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COMPARING THE EFFECTS OF CONVENTIONAL AND PASTURED POULTRY PRODUCTION SYSTEMS ON THE STRESS LEVELS OF BROILERS

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Abstract

The objective of this study was to measure stress levels in broilers raised on a pasture production system (PPS) and a conventional production system (CPS) by evaluating lymphoid organ weights, white blood cell profiles (WBCP), total white blood cell counts (TWBC), and heterophil to lymphocyte ratios (HLR). Broilers were brooded indoors for 3 weeks then divided into 2 groups; one placed on pasture while the other remained indoors. Blood was collected at weeks 3, 5, and 7 via brachial venipuncture. After 49 days, birds were slaughtered and lymphoid organs harvested. Results showed no differences between treatments for lymphoid organs, TWBC, or WBCP. Eosinophils were higher ($P < 0.05$) in CPS (333.33) compared to PPS birds (148.00) at week 5. The HLR was lower ($P < 0.05$) in PPS broilers at week 5 (0.40) than weeks 3 (0.44) and 7 (0.43). The PPS broilers experienced less stress overall than CPS birds.

Keywords: Conventional Production System, Broilers, Pasture Production System, Stress

Introduction

The exposure of chickens to stress is an inevitable event in poultry production. When stress levels pass a certain threshold, they can result in distress in the birds and have a negative impact on performance. Elevated stress levels are primarily brought on by conditions in which the birds are contained. Stress can be caused by many factors, for example, climatic stress (extreme environmental temperature and humidity); environmental stress (wet litter, poor ventilation); nutritional stress (insufficient nutrients in feed); physiological stress (rapid growth rate); physical stress (catching, injections, transportation); social stress (overcrowding); psychological stress (fear, rough management); and pathological stress (exposure to infectious agents). Extreme heat and increased stocking densities are two main stressors that have been commonly investigated (Bartlett and Smith, 2003; Heckert et al., 2002; Thaxton et al., 2006; Virden and Kidd, 2009). Because of feathering and lack of sweat glands in poultry, heat loss is limited which can lead to elevated body temperatures. As a result, broilers hyperventilate and take dirt baths in efforts to cool their bodies. Increased stocking density has also raised concerns about animal welfare. This in turn has led to a specified maximum stocking density in certain countries (Guardia et al., 2011).

Blokhuis et al. (2000) stated that outdoor systems can decrease stress conditions and increase bird comfort. This is likely due to the broilers being in a more natural environment. In conventional production systems, broilers raised on litter are exposed to built-up ammonia, dust, dander and debris, which can lead to respiratory problems for the birds, resulting in a decrease in performance qualities (Lay et al., 2011). Other possible

stress factors such as extreme temperatures, photoperiod, and light intensity are uncontrollable variables associated with outdoor (pasture, free-range, and organic) poultry production systems (Fanatico et al., 2005; Wang et al., 2009). In addition, predation can potentially be a major issue in outdoor production systems leading to even higher stress levels.

Although the literature suggests that birds reared outdoors are likely to be less stressed, there is still a lack of literature comparing stress based on production systems. Further research is needed evaluating the effects of different production systems on various stress indicators (corticosterone, heterophil to lymphocyte ratio, and lymphoid organ weights). Thus, the objective of this study was to measure stress levels in broilers raised on pasture and conventionally by evaluating lymphoid organ weights, white blood cell profiles (WBCP), total white blood cell counts (TWBC), and heterophil to lymphocyte ratios (HLR).

Literature Review

Increased stress in broilers can be identified through a number of methods. When under duress, corticosteroids, which are hormones produced by the adrenal glands, are released in the blood. Corticosterone is the primary corticosteroid hormone in birds (Virden and Kidd, 2009). Thus, measuring the level of corticosterone in the blood gives a quantitative indication of stress on the birds. Stress levels can also be identified by examining the total and differential white blood cell counts. There are five major types of white blood cells: heterophils, lymphocytes, basophils, eosinophils, and monocytes. Each plays a specific and important role in the immune system, protecting the body from foreign substances and repairing tissues (Campbell, 1995).

Moreover, examining the leukocyte profile provides vital information on the overall health of the bird and the susceptibility of contracting diseases. Heterophils and lymphocytes are the most common white blood cells found in most avian species. The ratio of heterophil to lymphocyte (HLR) has been identified as a reliable indicator of stress (Campbell, 1995; Carlo, 2013; Davis et al., 2008; Heckert et al., 2002). Broilers with elevated corticosterone levels have demonstrated depressed lymphocyte numbers (Glick, 1967; Siegel and Latimer, 1970). This in turn, will result in a higher ratio of heterophils to lymphocytes, resulting in an increased HLR (Siegel, 1995). In addition, the primary (thymus, bursa of fabricius) and secondary (spleen) lymphoid organ weights can be examined and correlated to stress levels. Low lymphocyte numbers are likely due to the regression of lymphoid tissue caused by the presence of corticosterone for prolonged periods (Siegel, 1971). Moreover, several studies have shown decreased lymphoid organ weights in broilers following corticosterone administration (Gross et al., 1980; Puvadolpirod and Thaxton, 2000a; Puvadolpirod and Thaxton, 2000b).

Campo et al. (2008) analyzed the effects of production systems and cold stressors on the HLR. Birds reared with outdoor access had significantly lower HLRs, indicating that these broilers were less stressed than those housed indoors in deep litter. Heckert et al. (2002) investigated the effects of stocking density and perch availability under simulated commercial production conditions on the immune status of broilers. These authors

evaluated the lymphoid organ weights, HLR, and lymphocyte blastogenesis. Results showed lymphoid organ weights, specifically bursa of fabricius weight, were the best indicator of stress. As stocking density increased, bursa of fabricius weight significantly decreased indicative of increased stress levels. Tong et al. (2014) conducted a study evaluating number of days on pasture and the effect on growth performance, carcass yield, meat quality, and lymphoid organ weights. Birds were kept on pasture for 0, 7, 14, and 21 days. There were no effects on weight of the liver, spleen, and bursa of fabricius. However, thymus weights did show an increasing then decreasing quadratic response to increasing days on pasture.

Materials and Methods

Experimental Birds, Diet and Housing

This research was conducted at the Poultry Unit of the George Washington Carver Agricultural Experimental Station at Tuskegee University in Tuskegee, Alabama 36088. The study was performed during the summer of 2013, from June - August. Three hundred and sixty 1-day-old male Cornish Rock broilers were purchased from Murray McMurray Hatcheries (P.O Box 458, 191 Closz Dr., Webster City, Iowa 50595). Upon arrival, chicks were wing-banded for identification, weighed, and randomly assigned to one of six pens prepared for brooding. Brooding pens were approximately 12 ft x 14 ft (3.66 m x 4.27 m). Each pen contained a 250-Watt infrared fluorescent brooding lamp to provide the chicks with adequate heat. Pens also contained bedding material of wood shavings approximately three inches thick to provide cushioning for the chicks. In addition, chick feeders and drinkers were placed in each pen. A commercial broiler ration, Nutrena® NatureWise meat birds crumble feed (P.O. Box 5614, Minneapolis MN, 55440), purchased from H. A. Vaughan Feed Store (106 West Lee Street, Tuskegee, Alabama 36083) was fed to the birds through the duration of the study. Feed and water were provided ad libitum to the birds. Table 1 shows the chemical composition of the feed offered as provided on feed label.

Table1. Chemical Composition of Diet

Macronutrients	
Crude Protein (%)	22.0
Lysine (%)	1.0
Methionine (%)	0.37
Crude Fat (%)	2.5
Crude Fiber (%)	6.0
Calcium (%)	0.9 - 1.4
Phosphorus (%)	0.6
Salt (%)	0.25 - 0.65
Sodium (%)	0.15 - 0.22

*Chemical composition as listed on label of feed bag

Experimental Procedure

This study utilized two treatments, the pastured poultry production system (PPS) and the conventional production system (CPS). Each treatment was replicated three times (60

birds per replication = 180 birds per treatment = 360 total). The birds were randomly assigned to treatment groups and brooded indoors for three weeks. After brooding, one treatment was moved into three polyvinyl chloride (PVC) pens on pasture while the other treatment remained in three indoor pens. The indoor pens were modified to be similar in size to the PVC pens measuring 10 ft. x 12 ft. (3.05 m x 3.66 m). The PVC pens were approximately 3 ft. high (0.9 m) with wire fencing around all sides. A tarp covered approximately two-thirds of the top of the pen providing shade and shelter for the birds. Access into the PVC pens was available via a top swinging door. All pens were equipped with hanging feeders and automatic drinkers. Weights and feed intake were recorded on a weekly basis. The study was conducted for a total of 49 days.

Blood Collection

At week 3, before the birds were taken out to the pasture pens, approximately 2 mL of blood was collected via brachial venipuncture from five, randomly selected birds from each replication (15 birds per treatment) to get a baseline white blood cell profile. Blood samples were taken again at weeks 5 and 7 from the same 15 birds from each treatment to observe if time on pasture impacted stress levels. Blood samples were collected into K2 EDTA vacutainer tubes. Immediately following blood collection, microscope slides were smeared and taken to a lab at the Tuskegee University School of Veterinary Medicine where they were stained with a Wescor Aerospray 7120 Hematology Slide Stainer using Wright's stain. Total white blood cell count and white blood cell profile were performed using a Nikon light microscope at a magnification of 100X, according to a procedure adapted from Fudge (2000), and heterophil to lymphocyte ratios were then determined.

Harvesting Lymphoid Organs

After final weights were recorded on day 49, feed was removed from all pens in preparation for slaughter and processing on day 50. On the day of slaughter, the birds were weighed to obtain pre-slaughter weights. They were then placed in killing cones, manually decapitated, and exsanguinated. The birds were dipped in 145°F (62°C) water for 30 seconds to loosen the feathers. Feathers were then removed using a batch de-feathering machine. The birds were eviscerated and the bursa of fabricius, thymus, and spleen were removed and weighed.

Statistical Analysis

The experimental design was a completely randomized design (CRD) with two treatments and three replications. Statistical analyses were performed using the General Linear Model procedure of SAS. Analysis of variance (ANOVA) tables were used for determining significant differences.

Results and Discussion

Table 2 shows the weights of the primary and secondary lymphoid organs. No significant differences were found in weights of the lymphoid organs between the CPS and PPS treatments. The results of this study are comparable to those of Tong et al. (2014) who found no effects on liver, spleen and bursa of fabricius when observing free-range broilers kept on pasture for 0, 7, 14, and 21 days. In the current study, the broilers were

on pasture for 28 days. Previous reports have suggested that outdoor production systems can decrease stress conditions and increase bird comfort (Blokhuis et al., 2000), due to the birds being in a more natural environment where they are exposed to fresh air and sunshine. However, this was not reflected in the results of the current study since the conventionally produced birds showed similar results.

Table 2. Primary and Secondary Lymphoid Organ Weights of Broilers Reared on Pasture and Conventional Production Systems

Lymphoid organs	Treatments (%)	
	CPS	PPS
Thymus	0.30 ± 0.01	0.32 ± 0.01
Bursa of Fabricius	0.14 ± 0.01	0.13 ± 0.01
Spleen	0.11 ± 0.01	0.10 ± 0.01

CPS=conventional production system, PPS=pasture production system

Table 3 provides the WBCP of broilers based on treatment. None of the white blood cells were found to be significantly higher based on treatments, however, it should be noted that the eosinophils were higher in broilers reared in the CPS (261.33) compared to those in the PPS (150.67). Eosinophils function in numerous inflammatory processes, especially allergic reactions and disorders (Campbell, 1995). Thus, eosinophils would be expected to be higher among CPS broilers where birds are said to be more exposed to stressors. Although no similar studies were found evaluating the WBCP based on production systems, the literature revealed studies comparing breeds and season. This current study was conducted during the summer months; therefore, there could be some connections to the heat of summer and the way the birds responded. Rajkumar et al. (2011) evaluated stress parameters in normal neck and naked neck broilers during the winter and summer. HLR, basophil, and eosinophil concentrations were found to be higher ($P < 0.05$) during the summer and among normal neck birds indicating that these birds were under stress.

The WBCP based on time is reported in Table 4. No significant differences were found among the different time periods (weeks 3, 5, and 7). However, significant differences were found between treatments for eosinophils at week 5, after being on pasture for 2 weeks. Because eosinophils function in inflammatory processes, such as allergic reactions and disorders (Campbell, 1995), the cell count tend to be higher in the CPS treatment. This is because the birds are more exposed to stressors like dust and dander compared to the PPS treatment where birds are reared outdoors in fresh air. In contrast, Abdi-Hachesoo et al. (2011) reported no effects on hematological parameters when comparing 8-week old male chickens of two different breeds, but the authors did not specify the type of production system used. However, white blood cell counts in the present study were similar to those found by Abdi-Hachesoo et al. (2011). In the present study, basophils were not found on blood smears at weeks 5 and 7, therefore, no values are shown. This is not uncommon because basophils make up less than 0.5% of white

Table 3. White Blood Cell Profile of Broilers Raised on Pasture and Conventional Production Systems

Leukocytes	Treatments			
	CPS		PPS	
	Number of Cells	(%)	Number of Cells	(%)
TWBC	14,705 ± 392		14,546 ± 392	
Basophil	3.55 ± 2.51	0.02	0.00 ± 2.51	0.00
Eosinophil	261.33 ± 30	1.84	150.67 ± 30	1.04
Heterophil	4,412 ± 142	29.99	4,071.11 ± 142	28.60
Lymphocyte	9,652 ± 229	65.53	9,768 ± 229	67.71
Monocyte	376 ± 130	2.62	556 ± 130	2.65

CPS=conventional production system, PPS=pasture production system
TWBC-total white blood cell count; %- percent of TWBC

blood cells. Moreover, because this cell type is relatively rare, basophils may not be on every slide (Histology Lab Manual, 2008).

Table 5 shows the HLR at weeks 3, 5, and 7 for each treatment. Differences were not significant between treatments; however, differences were significant based on time for the PPS broilers. After two weeks on pasture, the HLR decreased indicating that birds were less stressed than before they were put on pasture. Interestingly, stress levels increased after being on pasture for four weeks. This could be the result of weather and/or predation issues. During the last four weeks of the study (weeks 4 to 7) on pasture, temperatures averaged between 75-80°F (24-27°C) (Weather History of Tuskegee, Alabama (2013), which exceeds the thermoneutral zone of 64-75°F (18-24°C) for chickens (Bell and Weaver, 2002). As a result, the broilers may have experienced more stress during the last two weeks. It should also be noted that the PPS birds were attacked by a predator on one occasion which could have caused an elevation in the levels of the stress hormone, corticosterone, which may have depressed lymphocyte production causing an increase in the HLR in the birds.

Although not significant, the CPS birds showed the same trend as the PPS birds. This indicates that if high environmental temperature was the problem, then it was also hot for the birds in the conventional system. Henry (2002) stated that broilers perform best at 70°F (21°C). Although not significant, it is noteworthy that broilers reared conventionally showed higher HLRs overall than those reared on pasture. The results from this study are supported by Campo et al. (2008), who reported that hens with outdoor access were less stressed than those without outdoor access. Blokhuis et al. (2000) also stated that outdoor access can decrease stress levels in broilers.

Table 4. White Blood Cell Profile of Broilers Based on Treatment and Time

Leukocyte Profile	Treatments			
	CPS		PPS	
	Number of Cells	(%)	Number of Cells	(%)
TWBC				
Week 3	13,600 ± 963		12,227 ± 963	
Week 5	14,800 ± 529		14,933 ± 529	
Week 7	15,733 ± 422		14,533 ± 422	
Basophil				
Week 3	10.67 ± 7.54	0.07	0.00 ± 7.54	0.00
Week 5	-	-	-	-
Week 7	-	-	-	-
Eosinophil				
Week 3	275 ± 52	2.13	172 ± 52	1.20
Week 5	333 ± 57[a]	2.27	148 ± 57[b]	1.00
Week 7	176 ± 46	1.13	132 ± 46	0.93
Heterophil				
Week 3	4,125 ± 236	30.27	3,984 ± 236	29.53
Week 5	4,237 ± 270	28.67	4,007 ± 270	26.93
Week 7	4,873 ± 228	31.00	4,223 ± 228	29.33
Lymphocyte				
Week 3	8,747 ± 351	64.33	9,135 ± 351	65.87
Week 5	9,848 ± 465	66.47	10,341 ± 465	69.27
Week 7	10,360 ± 364	65.80	9,827 ± 364	68.00
Monocyte				
Week 3	424 ± 380	3.20	987 ± 380	3.40
Week 5	381 ± 61	2.60	437 ± 61	2.80
Week 7	324 ± 58	2.07	245 ± 58	1.73

CPS=conventional production system, PPS=pasture production system
TWBC-total white blood cell count; %-percent of TWBC
Columns with different superscripts indicate significant difference at $P < 0.05$.

Table 5. Heterophil to Lymphocyte Ratio of Broilers Raised on Pasture and Conventional Production Systems

Heterophil to Lymphocyte Ratio		
Weeks	Treatments	
	CPS	PPS
3	0.48 ± 0.03	0.44 ± 0.03[a]
5	0.45 ± 0.03	0.40 ± 0.03[b]
7	0.48 ± 0.03	0.43 ± 0.03[a]
Overall (wks 3-7)	0.47 ± 0.03	0.42 ± 0.03

CPS=conventional production system, PPS=pasture production system
Columns with different superscripts indicate significant difference at $P < 0.05$

Differences may exist when using HLR or lymphoid organ weights in assessing stress because of the sensitivity to specific stressors. Studies have shown that different stress identifiers are more accurate for different stressors (Gross and Siegel, 1983; Vleck et al., 2000). Thus, more research should be done in efforts to appropriately and accurately match stressors with stress identifiers.

Conclusion

Based on the results of this study, birds in both the PPS and CPS treatments exhibited the same level of stress indicators throughout the study. There were no differences in lymphoid organ weights. The white blood cell profile also showed no differences based on treatments. However, differences were observed for the white blood cell profile based on time. After being on pasture for 2 weeks, CPS broilers had a higher eosinophil count than that of PPS broilers. Differences were also identified in pasture broilers based on time for HLRs. Although no significant differences were found between the two treatments, it is noteworthy to mention that the pasture raised broilers were less stressed overall than the conventional broilers. Further research should be conducted to compare different stress identifiers to determine if specific identifiers are more accurate with specific stress types.

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