

Excessive fiber inclusion is also detrimental in part owing to the lack of appropriate endogenous carbohydrase enzymes (Anuradha and Roy, 2015) and limitations on the fermentation capacity of the host, and as a result, there is a balance to be struck with respect to delivery of the correct amount of fermentable carbohydrates. To address these problems and maximize fiber utilization, the feed industry adds exogenous carbohydrases to the diet. It has been shown that not only exogenous carbohydrase preparations partially depolymerize soluble nonstarch polysaccharides and thus reduce viscosity of digesta and release entrapped nutrients in cell contents (Craig et al., 2019) but also the end products of these enzymes may be equally important. As insoluble and soluble fiber is progressively depolymerized, smaller and more fermentable polysaccharides and oligosaccharides are produced, which markedly increases the amount of fermentable substrates (Kiarie et al., 2013; Vila, 2017; Bautil et al., 2019; Craig et al., 2019). This can modify the pattern of fermentation in the cecum such that the production of short-chain fatty acids (SCFA, i.e., acetate, propionate, and butyrate) is favored over that of amines and ammonia derived from protein putrefaction. An added benefit is that production of SCFA in place of amines and ammonia results in a lower pH, which not only deters pathogen growth but also leads to improvements in mineral absorption and stimulates mucus secretion (Williams et al., 2005; Parsaie et al., 2007; Bedford and Cowieson, 2012; Kiarie et al., 2013, 2014; Slavin, 2013; Vila, 2017; Makki et al., 2018; Bautil et al., 2020).

Despite the considerable levels of fiber in laying hen rations, its role in laying hen diets is inconclusive, with some research reporting positive effects of fiber inclusion on performance (Samli, 2006) but others reporting negative effects (Tabook et al., 2006; Guzman et al., 2015; Sadeghi et al., 2015). Wheat bran and sugar beet pulp (SBP) are 2 popular fiber sources produced as

by-products of the wheat flour and sugar beet industries, respectively. Wheat bran is the outer layer of the grain that makes up 13% of the grain weight. It is rich in insoluble nonstarch polysaccharides such as arabinoxylan (77%), beta-glucan, and cellulose (Banino, 2012). Sugar beet pulp is also a high-fiber ingredient (81.4%) rich in total non-starch polysaccharides (NSP, 77.9%). The majority is in the form of soluble fiber components (Banino, 2012) such as pectin-type polysaccharides, which are also highly fermentable by the intestinal microflora (Banino, 2012).

It seems likely that fiber plays an important role in both performance and health, particularly in the aged laying hen diet. However, the effect of soluble vs. insoluble fiber is less clear, and the potential of carbohydrase preparations to maximize their utilization is poorly understood. Thus, the aim of the present study was to evaluate the effects of 2 different fiber sources (wheat bran as an insoluble nonviscous and SBP as a soluble viscous fiber) and xylanase supplementation on production, egg quality, digestibility, and intestinal morphology in aged laying hens.

MATERIALS AND METHODS

Fiber Source Preparation and Analysis

Wheat bran and SBP were supplied by a local feed factory and ground into fine powder using a laboratory mill. The CP, ether extract, crude fiber, and ash contents of the ground powders were determined by use of standard laboratory methods (AOAC, 1995). Neutral detergent fiber was determined by the method of Soest and Wine (1967). Acid detergent fiber was measured by the method of Soest (1963). Total dietary fiber (TDF) and insoluble dietary fiber (IDF) were measured by the method of Lee et al. (1992), and then, soluble dietary fiber was determined by subtracting IDF from TDF.

Birds, Housing, and Feeding

This experiment was conducted at the Department of Animal Science, University of Kurdistan, Sanandaj, 416, Kurdistan, Iran. All animal care and use procedures were evaluated and approved by the University of Kurdistan Animal Care and Use Committee, which are in compliance with international guidelines (FASS, 1999). A total of 540 laying hens (Lohman LSL Lite from the University of Kurdistan, Dehghan Research Station laying hen flock, Kurdistan, Iran) at 90 wk of age, which had been in production for 70 wk, were used. The birds were weighed as a group at the beginning of the experiment and then distributed into three-tier conventional cages (60 × 60 × 50 cm³) equipped with gutter feeders and 2 nipple drinking systems per cage. Before the start of the experiment, egg production was recorded on a cage basis for 2 wk as a means to equilibrate initial performance across treatments. As a result, 60 cages with similar production (with an average of 71 ± 9.0%) were selected and allocated to treatments in a

completely randomized design in a 5×2 factorial arrangement. Each treatment had 6 replicates, each of which contained 9 birds. The feed (mash) and water were provided ad libitum, and the experiment lasted for 9 wk.

As presented in Table 1, the basal experimental diets were formulated to meet or exceed nutritional requirements (Lohman LSL Lite Handbook, 2016). A basal diet was fed as a control diet and then supplemented with either 3 or 6% wheat bran or 3 or 6% SBP, each diet fed with or without 100 mg/kg of xylanase preparation. The ingredient and nutrient composition of the diets is shown in Table 1. The environmental conditions of the house were adjusted as per the Lohman LSL Lite Handbook (2016). A 16L:8D light schedule was used, and RH and temperature kept at 65 to 75% and 22°C to 26°C, respectively. The xylanase enzyme was provided by AB Vista (Econase XT25; AB Vista Feed Ingredients, Marlborough, UK), which is an enzyme preparation with endo-1,4-beta-xylanase (xylanase) as the main activity. This enzyme preparation was produced using a strain of *Trichoderma reesei*, and its

main activity is a thermostable endoxylanase (minimum = 160000 BXU/g), which was added to the basal diets as granules.

Performance Measurements

BW was recorded on a cage basis at the start of the experiment ($1,730 \pm 65$ g) at 90 wk of age. Daily records were kept for egg production, feed consumption, egg weight, and mortality up to 99 wk of age. Based on these data, egg weight (g), hen-day production (%), egg mass (g/hen/day), daily average feed intake (g), and feed conversion ratio (FCR; average feed intake divided by egg mass) were calculated.

Egg Quality

Eggs were collected for measurements of egg quality over 2 consecutive days every 3 wk (93, 96, and 99 wk of age). All eggs were dipped in incremental dilutions of saline solution to determine specific gravity such that 6 saline solutions were required. These solutions

Table 1. Composition and calculated and analyzed nutrient contents (%) of experimental diets.

Ingredient	Control	Sugar beet pulp, %		Wheat bran, %	
		3	6	3	6
Sugar beet pulp	-	3.0	6.0	-	-
Wheat bran	-	-	-	3.0	6.0
Corn	66.56	63.21	59.85	63.63	60.70
Soybean meal	21.66	21.55	21.44	21.27	20.87
Soybean oil	0.24	0.66	1.09	0.59	0.94
CaCO ₃	9.39	9.38	9.38	9.41	9.42
Dicalcium phosphate	1.17	1.19	1.21	1.14	1.10
Common salt	0.24	0.23	0.24	0.24	0.24
Vitamin premix ¹	0.25	0.25	0.25	0.25	0.25
Mineral premix ²	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.13	0.14	0.15	0.13	0.13
L-Lysine	0.00	0.01	0.03	0.00	0.00
NaHCO ₃	0.11	0.11	0.12	0.11	0.10
Calculated analysis					
CP, %	14.64	14.64	14.64	14.64	14.64
ME, kcal/g	2.72	2.72	2.72	2.72	2.72
Calcium, %	3.91	3.91	3.91	3.91	3.91
Total phosphate, %	0.54	0.53	0.53	0.56	0.57
Avail phosphate, %	0.33	0.33	0.33	0.33	0.33
Total cysteine, %	0.22	0.21	0.21	0.22	0.22
Total lysine, %	0.70	0.700	0.700	0.701	0.702
Total methionine, %	0.35	0.36	0.36	0.35	0.35
TSAA, %	0.57	0.57	0.57	0.57	0.57
Na, %	0.14	0.14	0.14	0.14	0.14
Cl, %	0.18	0.18	0.18	0.18	0.18
K, %	0.64	0.63	0.61	0.66	0.68
DCAB, mEq/kg	174	171	168	179	184
Crude fiber, %	2.98	3.34	3.70	3.25	3.53
Analyzed compositions					
CP, %	15.69	15.71	15.81	15.46	15.65
Crude fiber, %	3.12	3.61	3.92	3.53	3.86
Total dietary fiber, %	10.98	12.63	12.80	12.04	12.10
Insoluble dietary fiber, %	8.04	8.94	8.74	8.92	9.09
Soluble dietary fiber, %	2.94	3.69	4.06	3.12	3.01

Abbreviation: DCAB, dietary cation–anion balance.

¹Provided per kilogram of diet: vitamin A, 8800 IU; vitamin D3, 2500 IU; vitamin E, 11 IU; vitamin K3, 2.2 IU; thiamin, 1.5 mg; riboflavin, 4 mg; nicotinic acid, 7.85 mg; pantothenic acid, 34.65 mg; pyridoxine, 2.46 mg; folic acid, 0.48 mg; vitamin B12, 4 mg; antioxidant, 400 mg.

²Provided per kilogram of diet: manganese, 74.4 mg; zinc, 64.67 mg; iron, 75 mg; copper, 6 mg; iodine, 0.86 mg; selenium, 0.2 mg; choline chloride, 200 mg.

had specific gravities of 1.000, 1.062, 1.070, 1.082, 1.090, and 1.102. The specific gravity of the salt solution in which each egg just floated was regarded as the specific gravity of that egg. The length and width of the eggs were measured to determine the egg shape index. After yolks and albumins were separated to determine their height, the relative weight of yolk and albumin, yolk-to-albumin ratio, Haugh unit, relative weight of dry shell, and egg surface were measured.

Blood and Serum Collection

Blood samples were taken from a brachial vein of one hen per replicate at 99 wk of age before slaughter and then centrifuged at $1,372 \times g$ for 15 min to separate sera and kept at -20°C until required for analyses. Serum cholesterol and uric acid levels were colorimetrically determined using commercial kits (Pars Azmoon Company, Tehran, Iran).

Carcass Traits

At the end of the ninth week (99 wk of age), the hens previously chosen for blood sample collection were weighed and killed by cervical dislocation. The empty proventriculus, gizzard (after removal of the surrounding fat), and pancreas were weighed; then, their weight relative to BW was measured, and data were expressed as a percentage of live BW. The length and weight of the empty duodenum, jejunum, ileum, and cecum were recorded. All weight and length data are expressed as a percentage of live BW.

Intestinal Morphology

For morphometric analysis (at 99 wk of age), approximately 3 cm of the intestine from the middle of the jejunum and ileum was collected, washed with physiological serum, and fixed in 10% formalin. Tissues were dehydrated, cleared, and impregnated with paraffin using an automatic tissue processor and then embedded in paraffin wax. Sections were cut ($6\ \mu\text{m}$) from the waxed tissue using a SRM 200 model microtome (Sakura Finetek Europe B.V., Alphen aan den Rijn, Netherlands). The sections were floated on prewarmed water (50°C) before mounting on slides to remove wrinkles. The slides were stained with hematoxylin and eosin stain, and then, morphometric indices such as villus height, crypt depth, villus height-to-crypt depth ratio, and villus surface area were determined at a magnification of $40\times$ using a light microscope. The mean values from 10 villi per sample were used as the average value for further analysis (Gunal et al., 2006).

Apparent Ileal Digestibility

Titanium dioxide (Merck KGaA, 64271 Darmstadt, Germany) was added to the diets 3 d before the end of the study as an indigestible marker to determine apparent ileal nutrient digestibility. The total ileum

contents of hens were squeezed into plastic bags and stored at -20°C until analysis. Organic matter, ash, and protein of all dried samples were determined (AOAC, 1995), and ileal digestibility was calculated by analysis of the marker using the method of Short et al. (1996).

pH of Intestinal Digesta

The procedure as reported by Nisbet et al. (1993) was used to measure the pH of digesta in the different parts of the GIT. Approximately 1 g of digesta from each of the proventriculus, gizzard, duodenum, jejunum, ileum, and cecum was each mixed with 9 mL of distilled water in a test tube, and their pH was immediately determined using a digital pH meter.

Statistical Analysis

All collected data were subjected to analysis of variance as a factorial 5×2 trial using the General Linear Model procedures of SAS Institute (2001) (SAS 9.2, Cary, NC). The cage was the experimental unit for production trait analysis, whereas the individual bird sample served as the experimental unit for small intestine morphology and blood serum trait analysis. Tukey's test was used to compare differences between means when the model was declared significant ($P < 0.05$).

RESULTS

Fiber Source Preparation and Analysis

The chemical composition of wheat bran and SBP samples used in the present study including CP, crude fiber, neutral detergent fiber, acid detergent fiber, TDF, IDF, soluble dietary fiber, ether extract, nitrogen-free extract, and ash is shown in Table 2 and is presented on a DM basis.

Performance

The effects of different dietary treatments on hen-day production, egg weight, egg mass, average feed intake, and FCR (feed intake/egg mass) are presented in Table 3. No significant interactions between the enzyme and ingredient were observed for hen-day production, egg weight, egg mass, average feed intake, and FCR. The hens fed with the diets containing either of the levels of SBP or 6% wheat bran, but not 3% wheat bran, had lower hen-day production than the control ($P < 0.05$). The addition of enzymes increased hen-day production ($P < 0.05$) across all treatments. Supplementation of 6% SBP decreased egg mass in comparison with the control ($P < 0.05$), and egg mass tended to increase with enzyme addition ($P = 0.06$). Adding fiber, with the exception of 3% wheat bran, resulted in a reduction in feed intake, which was to some extent recovered with the addition of enzyme. Addition of fiber or enzyme had no effects on egg weight or FCR.

Table 2. Chemical composition of wheat bran and sugar beet pulp on a DM basis (%).

Fiber source	Chemical composition, %									
	DM	CP	EE	Ash	CF	NDF	ADF	TDF	IDF	SDF
Wheat bran	87	13.13	1.92	3.79	12.21	59.33	11.85	46.87	40.78	6.09
Sugar beet pulp	90	9.33	1.62	6.12	14.64	43.98	31.58	62.22	41.99	20.23

Abbreviations: ADF, acid detergent fiber; CF, crude fiber; EE, ether extract; IDF, insoluble dietary fiber; NDF, neutral detergent fiber; SDF, soluble dietary fiber; TDF, total dietary fiber.

Egg Quality

The effects of treatment on egg quality are presented in [Table 4](#). Egg weight, egg shape index, yolk height, albumin height, shell thickness, relative yolk weight, relative albumin weight, yolk-to-albumin ratio, relative shell weight, Haugh unit, egg surface, and egg specific gravity of laying hens were not affected by fiber, enzyme, or the interaction.

Serum Biochemical Traits

Serum cholesterol and uric acid data are presented in [Table 5](#), and none were affected by fiber source, enzyme, or their interaction.

Carcass Characteristics

The effects of dietary treatments on organ and GIT section weight and length relative to BW are presented in [Table 6](#). The relative weight of the proventriculus, gizzard, pancreas, duodenum, jejunum, and cecum and relative length of the duodenum, jejunum, ileum, and cecum were not influenced by adding wheat bran, SBP, or enzyme to the diets. However, the weight of the ileum was greater ($P < 0.05$) in hens fed with diets containing 6% SBP than in hens fed with diets containing 3% wheat bran or 3% SBP. No enzyme or interactions were found significant for any GIT or organ weights or lengths.

Intestinal Morphology

Jejunal villus height, villus height-to-crypt depth ratio, and villus surface area were affected by dietary treatment ([Table 7](#)). Birds fed with the 3% wheat bran diet had greater villus height than those subjected to the control and all other treatments ($P < 0.05$). Although crypt depth was not affected by any treatment, the 3% wheat bran diet resulted in a higher villus height-to-crypt depth ratio than the control diet and the diets containing 6% wheat bran and 3% SBP ($P < 0.05$). Adding 3% wheat bran also increased ($P < 0.05$) villus surface area compared with the control diet and the diets containing 6% wheat bran and 6% SBP. Jejunal villus height, crypt depth, and villus height-to-crypt depth ratio were not affected by enzyme addition, but it did increase villus surface area ($P < 0.05$).

Ileal villus height and villus height-to-crypt depth ratio were not influenced by adding different levels of either of the fiber sources ([Table 8](#)). In contrast to the jejunum, the 3% wheat bran diet decreased villus surface area ($P < 0.05$). Ileal villus height and villus height-to-crypt depth ratio were not influenced by enzyme addition, but it did increase villus surface area ($P < 0.05$). The effect of enzyme \times ingredient interaction was observed only in ileum crypt depth ($P < 0.05$, [Table 9](#)), whereby the difference in crypt depth between the groups fed with 3 and 6% wheat bran diets was lost on enzyme addition.

Table 3. Effects of enzyme supplementation and dietary fiber source (wheat bran and sugar beet pulp) on production performance in laying hens from 70 to 79 wk of lay.¹

Treatments	Performance				
	Hen-day production, %	Egg weight, g	Egg mass, g/hen/day	Average feed intake, g	FCR, g/g
Enzyme level, mg/kg					
0	67.27 ^b	65.98	44.09	106.27 ^b	2.40
100	69.40 ^a	65.74	45.13	107.80 ^a	2.44
Ingredients					
Control	72.20 ^a	66.56	46.19 ^a	109.35 ^a	2.35
Sugar beet pulp, 3%	66.81 ^c	65.84	44.06 ^{a,b}	106.39 ^{b,c}	2.45
Sugar beet pulp, 6%	64.78 ^c	65.83	42.72 ^b	104.26 ^c	2.45
Wheat bran, 3%	70.65 ^{a,b}	65.64	45.69 ^a	108.94 ^{a,b}	2.42
Wheat bran, 6%	67.24 ^{b,c}	66.30	44.39 ^{a,b}	106.26 ^c	2.43
SEM	0.42	0.12	0.28	0.33	0.014
Probability					
Enzyme	0.009	0.32	0.06	0.02	0.280
Ingredients	0.001	0.12	0.0008	0.0001	0.112
Enzyme \times ingredients	0.50	0.19	0.68	0.22	0.350

^{a-c}Means in the same column and under each main effect with different letters are different ($P < 0.05$).

¹For enzyme level and ingredients, each mean represents the mean of 30 and 12 replicate cages of 9 birds each.

Table 4. Effects of enzyme supplementation and dietary fiber source (wheat bran and sugar beet pulp) on internal and external indices of egg quality in laying hens from 70 to 79 wk of lay.¹

Treatments	Egg quality											
	Egg weight, g	Egg shape index	Yolk height, mm	Albumin height, mm	Shell thickness, mm	Yolk weight, %	Albumin weight, %	Yolk-to-albumin ratio, %	Shell weight, %	Haugh unit	Egg surface (cm ²)	Specific gravity
Enzyme level, mg/kg												
0	65.53	75.20	16.04	6.81	0.37	29.90	60.10	50.14	8.90	80.23	93.07	1.08
100	65.34	75.81	15.96	6.77	0.37	29.75	60.27	49.84	8.90	79.86	93.09	1.08
Ingredients												
Control	65.04	75.02	16.00	6.81	0.367	29.89	60.08	50.30	8.92	80.27	92.82	1.08
Sugar beet pulp, 3%	65.32	75.15	16.01	6.85	0.368	29.82	60.15	50.08	8.86	80.53	92.86	1.08
Sugar beet pulp, 6%	65.59	75.53	16.00	6.79	0.369	29.77	60.31	49.56	8.92	80.13	93.31	1.08
Wheat bran, 3%	65.44	76.63	15.90	6.70	0.372	29.85	60.08	50.12	8.95	79.40	92.78	1.08
Wheat bran, 6%	65.80	75.21	16.09	6.80	0.372	29.81	60.30	49.88	8.83	79.86	93.64	1.08
SEM	0.16	0.15	0.208	0.026	0.0009	0.077	0.084	0.208	0.020	0.16	0.175	0.0001
Probability												
Enzyme	0.610	0.919	0.118	0.421	0.579	0.332	0.331	0.481	0.993	0.266	0.944	0.579
Ingredients	0.620	0.248	0.230	0.436	0.350	0.991	0.837	0.834	0.352	0.215	0.455	0.715
Enzyme × ingredients	0.357	0.250	0.652	0.982	0.370	0.062	0.112	0.079	0.151	0.979	0.236	0.835

¹For enzyme level and ingredients, each mean represents the mean of 30 and 12 replicate cages of 9 birds each.

Apparent Ileal Digestibility

Ileal organic matter and protein and ash digestibility were not influenced by the fiber source, but the diets containing 3% SBP increased ileal DM digestibility compared with the control diet and 6% wheat bran diets ($P < 0.05$, Table 10). Enzyme supplementation increased ileal DM, organic matter, and protein digestibility ($P < 0.05$). No interactions were observed for any measure of ileal digestibility.

pH of Intestinal Digesta

Proventriculus, gizzard, duodenum, jejunum, and ileum pH was not influenced by dietary treatments (Table 11), but cecal pH of the birds fed with 3% wheat bran and 3% SBP diets was lower than that of the control ($P < 0.05$). Enzyme supplementation reduced cecal pH ($P < 0.05$). No interaction was observed for all measurements of gastrointestinal pH.

DISCUSSION

Addition of either of the fiber sources to the diet appeared to depress hen-day production, intake, and egg mass in a dose-dependent manner, the effects being more evident with SBP. Sugar beet pulp is more of a soluble pectin-based fiber source, whereas wheat bran is more of an insoluble arabinoxylan fiber source, and hence, the modes of action of these different sources may differ. With regard to insoluble fiber addition to laying hen diets, our expectation was that the lower wheat bran inclusion level may be sufficient to stimulate gizzard action and aid in digestion of the rest of the diet (Hetland et al., 2003) without compromising digestion via either increased passage rate or viscosity, whereas the 6% wheat bran inclusion may prove excessive. However, even the lowest inclusion level of wheat bran tended to reduce rather than improve hen-day production (NS), and addition of 6% wheat bran resulted in the expected downturn in performance, possibly via the passage rate increasing beyond the digestive capacity of the bird.

Increased addition of SBP depressed hen-day production and egg mass and increased intake in a dose-dependent manner and in most cases numerically more so than wheat bran. Sugar beet pulp is more of a soluble fiber source as noted previously, but pectin-based rather than xylan-based fiber source, so it is interesting to see that the effects of this soluble pectin were similar to those of the insoluble wheat bran source in this regard. Sugar beet pulp addition (4%) to laying hen diets has previously (Guzman et al., 2016) been shown to depress production between 17 to 46 wk of age, and the same authors (Guzman et al., 2015) reported 4% SBP also reduced weight gain and increased FCR of pullets between 1 to 17 wk of age, suggesting this fiber source is detrimental to digestive efficiency at the rate used. Similar growth depressing effects have been noted by Sadeghi et al. (2015) in broilers when 30 g/kg of SBP

Table 5. Effects of enzyme supplementation and dietary fiber source (wheat bran and sugar beet pulp) on serum cholesterol (mg/dL) and uric acid (mg/dL) levels in laying hens at 79 wk of lay.

Treatments	Serum biochemical traits	
	Cholesterol, mg/dL	Uric acid, mg/dL
Enzyme level, mg/kg		
0	200.69	5.51
100	195.74	5.51
Ingredients		
Control	170.03	5.16
Sugar beet pulp, 3%	188.05	5.18
Sugar beet pulp, 6%	243.61	5.81
Wheat bran, 3%	189.82	5.79
Wheat bran, 6%	199.57	5.61
SEM	9.80	0.19
Probability		
Enzyme	0.79	0.99
Ingredients	0.17	0.70
Enzyme × ingredients	0.22	0.23

¹For enzyme level and ingredients, each mean represents the mean of 30 and 12 replicate cages of one sampled bird each.

or 30 g/kg of a SBP/rice husk blend was included in the diet or when potato peel (15%) or SBP (7%) was added to the diet (Abdel-Hafeez et al., 2018). It is presumed these pectins are not degraded by the host or fermented efficiently by the microbiome and perhaps exert their antinutritive effects by increasing intestinal viscosity.

Adding the enzyme compensated for all the losses in performance noted with the 3% wheat bran diet, but it clearly did not compensate for the loss in performance noted with the higher wheat bran inclusion level or the SBP inclusion. In this work, egg mass only tended to be increased with enzyme addition and inclusion of the fiber sources, and only the 6% SBP diet resulted in decrease of egg mass in comparison with the control diet ($P < 0.05$). The fact that the enzyme is principally a xylanase suggests that it will more likely be able to compensate for addition of a arabinoxyylan-rich fiber source such as wheat bran than a pectin-rich source

such as SBP, and indeed, the egg mass data suggest this to be the case. Nevertheless, the fact that the enzyme had an effect on production regardless of fiber source addition also suggests that there was adequate substrate in the basal corn-soy diet to allow for improvements in digestion of the diet as a whole.

Neither the addition of fiber sources nor the addition of enzyme influenced egg quality parameters. Similar results were reported by Samli (2006), who found that adding 0.5, 5, and even 10% rice bran to the diet of 22-week-old laying hens did not affect yolk weight, albumin weight, shell thickness, and shell weight. Given the addition of fiber-rich sources to the diets did not influence egg quality parameters, it is not surprising that addition of the enzyme had little effect as well.

Serum cholesterol and uric acid levels were not affected by dietary treatments. It has been assumed that modulation of microbial fermentation either via soluble viscous and fermentable fibers (SBP) or via insoluble, less fermentable fiber (wheat bran) would alter the gastrointestinal microbiome and thus change the rates of bile acid deconjugation and excretion and as a result fat digestion, which would be expected to influence serum cholesterol levels (Story and Kritchevsky, 1976; Parsaie et al., 2007; Ridlon et al., 2016). With respect to serum uric acid levels, it has been hypothesized that fiber inclusion in basal diet reduces ammonia production in the cecum, which may influence serum uric acid levels (Roberts et al., 2006). The work of this group suggested that distillers dried grains with solubles (10%), wheat middlings (7.3%), or soy hulls (4.8%) could reduce ammonia production in laying hen manure by up to 50% without adverse effects on egg production (Roberts et al., 2006).

The weight of the ileum was greater in hens fed with the 6% SBP diet than in those subjected to most other fiber treatments. This could be due to the high soluble fiber content of SBP delaying digestion and passage rate, which signals the bird to increase the gut size to facilitate more extensive nutrient extraction. Such a

Table 6. Effects of enzyme supplementation and dietary fiber source (wheat bran and sugar beet pulp) on relative weight and lengths of gastrointestinal organs to live BW (%) in laying hens at 79 wk of lay.¹

Treatments	Relative weight or length of gastrointestinal organs to live BW, %										
	Proventriculus	Gizzard	Pancreas	Duodenum	Jejunum	Ileum	Cecum	Duodenum length	Jejunum length	Ileum length	Cecum length
Enzyme level, mg/kg											
0	0.438	1.88	0.182	0.568	1.31	1.26	0.516	1.33	2.99	3.05	0.774
100	0.408	1.82	0.184	0.564	1.32	1.22	0.472	1.39	3.03	3.08	0.748
Ingredients											
Control	0.445	1.79	0.190	0.576	1.39	1.380 ^{a,b}	0.512	1.45	3.17	3.31	0.817
Sugar beet pulp, 3%	0.383	1.89	0.175	0.566	1.27	1.301 ^b	0.498	1.31	2.86	3.00	0.748
Sugar beet pulp, 6%	0.422	1.99	0.191	0.573	1.37	1.425 ^a	0.482	1.39	3.00	2.95	0.765
Wheat bran, 3%	0.417	1.73	0.175	0.507	1.23	1.183 ^b	0.444	1.31	3.07	3.07	0.753
Wheat bran, 6%	0.487	1.83	0.183	0.605	1.32	1.184 ^{a,b}	0.535	1.36	2.97	3.00	0.722
SEM	0.012	0.03	0.004	0.013	0.03	0.030	0.15	0.020	0.056	0.05	0.17
Probability											
Enzyme	0.140	0.330	0.830	0.880	0.900	0.490	0.08	0.211	0.738	0.840	0.470
Ingredients	0.250	0.120	0.540	0.250	0.660	0.030	0.21	0.330	0.520	0.240	0.540
Enzyme × ingredients	0.058	0.310	0.190	0.176	0.420	0.518	0.21	0.409	0.530	0.370	0.510

^{a,b}Means in the same column and under each main effect with different letters are different ($P < 0.05$).

¹For enzyme level and ingredients, each mean represents the mean of 30 and 12 replicate cages of one sampled bird each.

Table 7. Effects of enzyme supplementation and dietary fiber source (wheat bran and beet pulp) on jejunum villus height (μm), crypt depth (μm), villus height-to-crypt depth ratio, and villus surface (μm) of laying hens at 79 wk of lay.¹

Treatments	Jejunum morphology			
	Villus height, μm	Crypt depth, μm	Villus height-to-crypt depth ratio	Villus surface, mm^2
Enzyme level, mg/kg				
0	819.10	176.24	4.66	0.09 ^b
100	894.10	180.72	4.88	0.115 ^a
Ingredients				
Control	782.95 ^b	188.17	4.04 ^b	0.093 ^b
Sugar beet pulp, 3%	838.34 ^b	184.27	4.36 ^b	0.114 ^{a,b}
Sugar beet pulp, 6%	812.73 ^b	176.11	4.74 ^{a,b}	0.103 ^b
Wheat bran, 3%	1,093.12 ^a	175.33	5.76 ^a	0.135 ^a
Wheat bran, 6%	757.14 ^b	168.54	4.95 ^{a,b}	0.091 ^b
SEM	25.23	3.56	0.16	0.004
Probability				
Enzyme	0.07	0.54	0.46	0.03
Ingredients	0.0001	0.46	0.01	0.001
Enzyme \times ingredients	0.72	0.61	0.71	0.35

^{a-b}Values in the same column and under each main effect with different letters are different ($P < 0.05$).

¹For enzyme level and ingredients, each mean represents the mean of 30 and 12 replicate cages of one sampled bird each.

response has been noted in high-NSP diets, wherein the size of the small intestine increases in proportion with the viscosity of the diet to provide the required area for digestion and absorption (Craig et al., 2019).

Morphological parameters such as villus height, villus height-to-crypt depth ratio, and villus surface were significantly increased in the jejunum of hens fed with the 3% wheat bran compared with those subjected to control treatment and most other treatments, which suggests this level of bran was sufficient to optimize intestinal development, whereas increasing beyond 3% was clearly in excess. When considering only those diets containing additional fiber, the hen-day production data mirror these intestinal measurements. An adequate supply of fermentable carbohydrates can alter the activity of the microbiome such that the production of SCFA is optimized for gut health (e.g., high butyrate), protein fermentation is minimized, and, as a result, the presence of branched chain volatile fatty acids (VFA), ammonia, and other toxic compounds such as amines, short-chain phenols, sulfides, thiols, and indoles (Kieffer et al., 2016; Koh et al., 2016;

Williams et al., 2017) is minimized. Fermentable carbohydrates are most often soluble and of lower molecular weight, but they can be derived by hydrolysis and dissolution of insoluble cell wall material by enzymes such as xylanases. Because wheat bran has less soluble fiber content than beet pulp (Banino, 2012) and does not increase the viscosity of digestive contents perhaps as much as beet pulp, adding wheat bran to the diet may have improved morphological parameters by delivery of fermentable fiber without the antinutritive consequences of viscosity. The balance between fermentability and viscosity is probably important, and it is noteworthy that the addition of the enzyme, which would be expected to increase the concentration of shorter, more fermentable, soluble NSP (Parsaie et al., 2007; Bedford and Cowieson, 2012; Kiarie et al., 2013, 2014; Vila, 2017), tended to increase villus height and increased villus surface area. Taheri et al. (2016) reported that supplementation of barley-based broiler diets (11–12 d) with a multienzyme increased the jejunum villus surface area, which tends to support the aforementioned hypothesis. In contrast to our

Table 8. Effects of enzyme supplementation and dietary fiber source (wheat bran and beet pulp) on ileum villus height (μm), crypt depth (μm), villus height-to-crypt depth ratio, and villus surface (μm) in laying hens at 79 wk of lay.¹

Treatments	Ileum morphology			
	Villus height, μm	Crypt depth, μm	Villus height-to-crypt depth ratio	Villus surface, mm^2
Enzyme level, mg/kg				
0	598.92	161.38	3.66	0.071 ^b
100	636.20	155.22	3.75	0.076 ^a
Ingredients				
Control	657.98	154.41	3.64	0.068 ^{a,b}
Sugar beet pulp, 3%	629.51	162.43	3.65	0.086 ^a
Sugar beet pulp, 6%	602.44	163.94	3.68	0.079 ^{a,b}
Wheat bran, 3%	572.51	138.44	3.96	0.058 ^b
Wheat bran, 6%	625.35	172.29	3.59	0.076 ^{a,b}
SEM	18.66	3.04	0.13	0.003
Probability				
Enzyme	0.35	0.237	0.75	0.35
Ingredients	0.73	0.001	0.91	0.036
Enzyme \times ingredients	0.94	0.026	0.69	0.95

^{a-b}Values in the same column and under each main effect with different letters are different ($P < 0.05$).

¹For enzyme level and ingredients, each mean represents the mean of 30 and 12 replicate cages of one sampled bird each.

Table 9. The effect of enzyme \times ingredient interaction on ileum crypt depth in laying hens at 79 wk of lay.¹

Treatments	Enzyme, 100 mg/kg	Enzyme \times ingredients
		Ileum crypt depth
Control	–	164.93 ^{a,b,c}
Control	+	143.89 ^{a,b,c}
Sugar beet pulp, 3%	–	175.48 ^{a,b}
Sugar beet pulp, 3%	+	149.39 ^{a,b,c}
Sugar beet pulp, 6%	–	162.91 ^{a,b,c}
Sugar beet pulp, 6%	+	164.97 ^{a,b,c}
Wheat bran, 3%	–	143.19 ^{b,c}
Wheat bran, 3%	+	133.70 ^c
Wheat bran, 6%	–	184.16 ^a
Wheat bran, 6%	+	160.41 ^{a,b,c}

^{a-c}Values with different letters are different ($P < 0.05$).

¹For each treatment, each mean represents the mean of 6 replicate cages of one sampled bird each.

results, [Sadeghi et al. \(2015\)](#) found that 30 g/kg of SBP, 30 g/kg of rice husk, or a 30 g/kg of the mixture of the 2 fiber sources reduced duodenal and ileal villus height at 21 d, suggesting the antinutritive effects of excess soluble fiber overpowered the expected benefits from increased fermentable substrate in birds of this age.

In the ileum, the diet containing 3% wheat bran significantly decreased villus surface area (the exact opposite of its effect in the jejunum), which may be due to the significant beneficial effects noted in the jejunum, resulting in reduced need for absorptive capacity in the ileum.

Inclusion of 3% SBP significantly increased ileal DM digestibility compared with the control group. Similar to our finding, [Gonzalez-Alvarado et al. \(2010\)](#) added 30 g/kg of oat hull or 30 g/kg of SBP to broiler diets and showed that either of the fiber sources increased digestibility and nutrient retention (DM, organic matter, soluble ash, and nitrogen), but this effect was greater for oat hull supplementation. However, [Jimenez-Moreno et al. \(2013\)](#) noted that supplementation of 50 and 75 g/kg of SBP to broiler diets reduced digestibility of DM and organic matter, suggesting that there may be

Table 10. Effects of enzyme supplementation and dietary fiber source (wheat bran and sugar beet pulp) on apparent ileal digestibility in laying hens at 79 wk of lay.

Treatments	Ileal digestibility, %			
	DM	Organic matter	CP	Ash
Enzyme level, mg/kg				
0	91.13 ^b	66.36 ^b	60.15 ^b	38.53
100	91.81 ^a	68.74 ^a	61.57 ^a	40.89
Ingredients				
Control	91.06 ^{b,c}	67.91	60.95	40.84
Sugar beet pulp, 3%	92.38 ^a	68.83	62.16	44.00
Sugar beet pulp, 6%	92.03 ^{a,b}	65.77	59.99	35.92
Wheat bran, 3%	91.16 ^{a,b,c}	66.94	59.87	39.86
Wheat bran, 6%	90.74 ^c	67.64	61.33	37.95
SEM	0.16	0.45	0.34	0.97
Probability				
Enzyme	0.02	0.01	0.03	0.22
Ingredients	0.002	0.26	0.183	0.08
Enzyme \times ingredients	0.46	0.37	0.73	0.84

^{a-c}Values in the same column and under each main effect with different letters are significantly different ($P < 0.05$).

¹For enzyme level and ingredients, each mean represents the mean of 30 and 12 replicate cages of 9 birds each.

a limit to the inclusion level beyond which the negative effects outweigh the positive effects.

Enzyme supplementation significantly increased ileal DM, organic matter, and protein digestibility, probably via breaking down antinutritive polysaccharides and producing low-molecular-weight carbohydrates, which can be fermented by intestinal bacteria ([Vila, 2017](#)). Regardless, it is noteworthy that the apparent benefit in DM digestibility for the 3% SBP diet did not translate into superior performance, which is likely due to the effects it had on intake or utilization of digested nutrients for nonproductive purposes.

Addition of either 3% wheat bran or SBP significantly decreased the pH of cecal contents compared with the control diet, likely as a result of increased fermentation of the fiber to SCFA (such as acetate, propionate, and butyrate), which increased acidity. Such a response can have significant benefits including reducing the growth of pathogenic bacteria and increasing the absorption of minerals ([Kieffer et al., 2016](#); [Koh et al., 2016](#); [Williams et al., 2017](#)). In addition, elevated VFA production can stimulate the production and secretion of mucus, which acts as a protective barrier in the intestine ([Williams et al., 2005](#); [Slavin, 2013](#); [Makki et al., 2018](#)). Enzyme supplementation also significantly reduced cecal pH, likely owing to its ability to degrade insoluble, poorly fermented fiber into soluble and rapidly fermented fiber. [Morgan et al. \(2019\)](#) noted that adding a combination of 2% arabinoxylan and 16,000 units (BXU/kg) of the same enzyme used here in a wheat–soybean meal–based diet resulted in an increased concentration of total SCFA, acetic acid, butyric acid, and isovaleric acid in the cecum. However, reports on the effects of NSPases on SCFA levels in the large intestine are not consistent, and this may be due to the enzyme type and dose used and the main ingredients of the diet. It could also be due to the fact that SCFA concentrations are a point-in-time measurement of how much VFA is present, and it does not reflect the much more relevant point which is how rapidly the VFA are being produced and consumed. As a result, conflicting reports such as the report of [Taylor et al. \(2018\)](#) that adding 12,000 BXU/kg of xylanase to wheat-based diet had no significant effect on gizzard, ileum, and cecum pH and VFA production in the cecum is not surprising.

CONCLUSIONS

Although the addition of the different fiber sources reduced production performance, it was clear that this negative effect was not as marked with addition of the 3% level of wheat bran. The addition of 3% wheat bran did result in improved intestinal morphological parameters and reduced cecum pH. Thus, the addition of 3% wheat bran has the potential to improve the intestinal health of the older laying hen without a significant negative effect on laying performance, particularly in the presence of the enzyme, which restored performance and resulted in additional benefits in functional, morphological, and digestibility parameters.

Table 11. Effects of enzyme supplementation and dietary fiber source (wheat bran and beet pulp) on pH of gastrointestinal segments at 79 wk of lay.¹

Treatments	Gastrointestinal pH					
	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Cecum
Enzyme, mg/kg						
0	4.21	3.37	6.04	6.10	6.40	5.85 ^a
100	4.29	3.36	6.05	6.03	6.44	5.29 ^b
Ingredients						
Control	4.19	3.44	6.02	6.00	6.41	5.81 ^a
Beet pulp, 3%	4.35	3.44	6.08	6.08	6.42	5.45 ^b
Beet pulp, 6%	4.29	3.21	6.06	6.08	6.46	5.57 ^{a,b}
Wheat bran, 3%	4.23	3.27	6.01	6.03	6.26	5.47 ^b
Wheat bran, 6%	4.19	3.44	6.05	6.15	6.53	5.55 ^{a,b}
SEM	0.052	0.048	0.02	0.039	0.04	0.05
Probability						
Enzyme	0.83	0.43	0.77	0.83	0.29	0.01
Ingredients	0.41	0.95	0.78	0.39	0.59	0.0001
Enzyme × ingredients	0.46	0.81	0.56	0.84	0.17	0.65

^{a-b}Values in the same column with different letters are significantly different ($P < 0.05$).

¹For enzyme level and ingredients, each mean represents the mean of 30 and 12 replicate cages of one sampled bird each.

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DISCLOSURES

No potential conflict of interest was reported by the authors. Dr. M. R. Bedford is an employee of AB Vista Feed Ingredients (UK). The authors declare that his role in this project was not directional in the discussion of the results and findings from any commercial angle.

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