

# Effect of feed form, soybean meal protein content, and Rovabio Advance on poult live performance to 3 wk of age

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**ABSTRACT** Based on research reports, feed characteristics can increase poult growth via several factors. Two rearing experiments (**EXP**) were conducted to test the effects of feed form and ingredient quality in turkey poult. Bird performance and the duodenum, jejunum, ileum, and cecum morphology were observed in both EXP. Poults were reared in battery cages (48 cages in EXP 1 and 72 cages in EXP 2). Four dietary treatments with differing feed form and function factors were evaluated in EXP 1. A completely randomized block design with a  $2 \times 2 \times 2$  factorial arrangement of treatments consisting of 2 levels of fines, 2 soybean meal (**SBM**) sources, and 2 levels of an enzyme cocktail (Rovabio Advance) was tested in EXP 2. Poult BW, BW gain (**BWG**), feed intake (**FI**), and feed

conversion ratio (**FCR**) were determined in both EXP. Apparent metabolizable energy corrected for nitrogen (**AMEn**) was determined in EXP 2. Differences were considered to be statistically significant at  $P \leq 0.05$ . Feeding increased feed crumble particle size with fewer fines in the starter feed resulted in an increased BWG accompanied by an increased FI. Reduced feed fines reduced  $AMEn$  when the dietary enzyme cocktail was not present. The feed formulation with 60% CP SBM resulted in a lower FI and an improved FCR. The enzyme cocktail interacted synergistically with screening and fed SBM source factors on the  $AMEn$  and FCR. It was concluded that both the feed form and quality, as used in this study, affect poult performance.

**Key words:** feed form, feed quality, particle size, enzyme, soybean meal

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## INTRODUCTION

Increasing market demand for birds reared without the use of antibiotic growth promoters (**AGP**) such as “antibiotic-free programs” or “no antibiotics ever” is creating concerns and opportunities for the poultry industry (Gould, 2017; Rubin, 2014; Olenjnik, 2016). Consumers have stated preferences for reduced purchases of meat produced with antibiotics (Dibner and Richards, 2005; Polansek, 2014; Huffstutter and Baertlein, 2015; Calderone, 2017). Therefore, new goals are being developed to reduce the rearing of poultry, such as turkeys and broilers, using less antibiotics (Butterball, 2018; Perdue, 2018). Eliminating dietary inclusion of AGP can adversely affect digestibility by allowing the proliferation of enteric fermentative organisms (Montagne et al., 2003). Fermentative organisms proliferate in the small intestine because of the increased

digesta retention time when nonstarch polysaccharides (**NSP**) are present in the feed (Choct et al., 1996; Choct, 1997). Feed NSP values are especially crucial during the starter phase when young birds have an immature microbiota and their dietary level NSP from soybean meal (**SBM**) can be higher than for older birds (Choct et al., 2010). Thus, controlling the antinutritional effects of NSP in starter diets could have a significantly positive impact on AGP, antibiotic-free programs, and no-antibiotics-ever production systems.

An increased feed intake (**FI**) and reduced feed wastage in young birds can be encouraged by reducing the abundance of fines in the starter crumble feed at placement. An early FI is an essential factor affecting the poult performance and gut health (Noy et al., 2001), and the initial FI is also critical in the transition from a yolk-based diet to a feed-based diet (Uni and Ferket, 2004). Although there is an established understanding of the nutritional requirements for the starter phase, the evaluation of the abundance of fines fed to poults must be further examined. Corn and SBM-based diets contain high amounts of NSP, which are classified as antinutritional and can be detrimental to growth performance and health (Choct, 1997; Montagne et al., 2003). Although SBM contains a high

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amount of protein, it also includes a significant amount (35%) of indigestible carbohydrates (Choct et al., 2010). Less than 1% of SBM carbohydrates are considered starch; the rest are deemed free sugars and NSP (Choct et al., 2010). Consequently, grain and plant protein by-product NSP contents have a significant effect on the feed quality, enzyme efficiency, and poult growth performance. New varieties of SBM with high CP levels could be associated with reduced NSP content and thus result in better growth performance for turkey poult consuming high-protein starter feed. Moreover, this new variety of high-protein SBM should be tested with supplemental enzymes that are already used to reduce the adverse effects of dietary NSP.

The use of a high-CP SBM in the starter feed may enhance the growth performance characteristics, increase feed digestibility, and affect the duodenum, jejunum, and cecum morphology during the starter feed phase of turkeys. Crumble quality in this work is defined as the percentage of fines in the crumbles offered to the bird. Birds will prehend feed particles according to their beak size and mechanoreceptors in the beak (Moran, 1982). However, beak conditioning is a common practice in turkeys at the hatchery, resulting in a shortened upper beak with an effect to reduce injuries to birds by reducing bird-to-bird pecking. Beak conditioning may lead to consumption issues and feed wastage when excessive fines are present. Minipelleting or micropelleting the starter feed phase could reduce the abundance of fines and increase the particle size in comparison to crumbles. Alternatively, feed milling procedures to increase the particle size of crumbles with reduced fine content could be an option to consider.

The objective of this work was to determine the effects of the feed particle size, feed enzymes, and increased relative CP content of SBM on turkey poult performance, feed digestibility, and effects on the poult duodenum, jejunum, and cecum morphology.

## MATERIALS AND METHODS

### *Treatments and Experimental Design*

For the first experiment (EXP), treatment 1 (control) was an all-vegetable-based crumble feed. Treatment 2 (medicated three-way) was as treatment 1 but with the inclusion of 3 medications: amprolium (113.5 g per ton of feed), bacitracin (125 g per ton of feed), and penicillin (125 g per ton of feed). The United States Food and Drug Administration approval for the use of medications combined with penicillin was withdrawn in June 2015. However, this three-way feed treatment was used as an example of past industry practices for improving poult performance. The control and three-way feed were formulated based on breeder recommendations using typical commercial turkey industry corn-SBM-based diets. AlphaStart is a commercial proprietary feed with a combination of phytogenic, probiotic, and prebiotic feed additives (Devenish Nutrition LLC, Fairmont, MN). Nutrient composition is provided in Tables 1–3.

Treatment 3 (AlphaStart Crumble) was a mash AlphaStart turkey starter pelleted and crumbled at North Carolina State University (NC SU). Treatment 4 (AlphaStart Mini) was an outsourced AlphaStart 2-mm minipellet turkey starter. Treatments 3 and 4 were from the same basal mix, and test ingredients were hand-added. The hand-adds were checked against batch records. Although the manufacturer shared no specific nutritional or ingredient information, these diets were formulated to be isonitrogenous and isocaloric to treatments 1 and 2. The basal feed was minipelleted, achieving a 2,000- $\mu$ m pellet size at the Devenish feed mill in Maquoketa, IA. The control crumbles and three-way medicated feeds were formulated to be isocaloric and isonitrogenous with AlphaStart diets (Table 1). Treatment diets were randomly assigned to one of 48 cages of poult (7 poult/cage), with 12 replicate cages of poult per treatment. AlphaStart minipellet, AlphaStart crumble, and medicated three-way were fed from 0 to 14 d of age and then all pens of birds were fed treatment 1 (control crumble) from 14 to 21 d.

For EXP 2, a completely randomized block design with a  $2 \times 2 \times 2$  factorial arrangement of treatments was used. In EXP 2, 2 sources of SBM was used: a feed formulated with high CP SBM (60% CP SBM) and a feed formulated with a standard CP SBM (48% CP SBM). The second factor was the reduction of fines in the diet: reduced feed fines (RFF) and increased feed fines (IFF). The third factor was the presence (200 mL/mt) or absence of an enzyme cocktail containing arabinofuranosidases and xylanases (Rovabio Advance, Adisseo France S.A.S, Antony, France). Treatments diets were randomly assigned to one of 72 cages of poult (7 poult/cage), with 9 replicate cages of poult per treatment.

### *Housing and Management*

In both EXP, Petersime battery cages (Petersime Incubator Co., Gettysburg, OH) were used. The cages had wire floors, and each was equipped with a trough drinker and feeder and a thermostatically controlled cage heater. The ambient temperature was gradually decreased from 95°F at placement to 85°F at 21 d of age. Humidity was approximately 55%. Twenty-three hours of light per day was provided.

### *Poult Origin and Management*

Experiment 1 was conducted with 336 male Nicholas Select poult (Aviagen Turkeys, Lewisburg, WV) placed on the day of hatch. Poult were weighed individually at placement and on days 7, 14, and 21. Experiment 2 was conducted with 504 Nicholas Select female poult (Aviagen Turkeys) placed on the day of hatch. Poult were weighed individually at placement and on days 7, 14, and 21. The weight of each cage of birds plus culls and mortalities was used to determine the feed conversion ratio (FCR). Poult consumed feed and water ad libitum throughout the EXP. Birds were checked a minimum

**Table 1.** Diet composition in experiments 1 and 2.

Ingredient (%)	Starter experiment one <sup>1</sup>		Starter experiment 2 <sup>2</sup>	
	Control	Three-way Medicated <sup>2</sup>	Low-CP SBM <sup>3</sup>	High-CP SBM <sup>4</sup>
Corn	35.00	34.90	44.87	47.50
Wheat	10.00	9.97	2.50	3.00
Wheat middlings	0.00	0.00	1.00	6.00
Soybean meal	43.00	42.88	42.00	34.00
Soybean oil	5.00	4.99	2.00	2.03
Calcium carbonate	2.20	2.19	2.30	2.30
Monocalcium phosphate	2.55	2.54	3.30	3.30
Salt	0.25	0.25	0.23	0.23
Mineral mix <sup>5,6</sup>	0.15	0.15	0.20	0.20
Vitamin mix <sup>7</sup>	0.15	0.15	0.15	0.15
Selenium mix	0.05	0.05	0.05	0.05
Choline chloride	0.20	0.20	0.20	0.20
Lysine <sup>8</sup>	0.61	0.61	0.54	0.40
Methionine <sup>9</sup>	0.50	0.50	0.32	0.30
Sodium bicarbonate	0.20	0.20	0.25	0.25
Threonine <sup>8</sup>	0.15	0.15	0.10	0.10
Quantum Blue 5G	0.01	0.01	-	-
Amprolium <sup>1</sup>	-	0.05	-	-
Penicillin <sup>1</sup>	-	0.104	-	-
BMD 60 <sup>1:10</sup>	-	0.104	-	-
Ingredient total	100	100	100	100

Abbreviation: SBM, soybean meal.

<sup>1</sup>AlphaStart ingredient composition information was not provided.

<sup>2</sup>Two basal feeds were formulated with the 2 different sources of soybean meal, one with 48% CP and the other one with 60% CP.

<sup>3</sup>High-CP soybean meal, 60% CP.

<sup>4</sup>Low-CP soybean meal, 48% CP.

<sup>5</sup>In experiment 1, the mineral premix provided the following per kg of diet: manganese, 90 mg; zinc, 90 mg; iron, 60 mg; copper, 7.5 mg; iodine, 1.9 mg; cobalt, 0.75 mg.

<sup>6</sup>In experiment 2, the mineral premix provided the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg.

<sup>7</sup>Donated by DSM Nutritional Products; vitamin premix provided the following per kg of diet: vitamin A, 19,841 IU; vitamin D3, 5952 IU; vitamin E, 99 IU; vitamin B12, 0.06 mg; biotin, 0.38 mg; menadione, 6 mg; thiamine, 6 mg; riboflavin, 20 mg; pantothenic acid, 33 mg; vitamin B6, 12 mg; niacin, 165 mg; folic acid, 3 mg.

<sup>8</sup>Ajinomoto North America.

<sup>9</sup>Evonik North America.

<sup>10</sup>Bacitracin Methylene Disalicylate (BMD) 60 g per pound of feed.

of 4 times per day. All bird handling procedures were approved by the NCSU Institutional Animal Care and Use Committee.

### Feed Manufacturing

For EXP 1, both the control and the three-way medicated starter feeds were manufactured at the NCSU Feed Mill Education Unit. Corn was milled with a hammer mill using 6/8 screens (model 1522, Roskamp Champion, Waterloo, IA) generating corn with a particle size of 300  $\mu$ m. The basal diets for these 2 feeds were batched and blended in a counterpoise ribbon mixer (model TRDB126060, Hayes & Stolz, Fort Worth, TX) for 3 min of a dry mix followed by 90 s of a wet mix. The AlphaStart (in the mash form and already blended), the control, and the three-way medicated starters were then conditioned for 30 s at 175°F in a single pass conditioner (model C18LL4/F6, California Pellet Mill, Crawfordsville, IN). The feeds then were pelleted by a 30-HP pellet mill (model PM1112-2, California Pellet Mill, Crawfordsville, IN), using an 11/64"  $\times$  1 3/8" pellet mill die. The pellets of each feed were cooled in a counterflow cooler (model VK09X09KL, Geelen Counterflow

USA, Inc., Orlando, FL), and then crumbled (model 624s, Roskamp Champion). The treatment 4 diet, the AlphaStart minipellet, was provided by Devenish Nutrition (Devenish Nutrition LLC, Fairmont, MN).

For EXP 2, all feed were manufactured at the NCSU Feed Mill Educational Unit. Two basal feed formulations were used to create the 8 total combinations of feed treatments. One basal feed was manufactured with a 48% CP SBM and the other with 60% CP SBM. All treatment feeds were formulated to the same CP and amino acid levels. Celite (Celite, Millipore Sigma, Darmstadt, Germany) was added at 2% to both basal feeds as an indigestible marker to determine AMEn. Each basal feed was mixed in a counterpoise ribbon mixer (model TRDB126060, Hayes & Stolz), conditioned in a single pass conditioner (model C18LL4/F6), and pelleted in a 30-HP pellet mill (model PM1112-2), using an 11/64"  $\times$  1 3/8" pellet mill die. The pellets of each feed were cooled in a counterflow cooler (model VK09X09KL, Geelen Counterflow USA, Inc.), crumbled (model 624S, Roskamp Champion), and split into 4 batches for further processing. The first 2 batches were sent to a double-ribbon mixer (model SRM304, Scott equipment, New Prague, MN); the first was mixed for

**Table 2.** Estimated NSP<sup>1</sup> composition (%) for ingredients in experiment 2.

Antinutritional Factor	Corn <sup>2</sup>	Wheat <sup>2</sup>	Wheat middlings <sup>1,4</sup>	SBM	Total
			Feed formulated with 48% CP SBM <sup>2,5</sup>		
Soluble NSP	1.12	0.05	0.01	4.73	5.91
Insoluble NSP	1.70	0.16	0.23	2.42	4.50
Cellulose	0.76	0.03	0.07	3.36	4.22
Total NSP	3.63	0.24	0.31	10.50	14.68
			Feed formulated with 60% CP SBM <sup>3,6</sup>		
Soluble NSP	1.19	0.06	0.07	0.69	2.01
Insoluble NSP	1.81	0.19	1.36	0.35	3.71
Cellulose	0.81	0.04	0.40	0.49	1.74
Total NSP	3.85	0.29	1.84	1.54	7.51

Abbreviation: NSP, nonstarch polysaccharides.

<sup>1</sup>Values estimated using feed formulation and estimated ingredient nonstarch polysaccharides.

<sup>2</sup>Values adapted and estimated from [Jaworski et al. \(2015\)](#).

<sup>3</sup>Values adapted and estimated from [Choct et al. \(2010\)](#).

<sup>4</sup>Values adapted from [Choct et al. \(2010\)](#) corrected with analyzed values.

<sup>5</sup>Soybean meal source with 48% CP.

<sup>6</sup>Soybean meal source with 60% CP.

1.5 min with no additions, whereas the second had the enzyme (Rovabio Advance, 200 mL/metric ton in 1 L water) added before mixing. The other 2 batches were sent to a pellet shaker (model 2 × 4 two-decker general Roto-shaker, Andritz Sprout-Bauer, Inc, Muncy, PA) with U.S. Tyler 6 and 10 screens to reduce the abundance of feed fines. The screened diets were then sent to a mixer, and the enzyme was added to one batch. This entire process was completed for both SBM types, thus achieving 8 feed treatments.

## Feeding Program

One starter feed phase, by treatment, was provided to day 14 ad libitum to the poults in EXP 1. All feed allocations were weighed to the nearest gram. The weight of feed remaining in feeders at the end of each data collection period was recorded. All feeders were dumped at 14 d. All poults were then fed the treatment 1 control crumble feed from 14 to 21 d of age. Feed allocation and feed weigh backs were used to determine poult feed consumption. The FCR was calculated using all birds, including weights of mortalities and culled birds.

In EXP 2, each starter feed treatment was fed ad libitum to all birds from placement to 20 d of age. The weight of feed issued was recorded when added to feeders and at 7, 14, and 20 d of age to calculate the bird FI and FCR.

## Feed Analysis

For both EXP, proximate analysis (250 g) was outsourced for DM, CP, crude fat, minerals, and total lysine for samples of each dietary treatment feed (Carolina Analytical Services, Bear Creek, NC). For EXP 2, ingredient NSP calculations were based on analysis and published reviews ([Choct et al., 2010](#); [Jaworski et al., 2015](#)). The estimated NSP content for the 60% CP SBM was calculated with laboratory analysis and completed with minerals and NSP ratios assumptions by [Choct et al. \(2010\)](#). The total estimated NSP

content was calculated using the percentage of corn, wheat, wheat middlings, and SBM present in the formulation and each ingredient's estimated NSP content ([Table 2](#)). No analyses for test ingredients in any treatment were performed; however, all were hand-added directly to the mixer and confirmed against batch records.

## Particle Size Determination

For both EXP, the particle size and variation calculations were determined using the American Society of Agricultural and Biological Engineers standard 319.4 methodologies. Treatment composite samples were collected from each feed bag. Three subsamples were collected from each composite sample after being homogenized. From each subsample, 100 g of feed and 0.5 g of sieve aid were weighed for sieve analysis. The sieves used were U.S. sieves 4, 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270, and a collection pan. The group of sieves was shaken in a sieve shaker (Ro-Tap Model RX-29, W.S. Tyler Industrial Group, Mentor, OH) for 15 min. The feed present on each sieve was weighed in grams and analyzed per formulas described in the [American Society of Agricultural and Biological Engineers standard 319.4](#) methodologies for the log-normal mean particle size and SD. Pellets were defined as the feed retained on U.S. sieve number 4, crumbles were defined as the feed retained on U.S. sieve numbers 6 and 12, and fines were defined as all feed passing through U.S. sieve number 12.

## AMEn Sampling and Analysis

The AMEn analysis was performed only in EXP 2. Fecal samples (100 g) were collected from cage pans on 13, 14, and 15 d of age from every cage. On the third day of sampling, the 3 d of samples were mixed in a single bag for each pen of birds. The samples were then dried in a Blue M drying oven (General Signal, Blue Island, IL) for 48 h at 60°C. The dried samples were ground and

**Table 3.** Calculated nutrient contents (%) for diets in experiments 1 and 2.

Nutrient	Starter experiment one <sup>1</sup>		Starter experiment 2 <sup>2</sup>	
	Control	Three-way medicated	Low-CP SBM <sup>3</sup>	High-CP SBM <sup>4</sup>
CP	26.60	26.53	25.86	25.79
ME (kcal/kg)	2,983.00	2,975.00	2,820.00	2,836.00
Crude fat	7.14	7.12	4.39	4.48
Lysine	1.82	1.81	1.74	1.74
Methionine	0.82	0.82	0.65	0.64
Methionine + cysteine	1.18	1.18	1.01	1.01
Tryptophan	0.32	0.32	0.32	0.31
Threonine	1.08	1.08	1.03	1.01
Arginine	1.65	1.65	1.64	1.71
Valine	1.19	1.19	1.18	1.26
Calcium	1.45	1.45	1.52	1.51
Available phosphorus	0.72	0.72	0.75	0.76
Sodium	0.19	0.19	0.19	0.19
Chloride	0.21	0.21	0.19	0.20

<sup>1</sup>AlphaStart ingredient composition information was not provided.

<sup>2</sup>Two basal feeds were formulated with the 2 different sources of soybean meal, one with 48% CP and the other one with 60% CP.

<sup>3</sup>Low-CP soybean meal, 48% CP.

<sup>4</sup>High-CP soybean meal, 60% CP.

analyzed in duplicate for gross energy, protein, and insoluble ash (Celite, Millipore Sigma, Darmstadt, Germany) content to calculate the AMEn. The gross energy was determined using a plain jacket bomb calorimeter (model 1341, Parr Instrument Company, Moline, IL) using the Parr instrument procedure. The protein content was estimated by multiplying the nitrogen content of the samples by 6.25. The nitrogen content in the samples was analyzed by the LECO Truespec N analyzer (Leco Corporation, St. Joseph, MI) using the Dumas method. Insoluble ash content was determined (Vogtmann et al., 1975).

### Duodenum, Jejunum, Ileum, and Cecum Morphology

In EXP 2, one poult per cage was chosen randomly and sampled at 14 and 21 d of age for the duodenum, midgut, and cecum. Samples were prepared for histology and analyzed by Veterinary Diagnostics Pathology, LLC, at Fort Valley, VA.

In EXP 2, small intestine and cecum samples were collected from one bird per cage on 20 d of age. Samples were taken from the duodenal loop, jejunum, and ceca

**Table 4.** Feed nutrient analysis<sup>1</sup> (%) and feed physical characteristics for diets in experiments 1 and 2.

Nutrient	Experiment 1				Experiment 2							
	NCSU Control	Three-way Medicated	Alpha Crum <sup>2</sup>	Alpha Mini <sup>2</sup>	High-CP SBM <sup>3</sup>				Low-CP SBM <sup>4</sup>			
					IFF <sup>5</sup>		RFF <sup>6</sup>		IFF <sup>5</sup>		RFF <sup>6</sup>	
					NEnz <sup>7</sup>	WEnz <sup>8</sup>	NEnz <sup>7</sup>	WEnz <sup>8</sup>	NEnz <sup>7</sup>	WEnz <sup>8</sup>	NEnz <sup>7</sup>	WEnz <sup>8</sup>
Moist	13.57	13.59	13.07	13.03	13.51	14.56	14.12	14.15	14.01	14.16	13.49	13.62
Fat	6.16	5.80	4.03	4.77	3.43	3.55	3.33	3.50	3.73	3.70	4.17	3.63
Protein	26.69	26.57	26.73	25.55	25.73	27.39	29.66	29.35	28.30	27.00	27.76	29.03
Ash	7.18	7.08	7.06	6.96	8.39	8.50	8.69	8.80	8.69	9.00	8.54	8.21
P	0.79	0.93	0.84	0.84	0.85	0.8	0.92	0.89	1.03	1.00	0.95	0.79
Ca	1.39	1.45	1.27	1.30	1.49	1.58	1.46	1.40	1.67	1.77	1.47	1.56
Na	0.19	0.17	0.13	0.15	0.16	0.14	0.16	0.17	0.17	0.17	0.14	0.16
Lysine	1.95	1.98	1.92	1.93	-	-	-	-	-	-	-	-
Dwg <sup>9</sup>	919	930	1,102	2,440	1,655	1,188	1,508	1,666	1,337	1,420	1,702	1,436
Swg <sup>10</sup>	2.44	2.44	2.51	1.73	2.83	3.23	2.77	2.3	3.033	3.13	2.47	2.4
Crums <sup>11</sup>	71	69	71	-	57	44	70	72	50	56	78	61
Fines <sup>12</sup>	29	31	29	-	38	52	30	28	47	40	22	39

<sup>1</sup>Feed analysis was performed by Carolina Analytical Services (17570 NC Highway 902, Bear Creek, NC 27207).

<sup>2</sup>The AlphaStart Crumble and minipellet feeds were provided by Devenish Nutrition and came from the same batch.

<sup>3</sup>High-CP soybean meal, 60% CP.

<sup>4</sup>Low-CP soybean meal, 48% CP.

<sup>5</sup>Increased feed fine feed not screened by U.S. sieves 6 and 12.

<sup>6</sup>Reduced feed fine feed screened and retained by U.S. sieves 6 and 12.

<sup>7</sup>No enzyme cocktail added.

<sup>8</sup>200 ml/mt enzyme cocktail added, (Rovabio Advance, Adisseo France S.A.S, Antony, France).

<sup>9</sup>Geometric mean diameters of the particle by mass.

<sup>10</sup>The geometric SD of the particle diameter by mass.

<sup>11</sup>Crumbles determined as feed under U.S. sieve number 12.

<sup>12</sup>Fines determined in crumbles as feed over U.S. sieve number 12.

**Table 5.** Effect of feed treatment on poult performance (g) in experiment 1.

Treatment <sup>1</sup>	Age (day)				
	0-7	7-14	14-21	0-14	0-21
	BWG <sup>2</sup>				
Control crumble	116	250 <sup>b</sup>	417	365 <sup>c</sup>	773 <sup>b</sup>
Medicated three-way crumble	115	261 <sup>a,b</sup>	498	376 <sup>b,c</sup>	773 <sup>b</sup>
AlphaStart crumble	119	270 <sup>a</sup>	392	389 <sup>a,b</sup>	781 <sup>a,b</sup>
AlphaStart minipellet	122	276 <sup>a</sup>	414	398 <sup>a</sup>	812 <sup>a</sup>
SEM <sup>3</sup>	2.99	4.11	9.05	4.63	9.5
P-value	0.36	0.0004	0.17	0.0001	0.02
	Feed intake				
Control crumble	125	285 <sup>b</sup>	594	410 <sup>b</sup>	1004 <sup>a,b</sup>
Medicated three-way crumble	125	281 <sup>b</sup>	558	404 <sup>b</sup>	964 <sup>b</sup>
AlphaStart crumble	125	299 <sup>a,b</sup>	570	424 <sup>a,b</sup>	996 <sup>a,b</sup>
AlphaStart minipellet	127	309 <sup>b</sup>	598	435 <sup>a</sup>	1034 <sup>a</sup>
SEM <sup>3</sup>	1.94	5.0	13.9	6.0	16.00
P-value	0.91	0.001	0.14	0.004	0.02
	Feed conversion ratio (feed:gain)				
Control crumble	1.055	1.517 <sup>a</sup>	1.775	1.112 <sup>a</sup>	1.258 <sup>a</sup>
Medicated three-way NCSU crumble	1.094	1.425 <sup>b</sup>	1.764	1.079 <sup>b</sup>	1.224 <sup>b</sup>
AlphaStart crumble	1.059	1.410 <sup>b</sup>	1.778	1.085 <sup>b</sup>	1.251 <sup>a,b</sup>
AlphaStart minipellet	1.035	1.432 <sup>b</sup>	1.767	1.089 <sup>b</sup>	1.252 <sup>a,b</sup>
SEM <sup>3</sup>	0.025	0.020	0.037	0.007	0.009
P-value	0.43	0.003	0.99	0.001	0.05

<sup>a-c</sup>Means within a column lacking a common superscript differ ( $P \leq 0.05$ ).

Abbreviation: NCSU, North Carolina State University.

<sup>1</sup>Treatments were fed to birds until 14 d; from 14 to 21 d all, birds received the control feed.

<sup>2</sup>The BW at placement was not significantly different ( $59 \pm 0.5$  g).

<sup>3</sup>The SEM for  $n = 12$  cages of 7 birds each per treatment.

and then submerged in neutral buffered formalin. Samples were trimmed and tinted in slides at NCSU College of Veterinary Medicine. Slides were observed under a microscope and photographed and then measured with AmScope software (version 3.7, AmScope, Irvine, CA) corrected with a 4 $\times$  magnification. Measurements were for the villus height, crypt depth, and muscular thickness. Up to 10 subsamples were taken per measurement per slide.

## Statistical Analysis

Both EXP 1 and EXP 2 had a completely randomized block design. The effect of feed treatment on

performance parameters and intestinal tissue observations was determined using the GLM procedure of JMP Pro 12 (SAS Institute, Cary, NC) for ANOVA for EXP 1. Data for EXP 2 were analyzed using the PROC MIXED procedure from SAS, version 9.4. (SAS Institute, Cary, NC). For both EXP, the pen was the experimental unit for comparisons of performance, whereas for intestinal comparisons, the experimental unit was the bird. The intestinal subsamples and the person taking the measurements of the subsamples were nested in the bird using a Proc mixed model. For both EXP, significant differences in main effects were separated using the Tukey HSD test. A  $P \leq 0.05$  was used

**Table 6.** Treatment effects on AMEn<sup>1</sup> at 14 d (kcal/kg) and poult performance (g) in experiment 2.

SBM <sup>2</sup>	Fines <sup>3</sup>	ENZ <sup>4</sup>	AMEn <sup>1</sup>	BWG			Feed intake			FCR <sup>5</sup>		
			14 d	0-7 d	0-14 d	0-20 d	0-7 d	0-14 d	0-20 d	0-7 d	0-14 d	0-20 d
HSBM			2,978	93	289	552	111 <sup>b</sup>	367 <sup>b</sup>	750 <sup>b</sup>	1.201	1.275 <sup>b</sup>	1.350 <sup>b</sup>
LSBM			2,955	93	294	558	116 <sup>a</sup>	391 <sup>a</sup>	797 <sup>a</sup>	1.249	1.307 <sup>a</sup>	1.395 <sup>a</sup>
SEM <sup>6</sup>			39	2	4	7	2	4	8	0.014	0.005	0.009
P-value			0.14	0.97	0.33	0.50	0.03	<0.0001	0.004	0.064	<0.0001	0.001
	IFF		2994 <sup>a</sup>	90 <sup>a</sup>	284 <sup>b</sup>	541 <sup>b</sup>	113	373 <sup>b</sup>	761 <sup>b</sup>	1.253 <sup>a</sup>	1.313 <sup>a</sup>	1.387 <sup>s</sup>
	RFF		2939 <sup>b</sup>	95 <sup>b</sup>	300 <sup>a</sup>	569 <sup>a</sup>	113	384 <sup>a</sup>	786 <sup>a</sup>	1.187 <sup>b</sup>	1.270 <sup>b</sup>	1.359 <sup>b</sup>
	SEM <sup>6</sup>		39	2	4	7	2	4	8	0.014	0.005	0.009
	P-value		0.001	0.04	0	0	0.82	0.03	0.03	0.001	<0.0001	0.021
		WENZ	2986 <sup>a</sup>	95	292	560	113	378	778	1.185 <sup>b</sup>	1.284	1.368
		NENZ	2947 <sup>b</sup>	91	291	551	113	379	770	1.255 <sup>a</sup>	1.299	1.376
		SEM <sup>6</sup>	39	2	4	7	2	4	8	0.012	0.005	0.009
		P-value	0.02	0.1	0.85	0.30	0.78	0.86	0.49	<0.0001	0.051	0.510

<sup>a,b</sup>Means within a column lacking a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Apparent metabolized energy corrected for protein.

<sup>2</sup>Feed soybean meal factors, high-CP soybean meal 60% (HSBM), and low-CP soybean meal 48% (LSBM).

<sup>3</sup>Feed fine abundance factors, increased feed fines (IFF), and reduced feed fines (RFF).

<sup>4</sup>The enzyme in diet factors, 200 ml/mt enzyme cocktail added, Rovabio Advance, Adisseo France S.A.S, Antony, France (WENZ), and no enzyme added (NENZ).

<sup>5</sup>Feed conversion ratio corrected for mortality using BWG as the numerator.

<sup>6</sup>The SEM  $n = 36$  cages with 7 birds per factor.

to set a significant difference between the primary and interaction effects of the parameters analyzed.

## RESULTS

### Feed Analysis

Calculated nutrient content for NCSU-manufactured feed is presented in Table 3. Results for the nutrient analysis of all 4 feeds are presented in Table 4 and are reflective of the calculated nutrient content.

### Treatment Particle Size

In EXP 1, the particle size of the minipellets was twice that of the crumble feeds. The abundance of fines in the crumbled feeds was determined to be approximately 30%. The minipellet feed was not measured for fines, as few to none were observed (Table 4).

In EXP 2, RFF feed had a larger feed particle size (1,504  $\mu\text{m}$  compared with 1,474  $\mu\text{m}$ ) and a lower SD for its sample distribution than the IFF feed (Table 4). The IFF feed had a higher abundance of fines present in the feed. The RFF feed had a mean percentage of 30% fines and 70% crumbles, while IFF consisted of 44% fines, 52% crumbles, and 4% pellets.

### Performance Parameters

In EXP 1, no differences due to treatment were observed for the BW of the bird at placement ( $59 \pm 0.5$  g) or in any performance parameter at 7 d of age. The poult fed the AlphaStart minipellet had higher cumulative BW gain (BWG) at 14 d, followed by the birds fed the AlphaStart crumble (Table 5). It appears that there was possibly both a form and function advantage to the AlphaStart minipellet as compared with the control crumble, or the three-way medicated feeds. Although the pattern of the treatment means comparison for the BWG appear to be similar at 21 and 14 d, the means for treatments at 21 d were not as easily separated statistically as the means for treatments at 14 d. This may be potentially due to the substantial increase in the SEM at 21 d compared with the SEM at 7, 7 to 14, and 0 to 14 d. At 14 d, the birds fed the medicated diet had a lower FI, and the birds fed the control diet had a higher FCR than birds fed the other diets. As the EXP proceeded by only feeding the control feed from 14 to 21 d, birds previously fed the AlphaStart minipellet treatment consumed more feed and had a higher BW and a similar FCR than those fed the medicated diet (Table 5).

In EXP 2, no differences due to treatment were observed for the BW at placement ( $57 \pm 0.26$  g). Birds fed RFF had a significantly higher BWG than those fed IFF (Tables 6 and 7). The FI was significantly higher for birds fed the RFF and low CP SBM feed treatment diets than those fed the IFF and feed formulated with a high CP SBM treatment throughout the EXP (Tables 6 and 7). The poult FCR was significantly improved at 7 and 14 d for birds fed feed formulated

with the high CP SBM. At 20 d, feed with low and high SBM sources interacted with the dietary enzyme. Birds fed feed formulated with a low CP SBM had a significantly lower FCR when the enzyme was present compared with when the enzyme was not present. Birds fed a feed formulated with a high CP SBM had a lower FCR than when fed the low CP SBM regardless of the enzyme. Birds fed RFF had a lower FCR at 7 d than those fed IFF. At 14 and 20 d, the feed screening process interacted with the dietary enzyme. Birds fed RFF with the enzyme had a significantly lower FCR than when fed RFF without the enzyme, and IFF with or without the enzyme (Tables 6 and 7).

### Apparent Metabolizable Energy Corrected for Nitrogen

In EXP 2, birds fed feed formulated with either SBM source had a similar AMEn at 14 d. The feed screening process and enzyme factors had a first-order interaction with AMEn at 14 d. The RFF treatment resulted in a similar AMEn compared with the IFF treatment when the enzyme was present. Not having the enzyme lowered the AMEn at 14 d for birds fed RFF than for birds fed IFF (Tables 6 and 7).

### Duodenum, Jejunum, Ileum, and Cecum Morphology

In EXP 1, statistical differences were observed among the treatments at 14 d; however, they did not persist to 21 d (Table 8). Data were not consistent throughout EXP 1. Statistical differences were observed only in the jejunum crypt depth and villus height-to-crypt depth ratio at 20 d in EXP 2. Feed formulated with a high CP SBM resulted in a significantly shallower crypt depth (93 vs.  $104 \pm 4$   $\mu\text{m}$ ,  $P = 0.02$ ) and a higher villus height-to-crypt depth ratio (12 vs.  $10 \pm 0.5$ ,  $P = 0.003$ ). These data indicate a change in the number of enterocytes developed between feed SBM sources.

## DISCUSSION

Experiment 1 was considered a preliminary EXP. The treatments that can be compared directly are the NCSU control with the three-way medicated and the AlphaStart crumbles with the AlphaStart minipellet; however, there were similarities in the nutritional content. Therefore, statistical analysis was conducted across all treatments, and inferences were made which were used to determine the experimental design used in EXP 2.

The efficiency of feed prehension by the bird will determine the amount of energy and nutrients used related to the energy and nutrients gained by eating. Mash feeds are more difficult for the bird to prehend, possibly resulting in the bird expending more energy for eating and wasting more feed than when eating pelleted feeds (Jensen et al., 1962; Calet, 1965). Birds select the feed particle size depending on the size of the beak

**Table 7.** Treatment first-order interactions<sup>1</sup> on AMEn<sup>2</sup> at 14 d (kcal/kg) and poult performance(g) in experiment 2.

SBM <sup>3</sup>	Fines <sup>4</sup>	ENZ <sup>5</sup>	AMEn <sup>2</sup>		BWG		Feed intake			FCR <sup>6</sup>		
			14 d	0–7 d	0–14 d	0–20 d	0–7 d	0–14 d	0–20 d	0–7 d	0–14 d	0–20 d
HSBM	IFF		2976 <sup>a</sup>	91	282	541	111	365	740	1.229	1.297	1.365
HSBM	RFF		3012 <sup>a</sup>	94	295	556	109	369	756	1.169	1.251	1.336
LSBM	IFF		2995 <sup>a</sup>	89	285	541	116	382	771	1.284	1.328	1.401
LSBM	RFF		2882 <sup>b</sup>	97	308	582	116	400	817	1.205	1.289	1.377
SEM <sup>7</sup>			41	2.52	4.82	9.97	2.24	5.54	13.15	0.013	0.007	0.009
<i>P</i> -Value			<0.0001	0.29	0.32	0.18	0.55	0.21	0.25	0.610	0.631	0.782
HSBM		WENZ	288	96	291	554	112	369	759	1.164	1.271	1.358 <sup>b</sup>
HSBM		NENZ	2,968	89	286	543	109	365	737	1.234	1.277	1.343 <sup>b</sup>
LSBM		WENZ	2,983	94	297	565	115	388	785	1.213	1.296	1.371 <sup>a,b</sup>
LSBM		NENZ	2,926	93	296	558	118	394	802	1.276	1.320	1.408 <sup>a</sup>
SEM <sup>7</sup>			41	2.52	4.97	9.97	2.24	5.54	13.15	0.013	0.007	0.009
<i>P</i> -Value			0.24	0.18	0.74	0.87	0.16	0.31	0.14	0.859	0.238	0.007
	IFF	WENZ	2,995	91	283	543	113	372	762	1.231	1.315 <sup>a</sup>	1.389 <sup>a</sup>
	IFF	NENZ	2,961	89	284	539	114	375	749	1.282	1.310 <sup>a</sup>	1.378 <sup>a,b</sup>
	RFF	WENZ	2,993	98	305	576	113	385	783	1.146	1.252 <sup>b</sup>	1.341 <sup>b</sup>
	RFF	NENZ	2,917	92	298	562	113	384	790	1.228	1.287 <sup>a</sup>	1.373 <sup>a,b</sup>
SEM <sup>7</sup>			41	2.52	4.97	9.97	2.24	5.54	13.15	0.013	0.007	0.009
<i>P</i> -Value			0.19	0.48	0.44	0.62	0.91	0.80	0.44	0.411	0.011	0.026

<sup>a,b</sup>Means within a column lacking a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>No significant interaction was found between Fines\*SBM\*ENZ.

<sup>2</sup>Apparent metabolized energy corrected for protein.

<sup>3</sup>Feed soybean meal factors, high-CP soybean meal 60% (HSBM), and low-CP soybean meal 48% (LSBM).

<sup>4</sup>Feed fines abundance factors, increased feed fines (IFF), and reduced feed fines (RFF).

<sup>5</sup>The enzyme in diet factors, 200 ml/mt enzyme cocktail added, Rovabio Advance, Adisseo France S.A.S, Antony, France (WENZ), and no enzyme added (NENZ).

<sup>6</sup>The feed conversion ratio corrected for mortality using the BWG as the numerator.

<sup>7</sup>The SEM n = 18 birds per interaction.

(Moran, 1982), and as the beak grows, the preferred particle size increases (Portella et al., 1988). The particle size is also referred to as the feed form by some authors. Inferior feed form (i.e., decreased pellet quality) can be a significant limiting factor for performance (Quentin

et al., 2004). The first feed is an essential factor in early gut health and poult performance (Noy et al., 2001). This first feed is also part of a critical transition from a yolk-based diet to a solid-based diet (Uni and Ferket, 2004). The starter phase is where the crumble form

**Table 8.** Treatment effects on the small intestine morphology at 14 d ( $\mu\text{m}$ ) in experiment 1.

Treatment	14 d				21 d			
	Villus height	Crypt depth	V:C <sup>1</sup>	Muscularis thickness	Villus height	Crypt depth	V:C <sup>1</sup>	Muscularis thickness
	Duodenum							
Control crumble	1,711	136 <sup>a</sup>	13	1,847	2,217	147	15.7	2,363
Medicated three-way crumble	1,655	106 <sup>b</sup>	15	1,785	2,162	142	13.6	2,305
AlphaStart crumble	1,671	113 <sup>a,b</sup>	16	1,784	2,116	139	15.6	2,255
AlphaStart minipellet	1,750	135 <sup>a</sup>	14	1,886	2,110	147	14.7	2,257
SEM <sup>2</sup>	74	7	1	77	58	7	0.66	60
<i>P</i> -value	0.8	0.05	0.22	0.73	0.55	0.8	0.66	0.55
	Jejunum							
Control crumble	696 <sup>a</sup>	126 <sup>a</sup>	5.6	822 <sup>a</sup>	877	142	6.3	1,019
Medicated three-way crumble	427 <sup>b</sup>	91 <sup>b</sup>	4.8	518 <sup>b</sup>	827	129	6.5	955
AlphaStart crumble	481 <sup>b</sup>	96 <sup>b</sup>	5.2	576 <sup>b</sup>	855	140	6.3	996
AlphaStart minipellet	552 <sup>a,b</sup>	116 <sup>a,b</sup>	4.8	668 <sup>a,b</sup>	888	137	6.7	1,025
SEM <sup>2</sup>	45	8	0.3	50	65	9	0.4	71
<i>P</i> -value	0.001	0.009	0.16	0.001	0.91	0.76	0.87	0.9
	Ileum							
Control crumble	367	99	3.7	466	699	139	5.3	875 <sup>a</sup>
Medicated three-way crumble	311	90	3.5	401	640	133	4.9	806 <sup>a</sup>
AlphaStart crumble	381	101	3.8	482	730	150	4.9	879 <sup>a</sup>
AlphaStart minipellet	373	108	3.7	481	565	125	4.6	688 <sup>b</sup>
SEM <sup>2</sup>	28	6.5	0.3	32	52	9.5	0.3	59
<i>P</i> -value	0.33	0.31	0.85	0.28	0.13	0.33	0.53	0.05

<sup>a,b</sup>Means within a column lacking a common superscript differ ( $P \leq 0.05$ ).

<sup>1</sup>Villus height-to-crypt depth ratio.

<sup>2</sup>The SEM n = 36 cages with birds per factor.



and quality might be the most important. Favero et al. (2009) concluded that minipellets could be used after the second week of placement.

In the first EXP herein, birds fed the minipellet as the starter feed were observed to have a higher BWG due potentially to an increased FI and also having an improved FCR compared with birds consuming the control crumble feed. It is also possible that difference in feed ingredients in the AlphaStart contributed to differences in bird performance. However, given that the birds fed the AlphaStart crumble were intermediate in performance between the AlphaStart minipellet and the control crumble, the inferences made concerning the feed form seem at least potentially valid for EXP 1. Therefore, minipellets in EXP 1 were concluded to be an option to affect the feed form by increasing the feed particle size resulting in higher quality starter feed pellets. One potential disadvantage of the minipellet may be the cost of production. In most feed mills, minipelleting would require new equipment fitted to the feed mill. In addition, feed production may not be as energy or time efficient as standard crumble production. However, minipelleting cost determination and production efficiency were not conducted in this study.

Screened crumbled feeds, RFF (1.5 mm), were selected as an alternative to a minipelleted feed for the second EXP. Similar results were observed in advantages for the bird BWG and FI, and also for an improved FCR. Therefore, an RFF crumble starter diet might be comparable in effects to minipelleted starter diets. Minipellet and RFF crumbles share similar characteristics with pelleted feed. Pelleted feed results in lower feed wastage, is heat-treated, and has a lower abundance of fines, all of which have been shown to result in an increased FI, increased BWG, and improved FCR compared with feeding a mash feed (Calet, 1965; Nir et al., 1995; Amerah et al., 2007a; Amerah et al., 2007b; Zang et al., 2009; Dozier et al., 2010; Selle et al., 2010; Serrano et al., 2012; Serrano et al., 2013; Lanson and Smyth, 1955).

Ingredient quality is as, or more, important than feed form. The quality of ingredients received by the feed mill will dictate the quality of the produced feed. Cereals and SBM contain NSP and are widely used as the base for animal feeds. The presence of NSP in poultry diets increases the viscosity of the unstirred water layer of the gut mucosa and changes the morphology in the gut. Therefore, NSP can be detrimental to bird nutrient efficiency (Choct, 1997). A higher bird FI and an improved FCR for the feed formulated with a high CP SBM source could be due to the changes in the amount of NSP that the birds consumed. The effect may be further explained by the interaction of feed formulated with low CP SBM and the enzyme at 20 d in EXP 2. The enzyme cocktail used herein has arabinofuranosidases and xylanases, which act on components of the cell wall of the grain, thereby increasing the amount of available and digestible nutrients by reducing the amount of NSP and cell wall components. This enzyme was designed to be used in corn and SBM formulations. Thus, a low CP SBM source increases the

enzyme efficiency by increasing the enzyme-substrate ratio when corn and SBM are present in the formulation. The feed source of SBM used in this EXP did not affect the AME<sub>n</sub>. However, when a feed formulated with a low CP SBM was fed to the birds, it may have increased the number of fermentative organisms.

The presence of NSP in the diet is detrimental to poultry because it increases the digest time and the number of fermentative organisms in the small intestine (Choct et al., 1996; Choct, 1997). Birds fed RFF feed had a reduced AME<sub>n</sub> value, which may have been caused by a reduction in the transit time of the digesta. Nutrient digestibility depends on the digesta retention time. In broilers fed wheat-based diets, AME<sub>n</sub> increased linearly by increasing the whole tract transit time (Hughes, 2008). Transit time through the small intestine is reduced for diets with whole wheat when compared with ground wheat (Svihus et al., 2002).

Similarly, increasing the feed particle size by pelleting the feed reduces the transit time of the digesta (Sundu, 2008). Adding the enzyme to the RFF increased feed digestibility and led to similar AME<sub>n</sub> values as IFF with or without the enzyme. Feed formulated with a high CP SBM reduced the enlargement of the birds' crypts and increased the villus height-to-crypt depth ratio. The difference in the birds' crypt depths at the jejunum could be explained by the lower amount of soluble NSP in the feed formulated with high CP SBM. Soluble NSP increases the viscosity of the intestinal contents, thereby reducing the nutrient absorption efficiency of poultry (Choct et al., 1996, 2010; Choct, 1997; Montagne et al., 2003; Hetland et al., 2004; Hughes, 2008). The NSP may not only prevent the bird from absorbing nutrients but also may have changed the gut physiology (Choct et al., 2010). Dietary fiber increases the crypt-cell proliferation and reduces the villus height-to-crypt ratio (Montagne et al., 2003). A decrease in the villus height-to-crypt depth ratio is considered to be detrimental to digestion and absorption (Montagne et al., 2003).

In both EXP, the poult FI and BWG were improved because of an increased feed particle size and RFF during the starter phase. An increase in the feed particle size increased the FI and, therefore, increased nutrient intake, consequently increasing the BWG of the bird. The feed formulation containing a high CP SBM source could be used to improve poult FI, BW, and FCR by decreasing the percentage of NSP in the feed. Functional factors in the mini-pellet and dietary enzyme may have improved the digestibility of feed formulated with a low protein CP SBM and RFF by reducing the adverse effects of NSP in the intestinal tract. Therefore, improved feed form and ingredient quality improved poult performance during the starter period.

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