# Effects of dietary inclusion of a cocktail NSPase and β-mannanase separately and in combination in low energy diets on broiler performance and processing parameters

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**Primary Audience:** Nutritionists, Live Production Managers

## SUMMARY

Two experiments were conducted to investigate whether an additive effect on performance could be achieved with the inclusion of a β-mannanase and a cocktail NSPase in combination. The design of both experiments included 5 dietary treatments, each containing 8 replicates: positive control (PC), negative control (NC) with a reduction of 132 kcal/kg AME throughout the experiment, NC + β-mannanase, NC + NSPase, and NC + β-mannanase/NSPase. In experiment 1, birds were fed a predetermined amount of feed, while in experiment 2, diets were fed to a certain age. Experiment 1 dietary phases included starter (0.68 kg), grower (1.45 kg.), finisher (1.45 kg), and withdrawal (remaining feed required). In the NC on d 14 and 28, BW was reduced ($P < 0.05$). Increases ($P < 0.05$) in BW were observed with NSPase and the combination of β-mannanase/NSPase on d 14 and with all treatments on d 28. The FCR was increased ($P < 0.05$) through d 28 in the NC. The combination of β-mannanase/NSPase reduced the FCR through d 28 to levels similar to the PC. Experiment 2 dietary phases included starter (d 1 to 14), grower (d 15 to 27), and finisher (d 28 to 41). A reduction in BW was observed ($P < 0.05$) in the NC diet on d 14, 27, and 35. Increases in d 14 BW were observed with the individual inclusion of the NSPase and the combination of β-mannanase/NSPase. The combination of β-mannanase/NSPase increased ($P < 0.05$) BW compared to the NC on d 27 and 35. Increases ($P < 0.05$) were observed in the FCR in the NC for each dietary phase. The inclusion of NSPase reduced the FCR through d 41 compared to the NC. The combination of β-mannanase/NSPase reduced the FCR through d 41 compared to the NC. These data confirm that enzyme supplementation in a low energy diet improves performance and indicates that beneficial effects may be observed from the use of multiple enzymes.

**Key words:** β-mannanase, broiler, NSPase, performance

2015 J. Appl. Poult. Res. 24:489–501
http://dx.doi.org/10.3382/japr/pfv055

## DESCRIPTION OF PROBLEM

Polysaccharides, polymers of monosaccharides linked by glycosidic bonds, are major components of feed ingredients used for monogastric animals. Starch, a polymer of glucose units linked by α-(1-4) with a few α-(1-6) bonds, is digested in the small intestine of poultry through endogenous enzyme activity. Nonstarch polysaccharides (NSPs) are fibrous materials found in

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the plant cell wall that include celluloses, hemicelluloses, pectins, and oligosaccharides. Monogastric animals such as poultry do not possess endogenous enzymes capable of cleaving and digesting $\beta$ ($\alpha$)-linked NSPs. Research has shown that, aside from representing a potential source of energy, diets high in NSPs can lead to increased intestinal viscosity [1], a reduction in nutrient digestibility [2], and ultimately a reduction in performance with regard to BW and FCR [3, 4]. One method for combating the antinutritive properties of NSPs may be the inclusion of exogenous enzymes.

Enzymes that degrade NSPs (NSPase) hydrolyze bonds that are indigestible by monogastric animals, allowing for improvements in digestibility [5–7]. Increased intestinal viscosity causes alterations in the gut microflora and can reduce nutrient utilization; however, the proper use of NSPase can combat these negative effects [4]. Inclusion of NSPase in broiler diets has also been reported to improve growth performance [2, 6, 8]. These improvements in growth performance can be attributed to increased AME, ileal digestible energy, and dry matter retention [8–11] associated with exogenous enzyme inclusion. The majority of diets contain a variety of NSPs; thus, the most effective means of enzyme use may be a cocktail carbohydrase approach that varies in specificity. Research has shown that cocktail carbohydrase preparations can increase starch digestibility and broiler performance and improve FCRs [8].

Beta-mannans, such as glucomannans and galactomannans, in the plant cell wall of soybean meal, guar, and sesame meal may reduce nutrient bioavailability [12]. The exogenous enzyme $\beta$-mannanase hydrolyzes the high-molecular-weight and soluble $\beta$-galactomannan into mannooligosaccharides through enzymatic degradation. The inclusion of $\beta$-mannanase is a potential strategy for combating these negative effects. The inclusion of $\beta$-mannanase has been shown to improve the FCR, reduce the water-to-feed ratio, and reduce the dry fecal output of broilers by degrading $\beta$-mannans [13]. The inclusion of $\beta$-mannanase in broiler diets also increases AMEn [14] and BW gain and improves the FCR [14–16]. Mannans are components of the surface of multiple pathogens; thus, they can elicit an innate immune response diverting energy toward an immune response as opposed to growth, which has been termed a feed-induced immune response [17]. The inclusion of $\beta$-mannanase reduces lesion development following *Eimeria* sp. and *Clostridium perfrigens* challenges [18], indicating beneficial immunological effects. Additionally, $\beta$-mannanase supplementation increases relative immune organ weights, increases the concentration of serum Igm, and increases T-lymphocyte proliferation [12].

Williams et al. [17] conducted a study to determine whether an intermittent application of $\beta$-mannanase (d 1 to 21) and a cocktail blend NSPase (d 22 to 47) was advantageous compared with individual supplementation. During the grower 2, finisher, and withdrawal phases of this study (d 22 to 47), the individual inclusion of $\beta$-mannanase and NSPase and the intermittent application of $\beta$-mannanase and NSPase significantly increased BWs to levels that were similar to those under the PC diet, with the intermittent application yielding the highest observed BW. Similarly regarding BW gain, individual inclusion and intermittent application increased weight gain compared to the NC diet to levels that were comparable to those under the PC diet; however, the intermittent application of $\beta$-mannanase and NSPase further increased ($P < 0.05$) weight gain compared to the PC diet. Therefore, the objective of these two experiments was to determine whether an additive effect of co-administration of $\beta$-mannanase and NSPase would be observed on broiler growth performance under reduced energy diets. The two experiments were identical in treatment; however, they varied with regard to ingredient profile, duration of trial, time of year, NSPase used, and bird strain to determine whether observed outcomes were similar. Both NSPases used in these studies were previously shown to improve growth performance when included in low energy broiler diets [6,17].

## MATERIALS AND METHODS

### *Experiment 1*

To evaluate the effects of a cocktail NSPase and $\beta$-mannanase in low energy diets in

combination and separately on broiler performance and processing parameters, the experimental design was a randomized complete block design containing 5 dietary treatments. Each treatment consisted of 8 replicate pens containing 28 Cobb 500 straight-run broilers (total of 1,120 chicks). Chicks were wing banded and allotted to floor pens based on day-old chick weight for a 48-d assay period. Broilers were reared in floor pens that contained fresh pine shavings as bedding material, provided ad libitum access to dietary treatments and water, and provided age-appropriate supplemental heat. Animal care was in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). The diets were corn and soybean meal based and contained Distillers' Dried Grains with solubles (DDGS) up to 10% and meat and bone meal up to 5% (Table 1). Diets were formulated to be isonitrogenous and contained phytase[1] (250 FTU/kg for 0.10% available phosphorus). The dietary phases consisted of a starter (0.68 kg), grower (1.45 kg), finisher (1.45 kg), and withdrawal (remaining feed required), with diets being fed as a crumble during the starter phase and pellet throughout the grower, finisher, and withdrawal phases. Each phase was fed as allocation on a per-bird basis, and each phase allocation was adjusted for observed mortality per replicate pen.

The 5 treatment groups consisted of a positive control (PC) diet that was formulated to an industry standard diet, negative control (NC) diet that had a reduction in energy of 132 kcal/kg compared to the PC, NC supplemented with β-mannanase,[2] NC supplemented with cocktail NSPase,[3] and NC supplemented with β-mannanase/cocktail NSPase inclusion. Cocktail NSPase was added to the mixer prepelleting, and β-mannanase was spray applied postpelleting. Premixes including sand and NSPase (400 g/ton) were added to all NC treatments prior to pelleting. Beta-mannanase and both NSPases were included at a rate of 100 mL/ton (720 million units/L product) and 113.5 g/ton, respectively, for all dietary phases. The NSPase used in experiment 1 contained an activity that included 1,500 units of xylanase, 1,100 units of β-glucanase, 35 units of α-galactosidase, and 110 units of β-mannanase. As defined by the manufacturer, one unit of β-mannanase activity is the amount of enzyme that generates 0.72 μg reducing sugars per minute from a mannose-containing substrate at pH 6.6 and a temperature of 40°C. One unit of xylanase activity is defined as micromoles of total reducing sugars released per minute at 40°C and pH 4.5. Samples were collected during feed manufacturing for nutrient analysis. Crude protein was determined using method (AOAC 2000) by combustion (AOAC 990.03), total phosphorus determined by wet ash ICP (AOAC 985.01 M), acid detergent fiber determined using an ANKOM (ANKOM Technology, Macedon, NY, USA) digestion unit (AOAC 973.18), and an ether extraction to determine crude fat (AOAC 920.39). Body weights and FCRs were determined on d 14, 28, 42, and 47. On d 48, following an 8-h feed withdrawal period, 5 male and 5 female broilers from each pen were processed and deboned for carcass and breast yields (40 male and 40 female/treatment/400 total, or 35% of total placement). All carcasses were air chilled for 16 h prior to deboning to avoid influencing yield data based on differences in carcass water uptake.

### *Experiment 2*

The experimental treatment groups were identical to those of the previous experiment; however, each of the 8 replicates contained 40 sexed Ross 708 chicks/replicate placed at a 1:1 male-to-female ratio. Chicks were wing banded and allotted to dietary treatment based on chick weight (total of 1,600 chicks). Broilers were reared in floor pens that contained fresh pine shavings as bedding material, provided ad libitum access to dietary treatments and water, and provided age-appropriate supplemental heat. Animal care was in accordance with a protocol approved by the IACUC. All diets were corn and soybean meal based containing DDGS at 2.5% and meat and bone meal (MBM) at 5% and formulated to be isonitrogenous (Table 2). All diets contained phytase

[1]Optiphos® L, Huvepharma Inc., Peachtree City, GA.
[2]Hemicell® L, Elanco Animal Health, Greenfield, IN. β-mannanase from *Bacillus lentus*.
[3]Enzyvia LLC, Sheridan, IN. Activity included 1,500 units of xylanase, 1,100 units of β-glucanase, 35 units of α-galactosidase, and 110 units of β-mannanase.

**Table 1.** Dietary formulation, calculated nutrient content, and analysis of nutrients of positive control (PC) and negative control (NC) starter, grower, finisher, and withdrawal diets fed to market broilers (Experiment 1). Beta-mannanase[1] and NSPase[2] were added to the NC diet separately and in combination.[3]

| | Starter (%) | | Grower (%) | | Finisher (%) | | Withdrawal (%) | |
|---|---|---|---|---|---|---|---|---|
| Ingredient | PC | NC | PC | NC | PC | NC | PC | NC |
| Corn | 58.08 | 61.33 | 65.94 | 69.92 | 65.47 | 69.42 | 65.04 | 69.23 |
| Distillers' Dried Grains with Solubles | 6.08 | 5.14 | 2.33 | 1.15 | 10.00 | 8.91 | 10.0 | 10.0 |
| Dehulled soybean meal (48%) | 26.34 | 26.27 | 21.99 | 21.91 | 16.22 | 16.03 | 15.93 | 14.38 |
| DL-methionine (99%) | 0.27 | 0.26 | 0.24 | 0.24 | 0.19 | 0.19 | 0.19 | 0.20 |
| L-lysine- HCl | 0.28 | 0.28 | 0.33 | 0.33 | 0.29 | 0.29 | 0.28 | 0.32 |
| Fat-A/V blend | 2.50 | – | 2.85 | 0.15 | 3.45 | 0.75 | 4.20 | 1.45 |
| Pork MBM | 4.22 | 4.18 | 4.38 | 4.33 | 2.41 | 2.44 | 2.43 | 2.43 |
| Limestone | 1.17 | 1.30 | 1.00 | 1.00 | 1.11 | 1.10 | 1.10 | 1.12 |
| Monocalcium phosphate | 0.30 | 0.31 | 0.11 | 0.13 | – | – | – | – |
| Sodium chloride | 0.41 | 0.01 | 0.29 | 0.29 | 0.21 | 0.21 | 0.20 | 0.16 |
| Vitamins[4] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace minerals[5] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Coban 90[6] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | – | – |
| L-threonine 98 | 0.02 | 0.02 | – | 0.01 | – | 0.01 | 0.01 | 0.03 |
| Sodium bicarbonate | – | 0.56 | 0.19 | 0.20 | 0.30 | 0.31 | 0.31 | 0.37 |
| Phytase[7] | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Calculated Nutrient Concentration | | | | | | | | |
| Protein | 22.80 | 22.80 | 20.50 | 20.50 | 18.19 | 18.20 | 18.01 | 17.67 |
| Lysine | 1.29 | 1.29 | 1.20 | 1.20 | 1.02 | 1.02 | 1.00 | 1.00 |
| Methionine | 0.60 | 0.59 | 0.54 | 0.53 | 0.48 | 0.48 | 0.48 | 0.49 |
| TSAA | 0.92 | 0.92 | 0.83 | 0.83 | 0.75 | 0.75 | 0.75 | 0.75 |
| Threonine | 0.80 | 0.80 | 0.70 | 0.70 | 0.63 | 0.63 | 0.63 | 0.63 |
| Calcium | 0.95 | 1.00 | 0.85 | 0.85 | 0.75 | 0.75 | 0.75 | 0.75 |
| Available phosphorus | 0.45 | 0.45 | 0.40 | 0.40 | 0.35 | 0.35 | 0.35 | 0.35 |
| Total phosphorus | 0.62 | 0.62 | 0.55 | 0.55 | 0.49 | 0.49 | 0.49 | 0.49 |
| Sodium | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Crude fat | 5.24 | 2.77 | 5.48 | 2.80 | 6.41 | 3.74 | 7.14 | 4.51 |
| AME (kcal/kg) | 3108 | 2977 | 3154 | 3023 | 3198 | 3067 | 3240 | 3108 |
| Analyzed Nutrient Content | | | | | | | | |
| Crude protein | 22.5 | 23.7 | 18.3 | 20.2 | 18.0 | 18.2 | 17.6 | 18.1 |
| Crude fat | 4.54 | 3.81 | 5.12 | 3.66 | 6.41 | 3.99 | 6.99 | 5.17 |
| Total phosphorus | 0.65 | 0.60 | 0.54 | 0.59 | 0.51 | 0.51 | 0.48 | 0.50 |
| Acid detergent fiber | 3.37 | 3.56 | 2.35 | 2.25 | 2.84 | 3.35 | 3.05 | 2.61 |

[1]Hemicell® HT - Elanco Animal Health, Greenfield, IN (363.2 g/ton). Analyzed enzyme recovery was 87.1 MU/ton for starter, 93.4 MU/ton for grower, 91.7 MU/ton for finisher, and 89.1 MU/ton for withdrawal.

[2]Enzyvia LLC, Sheridan, IN. Analyzed xylanase recovery was 159 U/kg for starter, 167 U/kg for grower, 185 U/kg for finisher, and 181 U/kg for withdrawal.

[3]Analyzed $\beta$-mannanase recovery was 88.9 MU/ton for starter, 90.7 MU/ton for grower, 92.3 MU/ton for finisher, and 87.5 MU/ton for withdrawal. Analyzed xylanase recovery was 171 U/kg for starter, 163 U/kg for grower, 191 U/kg for finisher, and 187 U/kg for withdrawal.

[4]Vitamin premix added at this rate yields 22,045 IU vitamin A, 7,716 IU vitamin $D_3$, 91 IU vitamin E, 0.04 mg $B_{12}$, 11.9 mg riboflavin, 91.8 mg niacin, 40.4 mg d-pantothenic acid, 261.1 mg choline, 2.9 mg menadione, 3.50 mg folic acid, 14.3 mg pyroxidine, 5.87 mg thiamine, 1.10 mg biotin per kg diet. The carrier is ground rice hulls.

[5]Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium/kg diet. The carrier is calcium carbonate, and the premix contains less than 1% mineral oil.

[6]Active drug ingredient monesin sodium 90 g/lb (90 g/ton inclusion: Elanco Animal Health, Indianapolis, IN). As an aid in the prevention of coccidiosis caused by *Eimeria necarix, Eimeria tenella, Eimeria acervulina, Eimeria brunetti, Eimeria mivati,* and *Eimeria maxima*.

[7]Optiphos® L, Huvepharma, Inc., Peachtree City, GA.

**Table 2.** Dietary formulation, calculated nutrient content, and analysis of nutrients of positive control (PC) and negative control (NC) starter, grower, finisher, and withdrawal diets fed to market broilers (Experiment 2). Beta-mannanase[1] and NSPase[2] were added to the NC diet separately and in combination.[3]

| | Starter (%) | | Grower (%) | | Finisher (%) | |
|---|---|---|---|---|---|---|
| Ingredient | PC | NC | PC | NC | PC | NC |
| Corn | 60.01 | 36.16 | 62.25 | 65.55 | 67.06 | 70.35 |
| Distillers' Dried Grains with Solubles | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Dehulled soybean meal (48%) | 27.09 | 26.56 | 25.16 | 24.54 | 20.60 | 19.99 |
| Pork MBM | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| DL-methionine (99%) | 0.27 | 0.27 | 0.19 | 0.19 | 0.17 | 0.17 |
| Fat blended A/V blend | 2.65 | – | 3.00 | 0.30 | 3.15 | 0.45 |
| L-threonine 98 | 0.01 | – | – | – | – | – |
| Lysine HCL | 0.25 | 0.26 | 0.17 | 0.19 | – | 0.013 |
| BMD 50[4] | 0.05 | 0.05 | 0.05 | 0.05 | – | – |
| 3-Nitro-20[5] | 0.025 | 0.025 | 0.025 | 0.025 | – | – |
| Stafac 20[6] | – | – | – | – | 0.05 | 0.05 |
| Sodium bicarbonate | – | 0.16 | – | – | 0.09 | 0.11 |
| Monocalcium phosphate | 0.21 | 0.20 | – | – | – | – |
| Sodium chloride | 0.42 | 0.31 | 0.42 | 0.42 | 0.41 | 0.40 |
| Vitamins[7] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace minerals[8] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Coban 90[9] | 0.05 | 0.05 | 0.05 | 0.05 | – | – |
| Phytase[10] | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 | 0.013 |
| Calculated nutrient concentration | | | | | | |
| Protein | 22.87 | 22.88 | 21.97 | 21.97 | 19.93 | 19.93 |
| Lysine | 1.29 | 1.29 | 1.18 | 1.18 | 0.92 | 0.92 |
| Methionine | 0.60 | 0.60 | 0.51 | 0.51 | 0.47 | 0.47 |
| TSAA | 0.92 | 0.92 | 0.82 | 0.82 | 0.75 | 0.76 |
| Threonine | 0.80 | 0.79 | 0.76 | 0.76 | 0.69 | 0.69 |
| Calcium | 1.00 | 1.00 | 0.85 | 0.85 | 0.75 | 0.75 |
| Available phosphorus | 0.45 | 0.45 | 0.40 | 0.40 | 0.35 | 0.35 |
| Total phosphorus | 0.61 | 0.61 | 0.56 | 0.57 | 0.54 | 0.55 |
| Sodium | 0.20 | 0.20 | 0.20 | 0.20 | 0.22 | 0.22 |
| Crude fat | 5.24 | 2.69 | 5.63 | 3.03 | 5.87 | 3.27 |
| AME (kcal/kg) | 3108 | 2977 | 3154 | 3023 | 3198 | 3067 |
| Analyzed Nutrient Content | | | | | | |
| Crude protein | 22.0 | 23.1 | 20.9 | 20.6 | 18.3 | 18.6 |
| Crude fat | 6.90 | 3.88 | 6.45 | 3.90 | 6.67 | 3.79 |
| Total phosphorus | 0.58 | 0.63 | 0.57 | 0.56 | 0.52 | 0.55 |
| Acid detergent Fiber | 4.74 | 5.07 | 4.78 | 4.34 | 3.30 | 3.42 |

[1]Hemicell® HT – Elanco Animal Health, Greenfield, IN (363.2 g/ton). Analyzed enzyme recovery was 97.4 MU/ton for starter, 94.3 MU/ton for grower, and 91.8 MU/ton for finisher.

[2]Enzyvia LLC, Sheridan, IN. Analyzed xylanase recovery was 264 U/kg for starter, 276 U/kg for grower, and 255 U/kg for finisher.

[3]Analyzed β-mannanase recovery was 90.7 MU/ton for starter, 93.6 MU/ton for grower, and 89.9 MU/ton for finisher. Analyzed xylanase recovery was 259 U/kg for starter, 268 U/kg for grower, and 288 U/kg for finisher.

[4]Bacitracin methylene disalicylate active ingredient bacitracin methylene disalicylate 50g/lb (50 /ton inclusion: Alpharma Inc., Fort Lee, NJ) for increased rate of weight gain and improved feed efficiency.

[5]3-nitro-20 active ingredient 3-nitro-4hydroxyphenylarsonic acid 20g/lb (10g/ton inclusion: Alpharma Inc., Fort Lee, NJ) for growth promotion, improved feed efficiency, and improved pigmentation.

[6]Stafac 20 active ingredient virginiamycin 20g/lb (20g/ton inclusion: Philbro Animal Health, Fairfield, NJ) for increased rate of weight gain and improved feed efficiency.

[7]Vitamin premix added at this rate yields 22,045 IU vitamin A, 7,716 IU vitamin $D_3$, 91 IU vitamin E, 0.04 mg $B_{12}$, 11.9 mg riboflavin, 91.8 mg niacin, 40.4 mg d-pantothenic acid, 261.1 mg choline, 2.9 mg menadione, 3.50 mg folic acid, 14.3 mg pyroxidine, 5.87 mg thiamine, 1.10 mg biotin/kg diet. The carrier is ground rice hulls.

[8]Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium/kg diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[9]Active drug ingredient monesin sodium 90 g/lb (90 g/ton inclusion: Elanco Animal Health, Indianapolis, IN), as an aid in the prevention of coccidiosis caused by *Eimeria necarix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunetti*, *Eimeria mivati*, and *Eimeria maxima*.

[10]Optiphos® L, Huvepharma, Inc., Peachtree City, GA.

(250 FTU/kg—manufacturer's recommendation for 0.10% available phosphorus). The 5 dietary treatments were similar to those in experiment 1 with a PC diet, a NC diet with a reduction of 132 kcal/kg AME compared to the PC diet, NC diet supplemented with $\beta$-mannanase, NC diet supplemented with NSPase[4], and NC diet supplemented with a combination of $\beta$-mannanase/cocktail NSPase. Premixes including sand and NSPase (400 g/ton) were added to all NC treatments prior to pelleting. Beta-mannanase and both NSPases were included at a rate of 100 mL/ton (720 million units/L product) and 113.5 g/ton, respectively, for all dietary phases.

The NSPase in experiment 2 had an activity of 2,700 U/g of xylanase and contained $\beta$-glucananase and $\alpha$-galactosidase. Three dietary phases included starter (d 1 to 14), grower (d 15 to 27), and finisher (d 28 to 41), and diets were fed in crumble (starter) and pelleted (grower and finisher) form. Unlike experiment 1, diets were fed to 1 d of age as opposed to an amount per bird. Antibiotic growth promoters (AGPs) were used in the form of bacitracin methylene disalicylate (BMD) during the starter and grower phases at a concentration of 50 g/ton and virginiamycin during the finisher phase at a concentration of 20 g/ton. Samples were collected during feed manufacturing for nutrient analysis. Crude protein was determined using method (AOAC, 2000) by combustion (AOAC 990.03), total phosphorus determined by wet ash ICP (AOAC 985.01M), acid detergent fiber determined using an ANKOM digestion unit (AOAC 973.18), and an ether extraction to determine crude fat (AOAC 920.39). Body weights and FCRs were determined on d 14, 27, and 41 to evaluate performance. On d 42, following an 8-h feed withdrawal period, 8 male and 8 female broilers from each replicate pen were removed and processed for carcass and fat pad measurements (64 male and 64 females/treatment/640 total, or 40% of total placement).

## STATISTICAL ANALYSIS

All data for both experiments were analyzed via a one-way ANOVA, and means were deemed significantly different at $P < 0.05$. Means were separated using Duncan's multiple range test. All processing data were analyzed via a 5 (dietary treatment) × 2 (sex) factorial analysis of variance using the general linear model procedure in SPSS v.18.0. Main effect means were deemed significantly different at $P < 0.05$ and separated using Duncan's multiple range test when appropriate. In instances when significant interactions were present between dietary treatment and sex, data were analyzed using a one-way ANOVA and individual treatment means were determined to be statistically different at $P < 0.05$ and separated using Duncan's multiple range test. All percentage data, including processing yields and mortality, were subjected to an arcsin transformation prior to analysis.

## RESULTS AND DISCUSSION

### *Experiment 1*

The reduction in dietary energy in the NC diet significantly reduced d 14 BW compared to the PC diet (Table 3). The inclusion of $\beta$-mannanase did not impact d 14 BW compared to the NC diet. The individual inclusion of NSPase and combination of $\beta$-mannanase/NSPase increased ($P < 0.05$) d 14 BW compared to the NC to levels that were comparable to the PC diet. On d 28, a decrease ($P < 0.05$) in BW was observed in the NC compared to the PC diet; the individual inclusion of $\beta$-mannanase and NSPase and the combination of $\beta$-mannanase/NSPase increased ($P < 0.05$) BW compared to the NC diet to levels that were similar to the PC diet. No differences in BW were observed among any of the dietary treatments on d 42 and 47. The reduction in dietary energy in the NC diet increased ($P < 0.05$) the mortality corrected FCR through d 14 compared to the PC diet (Table 4). The combination of $\beta$-mannanase/NSPase reduced the d 14 FCR to levels that were comparable to the PC diet. Similar observations were observed through d 28 as the reduction in dietary energy increased ($P < 0.05$) the cumulative FCR in the NC diet compared to the PC diet. Inclusion of NSPase or $\beta$-mannanase alone did not reduce the FCR to a level similar to the PC broilers; however, the combination of $\beta$-mannanase/NSPase did reduce the FCR to levels that were similar to

[4]Enspira®, Enzyvia LLC, Sheridan, IN. Xylanase (2700 U/g) from *Aspergillus niger* and *Trichoderma reeset*; also contains $\beta$-glucananse and $\alpha$-galactosidase.

**Table 3.** BW of straight-run market broilers fed diets reduced in energy and supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination (Experiment 1).

| | (g) | | (kg) | | |
|---|---|---|---|---|---|
| Treatment | Day 0 | Day 14 | Day 28 | Day 42 | Day 47 |
| Positive control | 45.6 | 507[a] | 1.606[a] | 2.816 | 3.310 |
| Negative control | 45.6 | 466[c] | 1.541[b] | 2.783 | 3.246 |
| β-mannanase | 45.6 | 476[b,c] | 1.584[a] | 2.799 | 3.284 |
| NSPase | 45.6 | 495[a] | 1.593[a] | 2.811 | 3.297 |
| β-mannanase + NSPase | 45.6 | 491[a,b] | 1.590[a] | 2.807 | 3.318 |
| *P* value | 0.765 | <0.001 | 0.029 | 0.948 | 0.524 |
| Pooled SEM | 0.1 | 4 | 0.007 | 0.012 | 0.013 |

[a–c]Means in same column differ significantly at $P < 0.05$.

[1]Activity included 1,500 units xylanase, 1,100 units β-glucanase, 35 units α-galactosidase, and 110 units β-mannanase. Enzyvia LLC, Sheridan, IN (113.5 g/ton).

[2]Hemicell® L – Elanco Animal Health, Greenfield, IN (100 mL/ton).

**Table 4.** Cumulative mortality corrected FCR and mortality of straight-run market broilers fed diets reduced in energy and supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination (Experiment 1).

| Treatment | FCR d 1 to 14 | FCR d 1 to 28 | FCR d 1 to 42 | FCR d 1 to 47 | Total mortality (%) |
|---|---|---|---|---|---|
| Positive control | 1.235[c] | 1.449[c] | 1.752 | 1.851 | 6.3 |
| Negative control | 1.348[a] | 1.508[a,b] | 1.783 | 1.880 | 4.9 |
| β-mannanase | 1.356[a] | 1.503[a,b] | 1.767 | 1.881 | 7.1 |
| NSPase | 1.315[b] | 1.525[a] | 1.795 | 1.884 | 8.4 |
| β-mannanase/NSPase | 1.279[b,c] | 1.461[b,c] | 1.760 | 1.868 | 6.7 |
| *P*-value | <0.001 | 0.007 | 0.245 | 0.474 | 0.594 |
| Pooled SEM | 0.010 | 0.009 | 0.009 | 0.009 | 1.7 |

[a–c]Means in same column differ significantly at $P < 0.05$.

[1]Activity included 1,500 units of xylanase, 1,100 units of β-glucanase, 35 units of α-galactosidase, and 110 units of β-mannanase. Enzyvia LLC, Sheridan, IN (113.5 g/ton).

[2]Hemicell® L - Elanco Animal Health, Greenfield, IN (100 mL/ton).

that of the PC diet. No differences were observed in the cumulative FCR through d 42 and 47 or in mortality amongst any of the dietary treatments.

The reduction in energy in the NC diet negatively impacted processing parameters, including a decrease ($P < 0.05$) in live weight and carcass weight compared to the PC diet (Table 5). Processed broilers fed the reduced energy diet supplemented with the individual inclusion of NSPase and β-mannanase and the combination of β-mannanase/NSPase resulted in an increase ($P < 0.05$) in live weight and carcass weight compared to the NC diet to levels similar to those of the PC diet. An interaction between dietary treatment∗sex was observed in fat pad weight and tenderloin weight as differences were only observed in male broilers. A reduction ($P < 0.05$) in fat pad weight was observed in male broilers in the NC diet compared to the PC diet. The individual inclusion of NSPase and β-mannanase and the combination of β-mannanase/NSPase did not impact fat pad weight compared to the NC diet. The inclusion of NSPase in the reduced energy diet increased ($P < 0.05$) tenderloin weights in male broilers when compared to the control diets. The inclusion of β-mannanase/NSPase and NSPase alone in the reduced energy diet increased ($P < 0.05$) the main effect breast meat weight compared to the NC diet. No differences were observed with regard to female fat pad weights among any dietary treatment groups, suggesting that even the low energy diet was sufficient and met the female broilers' energy requirements. The reduction in energy in the NC diet negatively impacted carcass yield as the NC diet resulted in the lowest observed carcass yield (Table 6). The inclusion of NSPase and the combination of β-mannanase/NSPase in the reduced energy

**Table 5.** Processing parameters including live weight, carcass weight without giblets (WOG) weight, fat pad weight, breast weight, and tender weight of broilers fed reduced energy diet with addition of a cocktail NSPase[1] and β-mannanase[2] separately and in combination (Experiment 1).

| Treatment | Sex | Live Wt. (g) | WOG Wt. (g) | Fat Pad Wt. (g) | Breast Wt. (g) | Tender Wt. (g) |
|---|---|---|---|---|---|---|
| Positive control | Male | 3,694 | 2,667 | 58.3[a] | 661.3 | 143.5[b] |
| | Female | 2,998 | 2,183 | 51.9[a,b,c,d] | 531.7 | 125.2[c,d] |
| Negative control | Male | 3,648 | 2,594 | 45.2[d] | 647.1 | 140.3[b] |
| | Female | 2,915 | 2,120 | 51.0[a,b,c,d] | 513.1 | 121.3[d] |
| β-mannanase | Male | 3,642 | 2,632 | 49.5[b,c,d] | 656.7 | 141.3[b] |
| | Female | 2,977 | 2,162 | 48.4[b,c,d] | 524.4 | 126.3[c,d] |
| NSPase | Male | 3,771 | 2,741 | 46.7[c,d] | 697.5 | 152.2[a] |
| | Female | 3,018 | 2,212 | 56.5[a,b] | 543.1 | 128.9[c,d] |
| β-mannanase/NSPase | Male | 3,671 | 2,653 | 48.3[b,c,d] | 656.3 | 138.5[b] |
| | Female | 3,038 | 2,236 | 54.4[a,b,c] | 560.1 | 130.8[c] |
| Main Effects | | | | | | |
| Treatment | | | | | | |
| Positive Control | | 3,332[a] | 2,415[a,b] | 55.0 | 594.0[a,b,c] | 134.0 |
| Negative Control | | 3,235[b] | 2,327[c] | 48.4 | 571.6[c] | 129.6 |
| β-mannanase | | 3,315[a] | 2,401[b] | 49.0 | 591.6[b,c] | 133.9 |
| NSPase | | 3,379[a] | 2,465[a] | 51.9 | 617.1[a] | 140.0 |
| β-mannanase/NSPase | | 3,354[a] | 2,444[a,b] | 51.4 | 608.2[a,b] | 134.6 |
| Sex | | | | | | |
| Male | | 3,686[a] | 2,659[a] | 49.8 | 663.9[a] | 143.1 |
| Female | | 2,989[b] | 2,182[b] | 52.4 | 534.5[b] | 126.4 |
| *P*-value | | | | | | |
| Treatment | | 0.029 | 0.001 | 0.089 | 0.007 | 0.005 |
| Sex | | <0.001 | <0.001 | 0.107 | <0.001 | <0.001 |
| Treatment x sex | | 0.472 | 0.430 | 0.022 | 0.151 | 0.030 |
| Pooled SEM | | 21 | 16 | 0.9 | 5.0 | 0.9 |

[a–d]Means in same column differ significantly at $P < 0.05$.

[1]Activity included 1,500 units xylanase, 1,100 units β-glucanase, 35 units α-galactosidase, and 110 units β-mannanase. Enzyvia LLC, Sheridan, IN (113.5 g/ton).

[2]Hemicell® L – Elanco Animal Health, Greenfield, IN (100 mL/ton).

diet increased ($P < 0.05$) carcass yield compared to the NC diet. No main effect differences were observed in breast meat yield or tenderloin yield among the dietary treatments. An interaction between dietary treatment*sex was observed in fat pad yield, and differences were only observed in male broilers. The energy reduction energy in the NC diet reduced ($P < 0.05$) fat pad yield compared to the PC diet. The individual inclusion of NSPase and β-mannanase and the combination of β-D-mannanase/NSPase did not impact fat pad yield compared to the NC diet.

### *Experiment 2*

The energy reduction in the NC diet reduced ($P < 0.05$) d 14 BW in the NC compared to the PC (Table 7). The individual inclusion of NSPase and the combination of β-mannanase/NSPase increased d 14 BW to levels that were similar to the PC. The inclusion of β-mannanase did not impact d 14 BW compared to the NC. On d 27 and 35, a reduction ($P < 0.05$) in BW was observed in the NC diet compared to the PC diet. The individual inclusion of NSPase and β-mannanase did not increase BW compared to the NC diet; however, the combination of β-mannanase/NSPase increased ($P < 0.05$) BW compared to the NC diet to levels that were similar to the PC diet. As in experiment 1, BW was not impacted at the conclusion of the experiment on d 41 between the controls and enzyme-supplemented diets, however; the combination of β-mannanase/NSPase resulted in the highest observed BW on d 41. Inclusion of β-mannanase resulted in the lowest observed cumulative mortality during the experiment, which was reduced ($P < 0.05$) compared to the PC fed broilers with more than a 3% decrease in total mortality.

During the starter phase, the energy reduction in the NC diet increased ($P < 0.05$) the FCR compared to the PC diet (Table 8). While no

**Table 6.** Processing parameters including carcass yield, breast yield, tenderloin yield, and fat pad yield of broilers fed reduced energy diet with addition of a cocktail NSPase[1] and β-mannanase[2] separately and in combination (Experiment 1).

| Treatment | Sex | Carcass Yield (%) | Breast Yield (%) | Tenderloin Yield (%) | Fat pad Yield (%) |
|---|---|---|---|---|---|
| Positive control | Male | 72.2 | 24.8 | 5.4 | 2.21[a] |
| | Female | 72.8 | 24.4 | 5.8 | 2.37[a] |
| Negative control | Male | 71.2 | 24.9 | 5.4 | 1.74[b] |
| | Female | 72.7 | 24.2 | 5.7 | 2.39[a] |
| β-mannanase | Male | 72.3 | 25.0 | 5.4 | 1.88[b] |
| | Female | 72.6 | 24.2 | 5.9 | 2.23[a] |
| NSPase | Male | 72.7 | 25.4 | 5.6 | 1.70[b] |
| | Female | 73.3 | 24.6 | 5.8 | 2.54[a] |
| β-mannananse/NSPase | Male | 72.3 | 24.7 | 5.2 | 1.82[b] |
| | Female | 73.6 | 25.0 | 5.9 | 2.44[a] |
| Main effects | | | | | |
| Treatment | | | | | |
| Positive control | | 72.5[a,b] | 24.6 | 5.6 | 2.29 |
| Negative control | | 72.0[b] | 24.5 | 5.6 | 2.11 |
| β-mannanase | | 72.4[a,b] | 24.6 | 5.6 | 2.05 |
| NSPase | | 73.0[a] | 25.0 | 5.7 | 2.15 |
| β-mannanse/NSPase | | 72.9[a] | 24.9 | 5.5 | 2.13 |
| Sex | | | | | |
| Male | | 72.1[b] | 25.0[a] | 5.4[b] | 1.88[b] |
| Female | | 73.0[a] | 24.5[b] | 5.8[a] | 2.40[a] |
| *p*-value | | | | | |
| Treatment | | 0.015 | 0.559 | 0.443 | 0.249 |
| Sex | | <0.001 | 0.025 | <0.001 | <0.001 |
| Treatment x Sex | | 0.310 | 0.388 | 0.318 | 0.023 |
| Pooled SEM | | 0.1 | 0.1 | 0.03 | 0.04 |

[a,b]Means in same column differ significantly at $P < 0.05$.

[1] Activity included 1,500 units xylanase, 1,100 units β-glucanase, 35 units α-galactosidase, and 110 units β-mannanase. Enzyvia LLC, Sheridan, IN (113.5 g/ton).

[2]Hemicell® L – Elanco Animal Health, Greenfield, IN (100 mL/ton).

**Table 7.** Body weight of straight-run market broilers fed diets reduced in energy and supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination (Experiment 2).

| Treatment | Day 0 BW (g) | Day 14 BW (g) | Day 27 BW (kg) | Day 35 BW (kg) | Day 41 BW (kg) | Mortality |
|---|---|---|---|---|---|---|
| Positive control | 43.6 | 443[a] | 1.375[a] | 2.100[a] | 2.552[a] | 5.0[a] |
| Negative control | 43.5 | 423[b,c] | 1.322[c] | 2.055[b] | 2.510[a,b] | 3.1[a,b] |
| β-mannanase | 43.5 | 410[c] | 1.288[d] | 1.999[c] | 2.459[b] | 1.8[b] |
| NSPase | 43.5 | 430[a,b] | 1.330[b,c] | 2.051[b] | 2.505[a,b] | 2.8[a,b] |
| β-mannanase/NSPase | 43.6 | 438[a,b] | 1.354[a,b] | 2.095[a] | 2.556[a] | 4.7[a,b] |
| *P* value | 0.757 | 0.005 | <0.001 | 0.002 | 0.011 | 0.037 |
| Pooled SEM | 0.1 | 3 | 0.006 | 0.010 | 0.012 | 0.4 |

[a–c]Means in same column differ significantly at $P < 0.05$.

[1]Enspira® – Enzyvia LLC, Sheridan, IN (113.5 g/ton).

[2]Hemicell® L – Elanco Animal Health, Greenfield, IN (100 mL/ton).

differences were observed between the enzyme treatments, the individual inclusion of NSPase and β-mannanase did not impact the starter FCR compared to the NC diet, however; the combination of β-mannanase/NSPase reduced the FCR to levels that were comparable to those of the PC diet. The energy reduction in the NC diet increased ($P < 0.05$) grower FCR compared to the PC diet; the inclusion of enzymes did not impact grower FCR. Similar trends were observed in the finisher phase with the energy reduction in the NC diet increasing ($P < 0.05$) the FCR

**Table 8.** Mortality corrected FCR and cumulative FCR of broilers fed diets reduced in energy and supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination (Experiment 2).

| Treatment | FCR Starter (d 1 to 14) | FCR Grower (d 15 to 27) | FCR Finisher (d 28 to 41) | FCR d 1 to 27 | FCR d 1 to 35 | FCR d 1 to 41 |
|---|---|---|---|---|---|---|
| Positive control | 1.251[b] | 1.465[b] | 1.995[b] | 1.399[b] | 1.541[c] | 1.678[c] |
| Negative control | 1.296[a] | 1.510[a] | 2.056[a] | 1.446[a] | 1.580[a] | 1.737[a] |
| β-mannanase | 1.282[a] | 1.499[a] | 2.018[b] | 1.435[a] | 1.572[a,b] | 1.716[a,b] |
| NSPase | 1.285[a] | 1.4990[a] | 2.010[b] | 1.434[a] | 1.562[b] | 1.706[b] |
| β-mannanase/ NSPase | 1.272[a,b] | 1.502[a] | 1.994[b] | 1.432[a] | 1.565[b] | 1.699[b,c] |
| *P*-value | 0.047 | 0.049 | 0.019 | 0.001 | <0.001 | <0.001 |
| Pooled SEM | 0.004 | 0.005 | 0.008 | 0.004 | 0.003 | 0.005 |

[a–c]Means in same column differ significantly at $P < 0.05$.

[1]Enspira® – Enzyvia LLC, Sheridan, IN (113.5 g/ton).

[2]Hemicell® L – Elanco Animal Health, Greenfield, IN (100 mL/ton).

compared to the PC diet. The individual inclusion of NSPase and β-mannanase reduced FCR to levels that were similar to those of the PC diet. The combination of β-mannanase/NSPase reduced ($P < 0.05$) finisher FCR compared to the NC diet to levels that were similar to those of the PC diet. Cumulatively through d 27 an increase ($P < 0.05$) in the FCR was observed in the NC diet compared to the PC diet; the inclusion of enzyme did not impact the cumulative FCR compared to the NC diet. The energy reduction in the NC diet increased ($P < 0.05$) the cumulative FCR through d 35 compared to the PC diet. The individual inclusion of β-mannanase did not impact the FCR compared to the NC diet. The individual inclusion of NSPase and the combination of β-mannanase/NSPase reduced ($P < 0.05$) the FCR compared to the NC diet; however, the reduction did not reach the levels of the PC diet. At the conclusion of the trial on d 41, an increase ($P < 0.05$) in the cumulative FCR was observed in the NC diet compared to the PC diet. The individual inclusion of β-mannanase did not impact the cumulative FCR compared to the NC diet. The individual inclusion of NSPase reduced ($P < 0.05$) the FCR compared to the NC diet; however, the reduction did not reach levels of the PC diet. The combination of β-mannanase/NSPase reduced ($P < 0.05$) cumulative FCR to levels that were comparable to the PC diet.

Regarding processing parameters, the energy reduction in the NC diet reduced ($P < 0.05$) the live weight, carcass weight, and fat pad weight compared to the PC diet (Table 9). The individual inclusion of NSPase and β-mannanase did not impact live weight, carcass weight, or fat pad weight compared to the NC diet; however, the combination of β-mannanase/NSPase increased these parameters to levels that were similar to those in the PC diet. No differences were observed in carcass yield among any of the dietary treatments. The inclusion of NSPase in the reduced energy diet reduced ($P < 0.05$) fat pad yield compared to the PC diet. The individual inclusion of β-mannanase and the combination of β-mannanase/NSPase did not impact fat pad yield compared to the control diets.

The results of the two experiments confirm the negative impact of reducing dietary energy level because broiler performance was reduced. A decrease in BW was observed with the reduced energy NC diet compared to the PC diet in both experiments. A study conducted by Williams et al. [17] reported reductions in BW with reductions in the dietary energy by 132 kcal/kg in the NC diet compared to the PC diet. Similar results were observed by Coppedge et al. [6], with a decrease in BW when energy levels were reduced by 133 kcal/kg compared to the PC diet. The reduction of dietary energy resulted in an increase in the FCR in the NC diet in both experiments compared to the PC diet. O'Neill et al. [19] observed negative effects when reducing dietary energy, which resulted in an increase in the FCR through d 42. In experiment 2, feed consumption was not impacted with a decrease in dietary energy; however, BW and FCR were negatively impacted, indicating that broilers were unable to meet their dietary energy requirements to maximize growth. In efforts to recover or increase the caloric value and ameliorate the negative effects of low energy diets, exogenous enzymes are supplemented in broiler diets.

**Table 9.** Processing parameters of male, female, and straight-run broilers fed diets reduced in energy and supplemented with a cocktail NSPase[1] and β-mannanase[2] separately and in combination (Experiment 2).

| Treatment | Sex | Live Wt. (g) | WOG Wt. (g) | Fat Pad Wt. (g) | WOG Yield (%) | Fat Pad Yield (%) |
|---|---|---|---|---|---|---|
| Positive control | Male | 2,799 | 2,184 | 36.2 | 78.0 | 1.66 |
| | Female | 2,300 | 1,822 | 36.1 | 79.3 | 1.98 |
| Negative control | Male | 2,725 | 2,127 | 34.0 | 78.2 | 1.61 |
| | Female | 2,239 | 1,779 | 32.6 | 79.4 | 1.83 |
| β-mannanase | Male | 2,673 | 2,087 | 34.2 | 78.1 | 1.64 |
| | Female | 2,255 | 1,760 | 34.1 | 78.1 | 1.94 |
| NSPase | Male | 2,757 | 2,158 | 32.2 | 78.4 | 1.49 |
| | Female | 2,253 | 1,779 | 32.0 | 78.9 | 1.80 |
| β-mannanase/NSPase | Male | 2,768 | 2,168 | 34.7 | 78.3 | 1.60 |
| | Female | 2,334 | 1,848 | 34.2 | 79.0 | 1.86 |
| Main Effects | | | | | | |
| Treatment | | | | | | |
| Positive Control | | 2,553[a] | 2,007[a] | 36.2[a] | 78.6 | 1.81[a] |
| Negative Control | | 2,478[b] | 1,951[b,c] | 33.3[b] | 78.8 | 1.72[a,b] |
| β-mannanase | | 2,463[b] | 1,924[c] | 34.2[a,b] | 78.1 | 1.79[a] |
| NSPase | | 2,503[b] | 1964[b] | 32.1[b] | 78.7 | 1.65[b] |
| β-mannanase/NSPase | | 2,552[a] | 2,011[a] | 34.5[a,b] | 78.7 | 1.73[a,b] |
| Sex | | | | | | |
| Male | | 2,745[a] | 2,145[a] | 34.3 | 78.2[b] | 1.60[b] |
| Female | | 2,276[b] | 1,797[b] | 33.8 | 79.0[a] | 1.88[a] |
| *p*-value | | | | | | |
| Treatment | | <0.001 | <0.001 | 0.010 | 0.242 | 0.032 |
| Sex | | <0.001 | <0.001 | 0.513 | <0.001 | <0.001 |
| Treatment x Sex | | 0.203 | 0.534 | 0.979 | 0.276 | 0.911 |
| Pooled SEM | | 12 | 9 | 0.4 | 0.1 | 0.02 |

[a–c]Means in same columns with different superscripts are different at $P < 0.05$.

[1]Enspira® – Enzyvia LLC, Sheridan, IN (113.5 g/ton).

[2]Hemicell® L – Elanco Animal Health, Greenfield, IN (100 mL/ton).

The efficacy of enzyme inclusion is significantly affected by ingredient profiles and substrate availability. The inclusion of a cocktail NSPase containing several enzymatic activities can target multiple components of feedstuffs, possibly having a greater effect than individual supplementation, which targets one substrate [20]. Improvements in BW and FCR were observed with the addition of a cocktail NSPase. Williams et al. [17] observed increases in BW and reductions in FCR throughout the trial with the addition of NSPase in reduced energy broiler diets. Similar improvements in BW and FCR were observed by Coppedge et al. [6] with the addition of NSPase. Meng et al. [8] used an NSPase that contained enzymatic activity similar to that of the NSPase used in the present study, with reductions in FCR in broilers fed a reduced energy corn and soybean meal diet.

Beta-mannanase inclusion is a dietary strategy used to combat the negative impacts of dietary β-mannans. Beta-mannanase inclusion has been shown to improve FCR and reduce water-to-feed ratio and dry fecal output of broilers by degrading β-mannans [13]. The inclusion of β-mannanase in experiment 1 of the present study increased d 28 BW in the NC diet to levels that were comparable to those of the PC diet. In experiment 2, the inclusion of β-mannanase reduced the FCR during the finisher phase to levels that were similar to those in the PC diet. Increases in BW and reductions in FCR were observed by Williams et al. [17] with the inclusion of β-mannanase in reduced energy diets. Zou et al. [12] also reported improvements in BW in broilers fed corn and soybean meal diets supplemented with β-mannanase compared to the control. While no significant differences were observed in mortality between the PC and NC diets in either experiment, the addition of β-mannanase in the reduced energy diet in experiment 2 resulted in a significant reduction in mortality compared to the PC treatment group and had the lowest mortality of any dietary treatment.

Jackson et al. [18] observed that the inclusion of β-mannanase in broiler diets resulted in an increase in immunological activity, a significant reduction in coccidial lesion scores in the GI tract of the bird following an *Eimeria* sp. and *Clostridium perfringens* challenge, leading to an overall improvement in performance, perhaps accounting for the reduction in mortality in experiment 2. The lack of performance response in experiment 2 with the inclusion of β-mannanase may be associated with a lack of environmental challenge since broilers were reared on fresh pine shavings as opposed to used litter, which is close to industry conditions. A described mechanism of action for β-mannanase is associated with immunological activity that reduces inflammation in the GI tract [18] caused by the presence of these NSPs, which can trigger the innate immune system, causing a nonproductive immune response [21].

The objective of the present experiment was to determine whether the co-administration of β-mannanase/NSPase would result in a subadditive effect compared to individual supplementation. The addition of each enzyme individually to the low energy diet had positive results in both experiments on multiple parameters. However, the combination of β-mannanase/NSPase in the low energy diet resulted in more consistent and increased improvements in BW and FCR. The combination of β-mannanase/NSPase increased ($P < 0.05$) BW in experiment 1 through d 28 and throughout the entire experiment 2. The greater impact in experiment 2 could be related to multiple factors, including the use of a different commercial broiler strain, the NSPase used, the dietary ingredient profile, or the use of growth promoters in experiment 2 but not experiment 1. The combination of β-mannanase/NSPase reduced FCR in experiment 1 through d 28 to levels that were comparable to the PC diet, yielding lower FCR results compared to the individual supplementation of β-mannanase and NSPase. In experiment 2, the combination of β-mannanase/NSPase reduced the FCR in the starter and finisher dietary phases and cumulatively through d 35 and 41 to levels that were comparable to the PC diet, yielding a lower FCR than individual supplementation. Williams et al. [17] conducted a similar study evaluating an intermittent application of β-mannanase (d 1 to 21) and NSPase (d 22 to 47) compared to individual supplementation of each enzyme. Interestingly, the intermittent application of β-mannanase and NSPase increased ($P < 0.05$) weight gain from d 22 to 47 compared to the PC diet, whereas individual supplementation yielded results that were similar to those of the PC diet, producing a subadditive effect such as in the current trial. In the current trial, improvements were observed in BW and FCR, whereas Williams et al. [17] only reported additive improvements in BW.

The reduction of dietary energy in the NC diet reduced multiple processing parameters in both experiments. Similar results were observed by Williams et al. [17] with the reduction of dietary energy in the NC diet reducing all evaluated processing parameters, including live weight, carcass weight and yield, and fat pad weight and yield. The sole inclusion of β-mannanase and NSPase and the combination of β-mannanase/NSPase increased multiple processing parameters to levels that were similar to that of the PC diet. In experiment 2, the individual inclusion of β-mannanase and NSPase did not impact live weight and carcass weight compared to the NC diet; however, the co-administration of β-mannanase/NSPase significantly increased these parameters compared to the NC diet and the individual inclusion to levels that were similar to the PC diet. In the study conducted by Williams et al. [17], the intermittent application of β-mannanase and NSPase increased ($P < 0.05$) individual live weight compared to the PC diet, whereas individual inclusion yielded results similar to that of the PC diet, suggesting that a subadditive effect was observed such as in the present study.

In the present study, supplementing reduced energy diets with β-mannanase and a cocktail NSPase improved growth performance and yield in broilers. The results confirm the ability of exogenous enzymes to hydrolyze bonds of various substrates that are indigestible by monogastrics. These data demonstrate that the co-administration of enzymes targeting various substrates determined by ingredient profiles could be beneficial in corn- and soybean-meal-based broiler diets. Additionally, the improvements observed with the co-administration of NSPase and β-mannanase were consistent across both trials even when different ingredient profiles,

commercial broiler strains, and antibiotic growth promoters were used.

## CONCLUSION AND APPLICATIONS

1. Reducing dietary energy negatively affects broiler performance, including BW, FCR, and processing parameters.
2. Individual inclusion of β-mannanase and NSPase can improve growth performance in reduced energy diets.
3. The co-administration of β-mannanase/NSPase resulted in a more consistent and elevated improvement in growth performance compared to individual inclusion.

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