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Practical Strategies to Maximize Cockatiel Health and Broiler Chicken Performance

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Practical Strategies to Maximize Cockatiel Health and Broiler Chicken Performance

by

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Abstract

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There are various methods of processing avian diets. However, the two common methods of processing include grinding, batching, mixing, and feeding the resultant mash that is not thermally processed, extrusion processing, baking, and steam conditioning and pelleting. The method of processing utilized can greatly affect nutrient availability, therefore affecting the health and/or performance of the avian. The objective of the first study was to transition cockatiels (*Nymphicus hollandicus*) that were housed in a controlled experimental setting, with a standardized transition strategy, using two different commercially available cockatiel diets that were advertised as nutritionally complete. The transition strategy consisted of gradually increasing the ratio of complete diet to seed-based diet over a 12d period. True amino acid digestibility was determined for each complete diet and demonstrated that both diets contained highly digestible amino acids. Fourteen cockatiels individually housed in acrylic cages were transitioned to one of the complete diets (Diet A or B). Diet A and B differed in ingredient composition, ingredient particle size, analyzed nutrients, and method of processing (baked or extruded). It may be speculated that cockatiels were more accepting of baked Diet A during transition and Diet A more efficiently maintained BW. The objective of the second study was to determine the effects of pelleting, inclusion of a *Bacillus* derived keratinase, and bird sex on broiler performance. The keratinase was batched and mixed prior to steam conditioning and pelleting and therefore must be thermally stable to demonstrate efficacy. This study consisted of a 2 x 2 x 2 factorial arrangement of treatments in a randomized complete block design; there was either a ground pelleted or mash diet with or without keratinase supplementation fed to a pen of either male or female Cobb x Cobb 500 broiler chicks. Mash feed was short-term conditioned to a temperature of 82^{o}C and extruded through a 38.1 x 4.76 mm pellet die using a 40 HP California Pellet Mill. Males had enhanced broiler performance as compared to females. The thermal conditions of the pelleting process decreased broiler performance likely due to decreasing nutrient availability. Keratinase activity was reduced due to thermal conditions of pelleting that may have contributed to the lack of enzyme efficacy with respect to broiler performance.

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TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS iv

LIST OF TABLES v

LIST OF FIGURES vi

KEY vii

CHAPTER 1: *Literature Review* 1
- Cockatiel Nutrition 1
- Effect of Bird Sex on Broiler Performance 4
- Feed Manufacture, Feed Form, and Broiler Performance 4
- Keratinase Supplementation 6
- References 9

CHAPTER 2: *Cockatiel transition from seed-based diet to complete diets* 13
- Abstract 14
- Introduction 15
- Materials and Methods 16
- Results 19
- Discussion 20
- References 21
- Tables and Figures 22

CHAPTER 3: *Influence of pelleting on the efficacy of a Bacillus derived keratinase in broiler diets* 30
- Summary 31
- Description of Problem 32
- Materials and Methods 34
- Results and Discussion 37
- Conclusions and Applications 40
- References 41
- Tables and Figures 43

CURRICULUM VITAE 49

LIST OF TABLES

CHAPTER 2

TABLE 1. Selected nutrient analyses of the complete diets and the seed-based diet 22
TABLE 2. Cockatiel transition strategy from a seed-based diet to a complete diet over a 12d period 23
TABLE 3. Digestible Amino Acid Content.....24
TABLE 4. Plasma chemistry and hematology results prior to the feed transition period, d15, and d29.....25

CHAPTER 3

TABLE 1. Ingredients, calculated nutrients, and analyzed nutrients for Cobb 500 broiler starter diets 43
TABLE 2. Ingredients, calculated nutrients, and analyzed nutrients for Cobb 500 broiler grower diets 44
TABLE 3. Ingredients, calculated nutrients, and analyzed nutrients for Cobb 500 broiler finisher diets 45
TABLE 4. Feed manufacture descriptive data, physical attributes, and enzyme activity for Cobb 500 broiler starter, grower, and finisher diets 46
TABLE 5. The effect of sex, keratinase, and level of processing on d3-39 broiler performance .48

LIST OF FIGURES

CHAPTER 2

FIGURE 1. Complete diet consumption during the transition period (d1-11) 26
FIGURE 2. Seed-based diet consumption during the transition period (d1-11).....27
FIGURE 3. Total consumption of feed (complete diet and seed-based diet) over 28d 28
FIGURE 4. Cockatiel weight measured periodically over a 47d period 29

KEY

CHAPTER 1

1. Red Blood Cell Count - RBC
2. Packed Cell Volume - PCV
3. Aspartate Aminotransferase - AST
4. Creatine Phosphokinase - CPK
5. Uric Acid - UA
6. Feed Conversion Ratio - FCR
7. Live Weight Gain - LWG
8. Feed Intake - FI
9. Pellet Quality - PQ
10. Pellet Durability Index - PDI
11. Modified Pellet Durability Index - MPDI

CHAPTER 2

1. Feed Intake - FI
2. Body Weight - BW

CHAPTER 3

1. Feed Intake - FI
2. Live Weight Gain - LWG
3. Feed Conversion Ratio - FCR
4. Ground Pellet - GP
5. Positive Control Diet Formulation - PC
6. Negative Control Diet Formulation - NC

CHAPTER 1: LITERATURE REVIEW

1. COCAKTIEL NUTRITION

Cockatiels (*Nymphicus hollandicus*) are small granivores (seed consumers) in the order Psittaciforme. The selection of the seed in the diet depends on many variables, such as ease of husking, toxin content, amino acid balance, and overall calorie content. Cockatiels have such diet selectivity due to their ability to fly to different food sources. Therefore, cockatiels in the wild have the ability to balance their diet components, such as proteins and lipids. In order to balance their diet during lack of seed availability, cockatiels have been known to eat grasses, leaves, and even insects. Time periods in which cockatiels face seed scarcity include early winter and early spring. During these time periods, they also tend to consume seeds with a higher fat content [1].

Cockatiels kept in captivity do not have the same ability to be nutritionally selective as in the wild. In captivity, cockatiels cannot fly to different food sources, therefore can only consume what is provided. Traditionally cockatiels in captivity have been fed seed-based diets. Seed-based diets may have a poor amino acid balance or a poor protein to calorie ratio, which both promote fat deposition. Also, even a marginal deficiency in methionine or lysine can cause greater fat deposition. This increased fat deposition may not be as detrimental in the wild due to adequate exercise [1]. However, during captivity cockatiels have limited ability to fly or exercise, therefore obesity is more prevalent.

In captivity, cockatiels are generally fed nutritionally incomplete seed-based diets. These seed-based diets are also deficient in many essential nutrients, such as some amino acids, calcium, available phosphorus, sodium, and some vitamins and minerals [2-4]. Vitamin A and D_3, and calcium deficiencies are among the most commonly seen ailments in companion birds. Deficiencies in crude protein are not seen as often [4]. Despite protein deficiencies being

uncommon, many seed-based diets have adequate crude protein comprised of high levels of nonessential amino acids. Therefore, these diets need to be supplemented with a higher quality amino acid profile [5]. These nutrient deficiencies can be best remedied through transitioning the bird to a nutritionally complete diet [4].

However, many companion birds are neophobic to a new diet. Also, cockatiels tend to eat only the higher fat content seeds, such as the sunflower seeds, which are energy dense and nutritionally imbalanced; containing low calcium, protein, iodine and carotene [4]. Also, because cockatiels only eat to satisfy their energy needs, eating a highly energy dense feed decreases the cockatiels overall intake. The lower intake then contributes to inadequacies in other nutrients [5]. To overcome the issue of nutritionally imbalanced seed-based diets, companies have produced seed-based diets with several nutrients sprayed on seeds or new diets with seeds supplemented with pellets [4]. A pellet is a feed form achieved by extruding a steam conditioned mash diet through a die of a certain size. However, the sprayed on nutrients are on the hulls, which are not consumed, and the pellets may not be ingested due to the selective nature of the birds [4].

The newly introduced complete diet should be based on nutritional ecology and diets of wild Psittaciforme birds, knowledge of the gastrointestinal anatomy and physiology, and nutrient requirements of species. With this information being kept in mind, the likelihood of successfully transitioning the birds should be greater. There are many complete diets on the market for companion birds, which all differ in size, color, ingredient composition, nutrient profile, and processing. The beaks and gastrointestinal tract of a bird are specially designed for a certain type of feed and size and may reject unfamiliar feeds. Furthermore, the cockatiel prefers soft, young seeds instead of harder, more mature seeds [6]. Color affects the bird's selection of food. Previous studies show that birds have accepted diets of one color and rejected the same diet in

another color. Size of the diet also affects the acceptance of a new diet [4]. The nutrient profile of most complete diets on the market is determined using recommended amino acid requirements for poultry. These requirements are used for fast growth in poultry, which are harvested at a young age, but companion birds are raised for slow growth and longevity [1].

There have been several transition strategies used in previous studies. For example, there are some transition strategies that incorporate feedings throughout the day that offer either the seed-based diet or the new complete diet. These transition strategies never expose the bird to the two different feeds and that same time. However, it may more beneficial to have the bird exposed to both the seed-based and complete diet at the same time throughout the day as suggested in some other transition strategies. Therefore, the birds will become accustomed to a new diet gradually. Despite different types of transition strategies there are some commonalities. The most important commonality is that most transition strategies suggest gradually transitioning the birds to the new diet, as opposed to an abrupt change in diet. Gradual transitions over weeks or months are generally more accepted, [4] because as previously stated birds tend to be neophobic to new diets.

In order to monitor the health status during transition, a researcher may collect plasma or serum samples to analyze blood chemistry and hematology. The difference between serum and plasma is that plasma contains clotting factors and an anticoagulant not found in serum. Plasma is easier to collect and therefore more frequently used in avian clinical settings. Also, plasma collection results in a larger sample volume compared to serum. Ultimately, the choice between plasma or serum collection is determined by what sample the specific laboratory prefers [7]. For example, common parameters that are used to monitor health status include, red blood cell count (**RBC**), packed cell volume (**PCV**), glucose, calcium, phosphorus, cholesterol, aspartate

aminotransferase (**AST**), creatine phosphokinase (**CPK**), uric acid (**UA**), and many others. RBC and PCV can both be used to determine dehydration or blood loss. Increased blood glucose can suggest diabetes mellitus or general stress, which are common ailments in companion birds. Calcium, phosphorus, and cholesterol content in the blood can demonstrate various nutritional disorders, which are also very common in captive companion birds [8]. AST is used in conjunction with CPK in order to indicate presence of liver or muscle damage and further distinguish between the two types of tissue damage [9]. Increased UA can suggest renal disease, gout, dehydration, starvation, and many other health concerns. Decreased UA can suggest end-stage liver disease [8]. These parameters can be used in conjunction with many others to determine the overall health of the avian species in question.

2. EFFECT OF BIRD SEX ON BROILER PERFORMANCE

It is well documented that male broilers have improved performance compared to female broilers. Lilly and coauthors [10] examined the effects of bird sex on broiler performance. Males had enhanced feed conversion ratio (**FCR**), live weight gain (**LWG**), feed intake (**FI**), and breast weight a when compared to females. Also, Han et al. [11] found that males require a higher amount of lysine compared to females, due to increased muscle accretion. Similar results were found in numerous other publications.

3. FEED MANUFACTURE, FEED FORM, AND BROILER PERFORMANCE

Poultry diets were traditionally fed as a mash form. However, modern poultry feed is predominantly pelleted. The cost of this feed manufacture and the feed itself comprises about 60-70% of the total cost of broiler production [12]. However, there are many reported benefits of

pelleting, such as an improved FCR, LWG, and better handling characteristics, which may rationalize the high costs of feed manufacture. Also, there is a reported health benefit to pelleting, for instances the high heat and steam may act as a microbicide against common foodborne pathogens, such as *Salmonella sp.* [13].

Despite many researchers having found benefits of pelleting, evidence exists that pelleting can cause detriment to broiler performance. There are many speculations that the pelleting process decreases nutrient availability. The high temperature of pelleting can cause protein denaturation and maillard complexing [14]. Loar and coauthors demonstrated that FCR increases as conditioning temperature increases [15]. Previous research also demonstrates that birds fed ground pelleted diets had a higher FCR than those fed unprocessed mash diets [16].

The quality of the pellet produced can affect broiler performance [10]. Pellet quality (**PQ**) is the ability of the pellet to maintain its structural integrity from manufacture to feeding. PQ can be measured in a variety of ways, including pellet durability index (**PDI**) and modified pellet durability index (**MPDI**). PDI measures the ratio of fines to pellets after standardized mechanical handling [12]. MPDI is a harsher way of measuring PDI, in hopes of mimicking the harsh conditions of shipment, moving through the auger in a poultry house, etc. PDI and MPDI can be measured through the Pfost tumbling can method and through the New Holmen Pellet Tester method. Pellet quality can be affected by many variables, including ambient temperature, conditioning temperature, and production rate throughout the manufacturing process.

Particle size can also affect broiler performance. Particle size can be measured using a Ro-Tap particle size analyzer. With a smaller particle size, there is a greater surface area, which can increase digestibility [17]. However, broiler chickens have been shown to have a preference for larger particle sizes [18]. Increased digestibility may be due to increased ability of

endogenous and exogenous enzymes to act on the feed [19]. Also, a larger particle size will reduce prehension energy leading to enhanced broiler performance [20]. Although particle size is important, it is found to be more important in mash diets and not pelleted diets [21-27]. Despite published significant effects of particle size on broiler performance, some literature demonstrates that particle size does not affect performance [22, 27]. In order to achieve a specific particle size, the feed may have to be ground using a hammer mill or a roller mill. A hammer mill reduces particle size by striking the feed with a set of hammers until it can pass through a screen of a certain size. A roller mill achieves a certain particle size by compression force by moving the feed through a set of horizontal rollers a set gap distance [27]. The hammer mill produces a higher amount of fines [21], which could negatively affect performance.

Diet formulation can affect pellet quality, and consequently broiler performance. When added fat is increased, pellet quality is decreased due to lubrication of the pellet die leading to decreased pellet binding [29]. Increases in added moisture increase pellet quality, [30] due to increased starch gelatinization [31]. Also, moisture can affect protein denaturation during processing [30].

4. KERATINASE SUPPLEMENTATION

A traditional poultry diet is comprised of corn and soybean meal. Although corn contains a low amount of dietary protein, it is the main source of protein due to a high percentage in the diet. Also, despite soybean meal being highly digestible, it only comprises a low amount of protein in the diet [32, 33]. Therefore exogenous proteases have been incorporated in poultry diets in order to alleviate problems with protein digestibility. If an added exogenous protease allows for better protein digestion, the overall protein content of the feed can be lowered; contributing to lower

broiler production costs and less nitrogen excreted into the manure [34]. Lower nitrogen content in the manure reduces the amount of environmental pollution caused by the poultry industry. Also, better protein digestion by protease supplementation can improve broiler performance and carcass yields [35-37]. It is proposed that an exogenous protease makes a higher amount of dietary amino acids available to the broiler [37].

The exogenous proteases incorporated into poultry feeds work by hydrolyzing proteins. Specifically the proteases called keratinases are commonly used in the poultry industry. Of the bacteria derived keratinases, *Bacillus spp.* seems to possess the highest amount of different keratinases [38]. Keratinases have a broad range of activities, not only degrading insoluble keratin [39-41]. It is reported that reducing agents aid in the function of the keratinase; allowing for more substrate sites for the keratinase, ultimately enhancing degradation of the protein [42].

Keratinases production is often initiated by the presence of a keratin substrate, such as poultry feathers, in a cultivation medium containing a *Bacillus spp.* However, keratinases can be produced from non-keratinous substrates as well, such as soybean meal and casein [40-43]. A *Bacillus sp.* can be produced by growing the *Bacillus sp.* in a fermenter followed by centrifuging and collecting the supernatant. Furthermore, the supernatant is spray-dryed to isolate the keratinase in order to add it to a poultry diet in a dry form [37]. A specific assay frequently used to detect the activity of a keratinase is the azocasein assay. The keratinase hydrolyzes the azocasein and releases the azo-molecule from the casein. Resulting activity is determined by measuring the absorbance of the azo-molecule using spectrophotometry. The researcher may modify the assay in order to eliminate interference of the keratin substrate with the azocasein [44].

Most keratinases in broiler feeds are added at the mixer, which exposes them to the thermal conditions of pelleting; high temperature, pressure, and steam. Also, during extrusion through the pellet die the feed is exposed to harsh mechanical forces. Such conditions can denature the protein's native structure causing a highly disordered structure and loss of function of the keratinase [45]. Eeckhout and coauthors concluded that most enzyme denaturation during processing occurs during steam conditioning instead of during extrusion through the pellet die [46]. For example, a typical conditioning temperature for poultry feeds is 85^{o}C, however the optimal temperature for many *Bacillus spp.* keratinases is approximately 55^{o}C [38]. On the contrary, some studies conclude that enzyme deactivation is due to frictional heat and pressure associated with extrusion through a pellet die [47, 48]. Therefore, the thermal stability of the keratinase must be considered because poultry feed is predominately pelleted in the United States.

Previous studies on the effects of keratinases have shown various results. Stark and coauthors [49] have shown that keratinase supplementation improved LWG and FCR in low-protein diets as compared to diets with adequate protein, despite decreased keratinase activity in the feed. However, feed intake was not affected by the keratinase. In a study by Odetallah and coauthors [50], keratinase supplementation improved FCR and LWG in adequate and low-protein diets. Angel and coauthors [51] concluded that keratinase supplementation improved protein digestibility and consequently improved broiler performance. Freitas and coauthors [52] demonstrated that keratinase supplementation caused a slight improvement in FCR however did not improve LWG. Another study showed the effects of different proteases, one protease did not improve performance whereas another protease did improve performance [53].

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CHAPTER 2: COCKATIEL TRANSITION FROM SEED-BASED DIET TO COMPLETE DIETS

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ABSTRACT

The objective of the study was to transition cockatiels (*Nymphicus hollandicus*) that were housed in a controlled experimental setting, with a standardized transition strategy, using two different commercially available cockatiel diets that were advertised as nutritionally complete. Prior to the transition, cockatiels were fed a seed-based diet for 30d. The transition strategy consisted of gradually increasing the ratio of complete diet to seed-based diet over a 12d period. True amino acid digestibility was determined on each complete diet and demonstrated that both diets contained highly digestible amino acids. Fourteen cockatiels individually housed in acrylic cages were transitioned to one of the complete diets (Diet A or B). Diet A and B differed in ingredient composition, ingredient particle size, analyzed nutrients, and method of processing (baked or extruded). Daily feed intake (**FI**) of seed-based and complete diets was measured. Periodically throughout the transition period and post transition, body weight (**BW**) and measures of plasma chemistry were obtained. All cockatiels accepted the transition strategy irrespective of complete diet. Cockatiels transitioned to Diet A consumed significantly more of the complete diet and less of the seed-based diet during periods of measurement throughout transition. Total FI was significantly greater for cockatiels fed Diet B for one third of the measurement periods ($P < 0.05$). Cockatiel BW was generally not affected due to complete diet utilized during or after transition ($P > 0.05$). Plasma chemistry and hematology results did not differ between cockatiels fed the two complete diets ($P > 0.05$). It may be speculated that cockatiels were more accepting of Diet A during transition and Diet A more efficiently maintained BW.

Key words: transition, nutrition, seed-based diet, complete diet, *Nymphicus hollandicus*

INTRODUCTION

Psittaciforme birds kept as companion animals are often provided nutritionally incomplete seed-based diets and develop deficiencies in one or more essential nutrients.[1,2] Utilization of a complete diet based on nutritional ecology and diets of wild psittaciforme birds, knowledge of the gastrointestinal anatomy and physiology, and nutrient requirements of the species or related species may allow for greater success with the rearing and propagation of captive Psittaciforme birds.[3] If birds are currently reared on seed-based diets then a transition strategy to a complete diet should be considered. In addition, commercially available diets advertised as nutritionally complete vary greatly in ingredient composition, ingredient particle size, nutrient profile, and method of processing. Limited data exists on the effectiveness of different commercially available diets to transition Psittaciforme birds from nutritionally incomplete seed-based diets. The purpose of this study was to transition cockatiels (*Nymphicus hollandicus*) that were housed in a controlled experimental setting, with a standardized transition strategy, using two different commercially available cockatiel diets, advertised as nutritionally complete.

MATERIALS AND METHODS

True amino acid digestibility

A total of 16 cecectomized single comb white leghorn roosters placed in individual raised wire cages to comprise a completely random design. True amino acid digestibility was estimated through modified methodologies of Sibbald.[4] Roosters were fasted for 24 h then a 30 g sample of each diet was precision fed to eight replicate roosters. Excreta were collected 48 hrs after precision feeding. Excreta samples were lyophilized, weighed and ground. The feed and excreta samples were analyzed in a commercial laboratory for amino acid content.

Birds and housing

Fourteen cockatiels of approximately two years of age and from the same commercial breeder were used in the experiment. Cockatiels were individually housed in acrylic cages (52.7 cm x 43.2 cm x 40.6 cm) within an environmentally controlled room. Temperature was maintained at 24^{o}C and lighting was continuous for 12 h per day. Each cage contained an acrylic feed hopper designed to minimize feed wastage, an acrylic water fount, wooden perches, and various toys to enhance social and physical well-being. All cages were positioned on large plastic trays to capture any feed wastage and allow for accurate feed intake measurement. Cages were cleaned daily. Feeding behavior using complete diets was mimicked, and birds were handled daily. Birds were weighed twice per week throughout the experiment.

Transition strategy and diet

One of two commercially available complete cockatiel diets was assigned to seven replications of caged cockatiel. Treatment allocation was blocked by location of the cage in the room. All birds were provided a commercially available seed-based diet for ad libitum consumption, 30

days prior to the initiation of the transition period. Water was provided for ad libitum consumption throughout the experiment. Each diet used in the experiment was analyzed for selected nutrients (Table 1).

The transition strategy took place over a 12d period (Table 2). Diets were provided in 60g total allotments each morning of the transition period. On the following morning, the amount of seed-based diet and complete diet were recorded in both the feed hopper and catch tray. After the 12d transition period, the birds remained on complete diets and FI and BW measurements continued to be recorded for an additional 16 and 35d, respectively.

Blood collection and analysis

Approximately 0.5 ml of blood was collected from each bird's left jugular or medial metatarsal vein prior to the transition period, as well as on D15, and D29 of the experiment. The blood was collected at the same time each day and the collection was completed in the same amount of time to reduce variation. The samples were sent to a commercial analytical laboratory (Avian Exotic Clin Path Labs) to obtain values for plasma chemistry and hematology. Measurements included white blood cell count, red blood cell count, packed cell volume, mean corpuscular volume, hemoglobin, mean corpuscular hemoglobin concentration, total protein, heterophil/neutrophil percentage, lymphocyte percentage, basophil percentage, eosinophil percentage, monocyte percentage, calcium, phosphorus, lactate dehydrogenase, creatine phosphokinase, chloride, uric acid, glucose, cholesterol, aspartate aminotransferase, and bile acid. In the event there was an insufficient plasma volume to complete blood chemistry assays, statistical analysis was run on fewer replications. All procedures were approved by the West Virginia University Animal Care and Use Committee.

Statistical Analysis

Data were analyzed using a completely random and randomized complete block design for the digestibility and transition study respectively. Data were analyzed using the GLM procedure of Statistical Analysis System.[5] Alpha was designated as 0.05. Statistical analysis was performed at each time period that measured variables were collected during the experiment.

RESULTS

True amino acid digestibility data demonstrated that Diet A contained higher digestible arginine, leucine, isoleucine, tryptophan, alanine, histidine, proline, aspartic acid, and glutamic acid ($P < 0.05$, Table 3). Diet B contained higher digestible methionine, threonine, and lanthionine ($P < 0.05$, Table 3).

All cockatiels accepted the transition strategy irrespective of complete diet. Cockatiels transitioned to Diet A consumed significantly more of the complete diet and less of the seed-based diet during periods of measurement throughout transition (Fig 1 and 2). Total FI was significantly greater for cockatiels fed Diet B for one third of the measurement periods (Fig 3). Cockatiel BW

was generally not affected due to complete feed utilized during or after transition ($P>0.05$, Fig 4). Plasma chemistry and hematology results did not differ between cockatiels fed the two complete diets (Table 4).

DISCUSSION

Despite significant differences among digestible amino acids of the complete diets, a majority of the levels provided by each diet were within 90% of the recommended total amino acid levels of broiler chicken finisher diets.[6] These data suggest that the complete diets were likely not deficient in amino acid content. However, higher digestible amino acid levels may alleviate performance and health detriments due to stress.[7]

Cockatiel transition from a seed-based diet to each complete diet was successful based on consumption, maintenance of body weight, lack of change in plasma chemistry and hematology, and no observed morbidity or mortality. Based on complete diet and seed-based diet consumption per se, it may be speculated that cockatiels were more accepting of Diet A during transition and Diet A more efficiently maintained BW. Diet A differed from Diet B in terms of ingredient composition, ingredient particle size, nutrient profile, and method of processing. Perhaps these variations made Diet A more acceptable or enhanced nutritional value, thus improving efficiency of weight maintenance. Diet A was composed of larger ingredient particle size and formed into a larger feed kibble, providing greater diet structural component compared to the small particle size and kibble size of Diet B. Past research focusing on poultry suggests that birds have a requirement for structural components that lead to increased nutrient utilization and performance.[8] Another possibility for variation in Diet effect would be the method of processing for each diet; Diet A was baked and Diet B was extruded. Feed processing that combines shear forces, heat, residence time, and water may result in partial protein denaturation[9] and may result in changes in protein availability to a non-ruminant animal.[10] Indeed, differences in thermal processes between baking and extruding in the current study could have affected nutrient availability.

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Table 1. Selected nutrient analyses of the complete diets and the seed-based diet.[a]

Measurement	Seed-based diet	Diet A	Diet B
Protein, Kjeldahl (N x 6.25) (%)	12.1	15.4	16.9
Calcium (%)	0.291	1.19	0.423
Phosphorous (%)	0.386	0.519	0.449
Phytic acid, ion exchange (%)	0.854	0.791	1.00
Non- phytate phosphorus[b] (%)	0.145	0.296	0.167
Sodium (ppm)	300	1210	1240
Vitamin A retinol and esters (IU A/kg)	1016	3681	7119
Vitamin D_2 (IU D/g)	< 0.5	< 0.5	< 0.5
Vitamin D_3 (IU D/g)	< 0.5	1.25	1.05

[a]Diets were analyzed at NP Analytical Laboratories, St. Louis, MO 63164
[b]% NPP was calculated by (% Total Phosphorus - (0.282 x % Phytic Acid))

Table 2. Cockatiel transition strategy from a seed-based diet to a complete diet over a 12d period. A 60g total allotment of feed was provided each day.

Day	Seed-based diet (%)	Complete diet (%)
1	90	10
2	80	20
3	70	30
4	60	40
5	50	50
6	50	50
7	50	50
8	40	60
9	30	70
10	20	80
11	10	90
12	0	100

Table 3. Digestible Amino Acid Content.[a]

Digestible amino acid (%)	Diet A	Diet B	SEM	P-value
Taurine	0.0222	0.0401	0.0153	0.4227
Aspartic Acid	1.3263	1.1069	0.0193	<.0001
Threonine	0.5063	0.6606	0.0117	<.0001
Serine	0.5622	0.5774	0.0090	0.2519
Glutamic Acid	2.5529	1.9903	0.0246	<.0001
Proline	0.8763	0.6310	0.0135	<.0001
Lanthionine	0.0303	0.1492	0.0008	<.0001
Glycine	0.2161	0.4414	0.1150	0.1875
Alanine	0.7748	0.6949	0.0123	0.0004
Cysteine	0.2425	0.2540	0.0072	0.2769
Valine	0.7449	0.7219	0.0122	0.2029
Methionine	0.4029	0.4193	0.0029	0.0013
Isoleucine	0.6239	0.5862	0.0086	0.0077
Leucine	1.2903	1.0850	0.0151	<.0001
Tyrosine	0.4359	0.4331	0.0063	0.7590
Phenylalanine	0.7458	0.7449	0.0108	0.9549
Lysine	0.7360	0.7490	0.0243	0.7106
Histidine	0.3928	0.3191	0.0074	<.0001
Arginine	0.9209	0.8720	0.0142	0.0284
Tryptophan	0.1927	0.1321	0.0012	<.0001

[a]Amino acid profile was analyzed at the Agricultural Experiment Station Chemical Laboratories at the University of Missouri, Columbia, MO 65211

Table 4. Plasma chemistry and hematology results prior to the feed transition period, d15, and d29.

Measurement	Prior to transition				D15				D29			
	Diet A	Diet B	SEM	P-value	Diet A	Diet B	SEM	P-value	Diet A	Diet B	SEM	P-value
WBC[a]	9.0	7.4	0.6	0.0914	6.9	8.1	1.3	0.5126	6.0	5.7	A - 0.6 B - 0.5	0.3739
RBC[b]	3.1	3.2	A - 0.1 B - 0.2	0.8844	3.0	3.0	0.1	0.6753	3.0	3.2	0.1	0.6639
PCV[c]	57.7	61.1	3.1	0.4592	50.7	51.3	1.5	0.7927	53.4	56.6	A - 2.0 B - 1.5	0.7085
MCV[d]	184.8	181.3	A - 5.3 B - 7.5	0.6626	175.6	179.2	1.8	0.2320	178.0	184.0	3.1	0.4686
HGB[e]	16.7	17.1	A - 0.9 B - 1.0	0.7295	17.0	15.3	1.5	0.3398	16.9	17.0	0.3	0.4226
MCHC[f]	29.0	30.3	A - 2.8 B - 4.0	0.7338	32.6	29.1	2.9	0.3789	32.0	28.7	1.2	0.3097
TotProt[g]	2.4	2.5	0.1	0.7938	2.4	3.3	0.3	0.1009	3.5	2.7	A - 0.5 B - 0.4	0.1801
Het/Neut[h]	47.3	44.6	2.0	0.3726	52.1	51.6	4.1	0.9255	46.6	49.7	A - 2.5 B - 1.9	0.5557
Lympho[i]	45.9	49.1	2.8	0.4400	41.9	43.9	4.8	0.7769	48.2	44.7	A - 2.7 B - 2.0	0.4226
Baso[j]	4.9	3.9	0.5	0.2341	4.1	43.9	4.8	0.7769	3.2	4.0	A - 1.0 B - 0.7	0.3111
Eosino[k]	2.0	2.4	1.0	0.7663	1.9	0.9	0.9	0.4554	2.0	1.6	A - 0.7 B - 0.6	0.6885
Mono[l]	0.0	0.0	0.0	-	0.0	0.0	0.0	-	0.0	0.0	0.0	-
Cal[m]	8.6	8.7	0.2	0.5023	8.8	9.1	0.2	0.3167	8.8	8.8	0.1	0.7267
Phos[n]	5.9	6.1	0.8	0.8509	3.0	4.3	0.3	**0.0389**	4.6	5.0	A - 0.8 B - 0.6	0.7764
LDH[o]	490.4	341.9	190.5	0.6012	279.9	266.1	42.4	0.8268	197.0	292.9	A - 53.4 B - 39.8	0.1623
CPK[p]	134.7	352.6	107.0	0.1997	284.6	114.9	62.4	0.1028	103.4	252.4	A - 139.0 B - 103.6	0.3115
Chlor[q]	103.6	103.0	2.2	0.8581	101.7	103.4	1.3	0.3924	110.0	105.2	3.3	0.3555
UricAcid[r]	6.6	8.1	1.1	0.3905	7.3	8.6	1.3	0.5231	8.7	7.7	A - 1.4 B - 1.0	0.6983
Glucose[s]	385.3	382.0	10.7	0.8346	292.0	361.0	-	-	307.8	187.0	112.0	0.8420
Chol[t]	383.1	375.7	111.0	0.9638	353.6	397.4	107.3	0.7822	281.0	215.0	A - 40.0 B - 29.7	0.2757
AST[u]	644.1	374.1	254.4	0.4813	475.9	307.4	101.8	0.2863	391.0	262.6	A - 139.1 B - 103.6	0.4877
Bile[v]	77.3	75.1	21.6	0.9461	42.0	40.2	8.9	0.8940	74.5	45.1	A - 8.4 B - 6.3	0.0717

[a]White blood cell count ($x10^3$/ul)
[b]Red blood cell count ($x10^6$/ul)
[c]Packed cell volume (%)
[d]Mean corpuscular volume (fl)
[e]Hemoglobin (g/dl)
[f]Mean corpuscular hemoglobin concentration (g/dl)
[g]Total protein (g/dl)
[h]Heterophil/Neutrophils (%)
[i]Lymphocytes (%)
[j]Basophils (%)
[k]Eosinophils (%)
[l]Monocytes (%)
[m]Calcium (mg/dl)
[n]Phosphorus (mg/dl)
[o]Lactate dehydrogenase (U/l)
[p]Creatine phosphokinase (U/l)
[q]Chloride (mg/dl)
[r]Uric Acid (mg/dl)
[s]Glucose (mg/dl)
[t]Cholesterol (mg/dl)
[u]Aspartate aminotransferase (U/l)
[v]Bile Acid (µmol/

Figure 1. Complete diet consumption during the transition period (d1-11).

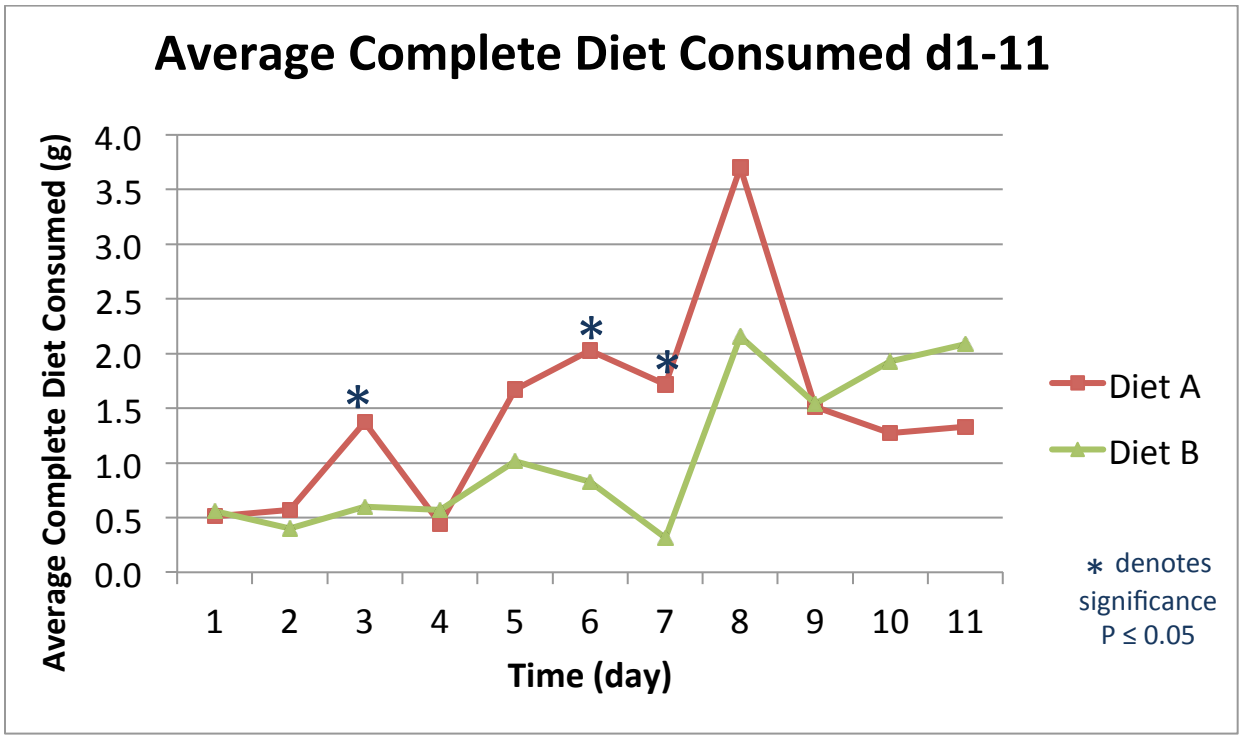


Figure 2. Seed-based diet consumption during the transition period (d1-11).

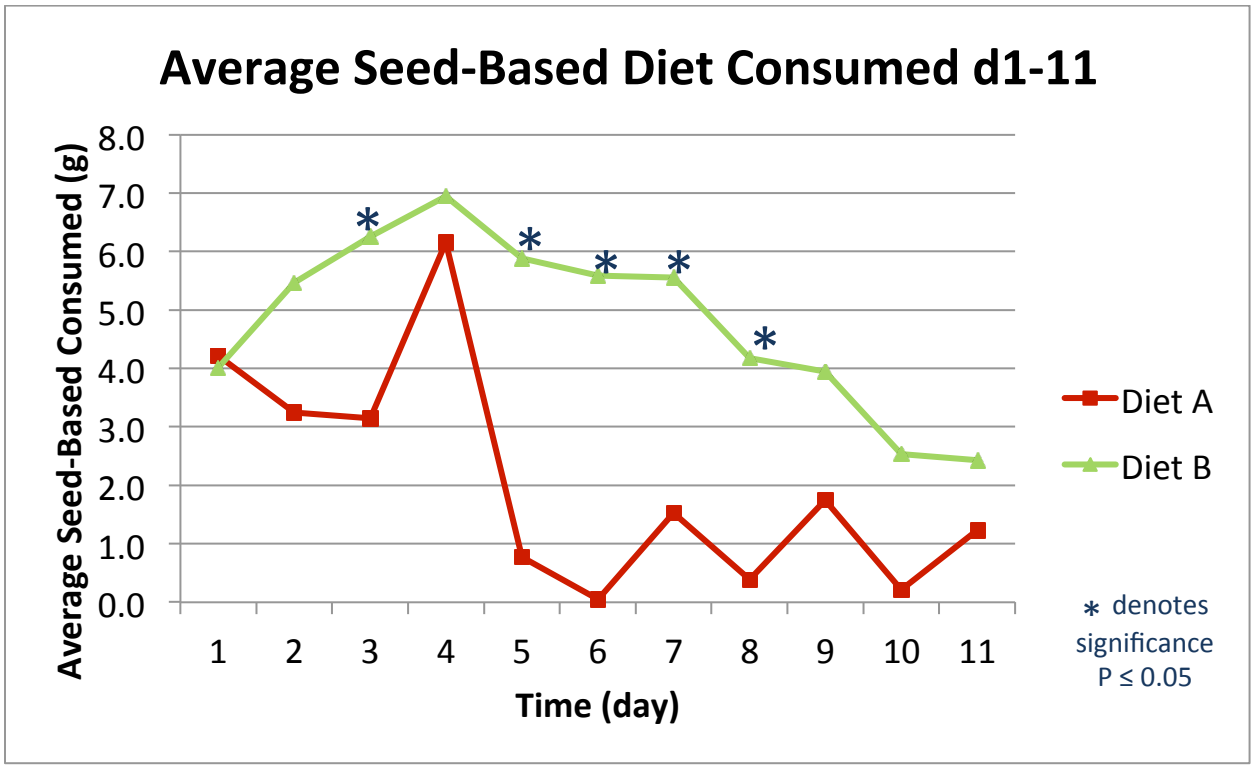


Figure 3. Total consumption of feed (complete diet and seed-based diet) over 28d.

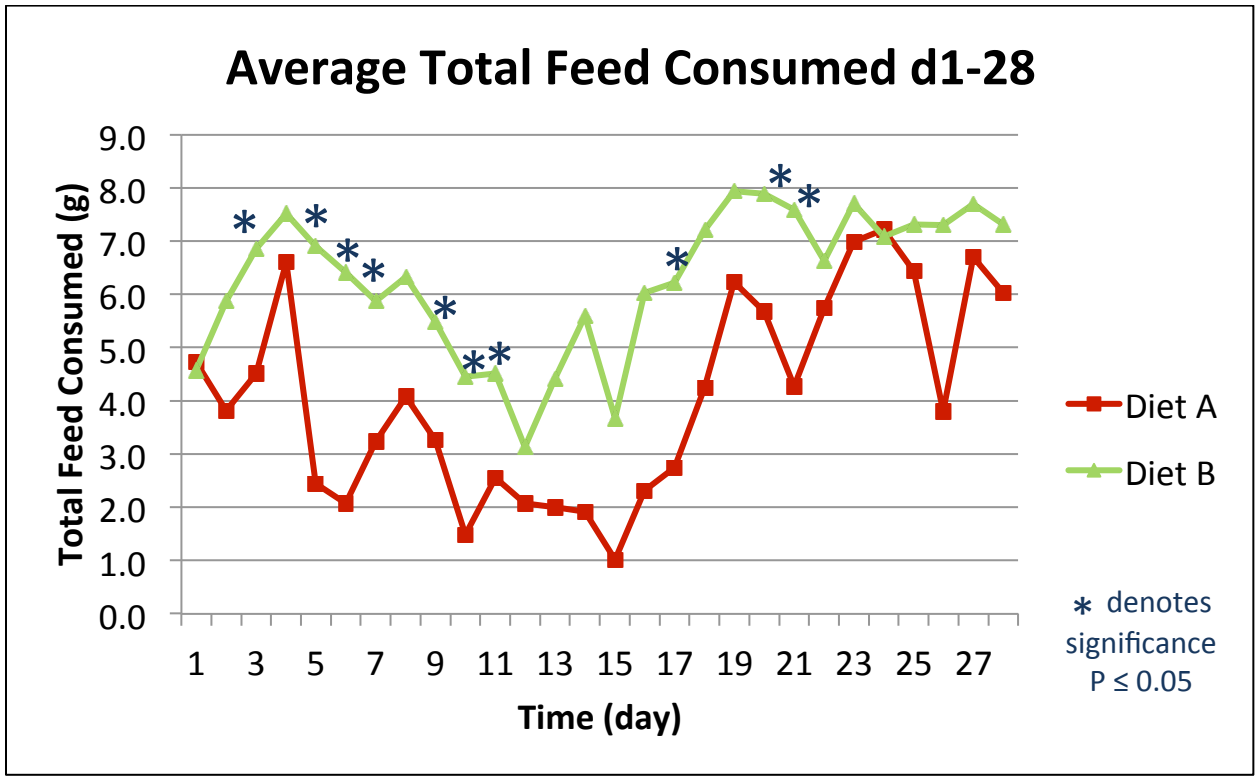
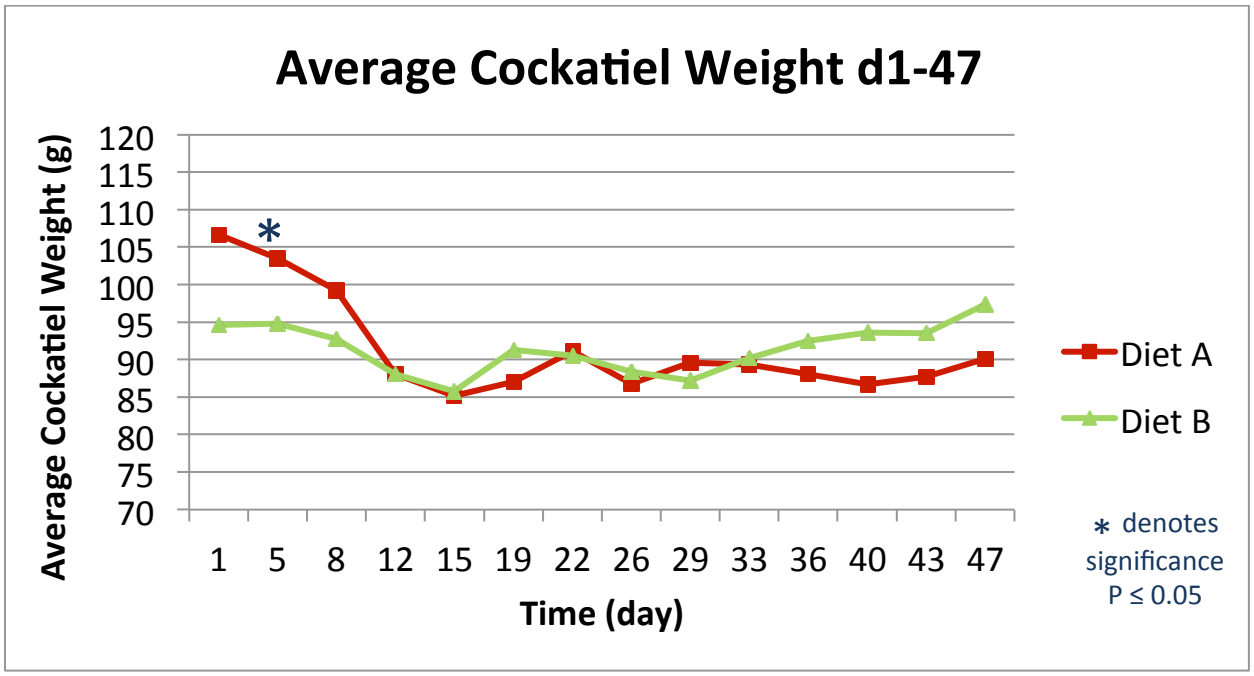


Figure 4. Cockatiel weight measured periodically over a 47d period.



CHAPTER 3: INFLUENCE OF PELLETING ON THE EFFICACY OF A *BACILLUS* DERIVED KERATINASE IN BROILER DIETS

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SUMMARY

The objective of this study was to determine the effects of pelleting, inclusion of a *Bacillus* derived keratinase, and bird sex on broiler performance. A majority of broiler feed in the United States is pelleted and contradictory data exists on its effects. A traditional corn and soybean based broiler diet is not completely digestible. Thus, a keratinase can be added to the diet to improve protein digestibility, ultimately enhancing broiler performance and decreasing production costs. However, the thermal conditions of the pelleting process can affect keratinase efficacy. In this study, males had an increased LWG and FI ($P<0.05$) compared to females, but a similar FCR ($P>0.05$). Broilers fed ground pellets as compared to mash had an increased LWG that was associated with a further increased FI ($P<0.05$). Consequently, ground pellet fed birds had an increased FCR ($P<0.05$) compared to mash fed birds. Overall, the inclusion of a keratinase did not affect performances variables ($P>0.05$), which may be due to the decreased activity. A multiple comparison test shows that males fed ground pellets supplemented with keratinase had an increased LWG and FCR as compared to the males fed the mash with keratinase, due to a further increased FI ($P<0.05$). These data were similar for females. This study demonstrates the superior performance of males to females and the pelleting process affects keratinase efficacy and broiler performance.

DESCRIPTION OF PROBLEM

Today's broiler production has 60-70% of its costs attributed to feed and feed manufacture. Therefore, strategies to reduce the cost of feed are being investigated. Broiler diets are mostly comprised of corn and soybean meal, that contribute the majority of crude protein to the diet. However, the native proteins in corn and soybean meal are not completely digestible [1-3]. Therefore, expensive synthetic amino acids are added to the feed to increase the amount of digestible amino acids. Furthermore, the addition of an exogenous keratinase has been shown to increase the nutrient digestibility of the feed, based on enhanced bird performance [4]. With increased nutrient digestibility of the corn and soybean meal, the amount of synthetic amino acids added to the diet can be reduced; therefore reducing the overall cost of the feed.

Despite the beneficial effects of an added keratinase, there may be issues with the stability of the enzyme during thermal conditions of pelleting. The high temperature, pressure, moisture, and mechanical forces can denature the structure of the protein, leading to loss of enzyme activity [5]. Thus, the thermal stability of the enzyme must be assessed because broiler diets are predominately steam conditioned and pelleted in the United States.

Research on pelleting predominantly shows the beneficial effects of pelleting, such as improved handling characteristics, improved hygienic quality, decreased ingredient segregation and selection, decreased feed wastage, increased breast yield, increased feed intake (**FI**) and live weight gain (**LWG**), and decreased feed conversion ratio (**FCR**) [6-11]. However, the pelleting process may be detrimental to nutrient availability. Loar and coauthors demonstrated that as conditioning temperature increases, FCR also increases [12]. Additional research has shown that birds fed ground pelleted diets had a higher FCR than those fed unprocessed mash diets [13].

The objective of the current study was to determine the effects of pelleting, inclusion of a *Bacillus* derived keratinase, and bird sex on broiler performance.

MATERIALS AND METHODS

Diet Preparations

Three diets for each phase of growth were formulated, which were either fed as a mash or ground pellet (**GP**), for a total of six dietary treatments. For the starter (d3 – 9; Table 1), grower (d10-21; Table 2), and finisher phases (d22-39; Table 3), the positive control (**PC**) and negative control (**NC**) diets were formulated to 100% and 85% of Agristat amino acid recommendations, respectively [14]. All feed was manufactured at the West Virginia University pilot feed mill [15] and descriptive data was recorded throughout (Table 4). Dry ingredients were batched then mixed for 10 minutes. All dietary fat was then added to the mixer [16] and the complete diet was mixed for an additional 10 minutes. The exogenous keratinase or sand was added at a recommended 0.058% inclusion to the NC or PC diets respectively and initially mixed with approximately 4 kg of ground corn prior to batching. Sand was added in order to ensure proper diet formulation ratio. Diets were steam conditioned at a commonly used temperature of 82.22^{o}C. During the manufacturing process, samples were taken for average hot pellet temperature, enzyme activity and pellet quality assays, such as pellet durability index, modified pellet durability index, and the New Holmen Pellet Tester (Table 4). All diets that were pelleted were ground using a hammer mill to a similar particle size as the unprocessed mash in order to eliminate feed form effects. All diets were analyzed for total amino acid profile [17] and proximate analysis (Tables 1-3). Enzyme activity was determined using an azocasein assay (Table 4) [18].

Bird Husbandry

A total of 1,680 male and 1,680 female Cobb x Cobb 500 [19] one-day-old broiler chicks were obtained from a commercial hatchery. [20] Chicks of each sex were kept separate and randomly allocated to one of 96 floor pens (0.69 x 2.44 m) that contained fresh pine shavings. Bird sex was incorporated into the study design to further justify any results in this study; it is well documented that males have superior performance. The pens were located in a cross-ventilated negative pressure house. The chicks were fed a common negative control diet until d3. On d3, the chicks were weighed by the pen and then adjusted to 35 chicks per pen and statistically similar pen weights. The six dietary treatments were randomly assigned to pens of either males or females in a randomized complete block design, for a total 12 treatments and 8 blocks. Blocking was based on pen location within the barn. Feed and water were provided for ad libitum consumption. Pens contained nipple drinkers [21] and the feed was provided in feed pans until d7. On d7, the chicks were transitioned to feed hoppers [22]. The initial temperature was set at 35^{o}C and gradually decreased throughout the study to maximize bird comfort. Lighting schedules were based on previous research and were adjusted prior to breast collection and weighing. Mortality was collected daily. All procedures involving the use of live birds were approved by the West Virginia University Animal Care and Use Committee.

Bird Performance

At the end of each growth phase (starter d9, grower d21, and finisher d39), the birds were individually weighed. Then, 10 birds within 10 g of the mean bird weight for the starter period, 6 birds within 10 g of the mean bird weight for the grower period, and 4 birds within 100 g of the mean for the finisher period were labeled. The following day, the labeled birds were individually weighed again and euthanized via cervical dislocation. Skinless breasts with bone-in were

removed and weighed for each bird. The breast was cut at the humeral-scapular joint and down the vertebral and sternal rib junction. Average breast yield per pen was then determined.

Statistical Analysis

Two separate analyses were performed. First, the overall full model (12 treatments) was analyzed as a randomized complete block design using one-way ANOVA. Then, the treatments in the 2 x 2 x 2 factorial arrangement were analyzed as a randomized complete block design using multi-way ANOVA. Significance was determined at $P \leq 0.05$. Comparisons of interest were further explored with linear contrasts. The statistical analyses were conducted using the GLM procedure of SAS [23].

RESULTS AND DISCUSSION

Descriptive Feed Manufacture Data and Keratinase Activity

For all growth phases, there were not any remarkable differences in the descriptive feed manufacture data variables between each diet (Table 4).

The expected descriptive keratinase activity in the mash diets was 450 U/g for an inclusion of 0.058%, however decreased activity in the mash was observed (Table 4). The azocasein assay determined the keratinase activity to be 431, 383, and 401 U/g in the mash diets for the starter, grower, and finisher diets, respectively. Keratinase activity post pelleting and grinding was decreased to 65%, 61%, and 68% of the mash activity for the starter, grower, and finisher diets, respectively. The decreased activity in the mash as well as post pelleting demonstrates the thermal instability of the keratinase.

Live Bird Performance

The main effect sex was significant for LWG and FI ($P<0.05$, Table 5), further describing that males had improved performance compared to females. However, no effect of sex on FCR was detected ($P>0.05$), due to similar trends in LWG and FI for males and females. No differences in breast yield data were detected at any growth phase ($P>0.05$) (Data not shown). Mortality percentage was not remarkable or significant ($P>0.05$).

The broilers fed ground pellets as compared to mash demonstrated an increased LWG, however showed a FI larger than the LWG ($P<0.05$, Table 5). Therefore, there was an increased FCR for broilers fed ground pellets ($P<0.05$). Previous research also showed the detriment of pelleting on broiler performance. A recent study by Shipe and coauthors showed an increased FCR for the ground pelleted diets as compared to the mash diets ($P<0.05$) [13]. Also, this study

showed a further increase in FCR when the diets were double pelleted then ground ($P<0.05$). It can be speculated the increase in FCR demonstrated in this study could have been attributed to the thermal conditions of the pelleting process. Previous research indicates that the pelleting process can lead to decreased amino acid digestibility in broiler chickens [12, 24].

Perhaps the detriment in broiler performance seen in this study is due to increased FI. The birds fed NC diets increased FI in order to remedy the inadequate amino acid content found in those diets. Despite increased FI, the broilers fed the NC diets were not able to overcome the decrease in amino acid content and indistinguishable performance to those fed the PC diet. Also, the pelleting process could have further decreased the amino acid content of the NC diets leading to a further increased FI and detriment to performance.

Overall, the main effect of keratinase was not significant for any performances variables ($P>0.05$, Table 5), perhaps in part to low enzyme recovery. However, the males fed ground pellets supplemented with the keratinase had an increased LWG and further increased FI as compared to the males fed the mash with keratinase, according to a multiple comparison ($P<0.05$). Thus, there was an increased FCR for the males fed ground pellets with keratinase compared to males fed mash with keratinase ($P<0.05$). These data were also similar for the females. It may be speculated that a traditional corn-soybean based diet is not an optimal substrate for the keratinase. It can be concluded that the thermal conditions of the pelleting process and grinding affected the keratinase recovery and consequent efficacy. Previous research has also shown that feed manufacture can decrease enzyme efficacy [25, 26].

Comparisons made using linear contrasts determined birds fed NC diets had decreased performance as compared to those fed PC diets. A comparison of all males fed mash demonstrated an FCR of 1.48 for those fed an the NC diet supplemented with keratinase to be

indistinguishable from an FCR of 1.54 for the NC diet not supplemented with keratinase (P>0.05). Furthermore, for males fed mash, the FCR of 1.42 for PC diets was significantly decreased compared to the FCR for those fed the NC diets (P=0.0125). Further comparisons demonstrated a similar trend for the females fed mash. In females, the FCR for the NC diet with keratinase, NC diet without keratinase, and PC diet was 1.51, 1.52, and 1.47, respectively (P=0.0881). Such comparisons demonstrate that birds fed PC diets showed a tendency to decrease FCR as compared to those fed NC diets, regardless of keratinase supplementation. The increased FCR for the NC diets is due to the inadequate digestible amino acid content within the diet formulation. Furthermore, these comparisons show that the keratinase had the potential to show positive effects, however due to decreased activity there were not any significant main effects.

CONCLUSIONS AND APPLICATIONS

1. Males demonstrated enhanced broiler performance as compared to females.
2. Keratinase did not endure the thermal conditions of feed manufacture and pelleting, shown through decreased activity. Therefore, keratinase did not enhance broiler performance in the mash or ground pelleted diets.
3. The thermal conditions of the pelleting process decreased overall broiler performance.

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27. **Table 1. Ingredients, calculated nutrients, and analyzed nutrients for Cobb 500 broiler starter diets.**

	NC[1] + Enzyme Mash	NC + Enzyme GP[2]	NC + Sand Mash	NC + Sand GP	PC[3] Mash	PC GP
Ingredients (%)						
Corn	60.82	60.82	60.82	60.82	56.34	56.34
Soybean Meal (48%)	28.95	28.95	28.95	28.95	32.81	32.81
Animal and Vegetable Blend Fat	2.83	2.83	2.83	2.83	3.46	3.46
Celite	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium Phosphate	1.53	1.53	1.53	1.53	1.51	1.51
Limestone	1.23	1.23	1.23	1.23	1.22	1.22
Meat and Bone Meal	1.12	1.12	1.12	1.12	1.12	1.12
Salt	0.32	0.32	0.32	0.32	0.36	0.36
DL Methionine	0.31	0.31	0.31	0.31	0.31	0.31
NB 3000 (vitamin/trace mineral)	0.25	0.25	0.25	0.25	0.25	0.25
L Lysine	0.19	0.19	0.19	0.19	0.23	0.23
Sodium Bicarbonate	0.18	0.18	0.18	0.18	0.20	0.20
L Threonine	0.08	0.08	0.08	0.08	0.11	0.11
Chlorotetracycline	0.025	0.025	0.025	0.025	0.025	0.025
Versazyme or Sand	0.058	0.058	0.058	0.058	0.058	0.058
Calculated Nutrients (%)						
Metabolizable Energy (kcal/kg)	3031	3031	3031	3031	3031	3031
Crude Protein	19.67	19.67	19.67	19.67	21.26	21.26
Digestible Lysine	1.06	1.06	1.06	1.06	1.18	1.18
Digestible Methionine + Cysteine	0.83	0.83	0.83	0.83	0.91	0.91
Digestible Threonine	0.69	0.69	0.69	0.69	0.77	0.77
Calcium	0.96	0.96	0.96	0.96	0.96	0.96
Available Phosphorus	0.45	0.45	0.45	0.45	0.45	0.45
Sodium	0.20	0.20	0.20	0.20	0.20	0.20
Analyzed Nutrients (%)[4]						
Gross Energy (kcal/kg)	3983	3897	4012	3885	4022	3949
Crude Protein	19.06	19.28	19.67	19.53	20.62	20.14
Lysine	1.21	1.17	1.34	1.19	1.24	1.23
Methionine + Cysteine	0.83	0.89	0.95	0.83	0.95	0.82
Threonine	0.75	0.70	0.79	0.72	0.77	0.75
Calcium	0.830	1.03	0.933	0.862	0.957	0.899
Phosphorous	0.833	0.76	0.802	0.802	0.863	0.833
Phytic acid, ion exchange	0.647	0.701	0.675	0.657	0.729	0.639
Non-phytate phosphorus[5]	0.412	0.487	0.449	0.431	0.486	0.404
Sodium	1860	2180	1500	1900	2330	1900

[1]Negative Control Diet Formulation.
[2]Ground Pellet.
[3]Positive Control Diet Formulation.
[4]Gross energy was analyzed at University of Arkansas Central Analytical Laboratory, Fayetteville, AR. Crude protein and amino content were analyzed at the University of Missouri Feed Labs, Columbia, MO. Calcium, phosphorus, phytic acid, ion exchange, and sodium were analyzed at N P Analytical Laboratories, St. Louis, MO.
[5]As directed by NP Analytical Labs. % NPP was calculated by (% Total Phosphorus - (0.282 x % Phytic Acid)).

Table 2. Ingredients, calculated nutrients, and analyzed nutrients for Cobb 500 broiler grower diets.

	NC[1] + Enzyme Mash	NC + Enzyme GP[2]	NC + Sand Mash	NC + Sand GP	PC[3] Mash	PC GP
Ingredients (%)						
Corn	66.85	66.85	66.85	66.85	61.04	61.04
Soybean Meal (48%)	21.13	21.13	21.13	21.13	26.20	26.20
Animal and Vegetable Blend Fat	5.00	5.00	5.00	5.00	5.00	5.00
Dicalcium Phosphate	2.60	2.60	2.60	2.60	3.36	3.36
Celite	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.58	0.58	0.58	0.58	0.57	0.57
Meat and Bone Meal	0.55	0.55	0.55	0.55	0.51	0.51
Salt	0.28	0.28	0.28	0.28	0.32	0.32
DL Methionine	0.27	0.27	0.27	0.27	0.28	0.28
NB 3000 (vitamin/trace mineral)	0.25	0.25	0.25	0.25	0.25	0.25
L Lysine	0.20	0.20	0.20	0.20	0.18	0.18
Sodium Bicarbonate	0.11	0.11	0.11	0.11	0.11	0.11
L Threonine	0.07	0.07	0.07	0.07	0.07	0.07
Coban	0.05	0.05	0.05	0.05	0.05	0.05
Versazyme or Sand	0.058	0.058	0.058	0.058	0.058	0.058
Calculated Nutrients (%)						
Metabolizable Energy (kcal/kg)	3096	3096	3096	3096	3096	3096
Crude Protein	18.30	18.30	18.30	18.30	20.30	20.30
Digestible Lysine	0.94	0.94	0.94	0.94	1.05	1.05
Digestible Methionine + Cysteine	0.75	0.75	0.75	0.75	0.83	0.83
Digestible Threonine	0.61	0.61	0.61	0.61	0.68	0.68
Calcium	0.86	0.86	0.86	0.86	0.86	0.86
Available Phosphorus	0.42	0.42	0.42	0.42	0.42	0.42
Sodium	0.19	0.19	0.19	0.19	0.19	0.19
Analyzed Nutrients (%)[4]						
Gross Energy (kcal/kg)	3751	3657	3769	3661	3817	3737
Crude Protein	17.4	16.8	16.2	17.0	19.3	20.6
Lysine	1.09	0.98	1.05	0.98	0.99	0.99
Methionine + Cysteine	0.71	0.71	0.85	0.71	0.73	0.73
Threonine	0.68	0.64	0.68	0.62	0.70	0.65
Calcium	0.952	1.00	0.892	0.971	0.897	0.972
Phosphorous	0.666	0.641	0.627	0.672	0.623	0.634
Phytic acid, ion exchange	0.736	0.692	0.671	0.662	0.721	0.707
Non-phytate phosphorus[5]	0.458	0.446	0.438	0.485	0.420	0.437
Sodium	1780	2020	1830	1670	1730	1730

[1]Negative Control Diet Formulation.
[2]Ground Pellet.
[3]Positive Control Diet Formulation.
[4]Gross energy was analyzed at University of Arkansas Central Analytical Laboratory, Fayetteville, AR. Crude protein and amino content were analyzed at the University of Missouri Feed Labs, Columbia, MO. Calcium, phosphorus, phytic acid, ion exchange, and sodium were analyzed at N P Analytical Laboratories, St. Louis, MO.
[5]As directed by NP Analytical Labs. % NPP was calculated by (% Total Phosphorus - (0.282 x % Phytic Acid)).

Table 3. Ingredients, calculated nutrients, and analyzed nutrients for Cobb 500 broiler finisher diets.

	NC[1] + Enzyme Mash	NC + Enzyme GP[2]	NC + Sand Mash	NC + Sand GP	PC[3] Mash	PC GP
Ingredients (%)						
Corn	72.01	72.01	72.01	72.01	67.44	67.44
Soybean Meal (48%)	16.17	16.17	16.17	16.17	20.13	20.13
Animal and Vegetable Blend Fat	5.00	5.00	5.00	5.00	5.00	5.00
Dicalcium phosphate	2.76	2.76	2.76	2.76	3.36	3.36
Celite	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.49	0.49	0.49	0.49	0.48	0.48
Meat and Bone Meal	0.47	0.47	0.47	0.47	0.45	0.45
Salt	0.18	0.18	0.18	0.18	0.22	0.22
DL Methionine	0.28	0.28	0.28	0.28	0.28	0.28
NB 3000 (vitamin/trace mineral)	0.25	0.25	0.25	0.25	0.25	0.25
L Lysine	0.17	0.17	0.17	0.17	0.16	0.16
Sodium Bicarbonate	0.06	0.06	0.06	0.06	0.06	0.06
L Threonine	0.05	0.05	0.05	0.05	0.06	0.06
Chlorotetracycline	0.05	0.05	0.05	0.05	0.05	0.05
Versazyme or Sand	0.058	0.058	0.058	0.058	0.058	0.058
Calculated Nutrients (%)						
Metabolizable Energy (kcal/kg)	3163	3163	3163	3163	3163	3163
Crude Protein	16.20	16.20	16.20	16.20	17.78	17.78
Digestible Lysine	0.80	0.80	0.80	0.80	0.89	0.89
Digestible Methionine + Cysteine	0.62	0.62	0.62	0.62	0.69	0.69
Digestible Threonine	0.53	0.53	0.53	0.53	0.59	0.59
Calcium	0.80	0.80	0.80	0.80	0.80	0.80
Available Phosphorus	0.40	0.40	0.40	0.40	0.40	0.40
Sodium	0.18	0.18	0.18	0.18	0.18	0.18
Analyzed Nutrients (%)[4]						
Gross Energy (kcal/kg)	3837	3795	3796	3744	3737	3865
Crude Protein	16.1	15.0	16.3	16.2	19.3	18.7
Lysine	0.85	0.92	0.93	0.84	0.95	1.03
Methionine + Cysteine	0.56	0.71	0.61	0.58	0.67	0.69
Threonine	0.54	0.58	0.59	0.57	0.61	0.64
Calcium	0.830	0.781	0.798	0.835	0.760	0.809
Phosphorous	0.656	0.624	0.641	0.654	0.629	0.656
Phytic acid, ion exchange	0.845	0.764	0.701	0.777	0.827	0.811
Non-phytate phosphorus[5]	0.418	0.409	0.443	0.435	0.396	0.427
Sodium	1370	1740	1640	1590	1540	1640

[1]Negative Control Diet Formulation.
[2]Ground Pellet.
[3]Positive Control Diet Formulation.
[4]Gross energy was analyzed at University of Arkansas Central Analytical Laboratory, Fayetteville, AR. Crude protein and amino content were analyzed at the University of Missouri Feed Labs, Columbia, MO. Calcium, phosphorus, phytic acid, ion exchange, and sodium were analyzed at N P Analytical Laboratories, St. Louis, MO.
[5]As directed by NP Analytical Labs. % NPP was calculated by (% Total Phosphorus - (0.282 x % Phytic Acid)).

Table 4. Feed manufacture descriptive data, physical attributes, and enzyme activity for Cobb 500 broiler starter, grower, and finisher diets.

	Feed Mill Descriptive Data				Physical Attributes of Feed				Enzyme Activity
	Goal Cond. Temp[1] (°C)	Production Rate[2] (tonne/hr)	HPT[3] (°C)	Observed Amperage of Pellet Mill[4] (A)	Particle Size[5] (µm) [σ]	PDI[6] (%)	MPDI[7] (%)	NHPT[8] (%)	Activity[9] (U/g) (% Recovery)
Starter Phase									
NC[10] + Enzyme Mash	-	-	-	-	999 [1.83]	-	-	-	431
NC + Enzyme GP[11]	82.22	0.8355	76.89	16.5 - 17.4	966 [1.77]	86.56	78.33	77.00	284 (65%)
NC + Sand Mash	-	-	-	-	999 [1.83]	-	-	-	-
NC + Sand GP	82.22	0.7430	76.61	16.7 - 17.3	1011 [1.86]	86.45	77.94	78.00	-
PC[12] Mash	-	-	-	-	1006 [1.87]	-	-	-	-
PC GP	82.22	0.7688	76.00	16.7 - 17.5	1129 [1.82]	-	-	-	-
Grower Phase									
NC[10] + Enzyme Mash	-	-	-	-	682 [1.94]	-	-	-	383
NC + Enzyme GP[11]	82.22	0.7882	77.67	17.5 - 18.0	808 [1.99]	89.86	82.14	87.00	235 (61%)
NC + Sand Mash	-	-	-	-	671 [1.83]	-	-	-	-
NC + Sand GP	82.22	0.8134	77.39	17.6 - 18.0	814 [1.89]	86.74	81.37	86.20	-
PC[12] Mash	-	-	-	-	698 [1.92]	-	-	-	-
PC GP	82.22	0.7163	76.72	17.0 - 17.3	885 [1.94]	88.31	79.33	84.80	-
Finisher Phase									
NC[10] + Enzyme Mash	-	-	-	-	914 [2.08]	-	-	-	401
NC + Enzyme GP[11]	82.22	0.8244	80.33	18.2 - 18.8	791 [2.03]	90.94	86.71	90.80	274 (68%)
NC + Sand Mash	-	-	-	-	901 [2.00]	-	-	-	-
NC + Sand GP	82.22	0.8521	80.72	18.4 - 18.8	857 [2.00]	91.53	85.51	89.00	-
PC[12] Mash	-	-	-	-	869 [1.95]	-	-	-	-
PC GP	82.22	0.8505	78.00	17.9 - 18.3	801 [1.92]	88.17	81.76	86.20	-

[1]Goal conditioning temperature. Temperature was measured with a probe thermometer inserted in the stream of conditioned feed in the chute that connects the end of the conditioner to the pellet die chamber.
[2]Production rate is an average of multiple collections of pellets directly after extrusion from the pellet die for a 1 minute collection period.
[3]Immediately after extrusion, pellets were collected into an insulated container and temperature was measured using a Fluke 51 II thermometer.
[4]Amperage was determined as an average range after multiple visual readings of a digital amperage meter over a 30 second interval.
[5]Particle size was determined with a Ro-Tap particle size analyzer model RX-29 type 110V 60H2, WS Tyler, Mentor, OH. One hundred grams of each crumbled diet was placed in a dust-tight enclosed series of stacked (No. 4, 6, . . .) American Society for Testing and Materials (ASTM) screens affixed to the Ro-Tap particle size analyzer and shaken for 10 min. The screens were then separated and weighed. Particle size was calculated by subtracting the weight of the screen from the final weight of screen and sample after shaking. The mean geometric particle size and log normal geometric standard deviation were calculated as described by McEllhiney, 1994. Multiple samples were assayed and averaged.

[7]Modified pellet durability index was determined was determined in a similar manner to pellet durability index with the exception of adding 5, 13-mm hex nuts to the pre-tumbled sample to obtain added pellet agitation.
[8]New Holmen Pellet Tester. Pellet durability index based on the New Holmen Pellet Tester that uses a sample of 100 g of pellets and air flow within a perforated chamber for 30 s.
[9]Activity was determined using an azocasein assay. The protease will cleave azocasein and release azo dye. Activity is then determined by measuring dye levels in the supernatant post centrifugation using a spectrophotometer at a certain wavelength.
[10]Negative Control Diet Formulation.
[11]Ground Pellet.
[12]Positive Control Diet Formulation.

Table 5. The effect of sex, keratinase, and level of processing on d3-39 broiler performance.

Diet	Bird Sex	Keratinase Inclusion	Level of Processing	LWG per Pen (kg)	FI per Pen (kg)	FCR[1]
Negative Control	Male	Yes	Ground Pellet	49.683^{abc}	76.624^{a}	1.542^{a}
			Mash	47.218^{de}	69.970^{c}	1.482^{cd}
		No	Ground Pellet	50.006^{ab}	76.041^{a}	1.521^{abc}
			Mash	48.614^{bcd}	74.740^{ab}	1.538^{ab}
	Female	Yes	Ground Pellet	44.144^{fg}	68.535^{c}	1.555^{a}
			Mash	41.865^{h}	63.195^{d}	1.509^{abcd}
		No	Ground Pellet	46.028^{ef}	71.078^{bc}	1.544^{a}
			Mash	42.362^{gh}	64.298^{c}	1.518^{abc}
Positive Control	Male	No	Ground Pellet	51.244^{a}	76.322^{a}	1.489^{bcd}
			Mash	50.444^{ab}	71.821^{bc}	1.424^{e}
	Female	No	Ground Pellet	47.670^{cde}	69.959^{c}	1.466^{de}
			Mash	42.360^{gh}	62.221^{d}	1.470^{cde}
ANOVA P-value				**<.0001**	**<.0001**	**<.0001**
Fisher's LSD[2]				2.268	4.103	0.052
SEM[3]				0.805	1.457	0.018
Marginal Means						
Negative Control	Male	-	-	48.880^{a}	74.343^{a}	1.521
	Female	-	-	43.600^{b}	66.777^{b}	1.531
	-	Yes	-	45.728	69.581	1.522
	-	No	-	46.752	71.539	1.530
	-	-	Ground Pellet	47.465^{a}	73.069^{a}	1.541^{a}
	-	-	Mash	45.015^{b}	68.051^{b}	1.512^{b}
Main Effects and Interactions						
Sex				**<.0001**	**<.0001**	0.3899
Keratinase				0.0977	0.0641	0.5108
Level of Processing				**0.0002**	**<.0001**	**0.0228**
Sex X Keratinase				0.7871	0.8966	0.4663
Sex X Level of Processing				0.3947	0.3196	0.5504
Keratinase X Level of Processing				0.8980	0.3492	0.0545
Sex X Keratinase X Level of Processing				0.3165	0.1070	0.2390

[1]Feed Conversion Ratio (Feed:Gain) was calculated using mortality weight.

[2]Fisher's Least Significant Difference.

[3]Standard Error of the Mean.

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EDUCATION

MASTER OF SCIENCE

Nutritional and Food Science — August 2012 - May 2014
West Virginia University — *Morgantown, WV*

Thesis: Practical strategies to maximize cockatiel health and broiler chicken performance

BACHELOR OF SCIENCE

Animal and Nutritional Sciences — August 2010 - August 2012
West Virginia University — *Morgantown, WV*
GPA: 3.67

Biology (Early Admissions Student) — August 2009 - May 2010
Harrisburg Area Community College — *Harrisburg, PA*
GPA: 3.07

AWARDS AND HONORS

Cum Laude, August 2012
West Virginia University

Dean's List, Spring 2011- Spring 2012
West Virginia University

Blue & Gold Scholarship, Fall 2010- Spring 2011
West Virginia University

PEER-REVIEWED MANUSCRIPTS

AL Foreman, JS Moritz, and JA Fallon. Cockatiel transition from seed-based diet to complete diets. J Avian Med Surg. 2013. (Accepted with minor revisions).

RESEARCH AND TEACHING EXPERIENCE

GRADUATE RESEARCH ASSISTANT — August 2012 - Present

- Contract study on exogenous keratinases
- Assisted with contract studies with various companies, including Verenium, Alltech, Virginia Poultry Grower's Coop, etc.
- Assisted with other studies with biofuel coproducts and poultry litter biochar
- Guest lecturer on Avian Blood Sampling Techniques in Principles of Lab Animal Science class (11/19/2013)

- Assisted with Doddridge County FFA Farm Tour. Presented feed mill application, broiler production, and processing (10/23/2013)
- Assisted with Tiger Cub Farm Visit (10/7/2013)
- Assisted with WVU Family Day at the Farm Poultry Exhibit (Fall 2012, 2013)
- Assisted with several poultry processing workshops throughout the state of West Virginia (Fall 2013)
- Assisted with Poultry Workshop at WVU Organic Field Day (8/15/2013)
- Attended Association of Avian Veterinarian's Annual Meeting in Jacksonville, FL (August 2013)
- A-STEM Summer Camp. Presented on Animal Research and Data Collection (with an emphasis on poultry research) (8/5/2013)
- Assisted with WV Poultry Exhibit at the WV State Fair (August 2013)
- Attended Poultry Science Association Annual Meeting in San Diego, CA (July 2013)
- Assisted with WV Career Development Event (Poultry Judging) (6/19/2013)
- Assisted with Preston County Ag Safety Day Poultry Exhibit (6/8/2013)
- Attended International Poultry Expo in Atlanta, GA (January 2013)
- Attended Moorefield Fall Poultry Educational Meeting (11/14/2012)

GRADUATE TEACHING ASSISTANT August 2012 - Present

- Assisted teaching AVS 275 - Companion Animal Science
- Assisted teaching ANPR 367 - Poultry Production
- Assisted teaching ANPR 369 - Poultry Production Laboratory

UNDERGRADUATE RESEARCH ASSISTANT December 2011 - August 2012

- Conducted study "Transition of Cockatiels from a Seed-Based Diet to One of Two Commercially Available Nutritionally Complete Diets"
- Led feed manufacture study on keratinases
- Assisted contract studies with various companies, including Verenium, Alltech, Lignotech, Virginia Poultry Grower's Coop, etc.
- Assisted with WV Poultry Exhibit at the WV State Fair (August 2012)

GRADUATE COURSES

- A&VS 699 - Journal Club ..... P
- AEM 545 - Food Microbiology ..... A
- AGBI 410 - Introduction to Biochemistry ..... B
- AGBI 512 - Nutritional Biochemistry ..... IP
- AGBI 610 - General Biochemistry C
- ANNU 696 - Graduate Nutrition Seminar ..... B
- STAT 511 - Statistical Methods 1 ..... A
- STAT 512 - Statistical Methods 2 ..... A
- STAT 513 - Design of Experiments ..... IP

SELECTED UNDERGRADUATE COURSES

- A&VS 150 - Introduction to Animal Science ..... A
- A&VS 251 - Principles of Animal Science ..... A
- A&VS 275 - Companion Animal Science ..... A
- A&VS 402 - Values and Ethics ..... A
- AEM 341 - General Microbiology ..... B
- ANNU 362 - Advanced Non-Ruminant Nutrition ..... A
- ANPH 301 - Introduction to Animal Physiology ..... A-
- ANPH 405 - Animal Physiology Laboratory ..... A
- ANPR 338 - Poultry Judging ..... A
- BIOL 115 - Principles of Biology ..... B
- BIOL 117 - Introductory Physiology ..... B
- BIOL 121 - Anatomy and Physiology I ..... B
- BIOL 122 - Anatomy and Physiology II C
- CHEM 115 - Fundamentals of Chemistry ..... B
- CHEM 116 - Fundamentals of Chemistry ..... A
- CHEM 233 - Organic Chemistry C
- CHEM 234 - Organic Chemistry ..... B
- CHEM 235 - Organic Chemistry Laboratory ..... A
- CHEM 236 - Organic Chemistry Laboratory ..... A
- HN&F 126 - Society and Food ..... A
- MATH 121 - Calculus I ..... A
- PLSC 105 - Plants and People ..... A
- PHYS 101 - Introductory Physics ..... B
- PHYS 102 - Introductory Physics ..... A-
- SPAN 203 - Intermediate Spanish 1 ..... A+
- SPAN 204 - Intermediate Spanish 2 ..... A+
- WMAN 150 - Principle - Conservation Ecology ..... A
- WMAN 160 - Ecology of Invading Species ..... A

SKILLS

- Domestic poultry handling and husbandry
- Companion bird handling and husbandry
- Poultry judging (Breeder and USDA standards)
- Feed manufacture
- Diet formulation with BRILL Feed Ration Balancer program
- Dry matter assay
- Kjeldahl analysis
- Precision-feeding
- Tibia and intestinal extraction
- Blood sampling
- Cecectomy surgery
- Experience with SAS and JMP statistical software
- Internet and Microsoft Office literate