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Research Article

Evaluation of Cocktail NSPase Inclusion in Reduced-Energy Corn-soybean Meal Diets on Live Growth Performance and Carcass Yield of Male Broilers

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Abstract

Background and Objective: The objective of the current study was to evaluate the performance of broilers fed reduced-energy diets with the inclusion of nonstarch polysaccharide-degrading enzyme (NSPase). **Materials and Methods:** Three separate performance trials were conducted, with two of these trials evaluating processing yields in addition to growth performance. Each experimental design consisted of three dietary treatments including a positive control (PC), negative control (NC) with a 132 kcal kg^{-1} AME reduction throughout the experiments when compared to the PC and the NC supplemented with NSPase (NC+NSPase). All diets were corn and soybean meal based and contained distillers dried grains with solubles (DDGS). Experiments 1 and 3 consisted of 3 dietary phases including a starter (days 1-14), grower (days 15-28) and finisher (days 29-42) phase. Experiment 2 consisted of 4 dietary phases including a starter (days 1-14), grower (days 15-28), finisher (days 29-35) and withdrawal (days 36-42) phase. All experiments consisted of 10 replicates per treatment. At the conclusion of experiments 1 and 2, 6 broilers from each replicate were processed for carcass weight without giblets (WOG), fat pad and yield measurements on day 43. **Results:** In all experiments, the inclusion of NSPase increased body weight and improved feed conversion ratio (FCR) while also improving processing yields in experiments 1 and 2. In these trials, the NC diet yielded the lowest processing yields, while the inclusion of NSPase increased ($p<0.05$) multiple processing parameters when compared to the low-energy diet. **Conclusion:** These data confirm the ability of NSPase inclusion to improve performance and processing parameters in reduced-energy diets.

Key words: Broilers, carbohydrase, exogenous enzymes, growth performance, low-energy diet, NSPase

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biofuels derived from grains such as corn have substantially increased in the United States with production expanding from 2 billion gallons in 2001 to 14 billion gallons in 2011[1]. This alternative fuel source has increased in popularity as it is a cleaner, more renewable energy source than conventional fossil fuels[2]. Most ethanol production derives from corn, which is the most abundant crop in the U.S., resulting in the redirection of its use away from livestock production. This change has led to further increases in the price of corn for use in livestock and poultry feed ingredients[3] as well as a greater presence of potential byproduct meal sources for dietary inclusion. Distillers dried grains with solubles (DDGS) are the byproduct of corn fermentation in the production of ethanol and have been incorporated in poultry diets at an inclusion rate of 2.5-5% as an alternative protein source[2]. While DDGS generally contain greater concentrations of protein, fat, vitamins and minerals when compared to whole kernel corn, several nutritional disadvantages limit higher inclusion levels[4]. Nutritional variability, nonstarch polysaccharide (NSP) content and physical quality of the diet become a concern when feeding DDGS[5,6]. The total NSP content of DDGS is approximately 4 times that of whole kernel corn, with the main constituents being 16% cellulose, 8% xylans and 5% arabinans[6,7]. It is often speculated that the higher NSP content and nutrient variability associated with DDGS may impede digestion and nutrient absorption, resulting in a negative impact on bird performance. The use of exogenous enzymes is a potential strategy to reduce the deleterious effects of NSPs and by-product inclusion by improving the nutrient utilization of DDGS when included in corn and soybean meal-based diets.

In the U.S., corn and soybean meal are the two major feed ingredients used in poultry diets. While the NSP content of these feedstuffs is low compared to that of DDGS, their contribution to the overall NSP content of the diet is considerable due to their high inclusion rate[8]. Approximately 90% of plant cell walls are composed of NSPs, which sequester vital nutrients such as starch, proteins and lipids. These fibrous biopolymers are linked by glycosidic bonds within the cell wall and surround the starchy endosperm and aleurone layers, interfering with nutrient digestibility and phytate dephosphorylation[9,10]. Corn and soybean meal contain approximately 10 and 20% NSP, respectively, according to CVB[11]. Common NSP found in SBM and corn consists of arabinose, xylose, raffinose, rhamnose and galactose[12]. These NSPs are known to possess antinutritional properties that decrease nutrient digestibility, prevent access to nutrients through encapsulation and reduce broiler performance[13].

To ameliorate the negative effect of NSPs, exogenous enzymes have been introduced to improve performance by hydrolyzing indigestible bonds in the plant cell walls into smaller fragments, allowing increased digestibility and improved bird performance[12]. It is hypothesized that while a single enzyme acts on a certain substrate, cocktail carbohydrases allow multiple enzymes to act on various substrates, improving nutrient digestion and absorption. Previous research has shown that carbohydrase products improve weight gain and feed conversion ratio (FCR) in broilers fed corn and soybean meal diets due to the increased ileal digestibility of protein and NSP[14,15]. The objective of this study was to evaluate the impact of a cocktail NSPase in low-energy diets containing DDGS on broiler growth performance and processing parameters.

MATERIALS AND METHODS

Experimental diets: The effect of NSPase (cocktail carbohydrase) inclusion on broiler growth performance and processing yields in reduced-energy diets was evaluated in three consecutive trials with a completely randomized block design containing 3 dietary treatments during a 42 days grow-out. The dietary treatments included a positive control (PC), negative control (NC) with a 132 kcal kg^{-1} ME reduction compared to the PC and the NC supplemented with NSPase. Diets were corn and soybean meal based with a 5% DDGS inclusion throughout the trial for experiment 1 and experiment 3. Experiment 1 included pork meat and bone meal at an inclusion rate of 3% for the starter, grower and finisher diets. Experiment 2 consisted of 5% DDGS inclusion during the starter phase with a 10% inclusion of DDGS for the remainder of the trial. PC diets were formulated to amino acid and energy levels of that found in a typical industry diet (Table 1-3). During feed manufacturing, treatments 2 and 3 were mixed as one large basal diet and divided equally prior to enzyme inclusion. All diets contained 250 FTU kg^{-1} of phytase. The NSPase was included at a rate of 113.5 g t^{-1} (2,700 U g^{-1} of xylanase from *Aspergillus niger* and *Trichoderma reesei*; also contains β-glucanase and α-galactosidase) for all dietary phases with recovery analysis in the footnote of each diet table. All diets were pelleted with the exception of the starter diet, which was pelleted and then crumbled. Diets were pelleted at a temperature range of 80-85°C with a conditioning time of approximately 12 sec. Samples were collected in duplicate during feed manufacturing for nutrient analysis. Crude protein was determined using AOAC by combustion (AOAC 990.03), total phosphorus was determined by wet ash ICP (AOAC 985.01 M),

Table 1: Dietary formulations and calculated nutrient content of the positive control (PC) and negative control (NC) fed to male market broilers in experiment 1

	Starter		Grower		Finisher	
Ingredients (%)	PC	NC	PC	NC	PC	NC
Corn	56.03	60.48	62.33	64.82	67.47	66.83
Dehulled soybean meal (48%)	29.88	28.28	24.70	24.39	19.82	22.79
DL-methionine (99%)	0.26	0.27	0.24	0.24	0.16	0.15
Dried distillers grain	5.00	5.00	5.00	5.00	5.00	5.00
Lysine HCl	0.23	0.27	0.21	0.22	0.19	0.09
Fat, A/V blend	3.42	0.50	2.69	0.50	2.63	0.50
Limestone	1.02	1.03	0.83	0.83	0.75	0.74
Monocalcium phosphate	0.39	0.39	0.18	0.17	0.11	0.09
Sodium chloride	0.41	0.37	0.29	0.29	0.18	0.29
Sodium bicarbonate	0.01	0.06	0.17	0.18	0.33	0.17
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Trace minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01
Pork meat and bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Calculated nutrient content						
Protein	22.50	22.45	20.50	20.50	18.50	19.78
dig-lysine	1.18	1.18	1.04	1.04	0.90	0.90
dig-methionine	0.57	0.57	0.53	0.53	0.43	0.43
dig-TSAA	0.85	0.85	0.80	0.80	0.67	0.68
dig-threonine	0.70	0.69	0.64	0.64	0.56	0.61
Calcium	0.92	0.92	0.80	0.80	0.75	0.75
Available phosphorus	0.45	0.45	0.40	0.40	0.38	0.38
Total phosphorus	0.57	0.57	0.51	0.52	0.48	0.50
Sodium	0.20	0.20	0.20	0.20	0.20	0.20
Apparent metabolizable energy (kcal kg^{-1})	3102.00	2970.00	3124.00	2992.00	3168.00	3036.00
Analyzed nutrient content						
Crude protein	22.20	22.70	21.70	20.60	17.20	18.80
Crude fat	4.93	3.49	5.30	2.90	5.09	3.45
Total phosphorous	0.66	0.65	0.67	0.58	0.49	0.55
Acid detergent fiber	4.20	4.30	3.70	3.10	2.90	2.80

[1]Vitamin premix added at this rate yields; Vitamin A: 11,023 IU, Vitamin D_3: 3,858 IU, Vitamin E: 46 IU, B_{12}: 0.0165 mg, Riboflavin: 5.845 mg, Niacin: 45.93 mg, d-pantothenic acid: 20.21 mg, Choline: 477.67 mg, Menadione: 1.47 mg, Folic acid: 1.75 mg, Pyroxidine: 7.17 mg, Thiamine: 2.94 and Biotin per kg diet: 0.55 mg. The carrier is ground rice hulls. [2]Trace mineral premix added at this rate yields; Manganese: 149.6 mg, Zinc: 125.1 mg, Iron: 16.5 mg, Copper: 1.7 mg, Iodine: 1.05 mg, Selenium: 0.25 mg, a minimum of 6.27 mg calcium and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil. [3]Active drug ingredient monensin sodium, 90 g lb^{-1} (90 g t^{-1} inclusion; Elanco Animal Health, Indianapolis, IN), as an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati* and *Eimeria maxima*. [4]Optiphos. Huvepharma-St. Louis, MO [5]Analyzed enzyme recovery was; Starter: 238 U kg^{-1}, Grower: 260 U kg^{-1} and Finisher: 193 U kg^{-1}

acid detergent fiber was determined using an ANKOM digestion unit (AOAC 973.18) and ether extraction was used to determine crude fat (AOAC 920.39)[16].

Experiment 1 and 2: On the day of hatch, 1,050 Cobb 500 male broiler chicks were allotted to floor pens and dietary treatments based on initial body weight (BW). Each treatment consisted of 10 replicates containing 35 birds per replicate pen. Chicks were provided age-appropriate supplemental heat and given access to feed and water *ad libitum.* Chicks were placed in 1.83×1.83 m rearing pens equipped with tube feeders and nipple drinkers with recycled litter as bedding material. Animal care was provided in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). The dietary program for experiment 1 consisted of 3 dietary phases with a starter diet being fed from 1-14 days of age, grower from 15-28 days of age and finisher from 29-42 days. Experiment 2 consisted of 4 dietary phases including a starter diet from 1-14 dys of age, grower from 15-28 days, a finisher diet from 29-35 days and a withdrawal from 36-42 days. All broilers and feed were weighed on the days of dietary changes for determination of average BW and feed consumption for the calculation of mortality-corrected feed conversion ratio (FCR). Upon completion of each trial (days 42), 6 broilers per replicate were randomly selected and processed to obtain processing yield data including carcass without giblet (WOG) and fat pad weight and yield. All broilers were bulk weighed on the evening of day 42 prior to an 8 h feed withdrawal period for processing on day 43. Six broilers from each replicate pen

Table 2: Dietary formulations and calculated nutrient content of the positive control (PC) and negative control (NC) fed to male market broilers in experiment 2

	Starter (%)		Grower (%)		Finisher (%)		Withdrawal (%)	
Ingredient	PC	NC	PC	NC	PC	NC	PC	NC
Corn	52.63	55.96	55.70	57.84	58.75	62.25	65.89	69.01
Dehulled soybean meal (48%)	35.22	34.62	28.12	27.74	25.28	24.45	18.52	18.11
DL-methionine (99%)	0.26	0.25	0.20	0.20	0.10	0.10	0.14	0.13
Dried distillers grain	5.00	5.00	10.00	10.00	10.00	10.00	10.00	10.00
Lysine HCL	0.14	0.15	0.12	0.21	0.05	0.07	0.22	0.22
Fat, A/V blend	3.25	0.50	2.83	0.50	3.25	0.50	2.82	0.11
Limestone	1.85	1.86	1.51	1.52	1.30	1.31	1.16	1.17
Sodium bicarbonate	0.00	0.00	0.00	0.00	0.01	0.03	0.32	0.32
Monocalcium phosphate	0.86	0.86	0.71	0.71	0.54	0.54	0.49	0.48
Sodium chloride	0.44	0.44	0.37	0.42	0.36	0.40	0.14	0.14
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.00	0.00
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrient content								
Protein	22.10	22.15	21.33	21.37	19.97	19.88	17.47	17.54
dig-lysine	1.18	1.18	1.07	1.07	0.88	0.88	0.85	0.85
dig-methionine	0.60	0.60	0.50	0.50	0.39	0.39	0.40	0.39
dig-TSAA	0.87	0.87	0.78	0.78	0.65	0.65	0.63	0.63
dig-threonine	0.77	0.77	0.66	0.66	0.62	0.62	0.53	0.53
Calcium	0.95	0.95	0.87	0.87	0.80	0.80	0.75	0.75
Available phosphorus	0.45	0.45	0.42	0.42	0.38	0.38	0.36	0.36
Total phosphorus	0.57	0.57	0.57	0.57	0.52	0.53	0.49	0.49
Sodium	0.20	0.20	0.20	0.20	0.18	0.20	0.18	0.18
Apparent metabolizable energy (kcal kg^{-1})	3102.00	2970.00	3124.00	2992.00	3168.00	3036.00	1450.00	1390.00
Analyzed nutrient content[5]								
Crude protein	22.20	22.70	21.70	20.60	17.80	18.60	16.60	16.80
Crude fat	4.93	3.49	5.30	2.90	6.31	4.55	6.25	4.24
Total phosphorous	0.66	0.65	0.67	0.58	0.49	0.58	0.57	0.64
Acid detergent fiber	4.20	4.30	3.70	3.10	4.10	4.00	3.30	3.30

[1]Vitamin premix added at this rate yields; Vitamin A: 11,023 IU, Vitamin D_3: 3,858 IU, Vitamin E: 46 IU, B_{12}: 0.0165 mg, Riboflavin: 5.845 mg, Niacin: 45.93 mg, d-pantothenic acid: 20.21 mg , Choline: 477.67 mg, Menadione: 1.47 mg, Folic acid: 1.75 mg, Pyroxidine: 7.17 mg, Thiamine: 2.94 mg and 0.55 mg biotin per kg diet. The carrier is ground rice hulls. [2]Trace mineral premix added at this rate yields 149.6 mg manganese, Zinc: 125.1 mg, Iron: 16.5 mg, Copper: 1.7 mg, Iodine: 1.05 mg, Selenium 0.25 mg, a minimum of 6.27 mg calcium and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil. [3]Active drug ingredient monensin sodium, 90 g lb^{-1} (90 g t^{-1} inclusion; Elanco Animal Health, Indianapolis, IN), as an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati* and *Eimeria maxima*. [4]Optiphos; Huvepharma, St. Louis, MO. [5]Analyzed enzyme recovery was 203 U kg^{-1} for starter, 197 U kg^{-1} for grower and 191 U kg^{-1} for finisher and 182 for withdrawal

(60 broilers/treatment) were removed and individually weighed before processing. Carcass weight WOG and fat pad weights were determined and yields were calculated following processing prior to emerging chilling.

Experiment 3: On the day of hatch, 900 Cobb 500 male broiler chicks were allotted to floor pens and dietary treatments based on initial BW. Each treatment included 10 replicates containing 30 birds per replicate pen. Chicks were provided age-appropriate supplemental heat and given access to feed and water *ad libitum.* Chicks were placed in 1.83-1.52 m rearing pens equipped with tube feeders and nipple drinkers with recycled litter as bedding material. Animal care was provided in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). The dietary program consisted of 3 dietary phases including a starter diet from 1-14 days of age, a grower diet from 15-28 days and a finisher diet being fed from 29-42 days of age. All broilers and feed were weighed on the days of dietary changes for determination of average BW and feed consumption for the calculation of mortality-corrected FCR.

Statistical analysis: All data were subjected to a one-way analysis of variance (ANOVA) using SPSS V 18.0 with significantly different means ($p\leq 0.05$) separated using Duncan's multiple range test. Percentage data (mortality and yield) were subjected to an arcsine transformation prior to statistical analysis.

RESULTS

Experiment 1: During the starter period, the caloric reduction in the NC diet did not reduce BW compared to that of the PC diet (Table 4); however, the supplementation of NSPase

Table 3: Dietary formulations and calculated nutrient content of the Positive Control (PC) and Negative Control (NC) fed to male market broilers in experiment 3

	Starter (%)		Grower (%)		Finisher (%)	
Ingredients	PC	NC	PC	NC	PC	NC
Corn	56.03	60.48	62.33	64.07	67.47	66.83
Dehulled soybean meal (48%)	29.88	28.28	24.70	24.39	19.82	22.79
DL-methionine (99%)	0.26	0.27	0.24	0.24	0.16	0.15
Dried distillers grain	5.00	5.00	5.00	5.00	5.00	5.00
Lysine HCL	0.23	0.27	0.21	0.22	0.19	0.09
Fat, A/V blend	3.42	0.50	2.69	0.50	2.63	0.50
Limestone	1.02	1.03	0.83	1.59	0.75	0.74
Monocalcium phosphate	0.39	0.39	0.18	0.17	0.11	0.09
Sodium chloride	0.41	0.37	0.29	0.29	0.18	0.29
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Trace minerals[2]	0.05	0.05	0.05	0.05	0.05	0.05
Coban 90[3]	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01
Calculated nutrient content						
Protein	22.50	22.15	20.50	20.50	18.50	19.78
dig-lysine	1.18	1.18	1.04	1.04	0.90	0.90
dig-methionine	0.57	0.57	0.53	0.53	0.43	0.43
dig-TSAA	0.85	0.85	0.80	0.80	0.67	0.68
dig-threonine	0.70	0.69	0.64	0.64	0.56	0.61
Calcium	0.92	0.92	0.80	0.80	0.75	0.75
Available phosphorus	0.45	0.45	0.40	0.40	0.38	0.38
Total phosphorus	0.57	0.57	0.51	0.52	0.48	0.50
Sodium	0.20	0.20	0.20	0.20	0.20	0.20
Metabolizable energy (kcal kg^{-1})	3102.00	2970.00	3124.00	2992.00	3168.00	3036.00
Analyzed nutrient content[5]						
Crude protein	21.90	20.50	19.60	19.90	17.10	18.60
Crude fat	6.10	4.17	5.87	3.66	6.19	4.26
Total phosphorous	0.66	0.57	0.60	0.63	0.56	0.55
Acid detergent fiber	3.20	2.70	3.30	3.00	2.70	2.80

[1]Vitamin premix added at this rate yields Vitamin A: 11,023 IU, Vitamin D_3: 3,858 IU, Vitamin E: 46 IU, B_{12}: 0.0165 mg, Riboflavin: 5.845 mg, Niacin: 45.93 mg, d-pantothenic acid: 20.21 mg, Choline: 477.67 mg, Menadione: 1.47 mg, Folic acid: 1.75 mg, Pyroxidine: 7.17 mg, Thiamine: 2.94 mg and 0.55 mg biotin per kg diet. The carrier is ground rice hulls. [2]Trace mineral premix added at this rate yields; Manganese: 149.6 mg, Zinc: 125.1 mg, Iron: 16.5 mg, copper: 1.7 mg, Iodine: 1.05 mg, Selenium: 0.25 mg, a minimum of 6.27 mg calcium and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil. [3]Active drug ingredient monensin sodium, 90 g lb^{-1} (90 g t^{-1} inclusion; Elanco Animal Health, Indianapolis, IN), as an aid in the prevention of coccidiosis caused by *Eimeria necatrix*, *Eimeria tenella*, *Eimeria acervulina*, *Eimeria brunette*, *Eimeria mivati* and *Eimeria maxima*. [4]Optiphos; Huvepharma, St. Louis, MO. [5]Analyzed enzyme recovery was 393 U kg^{-1} for starter, 299 U kg^{-1} for grower and 222 U kg^{-1} for finisher

Table 4: Average body weights, cumulative mortality-corrected feed conversion ratio (FCR) and processing parameters (± standard deviation SD) of male broilers fed low-energy diets with the inclusion of NSPase in experiment 1

Phase	PC	NC[1]	NC+NSPase[2]	PSEM
Body weight (kg)				
day 14	0.425±0.012[b]	0.435±0.009[ab]	0.438±0.016[a]	0.002
days 28	1.512±0.064[a]	1.460±0.060[ab]	1.431±0.063[b]	0.013
days 42	2.658±0.170	2.624±0.107	2.611±0.203	0.029
Mortality-corrected feed conversion ratio (feed : gain)				
Starter	1.232±0.018[b]	1.268±0.018[a]	1.246±0.034[b]	0.005
Grower	1.459±0.043[b]	1.510±0.051[a]	1.515±0.057[a]	0.011
Finisher	1.923±0.077	1.941±0.041	1.856±0.174	0.021
days 1-28	1.399±0.031[b]	1.442±0.034[a]	1.462±0.039[a]	0.008
days 1-42	1.625±0.016[b]	1.664±0.015[a]	1.634±0.055[ab]	0.007
Processing parameters				
Live weight (g)	2772.000±249[a]	2666.000±179[b]	2714.000±232[ab]	16.000
WOG weight (g)	2115.000±177[a]	2037.000±133[b]	2068.000±173[ab]	12.000
WOG yield (%)	76.400±1.5	76.400±1.7	76.200±1.7	0.100
Fat pad weight (g)	36.700±11.8[a]	32.200±9.5[b]	29.8.00±9.6[b]	0.800
Fat pad yield (%)	1.800±0.6[a]	1.600±0.4[b]	1.4.00±0.4[b]	0.100

[a-b]Treatment means within a row with different superscripts differ significantly at $p \leq 0.05$ [1]Energy level reduced by 132 kcal kg^{-1} compared to the PC [2]Inclusion of 113.5 g t^{-1} (2,700 U g^{-1} of xylanase from *Aspergillus niger* and *Trichoderma reesei*; also contains β-glucanase and α-galactosidase) of NSPase (Enspira; United Animal Health, Sheridan, IN)

increased ($p<0.05$) BW by 13 g compared to that of the PC diet. On day 28, no differences in BW were observed between the PC and NC diets. Furthermore, the addition of NSPase in the reduced-energy diet did not improve BW when compared to that of the NC diet alone. At the conclusion of the trial, no differences in BW were observed among the treatments. With regard to FCR, the reduction in energy in the NC diet compared to that of the PC diet resulted in a 3 and 5 point increase ($p<0.05$) during the starter and grower period, respectively (Table 4). The inclusion of NSPase in the energy-deficient diet improved ($p<0.05$) starter FCR compared to that of the NC diet to levels similar to that of the PC. Following the starter period, NSPase supplementation in the NC diet did not significantly impact FCR throughout the remainder of the trial. The reduction in dietary energy in the NC diet increased ($p<0.05$) cumulative day 1-28 FCR by 4 points compared to that of the PC diet. Although no differences in FCR were observed among the treatments during the finisher period, the reduction in energy in the NC increased ($p<0.05$) cumulative days 1-42 FCR by 4 points compared to that of the PC diet.

With regard to processing parameters, reducing the caloric content in the NC diet resulted in a 3.82% lighter ($p<0.05$) individual broiler live weight compared to that of the PC diet (Table 4). Inclusion of NSPase in the reduced-energy diet increased the live weight of processed birds to levels that were comparable to those in the PC diet; however, improvements were not significantly different from those of the NC diet. A similar trend was observed in WOG weight, with the PC exhibiting a 3.83% increase ($p<0.05$) in WOG weight compared to the reduced-energy diet; the addition of NSPase in the NC yielded intermediate results amongst dietary treatments. No differences in WOG yield were observed among the treatments. In both fat pad weight and yield, differences were observed between the PC and NC diets, with the PC yielding the highest ($p<0.05$) fat pad weight and yield. The inclusion of NSPase did not impact fat pad weight or yield when compared to the NC diet.

Experiment 2: The 132 kcal kg^{-1} ME reduction in the NC diet negatively impacted BW compared to that in the PC throughout the trial (Table 5). During the starter phase, the inclusion of NSPase increased ($p<0.05$) BW by 75 g compared to that of the NC diet. On day 28, the reduction in energy in the NC diet reduced BW by 340 g compared to that of the PC diet. The supplementation of NSPase recovered 190 g of days 28 BW ($p<0.05$) compared to that of the NC. Similar trends were observed on days 35 and 42, with the inclusion of NSPase increasing ($p<0.05$) BW by 300 and 363 g, respectively, compared to that of the NC diet. During the starter, grower and finisher phases of the experiment, differences in FCR were observed between the PC and NC diets, with the reduced-energy diet yielding the highest ($p<0.05$) observed FCR among dietary treatments. During the starter phase, the inclusion of NSPase did not impact FCR compared to that of the NC diet. However, NSPase addition during the grower phase reduced FCR to levels similar to that of the PC-fed broilers. In the finisher phase, the inclusion of NSPase in the reduced-energy diet improved ($p<0.05$) FCR by 13 points

Table 5: Average body weights and cumulative mortality-corrected feed conversion ratio (FCR) (±Standard deviation SD) of male broilers fed low-energy diets with the inclusion of NSPase in experiment 2

Phase	PC	NC[1]	NC+NSPase[2]	PSEM
Body weight (kg)				
day 14	0.410±0.013[a]	0.310±0.010[c]	0.385±0.018[b]	0.008
day 28	1.490±0.055[a]	1.150±0.046[c]	1.340±0.042[b]	0.027
day 35	2.208±0.043[a]	1.675±0.038[c]	1.975±0.100[b]	0.042
day 42	2.902±0.042[a]	2.339±0.076[c]	2.702±0.102[b]	0.454
Mortality-corrected feed conversion ratio (feed : gain)				
Starter	1.219±0.050[b]	1.300±0.048[a]	1.273±0.030[a]	0.001
Grower	1.477±0.113[b]	1.671±0.215[a]	1.543±0.088[ab]	0.030
Finisher	1.877±0.102[b]	2.000±0.137[a]	1.872±0.147[b]	0.025
Withdrawal	1.939±0.095[a]	1.870±0.113[ab]	1.835±0.081[b]	0.019
days 1-28	1.407±0.079[b]	1.563±0.143[a]	1.475±0.049[ab]	0.021
days 1-35	1.555±0.045[b]	1.694±0.078[a]	1.598±0.035[b]	0.015
days 1-42	1.645±0.043[b]	1.738±0.073[a]	1.657±0.031[b]	0.012
Processing parameters				
Live weight (g)	2923.000±199[a]	2564.000±224[c]	2762.000±212[b]	19.000
WOG weight (g)	2176.000±162[a]	1853.000±302[c]	2058.000±170[b]	15.000
WOG yield (%)	74.400±2.30[a]	72.120±9.72[b]	74.500±1.70[a]	0.200
Fat pad weight (g)	28.730±6.80[a]	21.300±8.01[b]	23.080±7.19[b]	0.600
Fat pad yield (%)	1.320±0.30[a]	1.140±0.34[b]	1.110±0.30[b]	0.100

[a-c]Means within columns with different superscripts differ significantly at $p\leq 0.05$ [1]Energy level reduced by 132 kcal kg^{-1} compared to the PC. [2]Inclusion of 113.5 g t^{-1} of NSPase (2,700 U g^{-1} of xylanase from *Aspergillus niger* and *Trichoderma reesei*; also contains β-glucanase and α-galactosidase) of NSPase (Enspira; United Animal Health, Sheridan, IN)

Table 6: Average body weights and cumulative mortality-corrected feed conversion ratio (FCR) (±Standard deviation SD) of male broilers fed low-energy diets with the inclusion of NSPase in experiment 3

Phase	PC	NC[1]	NC+NSPase[2]	PSEM
Body weight (kg)				
day 14	0.436±0.017[a]	0.365±0.022[b]	0.449±0.012[a]	0.008
day 28	1.448±0.066[a]	1.229±0.062[b]	1.412±0.048[a]	0.021
day 42	2.764±0.130[a]	2.519±0.140[b]	2.713±0.232[a]	0.036
Mortality-corrected feed conversion ratio (feed : gain)				
Starter	1.227±0.034[b]	1.325±0.086[a]	1.211±0.018[b]	0.013
Grower	1.448±0.025[b]	1.462±0.033[b]	1.520±0.045[a]	0.009
Finisher	1.926±0.096	1.895±0.094	1.923±0.110	0.018
days 1-28	1.384±0.021[b]	1.423±0.027[a]	1.426±0.030[a]	0.006
days1-42	1.642±0.031	1.664±0.036	1.662±0.041	0.007

[a-b]Data in columns with different superscripts differ significantly at $p \leq 0.05$. [1]Energy level reduced by 132 kcal kg^{-1} compared to the PC. [2]Inclusion of 113.5 g t^{-1} of NSPase (2,700 U g^{-1} of xylanase from *Aspergillus niger* and *Trichoderma reesei*; also contains β-glucanase and α-galactosidase) of NSPase (Enspira; United Animal Health, Sheridan, IN)

compared to that of the NC diet. Furthermore, birds fed diets containing NSPase exhibited an FCR similar to that of the PC during the finisher period. With regard to cumulative FCR, significant differences were observed through day 28, days 35 and 42 between the PC and NC fed broilers, with the reduction in energy yielding the highest ($p<0.05$) observed cumulative FCR at each evaluated cumulative time point. Regarding cumulative FCR through day 28, the supplementation of NSPase reduced FCR to levels comparable to those of the PC; however, FCR did not differ from the NC diet. Cumulative FCR through day 35 and 42 was reduced ($p<0.05$) with the supplementation of NSPase in the NC diet while achieving levels similar to those in the PC diet.

With respect to processing, the negative impact observed on BW correlated with a reduction in carcass weight and yield. Higher energy levels in the PC increased ($p<0.05$) fat pad weight and yield compared to those in the NC diet. The inclusion of NSPase in the reduced-energy diet increased ($P<0.05$) both WOG weight and yield compared to those in the NC diet (Table 5), with WOG yields being similar to those in the PC. Supplementation with NSPase did not impact fat pad weight or yield compared to those in the energy-deficient diet.

Experiment 3: The reduction in energy in the NC diet decreased ($p<0.05$) BW at all evaluated time points (days 14, 28 and 42) compared to that of the PC (Table 6). On day 14, the inclusion of NSPase in the reduced-energy diet elevated ($p<0.05$) BW compared to that of the NC diet to levels that were similar to those of the PC. The inclusion of NSPase increased ($p<0.05$) BW by 84 g compared to that of the NC, with a numerical increase of 13 g being observed beyond that of the PC. NSPase supplementation improved ($p<0.05$) BW compared to that of the NC by 13 and 7.2% on days 28 and 42, respectively, to levels that were similar to those in the PC diet. During the starter phase, an increase ($p<0.05$) in mortality-corrected FCR was observed, with the removal of energy in the NC diet increasing FCR by 10 points compared to the PC diet. The supplementation of NSPase in the low-energy diet reduced ($p<0.05$) FCR to levels comparable to those in the PC. With regard to the grower phase, no differences were observed between the PC and NC diets. Through day 28, differences in cumulative FCR (days 1-28) were observed between the PC and the NC diets, with the PC yielding the lowest ($p<0.05$) observed FCR. NSPase inclusion in the low-energy diet did not improve cumulative FCR (days 1-28) compared to that of the NC diet. During the finisher period and cumulative day 1-42, no differences in FCR were observed among treatments.

DISCUSSION

These data indicate that the inclusion of exogenous enzymes in corn-soy diets containing 5-10% DDGS enhances the utilization of nutrients while improving both performance and FCR of male broilers. The inclusion of NSPase in the diet allows the manufacturer to reduce the caloric value through fat substitution with cereal grains such as corn or through dilution with high-fiber ingredients such as DDGS. In all three experiments, a 132 kcal kg^{-1} ME reduction in the NC reduced performance parameters when compared to the PC. In a study conducted by Coppedge *et al.*[12], a 133 kcal kg^{-1} ME reduction impacted performance parameters, including a 20 g decrease in day 26 BW and an increase in FCR. The addition of a cocktail NSPase in the diet resulted in a 6 point improvement in starter and cumulative days 1-26 FCR. Similar results in broiler performance have been reported with the inclusion of carbohydrases in diets[12,17,18]. It is widely accepted that the inclusion of exogenous enzymes in corn-soy diets has the potential to increase the overall feeding value of the diet while also improving bird performance.

Non-starch polysaccharides (NSP) have been shown to encapsulate vital nutrients such as starch and amino acids, resulting in a negative impact on broiler performance[10,19]. The use of exogenous enzymes in corn-soy diets mitigates these negative effects by hydrolyzing indigestible bonds, releasing these nutrients for utilization. Furthermore, the mode of action involving NSPase enzymes includes the degradation of viscous NSPs as well as the liberation of encapsulated nutrients bound within the cell walls of various plant-based feedstuffs. The ability to release these inaccessible nutrients via NSPase supplementation allows greater digestion and absorption of protein, starch and energy, resulting in greater BW gain and improved feed efficiency[10,20]. While a significant BW response was not observed between the PC and NC during experiment 1, it can be hypothesized that the removal of energy from the NC diet increased feed intake, resulting in an FCR response instead of a BW effect. The addition of NSPase in the reduced-energy diet yielded a 13 g increase ($p<0.05$) in BW compared to that of the PC for the starter phase. Following the starter period, inclusion of cocktail NSPase in the NC did not impact BW throughout the trial. Similar results were observed by Bi and Chung[21], in which improvements in performance parameters were not observed when evaluating the efficacy of a cocktail carbohydrase supplemented in a reduced-energy (-3%) corn-soybean meal diet. The reduction in energy in the NC diet increased ($p<0.05$) FCR compared to the PC during the starter and grower phases and cumulatively through days 28 and 42. It can be assumed that the FCR response was attributed to an increase in feed intake due to the caloric reduction in the NC. Leeson *et al.*[22] demonstrated that feed intake increased linearly with decreasing dietary energy levels. Furthermore, Hidalgo *et al.*[23] noted that increasing dietary AME through fat supplementation improved FCR by reducing broiler feed intake. Avila *et al.*[24] observed similar results with an energy reduction of 120 kcal kg^{-1} in the NC diet increasing both feed consumption and FCR compared to the PC in 46 days-old broilers. In the current trial, the supplementation of NSPase in the low-energy diet improved FCR to levels comparable to that of the PC during the starter period and cumulatively through days 42. Klein *et al.*[25] observed similar results in two subsequent experiments when evaluating an NSPase in combination with a β-mannanase in low-energy (-132 kcal kg^{-1}) diets. The addition of both NSPase and β-mannanase improved starter (experiment 1) and finisher (experiment 2) FCR to levels equivalent to that of the PC diet. The supplementation of cocktail carbohydrases in the diet has the ability to target and act on multiple substrates releasing nutrients within the specified substrates for utilization. In experiment 2 of the current study, the reduction in energy in the NC diet yielded a consistent decrease in BW throughout the trial compared to that of the PC diet. The inclusion of cocktail NSPase in the reduced-energy diet increased ($p<0.05$) BW compared to that of the NC diet. These results were consistent with findings reported by Cowieson[26], in which a 28 days trial was conducted to evaluate the effects of a multicarbohydrase and phytase inclusion in reduced-energy (-180 kcal kg^{-1}) diets. Cowieson[26] reported that the inclusion of both enzymes at levels of 100 mg kg^{-1} yielded a 14% increase in BW compared to that of the NC diet. Additionally, Wu *et al.*[27] observed similar improvements in broiler performance with the inclusion of a carbohydrase in wheat-soy diets. With respects to FCR, a consistent increase was observed in the NC compared to the PC from the starter to the finisher phase. In the finisher phase (days 28-35), inclusion of NSPase in the reduced-energy diet improved FCR to levels similar to those in the PC diet. Similar results were observed by Cowieson *et al.*[28], in which a 7 point decrease in FCR was achieved in reduced-energy diets (-110 kcal kg^{-1}) through xylanase and glucanase supplementation at levels of 100 and 150 g t^{-1}, respectively, through day 42. O'Neill *et al.*[18] noted that a reduction of 100 kcal kg^{-1} of energy in NC diets increased ($p<0.05$) FCR when compared to the PC diet. The supplementation of a xylanase product at levels of 16,000 BXU kg^{-1} in the reduced-energy diet improved ($p<0.05$) (6 points) FCR at the conclusion of days 35.

In experiment 3, a decrease ($p<0.05$) in BW was observed in the reduced-energy diet throughout the trial compared to that of the PC diet. Supplementing NSPase in the low-energy diet improved ($p<0.05$) average BW by 14% compared to that of the NC diet alone. Cowieson *et al.*[28] also observed an improvement in broiler performance when administering a carbohydrase in an energy-reduced (-150 kcal kg^{-1}) diet. Results observed by Olukosi *et al.*[29] indicated that the inclusion of a multicarbohydrase (XAP; 650 U xylanase, 1650 U amylase and 4000 U protease) in a 28 days trial yielded heavier BWs when administered in a reduced-energy diet (-115 kcal kg^{-1}). Additionally, Francesch and Geraert[30] reported that the multi-enzyme supplementation of phytase and carbohydrase in reduced-energy diets improved broiler BW through days 21. With regard to FCR, a reduction ($p<0.05$) was observed between the PC and NC diets for the starter phase. Cocktail NSPase supplementation in the reduced-energy diet improved ($p<0.05$) FCR to levels similar to the PC while yielding the lowest FCR for that period. Olukosi *et al.*[29] also observed an improvement ($p<0.05$) in FCR in low-energy diets supplemented with cocktail carbohydrase on days 21. Following the starter phase, no further improvements were observed when comparing the reduced-energy diet with

NSPase inclusion to the NC diet alone. Coppedge *et al.*[12] observed similar results with NSPase inclusion improving FCR compared to that of the NC diet in the starter phase while failing to influence FCR in the grower and finisher periods. When supplementing exogenous enzymes such as NSPase in the diet, bird responses to enzyme addition are not entirely predictable as seen in the series of these three experiments. In each experiment, a response was observed; however, the response varied from a BW response only to an FCR response only. Factors that impact these variations in response could include enzyme source and dose, dietary ingredients and factors and bird characteristics including age and sex[20]. Each of these three experiments illustrated a positive benefit with exogenous enzyme inclusion with either an impact on FCR or BW being more apparent. Sources of the variation in the response associated with these three trials may be ingredient variation, an environmental effect, or chick quality. Each trial was conducted during different seasonal periods of the year in which both temperature and humidity may have impacted the bird's behavior and consumption and the subsequent responses observed. Although varying responses were observed with enzyme supplementation, the removal of energy (-132 kcal kg^{-1}) in the NC diet throughout all experiments seemed to successfully elicit a consistent response between the NC and PC with differences in performance parameters being significant in each experiment. When 4% energy was removed in the NC, broiler performance was impacted, with reductions in BW and increases in FCR being observed. Similar differences between the PC and NC were reported by Klein *et al.*[25] and Williams, *et al.*[31], in which a 132 kcal kg^{-1} reduction in energy-reduced BW and increased FCR were reported, demonstrating that a 4% reduction in dietary energy is sufficient for adequate separation between the PC and NC diets.

While the incorporation of exogenous enzymes in broiler diets have shown to increase broiler performance, studies have also indicated the ability of enzyme supplementation to improve processing yields as well[2,31,32]. In experiment 1, the reduction in energy in the NC diet decreased processing parameters, including live weight, carcass weight and fat pad weight and yield compared to the PC diet. Williams *et al.*[31] observed similar results, with the reduction in energy (-132 kcal kg^{-1}) in the diet decreasing both fat pad weight and yield. The inclusion of NSPase in the low-energy diet yielded live weights and carcass weights similar to those of the PC. In experiment 2, a reduction ($p<0.05$) in the NC was observed in all processing parameters (live weight, WOG weight and yield, fat pad weight and yield) when compared to the PC diet. The inclusion of NSPase in the reduced-energy diet improved ($p<0.05$) live weight, WOG weight and yield compared to those in the NC diet. Similar results were observed by Coppedge *et al.*[12], in which the inclusion of NSPase in reduced-energy diets improved both live BW and carcass weights. In the current study, the supplementation of a cocktail NSPase in reduced-energy corn-soybean meal diets with DDGS inclusion improved broiler performance and processing parameters. Observations conclude that the administration of NSPase in reduced-energy diets can compensate for caloric reduction while improving broiler growth and feed conversion.

CONCLUSION

The results of this study indicate that through NSPase supplementation, producers can successfully incorporate by-product ingredients such as DDGS while reducing the caloric value of the diet without negatively impacting broiler performance. The addition of NSPase in low-energy broiler diets has the ability to target various substrates, degrading essential linkages crucial for enhanced nutrient digestion, which can ultimately improve broiler performance parameters and reduce production costs.

SIGNIFICANCE STATEMENT

This study describes the impacts of NSPase on broiler performance and processing parameters when included in low-energy corn-soy diets containing DDGS. This study will help producers gain a better understanding of the beneficial impact of NSPase in broiler diets and further identify optimum inclusion levels of by-product ingredients such as DDGS. These findings are useful given the continuous focus on higher inclusions of by-product ingredients as well as dietary manipulation in order to reduce diet costs.

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