The effect of feed processing of novel unheated, low trypsin inhibitor soybeans on the performance of young female turkeys reared from hatch to 21 days of age

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ABSTRACT The objective of this study was to evaluate the effects of dietary inclusion of a novel low trypsin inhibitor soybean (**LTI**) fed as mash or crumbled pellet form on the productive performance and digestibility in turkey poults from hatch to 21 d. A total of 336 Hybrid Converter turkey poults were allocated in groups of 7 to 6 treatments, each with 8 replicate cages. Treatments were arranged as a 3 × 2 factorial with inclusion level of the low trypsin-inhibitor soybean (0, 20, and 40% LTI) and feed form (mash and crumbled pellet). A single batch of feed was mixed for each inclusion level and divided into 2 aliquots: one remaining as mash and the other conditioned at 82°C for approximately 30 s, pelleted and then crumbled. On d 7, 14, and 21 posthatching, BW, and feed intake **(FI)** were recorded and BW gain **(BWG)** and feed conversion ratio (**FCR**) calculated. Excreta samples were collected from d 19 to 21 and pooled by cage. At d 21, intestines were excised, pancreas weights recorded **(P_{RW})**, and ileal contents collected. There were no interactions (linear, $P > 0.05$) between LTI inclusion and feed form on BW, BWG, FI, or FCR at d 7 or 21. Increasing LTI resulted in a linear reduction in BW at 7, 14, and 21 d ($P < 0.006$). Poults fed crumbles were significantly heavier at d 21 than those fed mash feed ($P < 0.027$) with no interaction of LTI level with feed form. FCR was not significantly greater with increasing LTI. However, poults fed crumbles had a better, lower FCR that those fed mash feed from d 0 to 21 ($P < 0.018$). There was a significant interaction between feed form and LTI level at 14 d ($P < 0.031$), but not 7 or 21 d. Pancreatic hypertrophy (P_{RW}) increased linearly with increasing LTI ($P < 0.001$) with a significant linear interaction with feed form ($P < 0.001$). Poults fed crumbles had less pancreatic hypertrophy. At 21 d of age, dietary fat digestibility (**ALD**, %) was linearly reduced with increasing LTI ($P < 0.001$). However, poults fed crumbles had significantly better fat absorption than poults fed mash (91.2 vs. 85.8%) ($P < 0.001$), and there was a significant linear interaction between feed form and LTI level ($P < 0.001$). AME_n was significantly better for the poults fed crumbles compared to mash (3228 vs. 3132 kcal/kg) ($P < 0.001$), and there was a significant linear interaction between feed form and LTI level ($P < 0.001$). Based on the results this trial, it is possible to include up to 20% unheated full fat LTI soybeans into poult starter diets after pelleting. Pelleting improves nutrient utilization, allowing for greater incorporation of the LTI soybean in the crumbled diet compared to the mash diet.

Key words: turkey, unheated soybeans, trypsin inhibitor, anti-nutritional factor

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INTRODUCTION

Full fat soybeans (**FFSB**) offer an excellent source of both protein and fat for poultry diets. They have naturally high oil and protein content with a favorable amino acid profile for growth (Waldroup, 1982). Conventional soybean varieties, however, contain several antinutritional factors (**ANF**) that can negatively impact nutrient availability and protein quality for poultry (Liener, 1994). Trypsin inhibitors (**TI**) are one such ANF that disrupts digestive enzymes, impeding protein hydrolysis. The primary TI found in soybeans are Kunitz trypsin inhibitor (**KTI**) and Bowman-Birk trypsin inhibitor (**BBTI**), which comprise approximately 80 and 20%, respectively, of the total TI in soybeans (Shivakumar et al., 2015). While KTI (20kDa) primarily binds and inhibits trypsin, BBTI (6-21kDa) binds and inhibits both trypsin and chymotrypsin (Liener, 1994). Research has demonstrated that feeding

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increased levels of TI to poultry depresses growth rates and feed intake (Batal et al., 2000; Pacheco et al., 2014; Evans et al., 2019; Hoffmann et al., 2019). Fortunately, both KTI and BBTI can be inactivated by heat, though BBTI may be more resistant to deactivation due to the presence of multiple disulfide bonds (DiPietro and Liener, 1989). As a result, the majority of soy products are routinely heat processed for TI inactivation, with remaining residual up to 20% of KTI and BBTI (Friedman and Brandon, 2001). This practice, however, can negatively influence nutrient quality and is both energy-intensive and costly. In response, new soybean varieties are being developed that have reduced levels of TI, which could potentially eliminate or reduce the cost of heat treatment before feeding to poultry.

These new varieties of soybean can have dramatically reduced levels (~40%) of TI due to the identification and selection of accessions affecting KTI expression in the seed (Jofuku et al., 1989). Evans et al. (2019) compared 2 novel lines of low KTI soybeans (18.2 and 10.3 TIU/mg) to a commercial soybean (29.8 TIU/mg) in a turkey feeding trial. For comparison, a survey of U.S. feed mills (n = 192) reported an average trypsin inhibitor activity (TIA) of 3.06 mg/g or 5.8 TIU/mg for conventional solvent-extracted soybean meal (Chen et al., 2020). Researchers, including Evans et al., 2019), have demonstrated that while unheated KTI soybeans are nutritionally superior to conventional soybean lines, they remain substandard to processed soybean meal (Herkelman et al., 1993; Perez-Maldonado et al., 2003; Evans et al., 2019). This may be due to the presence of other ANF including BBTI. Successful further reductions in TI have been observed on KTI soybeans with steam treatment (Herkelman et al., 1993; Batal and Parsons, 2003), roasting (MacIsaac et al., 2005), and extrusion (Palacios et al., 2004 and Perilla et al., 1997). Little, however, is known about the effect of steam pelleting on KTI soybeans. Thus, the objective of this study was to determine whether steam pelleting could reduce residual TI levels and allow for greater inclusion of low TI soybeans in turkey diets.

MATERIALS AND METHODS

Animal handling protocols and procedures were approved by the Institutional Animal Care and Use Committee of North Carolina State University.

Experimental Design and Diets

A total of 336 female day of hatch turkey poults (Hybrid Converter, Hendrix Genetics, Ontario, Canada) were sourced from a commercial hatchery (Sleepy Creek Hatchery, Goldsboro, NC) and reared to 21 d. Poults were randomly distributed into groups of 7 and weighed prior to placement in 48 Alternative Design (Siloam Springs, AR) battery cages. Cages were randomly allotted to 1 of 6 dietary treatments resulting in 8 replicates per treatment. Feed and water were consumed ad libitum. Batteries were located in a climate-controlled room and equipped with dual nipple water drinkers, feeding trough, supplemental heater, and collection pan located beneath the floor of each cage. Artificial light was provided for 24 h for the first 7 d; and then adjusted to provide 23 h of light and 1 h of dark for the remainder of the study. Heat was provided for bird comfort and adjusted based on bird behavior. Temperatures were monitored twice a day to yield averages of 32 to 35°C for the first 7 d followed by 26 to 34°C for the next 14 d.

Dietary treatments were arranged as a 3 × 2 factorial with inclusion level of the low trypsin-inhibitor soybean (0, 20, and 40% **LTI**) and feed form (mash and crumbled pellet). Diets were formulated to meet or exceed the breeder recommendations (Hybrid Turkeys). For formulation purposes, a metabolizable energy value of 3300 kcal/kg was designated for the LTI soybean (variety 43T908, eMerge Genetics, Des Moines, IA) based on the value given to heat processed, full-fat soybeans (NRC, 1994). Maximum inclusion rate of the LTI soybean was limited to 40% based on the natural oil content and replaced solvent-extracted soybean meal on a protein basis in the experimental diets (Table 1). Soy oil

Table 1. Composition of dietary treatments with varying levels of unheated soybeans fed to turkey poults through d 21 posthatching.[1]

	LTI inclusion level[2]		
	0%	20%	40%
Ingredient, %	0% LTI	20% LTI	40% LTI
Corn	30.00	30.00	30.00
Soybean meal	52.70	36.99	21.29
LTI soybean	0.00	20.00	40.00
Soy oil	7.15	3.87	0.60
Dicalcium P, 18.5%	3.00	3.00	3.00
Cellulose	2.36	1.35	0.32
Limestone	1.70	1.70	1.70
Diatomaceous earth	1.70	1.70	1.70
DL-methionine	0.35	0.35	0.35
Sodium chloride	0.30	0.30	0.30
Sodium bicarbonate	0.25	0.25	0.25
L-lysine HCl	0.22	0.22	0.22
Mineral premix[3]	0.15	0.15	0.15
Vitamin premix[4]	0.10	0.10	0.10
Antioxidant	0.02	0.02	0.02
Total	100.00	100.00	100.00
Calculated analysis			
ME, kcal/kg	2890	2890	2890
Crude protein, %	28.1	28.1	28.1
Calcium, %	1.4	1.4	1.4
Available phosphorous, %	0.7	0.7	0.7
Total AA, %			
Lysine	1.80	1.80	1.80
Methionine	0.75	0.75	0.75
Cysteine	0.40	0.40	0.40
Threonine	1.03	1.03	1.03

[1]Treatments arranged as a 3 × 2 factorial with inclusion level of the unheated low trypsin inhibitor soybean (0, 20, and 40% LTI) and feed form (mash or crumbled pellet).

[2]Unheated low trypsin inhibitor soybean (LTI) replaced solvent extracted soybean meal on a protein basis.

[3]Provided per kg of mineral premix: Iron 20 mg; Copper 2.5 mg; Manganese 30 mg; Zinc 30 mg; Iodine 1.25 mg, Cobalt 1.0 mg, and 0.3 mg Se.

[4]Provided per kg of vitamin premix: Vit A 13,200 IU; Vit D3 3,960 IU; Vit E 66 IU; Vit B1 4 mg; Vit B2 13 mg; Vit B 6 8 mg; Vit B12 40 μg; Vit K3 4 mg; Nicotinic acid 110 mg; Pantothenic acid 22 mg; Folic acid 2.2 mg; Biotin 254 μg.

was used to maintain similar caloric density of diets across treatments, with decreasing levels needed as inclusion of the LTI soybean increased. Cellulose (Solka-Floc, International Fiber Corporation, North Tonawanda, NY) was included in diets to balance nutrient density across treatments, while diatomaceous earth (Celite, World Minerals Inc., Santa Barbara, CA) was included as an indigestible reference.

Feed Manufacturing and Physical Characteristics

Feed was manufactured in accordance with Current Good Manufacturing Practices (CGMP) at the North Carolina State University Educational Feed Mill (Raleigh, NC). Corn and unheated LTI soybeans were ground using a roller mill (RMS, Harrisburg, SD) furnished with double pairs of 305 mm diameter rolls measuring 406 mm long and two 15-hp motors. The approximate roll differentials were 1.5:1 on the top and 2:1 on the bottom. The roll gap or distance between roll sets was adjusted manually for whole corn and whole soybeans to yield finished diets with an estimated particle size of 700 microns. Whole corn was ground using a 0.64 mm gap between the top set of rolls and 0.56 mm gap between the bottom set, while the unheated LTI soybean was ground with 0.20 mm and 0.15 mm gaps, respectively.

Dietary treatments were mixed in a 3.6 m^3 counterpoise mixer (Hayes and Stolz, Fort Worth, TX) for 270 s. Dry ingredients were allowed to mix for 90 s prior to the addition of the liquid soy oil. After mixing, batches were divided into 2 aliquots: one to be fed as mash and the other to undergo further processing into a crumbled pellet. This was done to reduce variation in diets due to deviations in scaling or mixing. To create crumbles, mash feed was conditioned at 82°C for approximately 30 s and pelleted (PM1112-2, CPM, Crawfordsville, IN) using a 4.4 mm × 35 mm die with a L:D of 8.0. Cooled pellets were passed through a single set of rolls to crumble.

Postmanufacture, particle size and bulk density were determined for each diet. Particle size was analyzed according to dry sieving method ASAE S319.2 (ASAE, 1995). Analysis included 13 sieves, the addition of sieve agitators and 0.5 g of dispersing agent (silicon dioxide, model SSA-58, Gilson, Lewis Center, OH) per 100 g of sample with a 10 min run time. Sieve sizes ranged from 3350 to 53 μm. Bulk density of dietary treatments was calculated using the method described by .Clemenstson et al., 2010. Briefly, sample was poured into a funnel-shaped hopper centered over a 1 L measuring cup. The sample was allowed to freely flow into the measuring cup and excess material was systematically leveled off. The filled measuring cup was then weighed and the bulk density calculated by dividing the mass of the sample in the measuring cup by the volume of the measuring cup.

Prior to crumbling, pelleted treatments were analyzed to determine the fines content and pellet durability index (**PDI**). Two samples of pelleted feed were taken at the pellet die during manufacture, cooled for 10 min in an experimental counter flow cooler, and then allowed to rest for 24 h prior to analysis. Samples were weighed and sifted for separation of fines and pellets using a U.S. No. 6 sieve with 3.35 mm screen openings. Any recovered fines were weighed and fines content calculated by dividing the mass of recovered fines by the initial sample mass multiplied by 100. Sifted pellet samples were retained for subsequent PDI analyses using the tumble box and Holmen NHP100 (TekPro Ltd, Norfolk, UK) methods. For the standard tumble box method (ASAE Standard S269.4, ASAE Standards 2003a), 500 g of sifted pellets were placed in a revolving chamber for 10 min rotating at 50 rpm. After testing, the pellets were screened using a U.S. No. 6 sieve and their weight recorded. The modified tumble box followed the same procedures as outlined by the standard method, except five 12.7 mm hex nuts were placed in the rotating chamber to increase the abrasive pressure on the pellets during analysis. For the Holmen analytical method, 100 g of sifted pellets were placed in the perforated chamber of the NHP100 pellet tester. Pellets were then agitated with forced air at 70 mbar for the designated testing time of 30 or 60 s. After testing, the pellets were screened using a U. S. No. 6 screen and their weight recorded. PDI was then calculated for all methods as the mass of pellets retained on the sieve screen after analysis divided by the initial mass of the pellets multiplied by 100.

Sampling Procedures

To assess growth performance, BW and feed intake (**FI**) were recorded on d 7, 14, and 21 posthatching. BW gain (**BWG**) and feed conversion ratio (**FCR**) were calculated for each cage of birds for the respective period. Mortality by cage (number and weight of poult mortalities within each cage) was recorded daily throughout the experiment and FCR was corrected accordingly. Fresh excreta samples were collected on d 19, 20, and 21 and composited for each pen of birds, with care taken to avoid feed or feather contaminants. Composited excreta samples were frozen and stored at -20°C until analyses. On d 21, all birds were euthanized via cervical dislocation, weighed, and their intestines excised. Pancreases were carefully removed from the duodenal loop and individually weighed, with pancreas weight reported as a percentage of poult BW ($\mathbf{P_{RW}}$). Ileal contents, beginning 1 cm posterior to the Meckel's diverticulum and ending 1 cm prior to the ileal-cecal junction, were manually expressed into plastic collection tubes, pooled within each cage of birds, and frozen immediately at -20°C until analysis.

Chemical Analysis

Representative samples of the solvent extracted soybean meal and LTI soybean were analyzed (Table 2) by the University of Missouri Agricultural Experiment Station (Columbia, MO) for dry matter (AOAC Official Method 934.01, 2006), crude protein (AOAC Official Method 934.01, 2006), crude fat (AOAC Official Method 920.39(A), 2006), crude fiber (AOAC Official Method 978.10, 2006), ash (AOAC Official Method 942.05), gross energy via bomb calorimetry, and trypsin inhibitor activity (AACC Official Method 22-40, 1995). Representative feed samples were obtained for each dietary treatment and analyzed by the University of Missouri Agricultural Experiment Station (Columbia, MO) for dry matter (AOAC Official Method 934.01, 2006), crude protein (AOAC Official Method 990.03, 2006), crude fat (AOAC Official Method 920.39(A), 2006), crude fiber (AOAC Official Method 978.10, 2006), ash (AOAC Official Method 942.05), calcium (AOAC Official Method 985.01), phosphorous (AOAC Official Method 985.01, 2006), sodium (AOAC Official Method 985.01, 2006), trypsin inhibitor activity (AACC Official Method 22-40, 1995), urease activity (AACC Official Method 22-90, 1995 AACC, 1995), and complete amino acid profile (AOAC Official Method 982.30 E(a,b,c), 2006). To estimate the fat digestibility of the dietary treatments, 5 g of sample was placed in a cellulose extraction thimble measuring 20 mm in diameter and 50 mm long. The sample was rinsed with 25 mL of petroleum ether for 6 consecutive washes. Post ether addition, the sample rested for 30 min between each wash to allow the ether to completely drain from the thimble and the sample temperature to return to ambient. The sample was stirred in the thimble prior to washes 3 and 6. After each wash, the eluate of ether and fat was collected into a beaker of known weight. Once collected, beakers were placed in a fume hood for 24 h at ambient temperature to allow ether evaporation. Beakers were then placed in a forced-air drying oven at 105°C for 1 h. Remaining oil residue was quantified by weighing the beaker and subtracting the empty weight. The weight of the recovered fat was then divided by the initial sample weight and multiplied by 100. Results were cumulative, with Wash 6 comprised of the total fat recovery of the sample. The fat recovery was determined on as-fed samples and samples that had been ground through a 0.5 mm screen (Model ZM-200, Retsch GmbH, Retsch-Allee, 42871, Haan, Germany) prior to analysis.

Excreta samples were freeze-dried (Labconco, Kansas City, MO) for approximately 7 d and subsequently ground to pass through a 0.5 mm screen (Model ZM-200, Retsch GmbH, Retsch-Allee, 42871, Haan, Germany). Excreta and diet samples were analyzed for dry matter (AOAC Official Method 934.01, 2006), gross energy, nitrogen, and fat content. Gross energy content was determined on an adiabatic bomb calorimeter (IKA Works, Inc., Wilmington, NC) with benzoic acid as the calibration standard, while nitrogen content was measured via combustion analysis (LECO Corporation, St. Joseph, MI) with EDTA as the calibration standard. Crude fat content was determined using petroleum ether and Soxhlet extraction. All samples were analyzed for recovery of the indigestible marker based on the acid-insoluble ash procedure described in Vogtmann et al., 1975 for the determination of apparent metabolizations. Retained nitrogen (**N_{Ret}**) and nitrogen-corrected apparent metabolizable energy (**AME_n**) were calculated according to Hill et al. (1959), while apparent lipid digestibility (**ALD**) was calculated according to Kong and Adeola (2014).

Table 2. Analyzed nutrient content (DM basis) of soybean meal and unheated low trypsin inhibitor soybean fed to turkey poults through d 21 posthatching.[1]

	Soybean meal	LTI soybean
Moisture, %	10.15	8.18
Crude protein, %	47.45	37.10
Crude fat, %	1.06	18.32
Crude fiber, %	3.50	5.10
Ash, %	6.05	4.86
Gross energy, kcal/kg	3,315	4,213
Trypsin inhibitor, TIU/mg	2.0	10.1

[1]Treatments arranged as a 3 × 2 factorial with inclusion level of the unheated low trypsin inhibitor soybean (0, 20, and 40% LTI) and feed form (mash or crumbled pellet). Unheated low trypsin inhibitor soybean (LTI) replaced solvent extracted soybean meal on a protein basis in diets.

Pooled ileal digesta samples were freeze dried (Labconco, Kansas City, MO) for approximately 96 h and ground to pass through a 0.5 mm screen (Model ZM-200, Retsch GmbH, Retsch-Allee, 42871 Haan, Germany). Ileal digesta and diets were analyzed for dry matter (AOAC Official Method 934.01, 2006) and complete amino acid profile (AOAC Official Method 982.30 E(a,b,c), chp. 45.3.05, 2006) by the University of Missouri Agricultural Experiment Station (Columbia, MO). Samples were analyzed for indigestible marker recovery according to Vogtmann et al., 1975 . Apparent ileal digestibility of amino acids **(AID)** was calculated according to Kong and Adeola (2014).

Statistical Analysis

Data were analyzed using the GLIMMIX procedure in SAS v. 9.4 (SAS Institute Inc., Cary, NC) with pen as the experimental unit and the Tukey-Kramer adjustment for multiple comparisons. Treatments were analyzed as a 3 × 2 factorial with inclusion level of the low trypsin-inhibitor soybean (0, 20, and 40% LTI) and feed form (mash and crumbled pellet). Preplanned linear and quadratic contrasts were performed to characterize interactions and main effects as a result of increasing LTI inclusion. There were 8 replicates per treatment. Treatment effects were considered significant at $P \leq 0.05$ and marginally significant at $0.10 \leq P \geq 0.05$.

RESULTS

Dietary Treatments

Complete diets were analyzed for nutrient composition, particle size, bulk density, and pellet quality (Tables 3 and 4). Diets remained similar in crude protein with increased variation in crude fat. Mash diets

Table 3. Analyzed diet composition[1] (DM basis) and physical attributes of mash and crumbled pellet diets with varying levels of unheated soybeans fed to turkey poults through d 21 posthatching.[1]

	0% LTI[2]		20% LTI		40% LTI	
	Mash	Crumble	Mash	Crumble	Mash	Crumble
Moisture, %	10.01	10.31	10.34	10.15	10.19	9.49
Crude protein, %	30.13	30.84	30.15	30.62	30.80	31.00
Crude fat, %	8.49	7.87	9.27	9.26	8.87	9.32
Crude fiber, %	4.43	3.85	4.71	3.99	4.71	3.87
Ash, %	10.67	9.97	10.99	10.28	10.71	10.52
Calcium, %	1.58	1.38	1.59	1.47	1.49	1.53
Phosphorous, %	1.02	0.99	1.05	1.05	1.04	1.09
Sodium, %	0.20	0.18	0.22	0.19	0.21	0.19
Indispensable AA, %						
Arg	2.13	2.05	2.12	2.17	2.24	2.17
His	0.82	0.81	0.81	0.86	0.86	0.83
Ile	1.38	1.35	1.34	1.35	1.39	1.33
Leu	2.48	2.45	2.43	2.48	2.52	2.47
Lys	2.00	1.92	2.00	2.00	2.07	1.99
Met	0.72	0.67	0.77	0.69	0.79	0.69
Phe	1.53	1.52	1.51	1.54	1.58	1.52
Thr	1.19	1.17	1.17	1.21	1.22	1.19
Trp	0.32	0.35	0.27	0.35	0.28	0.36
Val	1.48	1.45	1.44	1.47	1.50	1.45
Dispensable AA, %						
Ala	1.39	1.38	1.36	1.39	1.40	1.40
Asp	3.26	3.19	3.20	3.28	3.36	3.23
Cys	0.41	0.41	0.40	0.42	0.41	0.41
Glu	5.20	5.13	5.14	5.32	5.37	5.23
Gly	1.28	1.28	1.27	1.28	1.33	1.29
Hyl	0.04	0.04	0.04	0.04	0.03	0.04
Orn	0.03	0.02	0.02	0.02	0.02	0.02
Pro	1.59	1.55	1.56	1.60	1.61	1.60
Ser	1.34	1.38	1.33	1.47	1.39	1.35
Tyr	1.00	0.96	0.99	1.02	1.04	1.00
Trypsin inhibitor, TIU/mg	1.04	1.36	2.98	3.46	4.01	4.05
Urease activity, ΔpH	0.00	0.00	1.10	0.65	1.96	1.28

[1]Treatments arranged as a 3 × 2 factorial with inclusion level of the unheated low trypsin inhibitor soybean (0, 20, and 40% LTI) and feed form (mash or crumbled pellet).

[2]Inclusion level of the unheated low trypsin inhibitor soybean (LTI) in the diet.

analyzed at 8.49, 9.27, and 8.27% crude fat as LTI inclusion increased, while crumbled diets had reported crude fat values of 7.87, 9.26, and 9.32%, respectively. An initial petroleum ether wash of the as-fed mash diets showed decreased fat recovery as LTI increased; however, after 6 consecutive washes, more total fat was recovered as LTI increased (Table 5). Grinding the samples prior to rinsing with petroleum ether yielded numerically greater fat recovery both after the initial Wash 1 and cumulatively after Wash 6, though this may not as accurately predict the accessibility of the fat for the bird when compared to analyzing fat recovery on as-fed samples. TIU and urease activity increased in mash diets as LTI increased from 0 to 40%, with 1.04, 2.98, and 4.01 TIU per mg of diet corresponding to urease activities of 0, 1.10, and 1.96, respectively. The TIU and urease activity in the crumbled diets were analyzed at 1.36, 3.46, and 4.05 TIU per mg of diet with corresponding urease activities of 0, 0.65, and 1.28, respectively. Particle size, or d_{gw}, remained similar among mash diets, ranging from 655 to 730 μm; however, crumbled diets had greater d_{gw} as LTI increased from 0 to 40% in the diet. Crumbled diets had fewer fines and greater PDI regardless of analytical method as LTI increased, indicating that pellet hardness may be responsible for difference in crumble quality evidenced by increasing d_{gw} with LTI inclusion. Bulk density remained similar among all dietary treatments for both mash and crumbled diets.

Growth Performance

There were no interactions (linear, $P > 0.05$) between LTI inclusion and feed form for BW, BWG, FI, or FCR at d 7 or 21 (Table 6). At d 14 there was a greater reduction (linear interaction, $P < 0.012$) in BW for LTI diets when fed as mash compared to crumble. BWG was reduced (linear, $P < 0.003$) with increasing LTI regardless of feed form at d 7 and 14, with no effect (linear, $P = 0.676$) on BWG from 14 to 21 d. However, residual effects from the first 2 periods resulted in an overall linear reduction (linear, $P < 0.009$) in BWG from 0 to 21 d as well as reduced (linear, $P < 0.006$) BW for the duration of the trial in response to increasing LTI regardless of feed form. Poults consuming crumbles gained more

Table 4. Physical attributes[1] of mash and crumbled pellet diets with varying levels of unheated soybeans fed to turkey poults through d 21 posthatching.[1]

	0% LTI[2]		20% LTI		40% LTI	
	Mash	Crumble	Mash	Crumble	Mash	Crumble
Bulk density, kg/m^3	676	609	637	593	611	629
Particle Size[3]						
d_{gw}, μm	739	934	732	1164	655	1637
S_{gw}	2.14	2.49	2.04	2.39	2.22	2.27
Fines, %	—	29.56	—	15.35	—	6.58
PDI,[4] %						
Standard tumble	—	59.37	—	83.50	—	91.71
Modified tumble	—	7.87	—	46.23	—	79.35
NHP100 at 30 s	—	20.35	—	68.72	—	86.10
NHP100 at 60 s	—	3.86	—	48.09	—	79.27

[1]Treatments arranged as a 3 × 2 factorial with inclusion level of the unheated low trypsin inhibitor soybean (0, 20, and 40% LTI) and feed form (mash or crumbled pellet).
[2]Inclusion level of the unheated low trypsin inhibitor soybean (LTI) in the diet.
[3]Geometric mean diameter (d_{gw}) and standard deviation (S_{gw}) calculated according ASAE standard S319.2 with agitators and dispersing agent.
[4]Pellet durability index (PDI) analyzed using the standard and modified tumble box methods according to ASAE S269.4 with five 12.7 mm hex nuts used for modification and the Holmen NHP100 (TekPro Ltd, Norfolk, UK) pellet tester set at 70 mbar forced air with 30 or 60 s run time.

Table 5. Cumulative fat recovery (%) of mash diets with varying levels of unheated soybeans fed to turkey poults through d 21 posthatching.[1]

	0% LTI[1]	20% LTI	40% LTI
As-fed[2]			
Wash 1	3.70	3.00	2.16
Wash 2	5.16	4.98	4.97
Wash 3	5.44	5.30	5.55
Wash 4	5.54	5.37	6.08
Wash 5	5.62	5.44	6.29
Wash 6[3]	5.72	5.80	6.49
Ground[4]			
Wash 1	4.93	4.03	4.21
Wash 2	6.39	5.56	6.91
Wash 3	6.63	5.79	7.49
Wash 4	6.79	5.70	7.70
Wash 5	6.90	5.97	7.97
Wash 6[3]	6.97	6.01	8.15

[1]Treatments arranged as a 3 × 2 factorial with inclusion level of the unheated low trypsin inhibitor soybean (0, 20, and 40% LTI) and feed form (mash or crumbled pellet).
[2]Diets analyzed as-fed with particle sizes from 655 to 739 μm.
[3]Recovered fat per wash is cumulative, wash 6 represents the total recovered fat after ether washes 1 through 6.
[4]Diets analyzed after being ground to pass through a 0.5 mm screen.

weight ($P < 0.001$) by d 7 and 21, with greater ($P < 0.027$) BW at d 7, 14 and 21 compared to poults consuming mash regardless of LTI inclusion. Increasing LTI inclusion in mash diets reduced (linear, $P < 0.026$) BWG overall, from d 0 to 21, and at d 7 and 14; while BWG was only reduced (linear, $P = 0.036$) when diets were crumbled. Increasing LTI reduced (linear, $P < 0.010$) feed consumption overall from d 0 to 21 and at 7 and 14 d regardless of feed form, with no evidence of difference (linear, $P > 0.101$) from 14 to 21 d. Poults consuming crumbles had greater (linear, $P < 0.033$) intake overall and at d 7 and 21 regardless of LTI inclusion. Increasing LTI inclusion in mash diets reduced (linear, $P < 0.021$) feed intake at d 7 and 14, though not at d 21 (linear, $P = 0.432$) or for the overall trial period (linear, $P > 0.090$). Increasing LTI in crumbled diets decreased (linear, $P < 0.013$) feed intake at d 14 in addition to reducing (linear, $P < 0.041$) intake for the overall trial period. There were no interactions (linear, $P > 0.244$) between LTI inclusion and feed form on FCR at d 7, 21, or the overall trial period; however, at 14 d, FCR was poorer (linear interaction, $P < 0.031$) when feeding increasing LTI in mash diets compared to crumbled diets. LTI inclusion had no effect (linear, $P > 0.082$) on FCR overall or at any period regardless of feed form. FCR was reduced ($P < 0.018$) in crumble diets compared to mash diets from 0 to 21 d; though no evidence (linear, $P > 0.060$) of a difference was observed for the individual periods at d 7, 14, or 21.

Digestibility

There was no interaction (linear, $P > 0.723$) between LTI inclusion and feed form on N_{RET} (Table 7). Crumble diets resulted in greater ($P < 0.008$) N_{RET} than mash diets, while there was no evidence (linear, $P > 0.552$) of a difference when increasing LTI. There was an interaction (linear, $P < 0.014$) between LTI and feed form on AME_n. Increasing LTI in mash diets reduced AME_n at a greater rate than when included in crumbled diets. There was an interaction (linear, $P < 0.001$) between LTI and feed form on ALD, which was linearly decreased as LTI increased regardless of feed form; however, pelleting improved digestibility over mash. Greater pancreas hypertrophy (P_{RW}) was observed with increased LTI; however, pelleted diets helped to reduce those weights (linear interaction; $P < 0.001$). There was no interaction (linear, $P > 0.452$) between LTI and feed form on AID of total amino acids (Table 8). Total amino acid digestibility was reduced as LTI inclusion increased (linear, $P < 0.001$). Mash diets had greater total amino digestibility when compared with crumbled pellets ($P < 0.023$). There was no interaction (linear, $P > 0.339$) between LTI and feed form on AID of indispensable amino acids, except for Tryp (linear, $P < 0.025$). Increasing LTI in mash diets reduced (linear, $P < 0.009$) AID of Tryp,

Table 6. Growth performance of turkey poults fed unheated low trypsin inhibitor soybeans in mash or crumbled diets from d 0 to 21 posthatching.[1]

	LTI soybean[2]												Probability, $P <$				
	0%		20%		40%			LTI soybean			Form					LTI[6]	
	M	C	M	C	M	C	SEM	0%	20%	40%	M	C	LTI[3]	Form[4]	LTI × Form[5]	M	C
BW, g																	
d0	57	58	57	58	58	58	0.7	57	58	58	57	58	0.576	0.661	0.557	0.418	0.985
d7	135	139	125	143	117	131	4.2	137	134	124	126	138	0.006	0.001	0.230	0.006	0.237
d14	321	309	276	303	252	284	8.2	315	289	268	283	298	<0.001	0.027	0.012	<0.001	0.037
d21	623	660	584	657	559	618	14.9	641	620	588	588	645	0.001	<0.001	0.477	0.004	0.052
BWG, g																	
d7	77	81	68	85	59	74	4.1	79	77	67	68	80	0.003	0.001	0.183	0.003	0.222
d14	173	169	147	155	135	152	5.6	171	151	144	152	159	<0.001	0.123	0.065	<0.001	0.036
d21	302	346	299	351	305	334	11.3	324	325	319	302	344	0.676	<0.001	0.505	0.859	0.444
d0−21	548	594	508	588	498	560	15.4	571	548	529	518	581	0.009	<0.001	0.604	0.026	0.122
FI, g																	
d7	104	110	101	113	109	98	6.5	114	105	98	100	111	0.007	0.023	0.573	0.021	0.116
d14	231	233	202	218	205	206	7.4	232	210	206	212	219	0.001	0.282	0.930	0.018	0.013
d21	493	525	482	507	477	494	17.4	509	495	486	484	509	0.101	0.033	0.581	0.432	0.122
d0−21	827	870	770	833	772	803	24.0	848	802	788	790	835	0.010	0.016	0.790	0.090	0.041
FCR, g/g																	
d7	1.44	1.43	1.45	1.34	1.59	1.40	0.08	1.44	1.40	1.49	1.50	1.39	0.470	0.107	0.244	0.184	0.750
d14	1.34	1.38	1.37	1.42	1.52	1.36	0.05	1.36	1.40	1.44	1.41	1.39	0.082	0.577	0.031	0.007	0.752
d21	1.56	1.53	1.65	1.46	1.57	1.48	0.07	1.54	1.55	1.52	1.59	1.49	0.771	0.060	0.674	0.928	0.609
d0−21	1.49	1.39	1.60	1.40	1.61	1.38	0.06	1.49	1.47	1.50	1.53	1.44	0.953	0.018	0.400	0.520	0.575

[1]At hatch, 336 turkey poults were placed in groups of 7 and reared to 21 d with 8 replicates per treatment. Treatments were arranged as a 3 × 2 factorial with inclusion level of the unheated low trypsin inhibitor soybean (0, 20, and 40% LTI) and feed form (mash or crumbled pellet).

[2]Unheated low trypsin inhibitor soybean (LTI) replaced conventional solvent extracted soybean meal on a protein basis and was fed as either mash (M) or crumble (C).

[3]Linear contrast testing the effect of increasing LTI inclusion. There were no significant quadratic effects ($P > 0.05$).

[4]Main effect of feed form.

[5]Linear contrast testing the interaction between increasing LTI inclusion and feed form. There were no significant quadratic effects ($P > 0.05$).

[6]Linear contrasts testing the effect of increasing LTI inclusion in mash (M) or crumble (C) diets, respectively. There were no significant quadratic effects ($P > 0.05$).

Table 7. Effect of unheated low trypsin inhibitor soybeans in mash or crumbled diets on retained nitrogen (N_{Ret}), apparent metabolizable energy (AMEn), apparent lipid digestibility (ALD), and relative pancreas weight (P_{RW}) of turkey poults at d 21 posthatching.[1]

	LTI soybean[2]													Probability, P <					
	0%		20%		40%			LTI Soybean			Form		LTI[3]		Form[4]	LTI × Form[5]	LTI[6]	
	M	C	M	C	M	C	SEM	0%	20%	40%	M	C	Lin	Quad			M	C
N_{Ret}[7]	2.65	2.83	2.73	2.78	2.67	2.89	0.163	2.74	2.76	2.78	2.68	2.84	0.552	0.940	0.008	0.723	0.864	0.503
AMEn, kcal/kg	3163	3198	3176	3256	3058	3231	36.6	3180	3216	3145	3132	3228	0.193	0.028	<0.001	0.014	0.009	0.383
ALD, %	91.5	93.5	86.4	91.4	79.6	88.8	0.80	92.5	88.9	84.2	85.8	91.2	<0.001	0.216	<0.001	<0.001	<0.001	<0.001
P_{RW}, g/g BW	0.34	0.32	0.43	0.38	0.54	0.44	0.009	0.33	0.41	0.49	0.38	0.44	<0.001	0.788	<0.001	<0.001	<0.001	<0.001

[1]At hatch, 336 turkey poults were placed in groups of 7 and reared to 21 d with 8 replicates per treatment. Treatments were arranged as a 3 × 2 factorial with inclusion level of the unheated low trypsin inhibitor soybean (0, 20, and 40% LTI) and feed form (mash or crumbled pellet).
[2]Unheated low trypsin inhibitor soybean (LTI) replaced conventional solvent extracted soybean meal on a protein basis and was fed as either mash (M) or crumble (C).
[3]Linear and quadratic contrasts testing the effect of increasing LTI inclusion.
[4]Main effect of feed form.
[5]Linear contrast testing the interaction between increasing LTI inclusion and feed form. There were no significant quadratic effects ($P > 0.05$).
[6]Linear contrasts testing the effect of increasing LTI inclusion in mash (M) or crumble (C) diets, respectively. There were no significant quadratic effects ($P > 0.05$) for either feed form.
[7]N_{Ret} expressed as g of N retained per 100 g of feed consumed.

while there was no observed difference (linear, $P > 0.663$) in crumbled diets. LTI inclusion reduced (linear, $P < 0.008$) AID of all other indispensable amino acids regardless of feed form. Pelleting diets reduced ($P < 0.046$) AID of indispensable amino acids regardless of LTI, with the exceptions of His and Thr where there was no evidence of difference ($P > 0.066$). There was no interaction (linear, $P > 0.139$) between LTI and feed form on AID of dispensable amino acids, except for Hyl (linear, $P < 0.017$). Increasing LTI in mash diets reduced (linear, $P < 0.001$) AID of Hyl, while there was no observed difference (linear, $P > 0.543$) in crumbled diets. LTI inclusion reduced (linear, $P < 0.014$) AID of all other dispensable amino acids regardless of feed form. Pelleting diets reduced ($P < 0.049$) AID of dispensable amino acids regardless of LTI, with the exceptions of Ala, Gly, Orn, and Ser where there was no evidence of difference (linear, $P > 0.058$).

DISCUSSION

LTI inclusion appeared to have a greater impact on poult performance early in the trial. BWG was linearly decreased at 7 and 14 d in response to increasing LTI, but poults gained the same at d 21. It is possible that the developing digestive systems of the younger poults struggled early on to digest the LTI diets, but were beginning to equilibrate as the trial was concluding. Based on the relevant literature, poult digestive systems continue developing through 21 d of age and have an increased ability to handle TI as they age (Krogdahl and Sell, 1988; Mian and Garlich, 1995). Perhaps, in the study herein, if birds were allowed to continue maturing, performance would have continued to improve. The improvements in BWG from 14 to 21 d were not substantial enough to compensate for prior losses and are reflected in lower d 21 BW for poults fed LTI diets. Poults consuming 20% LTI in crumbled diets experienced a 1% reduction in 21 d cumulative BWG compared to a 5.7% reduction in cumulative BWG for poults consuming a crumbled diet with 40% LTI. Poults consuming mash diets experienced cumulative reductions in BWG of 7.3% for 20% LTI and 9.1% for 40% LTI diets. Pelleting the diets with LTI resulted in less reduction in BWG compared to mash diets especially with 20% LTI. A similar, although inverse, pattern was observed for P_{RW}. It is possible that including more treatments, such as including 10 and 30% LTI diets, a more definitive response to dietary LTI could have been elicited and observed.

Feed intake was linearly reduced with increasing levels of LTI through 14 d of age. This agrees with work reported by other researchers who also observed reduced consumption with inclusion of LTI soybeans fed at various levels lower or within the same range of this trial (Chohan et al., 1993; Herkelman et al., 1993; Perez-Maldonado et al., 2003). Perez-Maldonado et al. (2003) observed a 10% reduction in 23-d chick consumption with 17% inclusion of an LTI soybean in mash diets,

Table 8. Effect of unheated low trypsin inhibitor soybeans in mash or crumbled diets on apparent ileal digestibility (%) amino acid in turkey poults at d 21 posthatching.[1]

	LTI soybean[2]												Probability, $P <$				
	0%		20%		40%			LTI soybean			Form					LTI[6]	
	M	C	M	C	M	C	SEM	0%	20%	40%	M	C	LTI[3]	Form[4]	LTI × Form[5]	M	C
Indispensable AA, %																	
Arg	82.8	78.2	80.4	77.7	76.9	73.9	1.8	80.5	79.0	75.4	80.0	76.6	<0.001	0.004	0.551	0.005	0.025
His	77.7	73.4	74.8	74.0	71.0	68.7	2.1	75.5	74.4	69.9	74.5	72.0	<0.001	0.061	0.518	0.006	0.036
Ile	76.2	71.8	72.2	69.3	67.3	64.6	2.1	74.0	70.7	65.9	71.9	68.6	<0.001	0.023	0.627	0.001	0.003
Leu	75.0	70.5	71.1	68.5	66.4	63.9	2.2	72.7	69.8	65.1	70.8	67.6	<0.001	0.025	0.561	0.002	0.007
Lys	76.6	70.7	72.8	69.6	68.0	64.7	2.7	73.7	71.2	66.4	72.5	68.4	0.001	0.018	0.535	0.007	0.036
Met	85.0	80.0	83.5	78.9	80.3	76.4	1.7	82.5	81.2	78.3	82.9	78.4	0.008	<0.001	0.717	0.037	0.078
Phe	76.6	72.5	73.1	70.5	68.9	65.5	2.1	74.5	71.8	67.2	72.9	69.5	<0.001	0.015	0.848	0.003	0.003
Thr	68.2	62.8	65.0	62.4	59.1	57.7	2.7	65.5	63.7	58.4	64.1	61.0	0.001	0.066	0.339	0.005	0.072
Trp	77.2	77.1	73.4	76.2	70.3	78.1	2.0	77.2	74.8	74.2	73.6	77.2	0.091	0.015	0.025	0.009	0.663
Val	72.7	67.7	68.3	65.7	62.8	60.8	2.5	70.2	67.0	61.8	67.9	64.8	<0.001	0.046	0.425	0.001	0.010
Dispensable AA, %																	
Ala	73.5	68.6	69.1	66.8	64.2	63.1	2.4	71.1	67.9	63.6	68.9	66.2	<0.001	0.078	0.305	0.002	0.037
Asp	74.4	69.5	71.6	69.7	68.5	66.0	2.0	71.9	70.6	67.2	71.5	68.4	0.003	0.017	0.447	0.012	0.089
Cys	54.2	45.8	51.6	48.7	44.2	43.2	3.2	50.0	50.1	43.7	50.0	45.9	0.014	0.049	0.139	0.009	0.436
Glu	80.1	76.1	78.1	76.2	75.3	73.1	1.6	78.1	77.2	74.2	77.8	75.1	0.003	0.012	0.475	0.013	0.083
Gly	70.5	65.9	67.2	64.5	62.1	60.7	2.2	68.2	65.8	61.4	66.6	63.7	<0.001	0.058	0.397	0.004	0.041
Hyl	81.9	58.9	74.9	65.6	56.3	55.3	7.3	70.4	70.2	55.8	71.0	59.9	0.002	0.005	0.017	<0.001	0.543
Orn	67.6	45.8	41.2	41.3	30.5	25.9	6.8	56.7	41.3	28.2	46.4	37.7	<0.001	0.063	0.141	<0.001	0.014
Pro	76.2	71.2	73.7	71.5	69.7	68.0	1.7	73.7	72.6	68.8	73.2	70.2	<0.001	0.011	0.224	0.002	0.089
Ser	74.1	71.5	71.0	71.0	66.3	64.2	2.0	72.8	71.0	65.3	70.5	68.9	<0.001	0.218	0.849	0.001	0.001
Tyr	76.9	71.7	71.9	69.0	66.7	64.3	2.2	74.3	70.5	65.5	71.9	68.3	<0.001	0.015	0.430	<0.001	0.003
Total	75.5	70.7	72.3	70.1	68.2	65.9	1.9	73.1	71.2	67.1	72.0	68.9	<0.001	0.023	0.452	0.004	0.036

[1]At hatch, 336 turkey poults were placed in groups of 7 and reared to 21 d with 8 replicates per treatment. Treatments were arranged as a 3 × 2 factorial with inclusion level of the unheated low trypsin inhibitor soybean (0, 20, and 40% LTI) and feed form (mash or crumbled pellet).

[2]Unheated low trypsin inhibitor soybean (LTI) replaced conventional solvent extracted soybean meal on a protein basis and was fed as either mash (M) or crumble (C).

[3]Linear contrast testing the effect of increasing LTI inclusion. There were no significant quadratic effects ($P > 0.05$).

[4]Main effect of feed form.

[5]Linear contrast testing the interaction between increasing LTI inclusion and feed form. There were no significant quadratic effects ($P > 0.05$).

[6]Linear contrasts testing the effect of increasing LTI inclusion in mash (M) or crumble (C) diets, respectively. There were no significant quadratic effects ($P > 0.05$).

while the variety utilized in this trial resulted in only a 7% reduction with 40% LTI inclusion in mash diets. While it is likely that the differences observed in feed intake can be largely attributed to the ANF in the LTI soybean, pellet quality may have also been a contributing factor to low intake of crumbled diets at 14 d. Increasing LTI inclusion from 0 to 40% resulted in measured standard PDIs of 59, 84, and 92% and corresponded to fines levels of 30, 15, and 7%, respectively. The increased pellet hardness of the LTI diets likely influenced the final crumble size of the diets. For comparison, mash diets averaged 709 μm, while for crumbled diets the particle size was measured to be 934, 1164, and 1637 μm as LTI inclusion increased. Thus, in addition to potential palatability issues with a harder crumble in the LTI diets, the crumble size may have also been a deterrent for the young poult.

Overall, pelleting LTI diets improved growth performance compared to mash LTI diets. Pelleting the diets increased the AMEn and ALD to a greater degree as LTI inclusion increased. This was in contrast to observed dietary TI, which was shown to increase from mash to pelleted diets. Measured TI levels increased from 1.04 to 1.36, 2.98 to 3.46, and 4.01 to 4.05 TIU per mg in mash and pelleted diets as LTI increased from 0, 20, and 40%, respectively. This increase remains unsupported by the digestibility data or pancreas weights, which were 13.6% smaller in crumbled diets than mash. Furthermore, Perez-Maldonado et al. (2003) observed a decrease in TI and P_{RW} with increased AMEn as diets were steam conditioned at 85°C for approximately 30 s prior to pelleting. Under the similar pelleting conditions of this trial, it may have been expected to observe a similar decrease in TI and least of all, the observed increase.

The improvements in ALD with pelleting are likely the result of increased availability of the oil from the soybeans. Carew et al. (1961) reported that mechanical processes such as pelleting help to rupture the fat cells in the soybean structure, making them more available for digestion by poultry. Thus, the impact of pelleting is much greater in diets containing increased levels of LTI with more unruptured fat cells. The addition of supplemental oil also likely affected ALD. Supplemental oil was decreased as LTI level increased due to the natural oil content of the soybeans. Poults consuming diets with greater supplemental oil had greater ALD in the mash diets. This would agree with fat availability predictions made using the petroleum ether washes, where greater fat was initially recovered in early washes with diets receiving supplemental oil addition, indicating possibly greater availability for digestion.

Digestible amino acids were linearly decreased with increased LTI inclusion and agree with findings by Han et al. others (1991). This may have been a result of increased endogenous losses of amino acids due to increased hypersecretion by the pancreas at increasing levels of LTI. Heat application during pelleting may have also decreased the availability of some of the amino acids, especially lysine (Sibbald, 1980). The reduction in digestible amino acids should be considered when formulating diets with synthetic amino acids to ensure proper protein utilization of the LTI soybean.

Based on the results of this study, pelleting diets can improve the growth performance of poults consuming LTI soybeans compared to those fed mash. Pelleting was shown to improve nutrient utilization, allowing for up to 20% inclusion of LTI soybean in the diet. It may be possible to further increase LTI inclusion in pelleted diets for more mature birds and warrants further investigation.

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DISCLOSURES

The authors declare no conflicts of interest.

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