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Effect of Dietary Spirulina platensis on Stress Levels and Growth of Female Broiler Chickens

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Effect of Dietary *Spirulina platensis* on Stress Levels and Growth of Female Broiler Chickens

Undergraduate Honors Thesis

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Abstract

This study measured the effects of dietary *Spirulina platensis* supplementation on heterophil to lymphocyte ratio, footpad lesions, and growth performance parameters. One hundred and eighty Ross 708 broiler females were randomly divided evenly into fifteen pens with three dietary treatments for a total of five replications per treatment (twelve birds per replicate) for five weeks. The different treatments were a control diet of 20% crude protein, a diet of 17% crude protein, and a diet of 17% crude protein with 10% *S. platensis* added to the feed. Body weight, feed intake, and mortality rate was not significantly different between treatments ($P>0.05$). Body weight gain was significantly less among broilers fed *Spirulina* in their diets ($P<0.05$) compared to the other two treatments. Feed conversion ratio was significantly greater for the birds fed *Spirulina* in their diets ($P<0.05$). Footpad scores and heterophil to lymphocyte ratio was not significantly different between treatments ($P>0.05$). These results indicate that adding *Spirulina* to broiler diets does not have any negative effects on the health of the broiler but could have negative impacts on the economic side of poultry production.

Chapter I: Introduction and Literature Review

In any industry, there are always people trying to figure out how to make that industry more productive while also maintaining proper ethics and remaining economically profitable. Sometimes these additions are profitable and ethical, but other times they are not. It is important to recognize how these variables are affected by the addition (or subtraction) of an element into the industry. This study will be looking at how using *Spirulina platensis* as a feed additive affects performance parameters, footpad scores, and heterophil to lymphocyte ratio among female broiler chickens. While algae as a feed additive is not a new concept, it is one that has produced a wide variety of results. Because of this variation in results, it is still being tested today.

Spirulina platensis algae has been considered as a feed additive for many years. In fact, even in 1949, it was being proposed by Spoehr and Milnerl that the protein content of algae could help in facing the global protein shortages (Narasimha, Venkataraman, Duggal, & Eggum, 1982). A study conducted in 2018 found that the biomass of *S. platensis* had 514.7 g/kg of crude protein (Tavernari, et al., Roza, Surek, Sordi, Silva, Albino, Migliorini, Paiano, & Boiago, 2018). *S. platensis* has a large protein content, and contains all essential amino acids (Alvarenga, et al., Rodrigues, Cantarelli, & Zangeronimo, 2011). Algae is a good source of lysine and threonine (Narasimha et al., 1982) Due to this high protein content of 65-70% dry matter, *S. platensis* is considered a promising source of nutrients and large array of vitamins and minerals (Farag, Alagawany, & El-Hack, 2016). The algae form can also have a positive impact on the environment. Using less crude protein in broiler diets will lower the number of soybeans needed to be transported (Harn, Dijkslag, & Krimpen, 2019). Ross and Dominy (1990) suggested that adding 1.5 to 12% of *Spirulina* into broiler diets could replace soybean meal as a protein source

while still maintaining proper growth rates and feed efficiency. This was confirmed in 2004 when it was claimed that *Spirulina* can be a substitute form of protein (Rogatto et al., 2004). When compared directly to soybean meal, *Spirulina* was found to have greater values of dry matter, gross energy, crude protein, mineral matter, calcium, and total phosphorus. The apparent metabolizable energy is higher in *Spirulina* compared to soybean meal by 98 kcal/kg for dry matter. *Spirulina* also had higher gross energy by 9.6% compared to soybean meal (Alvarenga et al., 2011). Because of this protein content, *S. platensis* could be used in broiler nutrition (Kasapidou, et al., Kargopoulos, Karampampas, Christaki, Florou- Paneri, & Nikolakakis, 2016). This was tested in 2018, and it was discovered that the digestibility of dry matter and nitrogen increased linearly when broilers were fed diets containing *Spirulina* (Park, Lee, & Kim, 2018). Lower crude protein diets can also reduce digestive issues (Harn et. al, 2019). One can assume that digestive problems can induce stress.

Stress is characterized as all biological reactions to physical, emotional, or mental stimuli that disturb an individual's homeostasis (Pacek and Palkovits, 2001; Reyna, 2006). Stress is helpful to maintain adaption and homeostasis, but in the long run it can accelerate disease processes (McEwen, 2000; Reyna, 2006). As stress increases, mental health decreases, meaning the relationship between stress and mental health is negative. Negative physical symptoms affecting an individual's well-being come from being stressed (Covarribias, 2017). Stress causes stress hormone levels to increase which causes the body to reprioritize metabolism to deal with the stress at home (Chrousos, 1998; Reyna, 2006).

Spirulina is known to have multiple health benefits including antioxidant properties and anti- inflammatory effects (Deng & Chow, 2010). These health benefits can carry over into stressful situations. When heat stressed, broilers fed 1% and 2% *Spirulina* gained more weight

compared to the control group (Mirzaie, Zirak-Khattab, Hosseini, & Donyaei-Darian, 2018). Another study found that heat stressed broilers fed *Spirulina* had greater feed intake than the control group (Zeweil, Abaza, Zahran, Ahmed, AboulEla, & Saad, 2016). This same study suggested that *S. platensis* improved growth performance and immunity and decreased the detrimental effects of stress while under heat stress. When heat stressed, broilers fed *Spirulina* had lower production of stress hormones (corticosterone). In fact, the lowest corticosterone levels on day 38 of the trial was found in the broilers fed 2% *Spirulina* (Mirzaie et al., 2018). In the same study, the birds fed *Spirulina* had a greater production of antibodies compared to the control groups on day 38. Increases in heterophil to lymphocyte ratios are accepted as indicating that poultry have experienced stress (Mirzaie et al., 2018). Heat stress lowers the production of white blood cells, including heterophils (Zeweil et al., 2016). The ratio of heterophil to lymphocytes was lower in broilers fed *Spirulina* compared to heat stressed broilers not fed *Spirulina* (Mirzaie et al., 2018). In a study by Gross in 1989, Leghorns who had corticosterone added to their diets had an increased heterophil to lymphocyte ratio. When fed corticosterone in their diets, the heterophil to lymphocyte ratio increased from 0.38 in the control to greater than 9 in those fed *S. platensis* (Gross, 1989).

Footpad dermatitis has been prevalent in poultry for several decades across Europe and North America (Spoehr, H. A., & Milner, 1949; Arnon, 1938; Weidner & Eggum 1966; Farrar, 1966; Waslien, Calloway, & Margen, 1968). One of the most common causes of stress in poultry is footpad lesions. Because of this, the severity of foot pad lesions has become a crucial gauge of animal welfare (Kihlberg, 1972; Jacobson, 1951; Mejbaum, 1939; Mitchell 1924). Lower water intake can decrease the severity of wet litter, the main cause of footpad lesions, and therefore increase animal welfare (Martland, 1985). One way to lower litter moisture is by decreasing

crude protein in the diet, which reduces the need for copious urine flow to excrete extra nitrogen, therefore keeping the litter drier (Elwinger & Svensson, 1996; Alleman & Leclercq, 1997; Baily, 1999). *Spirulina* provides another way to reduce nitrogen excretion, and as *Spirulina* supplementation increased, ammonia emissions decreased (Park et al., 2018). Harn et al. (2019) found that footpad scores decreased linearly as crude protein was decreased. Broilers had the worst footpad lesions when fed the control feed containing the highest level of crude protein (Harn et al., 2019).

Whenever something new is implemented in the poultry industry, one of the greatest concerns is usually going to be how this will affect the performance parameters. There is much variation in results from different studies regarding this question. Some studies have found that *Spirulina* decreases growth. In a study by Ross and Dominy (1990), birds receiving *Spirulina* grew slower than the control diet. There was growth depression by the first week for the birds receiving 20% Spirulina. There have also been studies in which no significant differences in performance parameters were detected when birds were fed *Spirulina.* In the study by Ross and Dominy (1990), birds fed *Spirulina* for 41 days grew "at a comparable rate" to the control group. This has been supported by various other studies. Marzaie et al. (2018) found that adding *Spirulina* to the diet had no significant impact on weight gain, feed intake, and feed conversion ratio from 17-38 days of age. Park et al. (2018) also indicated that there were no effects on feed intake and mortality between all the treatment groups. Mortality was also the same between all the test groups (Bonos et al., 2016). Additional studies have indicated that *Spirulina* increases performance parameters. Broilers fed *Spirulina* in their diets had improved growth performance, body weight gain, and feed conversion ratios. It also increased antioxidant enzyme activity and decreased the amount of ammonia gas excreted by broilers (Park et al., 2018). This variation in

results is not new. Ross and Dominy (1990) made a suggestion that still holds true today: the quality of the *S. platensis* plays a large role in its performance, and in turn, the broilers' performances.

Justification for the Study

With any possible new additive to the broiler industry, it is necessary to determine if the addition is both humane and economically beneficial. This study will allow us to delve deeper into these two issues and get closer to learning the answers to these questions. If this study concludes that adding *Spirulina platensis* to broiler feed lowers stress and allows the birds to grow faster, then adding this alga to commercial broiler feed could be justified. The commercial broiler industry is committed to finding economically beneficial, yet humane, ways to improve their production. This study could confirm that *Spirulina platensis* is an additive that would accomplish that goal for the broiler industry.

Chapter II: Methods

Design

This study utilized a quantitative true experimental design. Studies performed using true experimental design explore cause and effect relationships between independent and dependent variables while in a strongly controlled environment. There are multiple types of True Experimental Design Studies. More specifically, this study utilized a Pretest-Posttest Control Group Design. The Pretest-Posttest Control Group Design employs random assignments. Random assignment indicates that each subject had an even chance of being placed in each group, experimental or control. All groups are pretested, exposed to their respective treatment, and then post-tested (Sousa et al., Driessnack, & Mendes, 2007). This design made the most

sense for this trial for many reasons. There was a cause and effect relationship being tested between lowering the crude protein in the diet and supplementing it with algae. There was a control group (industry standard 20% crude protein feed) and two experimental groups (17% crude protein feed and 17% crude protein feed supplemented with 10% *Spirulina platensis*). There were both independent (diet) and dependent (weight, footpad score, and heterophil to lymphocyte ratio) variables. The birds were randomly assigned. Each bird had just as much of a chance to be in one treatment as the other birds. The groups were all weighed before testing began and weighed again after testing ended, resulting in both pretests and posttests.

Purpose

The purpose of this experiment was to determine the effect of *Spirulina platensis* on performance parameters, footpad dermatitis, and stress levels in broiler chickens.

Objectives

I. Describe bodyweight (BW) gain per bird for each treatment in growing broilers.

II. Describe Feed Conversion Ratio (FCR) per bird for each treatment in growing broilers.

III. Describe feed intake per bird for each treatment in growing broilers.

IV. Describe percent mortality for each treatment in growing broilers.

V. Describe the average footpad score for each treatment.

VI. Describe the stress levels of each treatment using heterophil to lymphocyte ratios.

VII. Determine the effect *Spirulina platensis* has on these variables.

Participation and Sampling

One hundred and eighty female broiler chicks (Ross x 708) were obtained from the Wayne Farms Hatchery in Danville, Arkansas to be used in this study. This number of subjects

was selected so there would be 12 birds in each of 15 pens, giving us five repetitions of three treatments. Before Day 1 (placement day), 15 3-foot x 3-foot floor pens were set up in a well-ventilated poultry house. Bedding was added, along with water lines, hanging feeders, and supplemental feeders. Industry standard starter feed was added to both the hanging feeders and the supplemental feeders. The house was preheated to a brooding temperature of 90 degrees Fahrenheit. The primary breeders' recommendations were followed regarding the temperature in the house throughout the trial. On the morning of Day 1 (February 4, 2020), the chicks were collected from the Wayne Farms Hatchery and transported to the University of Arkansas poultry farm. Upon arrival, the chicks were randomly placed in the pens with twelve birds per pen. After placement, it was ensured that at least two birds per pen could drink from the water lines. From Days 1-14, the birds were checked multiple times daily, and starter feed was kept in the supplemental feeders and in the hanging feeders. The water lines were raised as needed. At 6 AM on Day 15 (February 18, 2020), the supplemental feeders were removed from all the pens and labeled to correspond with the pen number. The starter feed was removed from the feeders, and the empty feeders were weighed on a scale. The weights were recorded in pounds. Bushel laundry baskets were labeled and weighed, and the weights were recorded in pounds. The birds in pen 1 were placed in one of the laundry baskets and weighed. The number of birds in each pen and the basket number were recorded. The weight of the birds was recorded in pounds. This was repeated for each pen. Thirty-five pounds of grower feed was weighed out and placed into the corresponding feeder. The feeder was placed back in the proper pen. This was repeated for each pen and trial. On February 28, 2020 and March 5, 2020, twenty pounds of feed was added to each feeder with the proper feed for each treatment. The date and amount of feed being added was recorded. The birds were checked on multiple times each day to check the water line levels

and for mortality. Any deceased birds found were collected and disposed of properly. At 11 PM on night 34, the feeders were raised out of reach of the birds to prepare them for processing the next morning.

Treatments/Instruments

The broilers were from a high yield Ross strain used in approximately half the U.S. broiler industry. The birds were evaluated to 35 days, which is typical for female Ross 708 birds destined for high-value bone-in markets (e.g., KFC®, Chick-fil-A, etc.). The water lines were industry standard water lines. The hanging feeders, supplemental feeders, and starter feed were also industry standard. The control group was fed an industry standard grower feed (20% crude protein). The negative control group was fed industry standard grower feed with less crude protein (17%). The third and final group was fed the same feed as the second group but supplemented with 10% algae (*Spirulina platensis*). A scale that can weigh up to fifty pounds was used to weigh the feed out and weigh the birds on Day 14. Six 1.5-bushel laundry baskets (24" x 17 3/8" x 10 3/8") were used to carry and weigh the chicks on Day 14. On Day 35, blood was collected from the wing vein using a 19 gauge needle into 5mL heparinized tubes.

Data Collection

At 6:30 AM on Day 35, blood from two birds per pen (10 per treatment) was collected. After blood collection, the birds were euthanized by cervical dislocation. This blood was used to determine heterophil/lymphocyte ratios by a Cell-Dyn automated hematology analyzer set for chicken blood. This automated hematology analyzer is owned by the United States Department of Agriculture group in the University of Arkansas Poultry Science Department. The remaining birds were transported to the University of Arkansas poultry processing plant where they were weighed before processing and given a new wing band number. During processing, the foot pads were scored as 0 (clear), 1 (moderate), and 3 (severe). The criteria used to score was 0-clear foot

pad; 1-small lesion on foot pad; and 3-lesion bigger than a dime on footpad. After processing, the feeders were weighed with the remaining feed in them. The weight of each feeder was subtracted from the weight of the feed plus the weight of the feeder to get the weight of the remaining feed. Using this weight and the weight of the birds recorded at processing, the FCR, feed intake, and BW gain per bird was calculated.

Data Analysis

The data from the measurable variables tested during the study were collected and recorded in a document on Microsoft Excel to be analyzed. Mean, standard deviation, correlation, and regression of the data were calculated for these variables (BW gain per bird, FCR, feed intake per bird, mortality, and footpad scores). Heterophil to lymphocyte ratios were determined by running a cell dyne to establish the number of white blood cells. The ratio was determined by dividing the concentration of heterophils by the concentration of lymphocytes.

Chapter III: Results

Results of research projects are crucial for fully understanding how the independent variables affected the dependent variables. By analyzing these results, conclusions can be made which can lead to advancements in the field that is being tested.

A variety of different variables were tested for how they were affected by both the subtraction of crude protein and the addition of *Spirulina platensis* into the broilers' diet. Table I shows the performance parameters of each treatment of female broilers. The table shows the body weight at processing, body weight gain throughout the trial (from days 15-35), feed conversion ratio (FCR), feed intake, mortality rate, and footpad score for each treatment. These treatments were the control (20% crude protein), low crude protein (17% crude protein), and low crude protein with algae (17% crude protein supplemented with 10% *Spirulina platensis*). As

evidenced by the table, there was no significant difference (P>0.05) in the body weight at processing, feed intake, and mortality rate between treatments. The feed conversion ratio was significantly (P<0.05) greater among the birds who were fed *S. platensis* compared to those who were fed the control diet and the low protein diet. This means that it took the birds with the algae added to their diets more feed for them to gain one pound of body weight compared to both the control and the low protein diet. This is in line with the results of the body weight gain. The broilers fed *S. platensis* gained significantly (P>0.05) less weight per bird than both the control and the low crude protein groups. Not only did the birds fed the algae gain less weight, but it took more feed for them to gain the little weight they did. The second part of the table contains the footpad results. There was no significant (P>0.05) difference in the footpad scores among treatment groups. This indicates that there was approximately the same amount of ammonia levels in the litter among all treatments and could also suggest that the birds were under similar amounts of stress due to the footpad scores being alike.

Table I

Performance and Processing Yield from female 35 day old broilers fed diets differing in CP and CP source[1]

Parameter	Control	Low CP	Low CP + algae	SEM	P value
Body Weight, kg	2.08	2.12	2.09	0.0333	0.2573
Day 15-35 Performance					
BW gain, kg/bird	1.55[a]	1.46[b]	1.36[c]	0.0648	0.0021
FCR, kg/kg[2]	1.66[c]	1.75[b]	1.82[a]	0.0415	0.0002
Feed intake, kg/bird	2.57	2.55	2.54	0.1025	0.8631
Mortality, %	0.00	0.00	0.03	0.0276	0.1107
Foot pad score, avg[7]	0.50	0.56	0.48	0.4472	0.9577

[1]Control diet contained 20.1% CP. CP was allowed to decrease to 17% in the Low CP and Low CP + algae diets resulting in L-Ile, L-Val, L-Arg, and L-Trp diet inclusions. The Low CP + algae diet allowed algae to replace 50% of soybean meal. Diets were formulated to identical minimum amino acid ratios to Lys.

[2]FCR=Mortality Corrected feed to gain ratio

[3]Breast=*Pectoralis major*

[4]Tender=*Pectoralis minor*

[5] Carcass parts are skin-on and bone-in

[6]Yields represent chilled carcass parts relative to live BW

[7] 0=none, 1=mild & 2=severe; means represent average score

Table II depicts the proportions of the different types of white blood cells found in the blood sample from the same study as the first table. The birds were once again female and thirty-five days old. The treatments were the same as well: control, low crude protein, and low crude protein with algae. The white blood cell count was determined using automated hematology, more specifically, a Cell-Dyn machine. The two white blood cells utilized in this study were the heterophils and the lymphocytes. The percent of both types of blood cells were taken and then divided (heterophil/lymphocyte) to get the ratio. There was no significant ($P>0.05$) difference in heterophil to lymphocyte ratios between the three treatments. Studies have shown that this ratio is a satisfactory way to measure stress of broilers. Because the P-value was high, this indicates that the birds were under relatively the same amount of stress across all treatments.

Table II

Proportions among different white blood cell populations in blood from female 35-day old broilers fed diets differing in CP and CP source[1] determined by automated hematology

Parameter[2]	Control	Low CP	Low CP + algae	SEM	P value
Heterophil/lymphocyte	0.87	1.29	0.94	0.209	0.353
Heterophils, %	41.25	49.47	39.83	5.194	0.395
Lymphocytes, %	48.17	41.21	45.96	3.810	0.442
Monocytes, %	2.92	3.00	3.98	1.023	0.725
Eosenophils, %	0.17	0.45	0.16	0.133	0.254
Basophils, %	7.49	5.88	10.07	2.454	0.498

[1]Control diet contained 20.1% CP. CP was allowed to decrease to 17% in the Low CP and Low CP + algae diets resulting in L-Ile, L-Val, L-Arg, and L-Trp diet inclusions. The Low CP + algae diet allowed algae to replace 50% of soybean meal. Diets were formulated to identical minimum amino acid ratios to Lys.
[2]Heterophil to lymphocyte ratio. White blood cells are expressed as relative total white blood cell percentages.

Discussion

It is important to compare results of this study with studies of the past. This enables us to gain a better perspective on why different variables were affected, as well as to understand how this study fits into the wider array of previous studies on this topic.

The results of this study were like results of studies in the past. The performance parameters were supported by previous studies. The potential use of *Spirulina platensis* as a feed additive for broilers is something that is reasonable from a health standpoint, but not practical economically. The overall health of the broilers would not be impaired by the addition of *S. platensis* into their diet. The heterophil to lymphocyte ratio was not significantly different ($P>0.05$) across all treatments, therefore, the results of this study indicate that the broilers were all under similar amounts of stress. Considering all the birds were kept in a temperature-controlled house with access to food and water 24/7, it makes sense that the heterophil to lymphocyte ratio would be similar among all treatments. The birds were reared in an environment that mimicked industry and minimized stress. This study suggests that the feed type does not influence stress if all other aspects of a bird's wellbeing are taken care of (temperature, water access, etc.). The birds also had similar footpad scores across all the treatments. This result was unexpected, as it was different from previous studies on footpad scores. Previous studies have indicated that severity of footpad lesions linearly correlate with the amount of crude protein in the diet (Harn et al., 2019). However, in this study, all treatments had a similar incidence of footpad lesions. One possible explanation for this discrepancy is the leaking of the water lines. The water lines were kept at the lowest pressure that would reach the end of the line, however, they still leaked. This would have increased the wetness of the litter across all treatments, and therefore, could have skewed the footpad scores to be similar for all treatments. While the use of *S. platensis* would not affect the health of the broilers, economically this is not plausible feed additive. Although there was not a significant ($P>0.05$) difference in feed intake and mortality rate between treatments, there was a significant ($P<0.05$) difference in the feed conversion ratio (FCR) and the weight gain across treatments. The broilers fed *Spirulina* in their

diets had a higher FCR than both the control group and the low protein group. Adding *Spirulina* to the diet also lowered the body weight gain for the birds in that treatment. *Spirulina* as a feed additive would not be economically sustainable because of the increased cost it would take to raise the birds to the desired weight. It would take more food and time (and more money) for the birds to gain the weight needed for processing. The conclusion of this study goes against what would be expected. Alvarenga et al. (2011) discovered that *Spirulina* has greater values of crude protein, gross energy, and other valuable nutrients. Knowing this information, one would assume that the broilers with *Spirulina* in their diets would at least compare to, if not out-perform, those fed the control diet and the low crude protein diet. Instead, they did the exact opposite. One possible explanation to this is the quality of the protein in the *Spirulina* compared to the protein quality of soybean meal. If the quality of protein found in soybean meal is higher than the protein found in *S. platensis,* then that could offer a possible explanation as to why the broilers fed the control diet out performed those fed the low crude protein diet supplemented with *Spirulina*. Differences in bird performance in diets supplemented with *Spirulina* could be attributable to dietary energy content and starch type, in addition to amino acid composition and digestibility.

Recommendations

Throughout the process of analyzing the results of this project, I realized there are many questions left unanswered by this study. Because of these unanswered questions, there are multiple directions in which future studies can stem from this project. First, I believe more studies need to be performed regarding how *Spirulina platensis* affects the performance parameters of broiler chickens. There is much disconnection between studies regarding how these performance parameters are affected by algae. Some studies indicate that the algae have negative effects on the performance parameters, while some show there is no effect on these parameters when broilers are fed algae. There are still other studies that show *Spirulina* improves

performance parameters. More studies need to be done on this topic to gain a better understanding of how these algae affect broiler chickens' performance parameters. Another aspect of this project that needs further research is how the algae feed additive affects footpad scores. Although I currently believe that leaking water lines were the cause of this study's results, more investigation would need to be done to verify this assumption. If studies were performed that showed a vast difference among footpad scores of different treatments, it could be concluded that the water lines were indeed the cause of the footpad scores in this study. However, if multiple other studies without water line issues supported the results of this study, it could be speculated that the water lines were not the cause of the similar footpad scores. A final area that needs more research is the economic impact of using *Spirulina* in widescale broiler production. Even if using algae is deemed useful in improving performance parameters, if it is not economically reasonable, it is not plausible to incorporate into widescale broiler production. Therefore, research needs to be performed to determine if using *Spirulina platensis* as a feed additive for broilers is economically feasible.

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