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Influence of Encapsulation of Supplemental Amino Acids on their Utilization in Broilers

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**Influence of Encapsulation of Supplemental Amino Acids on
their Utilization in Broilers**

Undergraduate Honors Thesis

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Table of Contents

Abstract	3
Introduction	4
Literature Review	7
Materials and Methods	12
Results	15
Discussion	18
Conclusion	20
Tables	22
References	25

Abstract

This study was developed to assess whether microencapsulation of amino acids (AA) improves their absorption to increase broiler growth performance and reduce nitrogen excretion compared to standard feed-grade AA. Five hundred and sixty Ross 708 male broilers were randomly distributed to 70 battery cages and reared for 21 days. Reported data is grouped into periods: 0-7 days, 0-14 days, and 0-21 days. A total of 5 treatment diets were fed: an industry-based control and 4 diets with decreased levels of methionine, lysine, and threonine in either encapsulated or free forms, with or without a botanical feed additive. Encapsulated AA increased ($P < 0.05$) body weight (BW) and BW gain (BWG) for all periods and increased feed intake (FI) compared to non-encapsulated AA for two of three periods but did not affect feed conversion ratio (FCR). Presence of the botanical additive decreased BW, BWG, and FCR for all periods, and decreased FI for two periods. Nitrogen retention was not different ($P > 0.05$) among treatments. Encapsulation had no effect ($P > 0.05$) on intestinal concentration of methionine, lysine, or threonine in the jejunum or ileum. Birds fed encapsulated AA gained more weight in the same amount of time as those fed free-form AA. Although birds fed encapsulated AA treatments consumed more feed, FCR was numerically slightly improved, although this was not significant. These results indicate that encapsulated AA may improve growth performance of young broilers, which could be economically beneficial in a commercial setting. Further research using floor pens instead of cages is required to determine the cause of these effects. Encapsulated AA did not affect nitrogen retention, indicating that the potential environmental and welfare benefits of encapsulated AA are minimal. Subsequent trials with a longer growing cycle and an industry applicable environment are warranted.

Introduction

Background

The world's population is expected to increase over the next 30 years from 7.7 billion to 9.7 billion in 2050, with a potential peak of 11 billion around 2100 (United Nations, 2019). Furthermore, it is predicted that over half of this growth will take place in nine countries, including India, Nigeria, and Pakistan, with India overtaking China as the world's most populous country around the year 2027 (United Nations, 2019). A high percentage of the citizens of these countries live in poverty. Even today, one in seven people globally do not have ready access to a diet sufficient in protein and energy (Godfray et al., 2010). The number of people who are food insecure will only increase with the rising global population, presenting an increasingly difficult challenge to feed them.

Poultry has long been one of the most efficient and affordable protein sources. Given the speed and quantity with which poultry can be produced, it will certainly play an important role in meeting growing demands. In addition, poultry meat is not subject to many religious dietary restrictions that beef and pork have, making its importance even greater in certain countries, such as India. If the production of poultry can be made more efficient than it is today, the cost of poultry to consumers could decrease, potentially allowing more people with lower incomes to have access to a safe and affordable source of protein.

In commercial broiler chicken production, especially in the United States, feed costs alone can account for upwards of 70% of the total expenses of production (Ravindran, 2013). Of this cost, a large part is due exclusively to protein sources, especially soybean meal. Therefore,

reducing the amount of intact protein sources such as soybean meal in a poultry diet would reduce overall feed costs. This would decrease the overall cost of poultry production and could lower the end cost of products to consumers.

However, broiler chickens do not simply require protein, but rather many essential amino acids (AA) that makeup proteins within feedstuffs. In a typical broiler diet, most AAs, except for methionine, lysine, and threonine, are supplied in sufficient quantities from intact protein sources such as corn and soybean meal. To make up for the difference in the AA that are not sufficiently supplied by the primary feedstuffs, individual AA, typically purified in liquid or powder form, are supplemented in the feed. This brings the content for each essential AA up to the required levels for growth, without costly excesses.

Feeding a diet with reduced crude protein through AA supplementation can have several benefits. First, as protein is the most expensive dietary component, reducing its use can be economically beneficial. And from an environmental standpoint, reducing the amount of crude protein can lead to reduced nitrogen excretion. Prior research (Aletor et al., 2000; Bregendahl et al., 2002; Gomide et al., 2011), Belloir et al., 2017 reports that a 1% decrease in crude protein content can reduce nitrogen in the litter by approximately 10%. This can have major benefits when litter is used as fertilizer on agricultural land, as its nitrogen content often exceeds crop requirements (Sharpley, 1997) and can lead to eutrophication of surface water bodies. Reducing crude protein can also benefit animal welfare by improving litter quality (Harn et al., 2019). Excess nitrogen in litter creates ammonia, which can lead to respiratory issues and footpad lesions.

Problem Statement

In order for protein synthesis to take place, all AA required for that particular protein must be available at the same time. Because supplemental AA are not bound to anything that must be digested they may be absorbed rapidly, whereas those sourced from intact proteins may have a slower uptake due to digestion. As the amount of intact protein is lowered in a diet, the amount of AA that must be supplemented increases, potentially increasing the disparity in the rate of absorption between those supplemented and those derived from intact protein sources. If the rapid uptake of supplemented AA exceeds the rate at which protein synthesis can occur, these excess AA will be catabolized, and the nitrogen excreted.

Purpose of Study

This study was conducted to investigate whether the uptake of supplemented AA can be slowed via microencapsulation within a lipid matrix. Lysine is a major driver of muscle accretion, specifically *Pectoralis major*, or breast muscle. Breast muscle is usually the most valuable portion of a broiler, making lysine a nutrient worth investigating. Along with lysine, methionine and threonine are typically the most limiting AA in diets. These three amino acids will be encapsulated in palm oil, requiring them to undergo digestion once ingested. This may slow down the absorption of the supplemental AA within, maintaining the benefits of deriving AA from intact protein while allowing the dietary intact protein content to be reduced. Additionally, to investigate combination of encapsulated AA with other commonly microencapsulated feed additives, such as phytogenic, or botanical, ingredients, AA encapsulation will be tested in the presence or absence of a botanical feed additive.

Research Objectives

1. Evaluate whether increased microencapsulation of essential AA will lead to improved nutrient utilization and growth performance (body weight gain, breast yield, and feed conversion).
2. Determine if increased microencapsulation of essential AA will reduce the amount of nitrogen excreted into the litter.

Literature Review

While the literature addressing microencapsulation of additives in broilers is sparse, many studies have been conducted in areas that can be related to the subject. Research on amino acid (AA) absorption, digestion rates, and microencapsulation in other fields can provide useful information for this experiment.

Increasing Demand

The world's population is expected to rise to around 9.7 billion by the year 2050 (United Nations, 2019). However, even at current population and production levels, nearly one in seven people in the world do not have access to a diet sufficient in nutrients, particularly quality protein (Godfray et al., 2010). Protein can be obtained from a number of agricultural products, the most common being beef, pork, and poultry. Of these, poultry will be a leader in providing protein for the world's population. Poultry is much more efficient to produce than beef or pork, as fewer resources are required to produce an equivalent amount of food. This makes poultry a more affordable option, which is important as the majority of future population growth will occur in developing countries where people already struggle to feed themselves and their families. Additionally, some religions and cultures prohibit the consumption of beef and pork products,

deeming poultry and eggs one of the only animal options. Given all these factors, decreasing the end cost of poultry products will be beneficial to feeding the world.

Feed Cost

Proper nutrition is one of the most important aspects of successful poultry production. Diets are typically formulated to meet all nutritional requirements of poultry, while using the fewest and most affordable ingredients possible. Every effort is made to reduce the cost of feed. Even so, of all inputs associated with poultry production, feed costs alone can amount to nearly 70% of the total, with approximately 95% of feed costs due solely to meeting energy and protein requirements of the bird (Ravindran, 2013). Typical ingredients to meet these requirements include intact protein sources, particularly soybean meal. These intact proteins are vital to creating a nutritious diet, but they also represent the most expensive ingredients used.

Benefits of Reducing Intact Protein

There are many benefits to reducing the use of intact proteins in poultry feed. As stated above, proteinaceous feedstuffs are some of the most expensive ingredients. Reducing their use could potentially reduce the total cost of feed, leading to lower costs to consumers. Another benefit is reduced nitrogen excretion, which can have severe environmental impacts when it is surface applied and lost to the air, soil, and water. When an animal has excess protein in its diet, any that is unmetabolized is broken down into its elements. Some of these are retained while others are excreted. Nitrogen is one of those excreted, and reducing the amount of protein in a diet is known to reduce the amount of excreted nitrogen (Kidd & Kerr, 1996). Reducing dietary crude protein through amino acid supplementation could have positive implications for animal welfare as well. Nitrogen excretion can lead to excessive levels of ammonia in the litter, which can cause sores to

develop on the feet (paws) of birds. Excess nutrients in the intestinal tract can also promote bacterial growth, including *Clostridium perfringens*. *C. perfringens* leads to the development of a disease known as necrotic enteritis in chickens, and its growth has been shown to increase with increased levels of glycine in the intestinal tract (Dahiya et al., 2007).

Essential Amino Acids

Essential and nonessential AA serve as the components used to build proteins in poultry. Different proteins require different AA, and as such there are many that are required in a diet. Most of these can be sufficiently sourced from primary feed ingredients or corn and soybean meal, but the first three major limiting AA for poultry fed corn and soybean meal are methionine, lysine, and threonine (Fernandez et al., 1994). These three are usually not supplied by these ingredients in sufficient amounts to meet the needs of the bird, and as such, part of the ingredient needs are met in a synthetic crystalline form.

Digestibility

For a protein to be produced in the body, all the AA required must be present at the same time. In a diet using crystalline additives, this can become an issue. Amino acids sourced from intact proteins such as soybean meal are absorbed more gradually due to the time required for the protein to be digested. However, any supplemented AA is absorbed rapidly because it does not need to be digested. In a trial of 6,800 broiler chickens fed two diets with either slowly or rapidly digestible starch and varying levels of supplemental lysine, it was found that those fed slowly digestible starch had improved protein and energy utilization, resulting in greater weight and better feed conversion (Weurding et al., 2003). Because of this, the use of intact protein for the majority of AA needed for a diet remains the most effective method for feed formulations. It has also been

demonstrated that increasing the amount of time birds have access to feed improves digestibility of AA, indicating that slowing ingestion leads to slower digestion, which in turn improves intake of nutrients (Yin et al., 2019).

Limitations of Reducing Crude Protein

As indicated above, reducing the crude protein content in a diet has far-reaching ramifications for the usable AA content of that diet. If the amount of protein from primary feed ingredients is reduced, even more AA must be supplemented in addition to the three major limiting AA. It has been theorized that the sudden influx of these supplemented AA increases the catabolism of AA from both the diet and body to maintain an AA profile balance in the plasma (Aftab et al., 2006). This would further increase the disparity in digestibility and absorption rates between intact protein and crystalline AA, leading to even more asynchronous use of AA and increased excess.

Microencapsulation

Microencapsulation of AA presents itself as a promising method for decreasing intact protein use while maintaining the benefits of slower absorption. In microencapsulation, the desired supplemental nutrient (in this case a synthetic or crystalline AA) is encapsulated in a fat or a fat-like substance. This encapsulation causes the supplemented AA to go through digestion just as protein-bound AA would, as opposed to being absorbed almost immediately as a pure crystalline additive. Research is limited in this area, but one trial with chickens found that microencapsulated blends of organic acids and essential oils improved growth and gut health, suggesting that the capsule protected the blend from early, rapid absorption (Gheisar et al., 2015). In a different trial, encapsulated crystalline glycine was found in greater quantities further along the gastrointestinal

tract than a similar diet containing non-encapsulated crystalline glycine, as shown in Figure 1 (Dahiya et al., 2007).

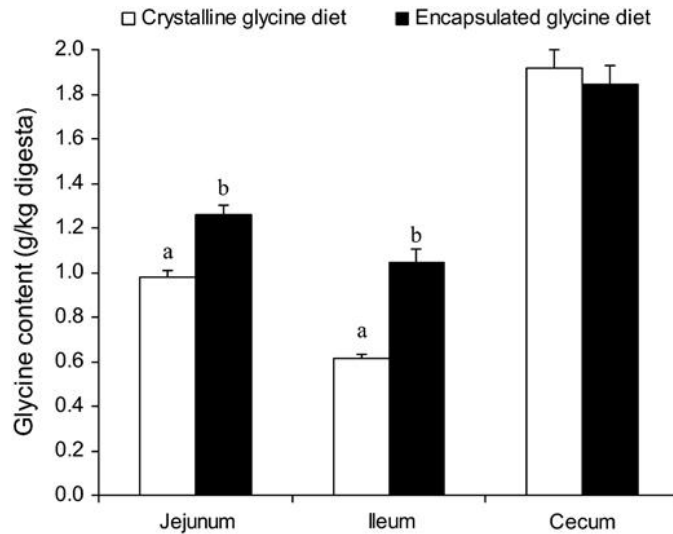


Figure 1: Effect of fat encapsulation on glycine concentration of digesta at different intestinal locations (jejunum, ileum, and cecum) in broiler chickens. Bars represent the mean \pm SEM, $n = 6$. At each site, bars labeled with different letters differ, $P < 0.05$ (adapted from *Dahiya et al., 2007*).

Microencapsulation has also been researched in other fields, with results that can be related to poultry. In humans, avian immunoglobulin IgY is being researched as an alternative treatment for enteric infections, but it is sensitive to the human gastrointestinal system. In an effort to counteract this, an experiment was conducted using pigs as a model of the human system, where the IgY was encapsulated in a pH-sensitive methacrylic acid copolymer (Kovacs-Nolan & Mine, 2004). The results showed that encapsulated IgY retained more activity than non-encapsulated IgY, indicating that it survived digestive conditions longer. In fish diets, protein-walled microencapsulation of specific AA performed better than a gelatin bound diet with the same ingredients (Yufera et al., 2001). The encapsulated diet suffered significantly less leaching, where nutrients from the diet are lost to dissolution into the water. Despite limited research on the subject, microencapsulation has been shown to have significant benefits and appears to be a promising solution to reducing crude protein in poultry diets while retaining the ability to slow the release of supplemented AA.

Materials and Methods

An experiment such as this with potentially major implications for the poultry industry must have a clear plan for how the experiment will be carried out. The methodology chosen for this study allowed for robust data collection and analysis and is replicable for any future investigators.

Research Objectives

Two research objectives were considered in this experiment. The first was to evaluate whether increasing microencapsulated AA will lead to an increase in growth performance. This was determined by measuring live weight gain and feed conversion throughout the trial, and overall pen weights after 21 days. The second objective was to determine if increased microencapsulation of essential AA reduces the amount of nitrogen excreted into the litter. This was analyzed by measuring the nitrogen content of the excreta for each treatment against the amount of nitrogen consumed over a set period.

Design of Study

This study followed an experimental design, with multiple treatments of subjects (broiler chickens) assigned based on a randomized complete block design. True experimental design allows for more control over the experiment and increases the overall validity (Williams, 2007). There were 5 dietary treatments, with 14 replicate cages of 8 birds each. These 5 treatment groups are outlined in Table 1. Nutrient compositions of each diet are provided in Table 2.

Table 1: Description of treatment diets

Treatment	n	Diet Type
1; Control	14	Industry control (1.28% dLys and recommended AA:Lys ratios)
2; Free AA - Botanical	14	Reduced AA density diet and standard AA
3; Enc. AA - Botanical	14	Reduced AA density diet and encapsulated AA

4; Free AA + Botanical	14	Reduced AA density diet and standard AA + botanical
5; Enc. AA + Botanical	14	Reduced AA density diet and encapsulated AA + botanical

These 5 diets consisted of 2 different AA density levels (industry control and “reduced”), each formulated with either crystalline or encapsulated (i.e. AA type) lysine, methionine, and threonine AA supplements, all fed in a crumbled form. Two more diets were tested to evaluate a botanical feed additive with both the crystalline and encapsulated reduced AA levels. Formulations included peanut meal to increase the required amount of supplemental amino acids to meet their minimum digestible levels. All variables other than those tested were controlled as best as possible, so that any potential results could be attributed to the independent variables. This trial protocol was approved by IACUC #21008.

Participants and Sampling

As stated above, there were 5 dietary treatments, with 14 replication cages of 8 birds each. This brings the total number of subjects to 560 distributed equally among 70 battery cages, each equipped with a trough feeder and 2 nipple drinkers. All birds were male from the Ross 708 commercial genetic line. These birds were reared from 0 to 21 days, after which they were euthanized and sampled. Fifteen birds were culled throughout the trial due to various ailments, 6 of which were from Treatment 1, and most were likely due to heat stress. However, because there were 14 replications of each trial with 8 birds in each, these few losses do not decrease the power of the study.

Rigor

Rigor was ensured by the experimental design itself, with randomized complete block assignment, eliminating any bias that could be caused by grouping the birds manually. In addition,

true experimental design allows for more control over external variables. Some of these include each group having the same space, same feeding routine, similar bird characteristics, and same temperature. All of these factors increase the overall validity of the experiment.

Data Collection

Live pen weights were collected periodically throughout the trial at 0-, 14-, and 21-days post-hatch. Feed consumption was measured weekly on the same days to calculate feed conversion over each period. Any mortalities were accounted for in both overall group weight and feed intake for that week. The feed for each treatment was carefully formulated based on industry standards, reduced AA density, and microencapsulation. Excreta was collected from the cages over the final 48 experimental hours, and each sample was analyzed for nitrogen content. After 21 days, those remaining of the initial 560 birds were euthanized and sampled. During the sampling process, the carcasses were weighed, and various organs, including the breast muscle and liver, from each bird were weighed individually. Weights and lengths of the duodenum, jejunum, and ileum were collected. Blood samples were taken from 2 birds in each cage and analyzed for total protein and uric acid content. Digesta was collected from the jejunum and ileum and analyzed for AA content to determine if encapsulation influenced the distance AA reaches in the gastrointestinal system.

Data Analysis

The 5 experimental treatments were based on a control plus a factorial arrangement of amino acid encapsulation (with or without encapsulation of lysine, methionine, and threonine) × botanical inclusion (with or without). Cages served as the experimental unit and cage location was used as the blocking factor (and was considered a random effect). Growth performance and organ measurements were based on 14 replicate cages of each treatment, whereas all other measurements

were based on 7 replicate cages. Data were analyzed by one-way ANOVA using SAS 9.4 (SAS Institute, Cary, NC). Pre-planned contrasts were used to evaluate the effect of AA density (Treatment 1 vs Treatment 2), main effects of AA encapsulation (Treatments 2 and 4 vs Treatments 3 and 5) and botanical inclusion (Treatments 2 and 3 vs Treatments 4 and 5), and the AA encapsulation \times botanical interaction. Statistical significance was considered at $P < 0.05$.

Results

There were no interactions between AA encapsulation and botanical inclusion on any measurement. Therefore, only the main effects of these factors will be discussed.

Live Performance

Overall pen weights and feed consumption were converted to a per-bird basis, reported as body weight (BW) and feed intake (FI), respectively. These values were then used to calculate body weight gain (BWG) and feed conversion ratio (FCR) over the 7-day period. Body weight, FI, BWG, and FCR for day 0-7, 0-14, and 0-21 are reported in Table 3. Lowering AA density did not influence BWG for day 0-7 ($P > 0.05$), but lower AA density did decrease BWG for day 0-14 ($P = 0.008$) and day 0-21 ($P < 0.001$). Lower AA density decreased FI for day 0-21 ($P = 0.029$) only and increased ($P < 0.001$) FCR for all periods.

Regarding AA type, encapsulation of AA increased BW and BWG for all periods ($P < 0.05$). Encapsulated AA increased FI for day 0-14 ($P = 0.010$) and day 0-21 ($P = 0.008$) only. Encapsulated AA had no significant effect on FCR for any of the reported periods ($P > 0.05$).

The botanical feed additive decreased all parameters (BW, BWG, FI, & FCR) for all periods ($P > 0.05$).

Organ Weights

Organ weight data are presented in Table 4. Lowering AA density resulted in lower Pectoralis major weights, both as an absolute weight ($P = 0.004$) and as a proportion of total body weight ($P = 0.033$) but did not influence ($P > 0.05$) other organ weights. Amino acid type or botanical feed additive did not influence ($P > 0.05$) absolute or relative weight of any organs.

Intestinal Amino Acid Content

Lysine, methionine, and threonine content of ileal and jejunal digesta is presented in Figures 2, 3, and 4, respectively. Reducing dietary AA density lowered jejunal lysine ($P = 0.031$), jejunal threonine ($P = 0.016$), and ileal threonine content ($P = 0.030$). There were no effects ($P > 0.05$) of AA encapsulation or botanical feed additive inclusion on intestinal AA content.

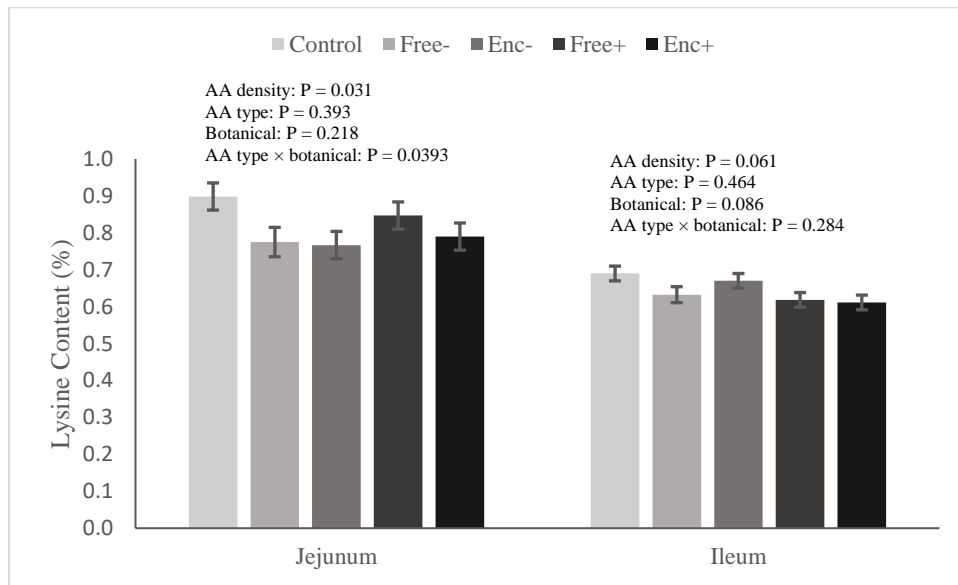


Figure 2: Comparison of jejunal and ileal lysine content (%). Presence/absence of botanical denoted by +/-, error bars represent \pm SEM

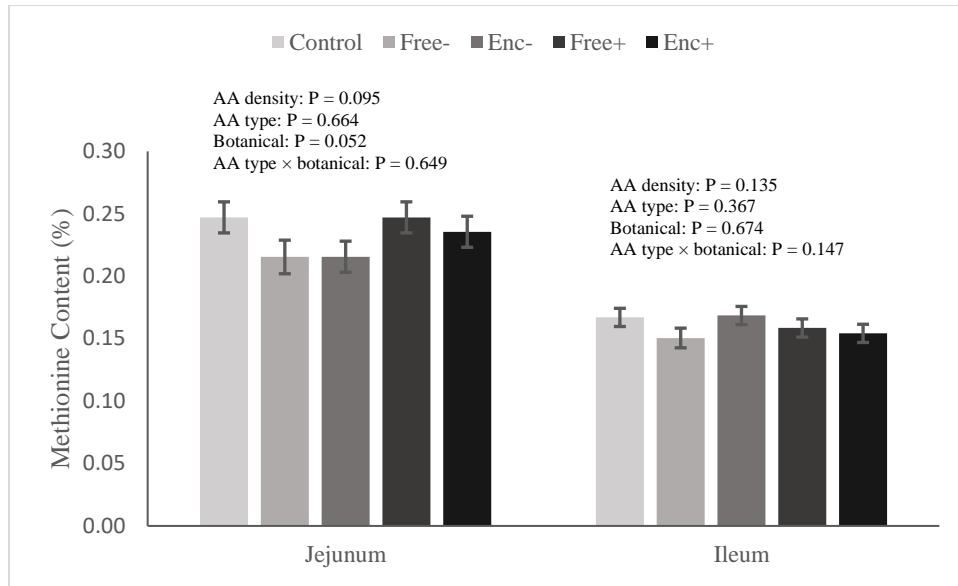


Figure 3: Comparison of jejunal and ileal methionine content (%). Presence/absence of botanical denoted by +/-, error bars represent ±SEM

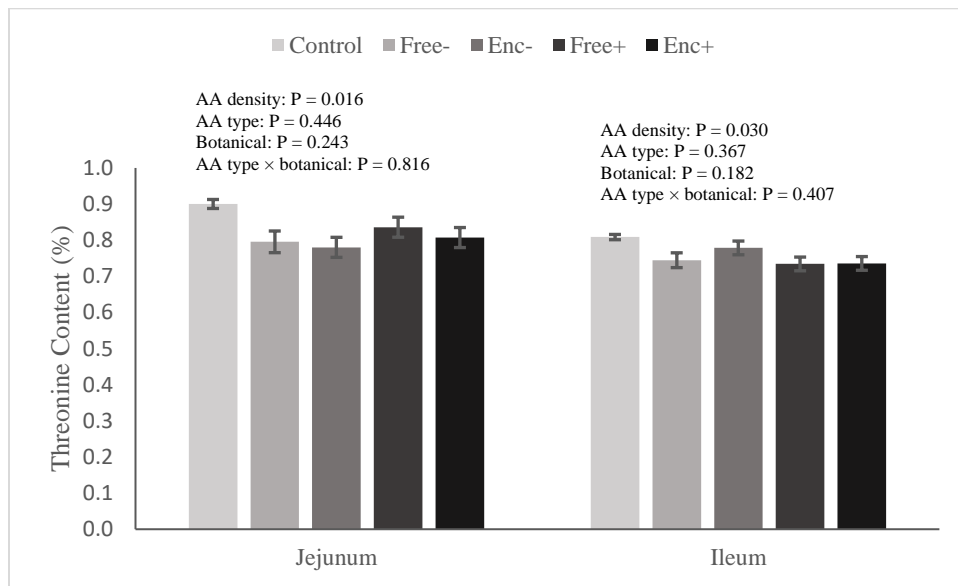


Figure 4: Comparison of jejunal and ileal threonine content (%). Presence/absence of botanical denoted by +/-, error bars represent ±SEM

Plasma Analyses

Plasma total protein (TP) and uric acid (UA) data are summarized in Table 5. However, AA density, AA type, and botanical additive showed no significant effects ($P > 0.05$) on plasma TP or plasma UA.

Nutrient Retention

Diets with lower AA levels increased dry matter (DM) retention ($P = 0.002$), which ranged from 76.149% in the control treatment to 78.233% in the free-form, reduced AA density treatment with botanical (data not reported). AA type and botanical did not influence ($P > 0.05$) DM retention. Nitrogen retention was also not affected by any parameter ($P > 0.05$), as represented in Figure 5.

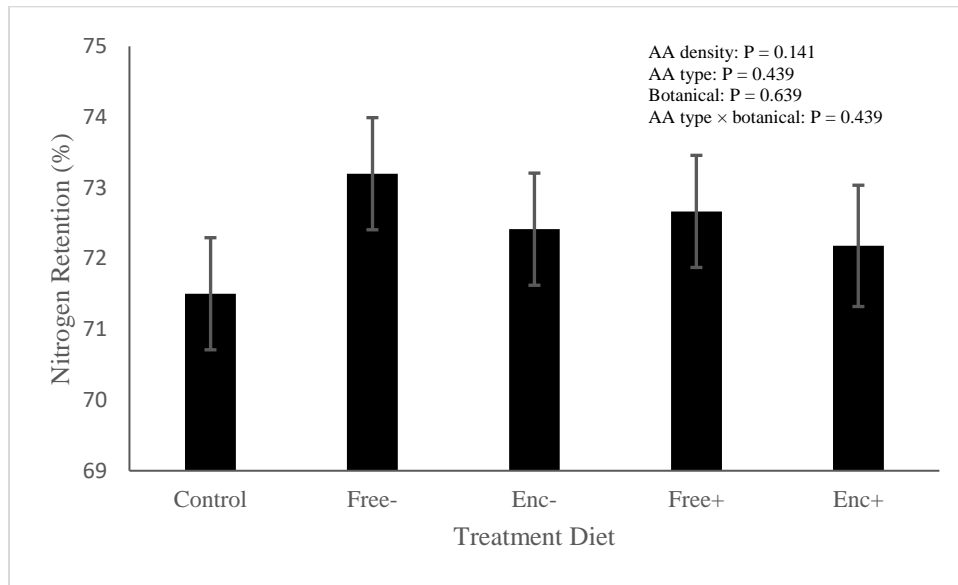


Figure 5: Nitrogen retention (%) for five experimental diets. Presence/absence of botanical denoted by +/-, error bars represent \pm SEM

Discussion

The goal of encapsulation was to delay absorption of AA to synchronize AA availability for protein synthesis in the bird. Diets with reduced AA density were used in this trial to increase

sensitivity to differences in AA utilization between encapsulated and free-form AA. Decreasing AA density decreased BW and BWG for day 0-14 and 0-21, and increased FCR for all periods ($P < 0.05$). In addition, *Pectoralis major* weights were lower in response to decreased AA density ($P < 0.05$). It is expected that reduced dietary AA will inhibit growth and muscle deposition, particularly in younger birds (Kidd et al., 2005), which was further confirmed by the results of this trial.

If encapsulation slowed down AA absorption, it was expected that higher contents of AA would be detected in the jejunum and/or ileum as it could be transported further before being absorbed. However, the data collected from intestinal digesta samples indicated encapsulation had no effect ($P > 0.05$) on intestinal AA content. This suggests that encapsulation does not delay absorption of supplemented AA. This contrasts with data by Dahiya who showed encapsulated glycine was found further along the intestinal tract than non-encapsulated glycine (Dahiya et al., 2007). Differences in the results of these two studies may be due to observation of different AA or different methods of encapsulation.

Encapsulation of AA increased BW, BWG, and FI throughout the trial ($P < 0.05$), except for FI for day 0-7 ($P = 0.072$). Final BW of birds fed reduced AA density in encapsulated form were higher than birds fed reduced AA density in free-form, but birds fed encapsulated AA also consumed more feed. Data collected does not indicate why this result occurred. As such, future trials accounting for different sample types are likely necessary to determine the reason encapsulated AA influence growth performance. Although FCR numerically decreased in encapsulated AA treatments, there was no statistical difference ($P > 0.05$). Further experimentation may be warranted using more treatments to precisely investigate effects of encapsulation on FCR in broilers in more industry-relevant conditions.

In the blood samples taken, it was expected that improved AA utilization due to encapsulation would result in increased TP and decreased UA content due to better synchronization of protein synthesis and reduced catabolism of excess AA. However, there were no significant effects ($P > 0.05$) of encapsulation on plasma TP. In addition to no differences in FCR, it can be inferred that encapsulation of AA had no significant effect on AA absorption rates and subsequent utilization.

In addition, encapsulated AA did not have any significant effect ($P > 0.05$) on dry matter or nitrogen retention. This suggests that encapsulated AA is not an effective method of reducing nitrogen content in broiler excreta and/or litter, and as such its environmental and welfare benefits appear to be minimal.

Although not a central focus of this paper, the botanical additive appeared to have a detrimental effect on growth performance. Body weight, BWG, and FI were all lower in treatments including the botanical ingredient, and FCR was higher. Based on these data, this botanical additive is likely not beneficial to commercial broiler production.

Conclusion

Among broilers fed experimental diets with reduced AA levels, those fed encapsulated AA displayed improved BW and BWG, resulting from increased FI. However, there was no statistical difference in FCR. Encapsulated AA did influence growth performance, but had no effect on any other sample type observed. Digesta AA concentration, plasma indicators of protein utilization, breast muscle deposition, and nitrogen retention indicated no differences in AA utilization with encapsulation. Further experimentation observing other aspects of growth and yield are likely necessary to determine the cause of this difference in growth performance. Although FI was

increased, broilers in this trial gained more weight in the same amount of time with no difference in FCR. In a commercial setting, birds could potentially be grown larger in the same amount of time by utilizing dietary encapsulated AA. This could allow integrators to produce more meat with lower end costs to consumers, thus helping more people afford quality food. However, this claim should be verified by future experiments in floor pens to increase similarities with commercial production.

It does not appear that encapsulated amino acids are of much use in reducing excreted nitrogen. Excreta and litter produced by birds fed encapsulated amino acids would likely contain similar levels of nitrogen as that from birds fed free-form amino acids, resulting in no difference in nitrogen runoff and subsequent waterway pollution after being spread on agricultural fields. However, reducing the amount of intact protein in diets could have an environmental benefit on the front-end, as lower demand for harvested crops could lessen agriculture's impact on fertile soil and decrease demand for resources.

Future research could benefit from trials conducted with more subjects and treatments to increase the number of samples collected. Many parameters appeared to be improved by encapsulated AA, but *P*-values did not reach significance, potentially due to the limited extent of this trial. In addition, subsequent trials extending beyond 21 days and using floor pens instead of cages are warranted to further investigate the potential advantages and practicality of utilizing encapsulated AA in commercial broiler diets.

Tables

Table 2. Ingredient and calculated nutrient composition (% , unless otherwise noted) of experimental diets fed to broilers from 0 to 21 d post-hatch

Ingredient, as-fed	- Botanical			+ Botanical	
	Control	Free	ENC	Free	ENC
Corn	49.65	56.49	56.49	56.49	56.49
Soybean meal (48%)	35.89	29.89	29.89	29.89	29.89
Peanut meal (44%)	7.50	7.50	7.50	7.50	7.50
Soy oil	3.73	2.35	2.35	2.35	2.35
Limestone	1.06	1.09	1.09	1.09	1.09
Dicalcium phosphate	0.95	0.98	0.98	0.98	0.98
Sodium chloride	0.39	0.37	0.37	0.37	0.37
Sodium bicarbonate	-	0.04	0.04	0.04	0.04
DL-Met (99%)	0.31	0.26	-	0.26	-
L-Lys-HCl (78.8%)	0.10	0.13	-	0.13	-
L-Thr (98.5%)	0.10	0.09	-	0.09	-
Timet (55% DL-Met)	-	-	0.47	-	0.47
ReLys (33% L-Lys)	-	-	0.31	-	0.31
Micotinic Thr (50% L-Thr)	-	-	0.18	-	0.18
AviPlus botanical (500 ppm)	-	-	-	0.05	0.05
Hydrogenated palm oil	0.04	0.51	0.04	0.47	-
Inert Filler	0.01	0.01	0.01	-	-
UofA TM (0.01%; Max = 0.12%)	0.10	0.10	0.10	0.10	0.10
Tyson 2x Broiler Vit	0.08	0.08	0.08	0.08	0.08
Choline chloride (60%)	0.07	0.09	0.09	0.09	0.09
OptiPhos2000 (0.250 lb/ton)	0.01	0.01	0.01	0.01	0.01
Nutrient composition, calculated unless otherwise noted					
AME _n , kcal/kg	3,058	3,058	3,058	3,058	3,058
CP	24.18	21.78	21.78	21.78	21.78
Digestible Lys	1.28	1.15	1.15	1.15	1.15
Digestible Met	0.65	0.57	0.57	0.57	0.57
Digestible Thr	0.86	0.77	0.77	0.77	0.77
Total Ca	0.90	0.90	0.90	0.90	0.90
Available P	0.45	0.45	0.45	0.45	0.45

Abbreviations: CTL = control; C AA = crystalline AA; E AA = encapsulated AA; C AA+ = crystalline AA w/ botanical; E AA+ = encapsulated AA w/ botanical.

Table 3. Live performance of broilers fed reduced AA density diets with added DL-Met, L-Lys·HCL, and L-Thr in free or encapsulated (ENC) form with or without a botanical feed additive

Item	Control	- Botanical		+ Botanical		SEM	<i>P</i> -values			
		Free	ENC	Free	ENC		AA Density	AA Type	Botanical	Interaction
0 to 7 d										
D 7 BW, g	187	186	192	181	184	2.0	0.725	0.024	0.002	0.433
BWG, g	143	142	148	137	140	1.9	0.814	0.025	0.002	0.498
FI, g	139	143	148	141	143	2.0	0.147	0.072	0.043	0.443
FCR, g:g	0.979	1.016	1.001	1.026	1.025	0.007	0.002	0.239	0.013	0.308
0 to 14 d										
D 14 BW, g	519	496	512	478	492	6.0	0.008	0.011	0.002	0.893
BWG, g	475	453	468	434	448	5.9	0.008	0.012	0.002	0.876
FI, g	511	509	525	499	511	5.5	0.864	0.010	0.027	0.787
FCR, g:g	1.076	1.135	1.124	1.149	1.143	0.005	<0.001	0.108	0.002	0.557
0 to 21 d										
D 21 BW, g	1007	927	963	893	928	12.1	<0.001	0.004	0.004	0.967
BWG, g	963	883	919	849	884	12.0	<0.001	0.004	0.004	0.963
FI, g	1123	1084	1117	1055	1089	12.8	0.029	0.008	0.024	0.942
FCR, g:g	1.166	1.229	1.215	1.243	1.235	0.006	<0.001	0.062	0.004	0.641

Table 4. Absolute and relative organ weights of broilers fed reduced AA density diets with added DL-Met, L-Lys·HCL, and L-Thr in free or encapsulated (ENC) form with or without a botanical feed additive

Item	Control	- Botanical		+ Botanical		SEM	<i>P</i> -values			
		Free	ENC	Free	ENC		AA Density	AA Type	Botanical	Interaction
P. major, g	148.07	123.96	131.29	112.40	120.26	5.675	0.004	0.186	0.051	0.964
P. major, % BW	14.58	13.45	13.65	12.50	13.25	0.367	0.033	0.205	0.071	0.458
Liver, % BW	2.54	2.44	2.52	2.50	2.48	0.081	0.398	0.687	0.875	0.495
Fat pad, % BW	0.48	0.59	0.65	0.64	0.65	0.039	0.061	0.342	0.544	0.520
Pancreas, % BW	0.26	0.28	0.28	0.29	0.28	0.011	0.392	0.655	0.798	0.308
Duo., % BW	0.96	1.06	0.99	1.06	1.05	0.038	0.064	0.312	0.405	0.391
Jej., % BW	1.81	1.90	1.85	1.84	1.77	0.054	0.210	0.255	0.187	0.932
Ileum, % BW	1.21	1.21	1.15	1.19	1.18	0.039	0.988	0.348	0.849	0.498

Table 5. Plasma total protein (TP) and uric acid (UA) content of broilers fed reduced AA density diets with added DL-Met, L-Lys·HCL, and L-Thr in free or encapsulated (ENC) form with or without a botanical feed additive

Item	Control	- Botanical		+ Botanical		SEM	<i>P</i> -values			
		Free	ENC	Free	ENC		AA Density	AA Type	Botanical	Interaction
Plasma TP, g/dL	2.514	2.343	2.500	2.371	2.371	0.110	0.281	0.482	0.654	0.482
Plasma UA, mg/dL	11.729	10.471	9.100	10.000	8.629	0.855	0.307	0.120	0.586	1.000

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