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Influence of L-carnitine on blood values of broiler chickens exposed to 16l:8d and 8l:16d photoperiod regimes

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Abstract

Different photoperiod regimes demonstrate promising potential in improving carcass weight and reduction of body fat. However, attaining normal blood values in the presence of feed supplements under a certain photoperiod regime has not been explored yet in broilers. The study investigated the effects of 200 ppm L-carnitine and photoperiod on hematological values of broilers (12-day old, n=60). A 2×2 factorial design in RCBD was carried out to produce four treatment groups (15 replicates/ group); T_1 = 16 hours light:8 hours dark without L-carnitine, T_2 = 16L:8D with L-carnitine, T_3 = 8L:16D with L-carnitine and T_4 = 8L:16D without L-carnitine. At 42 days, interaction effects of photoperiod and L-carnitine were significant for RBC count, hemoglobin and packed cell volume ($p<0.05$). Abdominal fat was lower by 1.66% and 1.51% in L-carnitine-supplemented chickens exposed to 16L:8D and 8L:16D compared to unsupplemented treatment groups (T_1 and T_4), respectively. Results also show that in all blood parameters studied, RBC (1.57-2.24×10^6/µL) was found below the reference range (2.5-3.5×10^6/µL) while lymphocyte differential count was higher (68.5-81.2%) than the reference range (45-70%). Eosinophil and monocyte were also found to be lower than the reference values, while hemoglobin, PCV, MCV, MCH, MCHC, heterophil, basophil, clotting time and bleeding time were within the normal range for chickens. The study concluded that supplementation of L-carnitine improved production performance as evidenced by lower % abdominal fat than an unsupplemented group. L-carnitine works better at a photoperiod of 16L:8D by improving RBC count, hemoglobin and packed cell volume (PCV).

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Introduction

L-carnitine is a water-soluble quaternary amine that occurs naturally in microorganisms, plants and animals (Bremer, 1983; Baumgartner and Blum, 1993; Sarica *et al*., 2005). A methylated amino acid roughly similar to choline, L-carnitine is biosynthesized *in vivo* from lysine and methionine (Rebouche and Paulson,1986) in the presence of ferrous ions and 3 vitamins; ascorbate, niacin and pyridoxine (Bieber, 1988; Feller and Rudman, 1988; Rebouche, 1991). L-carnitine covers an important role in lipid metabolism, acting as an obligatory cofactor for beta-oxidation of fatty acids by facilitating the transport of long-chain fatty acids across the inner mitochondrial membrane as acylcarnitine esters. Dietary L-carnitine supplementation promotes the β-oxidation of these fatty acids to generate adenosine triphosphate (ATP) and improves energy use (Rabie *et al.*, 1997 and Neuman *et al.*, 2002). In recent years, attempt to investigate the interaction effect of L-carnitine supplementation and photoperiod were only reported in a few papers: (a) testing the effect on pig's sperm quality (Yeste *et al.,* 2010) and (b) evaluating daily hypothalamic β-endorphin in rats (Genazzani *et al.,* 1990). Up until this time, this paper is the first of its kind to investigate the combined effect of these 2 factors (L-carnitine supplementation and photoperiod regime) in broiler chickens.

Photoperiod is the length of time to which birds are exposed to light each day (Abbas *et al.,* 2008). It is usually the sum of their exposure to sunlight and artificial light (Scheideler, 1989). Recent research comparing 12L:12D, 16L:8D and 20L:4D lighting schedules demonstrated clearly that longer periods of darkness prevent regular access to feed and consequently reduce feed intake and limit growth (Classen, 2004). Classen *et al.,* (1991) indicated that early growth rate was significantly reduced by longer periods of darkness, but gain from 14 to 35 days, as well as final bodyweight, were not affected by lighting programs. Feed conversion ratios were also higher for 12L:12D and two 6L:6D periods for each 24 hour period than 12 (1L:1D) periods per 24 hour period. Decreased photoperiods are reported, however, to decrease susceptibility to metabolic diseases such as ascites associated with pulmonary hypertension syndrome, sudden death syndrome, tibial dyschondroplasia and other skeletal disorders (Classen and Riddell, 1989; Classen *et al.*,1991; Renden *et al.*,1991; Petek *et al.,* 2005). These findings support the claim that regardless of the light regime used, restricted lighting programs enhance broiler production through improvements in body weights, feed efficiency, immune status and health as a result (Apeldoorn *et al.*, 1991; Fairchild, 2003).

Aside from production performance that was evaluated in a related paper (i.e., where body weight gain, feed conversion ratio and % abdominal fat were evaluated), one component of assessing the overall health of a broiler chicken is to examine the hematological values (Jain, 1993; Harrison and Lightfoot, 2006; Samour, 2006). These values are essential in support of clinicopathological diagnosis and in evaluating nutritional deficiencies, if applicable (Simaraks, 2004). Blood chemistry is also necessary to evaluate cellular changes (Ritchie *et al.,* 1994), such as comparing the clinical status of healthy vs. unhealthy animals (Phillips, 1999). Criteria such as species, age, sex, breed, medication taken, sampling site and sample size storage conditions were noted to be the factors associated with the level of these hematologic values in experimental broiler chickens (Phillips, 1999). In this study, these values will likely reveal interesting patterns and trends in the data and may reveal relationships between the two factors under investigation: photoperiod and supplementation of feeds with L-carnitine.

This study determined the effect of L-carnitine and photoperiod on hematological values of broiler chicken. Specifically, this research aimed to (1) determine the effect of 200 ppm L-carnitine on hematological values (i.e., RBC, hemoglobin, red cell indices, packed cell volume, WBC differential, H / L ratio, platelet count, clotting time and bleeding time) of broilers exposed to varying photoperiod; (2) determine which is the more effective photoperiod on hematological values of broilers with or without 200

ppm L-carnitine supplementation; and (3) determine interaction effects of 200 ppm L-carnitine and photoperiod on hematological values of broilers.

Materials and methods

Experimental design

The study employed a 2-factor factorial experiment in a randomized complete block design (RCBD), resulting in 4 treatment groups receiving both factors A & B (photoperiod and L-carnitine, respectively) randomly distributed per treatment as follows: T_1 = 16 hours light:8 hours dark without L-carnitine, T_2 = 16L:8D with L-carnitine, T_3 = 8L:16D with L-carnitine and T_4 = 8L:16D without L-carnitine. There were 15 experimental broiler chicks per treatment group, where 5 chicks comprise a replicate, and each chicken represent a sample.

Experimental animals

One day-old, healthy, straight-run commercial broiler chicks (n=60, Starbro®) were utilized in the current study and were kept for 6 weeks. Chicks were housed on the meshed wire floor and given water *ad libitum*. Specifically, two identical broiler houses were made, each having a floor area of 2.6 m^2 (2.6m × 1 m) where the first house covers cages T_1 and T_2 while the second house covers cages T_3 and T_4, respectively.

Actual broiler management practices

Brooding: Brooding (day 1-12) started after hatching, where all chickens received 24h lighting for the first 3 days with a brooding temperature of 33°C. Upon arrival to the site, animals were provided with 25% sugar solution, and feeding was administered *ad libitum* (chick booster crumble, Purina Bio1®). These feeds were given at 7:00 AM, 12 noon and 7:00 PM, respectively, taking into account the total amount of feeds consumed by the 60 chicks daily (Table 1).

On days 4-12, the brooder cage was exposed to 23 hours of light and 1 hr dark period (lights were off from 8:00 AM to 9:00 AM daily). This preliminary photoperiod (Gillespe, 1995) is necessary to provide the young chicks a longer time to feed and adjust to the source of water. Likewise, 10 watts incandescent bulb hanged 18 inches (47.5 cm) from the litter was utilized in the entire experimental period (Gillespe, 1995). On days 5-9 and 10-12, the lamp was raised from 18 to 20 inches and 18 to 22 inches off the littered floor, respectively, to reduce light intensity. Brooder guards, as suggested by Gillespe (1995), made of corrugated cardboard that is 30 cm tall and 78 cm in diameter, were used. Contrary to the growing period, L-carnitine was not yet added to the drinking water since half of the broiler chicken (n=30) belonged to the L-carnitine untreated group T1 and T4, respectively). All the broiler chickens were given a clean tap water *ad libitum* within the 12 day brooding period.

Growth period (day 13 to day 42): The brooder guard was removed and the 10 watts bulb was raised from 22 inches to 24 inches off the floor. During the segregation of the 60 chicks into 4 treatment groups, the farm assistant randomly assigned a replication number written on the left leg using a permanent marker. This was necessary for easy tracking of samples. Feeds and drinking water were given *ad libitum* except for the extra water supplemented with L-carnitine where only cages T2 and T3 could utilize. Carniking (www.lonza.com), a company that manufactures L-carnitine, stressed that poultry should not exceed 0.02% (200 ppm) of L-carnitine in feed. Thus, instead of mixing the encapsulated commercially-available L-carnitine, the researcher decided to mix it with drinking water to achieve a homogeneous solution. Each capsule (300 mg/capsule @ 30 capsules/bottle) was dissolved in 1.66 liters of water (200 ppm = 200 mg L-carnitine in 1 L of water), mixed thoroughly and divided the solution into 2 equal parts (0.83 L each), one given to T2 and the other part was given to T3 daily.

Like in the brooding stage, feeds were administered at 7:00 AM (0700), 12 noon (1200) and 7:00 PM (1700). The same schedule of measuring feeds left was followed. Photoperiod specified as factor A, like L-carnitine, was adjusted at day 13. T1 and T2 received 16 light and 8 dark periods daily where lights are turned on at 7:00 AM to 11:00 PM and off from 11:01

PM to 6:59 AM the next day. On the contrary, T3 and T4 followed the reverse photoperiod of 8 hours light and 16 hours dark period where lights were on from 7:00 AM to 3:00 PM and turned off from 3:01 to 6:59 AM in the next day. The schedule for measurement of weight weekly was the following: day 8, day 15, day 22, day 29, day 36 and day 43, representing weeks 1, 2, 3, 4, 5 and 6, respectively.

Data gathering procedure

Blood parameters: Collection of blood was held in the morning, from 7:00 AM to 8:00 AM on the 43rd day of the experiment. Blood was collected from the right jugular vein (v. jugularis dextra) using a gauge 21 needle and a 3 ml disposable syringe. At least 3-6ml of blood was collected per 100g body weight (Sturkie, 1965), following the recommended methodology of Samour (2006). After collection, the needle was removed, and the blood was gently deposited into a 0.5-1.0ml commercially available pediatric blood storage tube containing an anticoagulant (ethylenediaminetetra-acetic acid [EDTA], ~1.5mg/ml of blood). The vial was gently shaken and placed in an iced chest to be processed and analyzed within 24h after collection (Maala *et al.*, 2002). Immediately after collection, a drop was placed over a clean glass slide for a blood smear. Upon arrival in the laboratory, the blood samples were immediately analyzed. Specific procedures and laboratory protocols for the blood counting (RBC, WBC differential count and platelet count) were followed after Harrison and Lightfoot (2006) and Samour (2006).

Analysis of data

Data were encoded in the Windows Microsoft Excel® and were transferred to the data file of the Statistical Package for the Social Sciences (SPSS) software, Version 20. Data were labeled for identity and were analyzed using the SPSS Analysis of Variance program for the 2-factor experiment (2-way ANOVA). The main effects between subjects/factors were compared by Tukey's honestly significance test, which is a component program of SPSS.

Results and discussion

Hematological profile

In general, blood examination is performed for several reasons as a screening procedure to assess the general health of an animal (Jain, 1993). Hematological values are also important to clinicopathological diagnoses such as traumatic injury, parasitism, organic disease, bacterial septicemia and nutritional deficiencies (Simaraks, 2004). Blood chemistry is also necessary to evaluate cellular changes (Ritchie *et al.*,1994) and hematological values are useful in determining the health and general condition of chickens.

Table 1. Proximate analysis of the commercial feeds given to all experimental broiler chicken (n=60).

Chick booster crumble (Bio 100 Purina Feeds)	Broiler starter crumble (Bio 200 Purina Feeds)	Broiler finisher crumble (Bio 300 Purina feeds)
1st and 2nd week	3rd and 4th week	5th and 6th week
Crude protein – 21.5 %	Crude protein – 19.5 %	Crude protein - 17.0 %
Crude fat - 3.0 %	Crude fat - 4.0 %	Crude fat - 4.0 %
Crude fiber - 5.0 %	Crude fiber - 5.0 %	Crude fiber - 2.0 %
Moisture -13.0 %	Moisture - 13.0 %	Moisture - 13.0 %

*Source: Proximate Analysis, Purina Feeds Corporation, Philippines.

Once hematologic data are obtained from a sample animal, evaluation follows. However, in order to evaluate the data, this can be compared with other data from related animals that are clinically healthy (Phillips, 1999). These data for comparison are generally arranged into a reference range which accounts for both the low and high extremes of the values that are found in healthy animals. Criteria such as species, age, sex, race, medication taken, stress, parasitism were noted to be the factors associated

with the level of these hematologic values in experimental broiler chickens (Khan and Zafar, 2005; del Pilar *et al.,* 2001).The hematological profile of the broiler chickens (n=60) is shown in groups in Tables 2, 3, 4, 5 and 6. Table 2 below shows the mean RBC, hemoglobin and PCV from the four treatment groups.

Red blood cells

RBC count was not significantly different from the values of T1, T3 and T4, whereas T2 was markedly different from the three previously mentioned treatments ($p<0.05$). Across treatment, RBC count ranged from $1.57\pm0.41\times10^6$ /µL to $2.24\pm0.24\times10^6$ /µL (Table 2). This range of values is still lower compared to the Japanese quail (Albarece, 1963) value of 2.93 to 4.32×10^6 /µL. The interaction effect of photoperiod and L-carnitine was significant ($p<0.05$), indicating a compounded effect of these two factors in the RBC values of broiler chickens.

It is also noticeable that all the RBC values from the four treatments were below the reference value of $2.5\text{-}3.5\times10^6$ /µL (Jain, 1993). Similarly, RBC values from this present experiment were also lower than the mean RBC of locally-raised Philippine fighting cocks (Maala *et al.*, 2002).

Table 2. Red Blood Cells, Hemoglobin, and Packed Cell Volume (Mean±SEM) of chickens reared in two photoperiod conditions with and without L-carnitine (n = 60).

Photo-period	Treatments (w/ & w/o L-carnitine)	Hematological profile		
		Red Blood Cells ($x10^6$/µL)	Hemoglobin (g/dL)	PCV (%)
A_1 (16 L: 8 D)				
	T_1 - No L-Carnitine	1.57 ± 0.41^a	6.27 ± 1.10^a	20.47 ± 3.72^a
	T_2 - 0.02% (200 mg/L) L-carnitine	2.24 ± 0.24^b	7.27 ± 1.67^a	23.93 ± 5.58^a
A_2 (8 L: 16 D)				
	T_3 - 0.02% (200 mg/L) L-carnitine	1.84 ± 0.35^a	6.20 ± 1.47^a	20.33 ± 4.95^a
	T_4 - No L-Carnitine	1.83 ± 0.17^a	6.73 ± 1.10^a	22.00 ± 3.64^a
Interaction effect ($p<0.05$)	L-carnitine vs. Photoperiod	sig.	sig.	sig.

Different letter superscripts within columns indicate significant difference ($p<0.05$).

The implication of a low level of RBC in the broiler chickens could be attributed to the low production of RBC in the red bone marrow of the long bones (Mader, 2004). Despite the L-carnitine supplementation on T2 and T3, chickens' RBC was still below the normal range. This could be associated with low iron content in the diet since they were not given any nutritional supplement aside from L-carnitine in the whole experiment period. Another reason for the lower RBC count was the fact that the animals were not fed prior to blood collection. Still, another possible reason is the presence of intestinal parasites (i.e., roundworms seen during the evisceration) since all birds were not given a dewormer during the entire experimental period. Mader (2004) also noted that when there is an insufficient number of RBC or if the RBC has low hemoglobin content, the individual animal suffers from iron-deficiency anemia. However, some research conducted in the past revealed that L-carnitine could enhance the stability and integrity of red blood cell's membrane by participating in the regulation of membrane's phospholipids subjected to oxidative stress or damage (Lonza Inc., www.lonza.com), but was not observed to enhance RBC production in this experiment.

Hemoglobin (Hb)

The mean hemoglobin of broiler chickens ranged from 6.20 to 7.27 g/dL in this experiment (Table 2).

Hemoglobin concentration across the four treatments was insignificant to each other if compared ($p<0.05$). These values are also lower than the Philippine fighting cocks such as Talagudin, Texas, or imported fighting cocks (Maala *et al.*, 2002). Similarly, the Hb values in the broiler chickens are also lower than those of Philippine turkeys (Escudero and Jovellanos, 1965 as cited by Maala *et al.*, 2002) but still lower compared to that of Philippine native ducks (Escudero and Mansilla, 1965 as cited by Maala *et al.*, 2002).Among the four treatment groups, only T2 (7.27±1.67 g/dL) made it to the reference range established by Jain (1993) for clinically-healthy chickens, which is 7.0 to 13.0 g/dL. A normal Hb value can be associated with the amount of RBC since each RBC contains about 200 million hemoglobin molecules. A low RBC count would indicate the low availability of Hb to carry oxygen and carbon dioxide, which is essential for several metabolic body processes (Mader, 2004).

The interaction effect between L-carnitine and photoperiod was found to be significant ($p<0.05$) for Hb values, an indication that both light and L-carnitine supplemented each other to produce a unified effect on the hemoglobin molecule of RBC.

Table 3. Red Blood Cell Indices (Mean Corpuscular volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration (Mean ± SEM) of chickens reared in two photoperiod conditions with and without L-carnitine (n=60).

Photo-period	Treatments (w/ & w/o L-carnitine)	Red blood cell index		
		MCH (pg)	MCV (fl)	MCHC (%)
A_1 (16 L: 8 D)				
	T_1 - No L-Carnitine	32.53±8.42^{a}	106.80±27.8^{a}	30.27±0.52^{a}
	T_2 - 0.02% (200 mg/L) L-carnitine	41.40±6.22^{b}	134.67±21.3^{b}	30.49±0.46^{a}
A_2 (8 L: 16 D)				
	T_3 - 0.02% (200 mg/L) L-carnitine	34.00±8.84^{b}	112.40±28.4^{a,b}	30.47±0.52^{a}
	T_4 - No L-Carnitine	37.13±6.75^{a,b}	121.20±22.7^{a,b}	30.40±0.51^{a}
Interaction effect ($p<0.05$)	L-carnitine vs. Photoperiod	ns*	ns	ns

Different letter superscripts within columns indicate significant difference ($p< 0.05$) & *ns – not significant
MCV –Mean corpuscular volume, MCHC – Mean corpuscular hemoglobin concentration
MCH – Mean corpuscular hemoglobin.

Packed cell volume (PCV) or hematocrit

Packed cell volume (PCV) is an important hematologic assay because it provides an easy and objective way of estimating the number of erythrocytes in the sample. It is also essential for the calculation of the mean corpuscular volume (MCV) and means corpuscular hemoglobin concentration (MCHC) (Samour, 2006).

The mean PCV obtained in the present work ranged from 20.33 to 23.93%. PCV values in the four treatments are statistically insignificant ($p>0.05$) from each other. Though still lower than the Philippine fighting cocks (Maala *et al.*, 2002) still, these present PCV are considered normal under the reference range of 22-25% (Jain, 1993) except T1 (20.47±3.72%) and T3 (20.33±4.95%). However, much higher PCV than in the present study was reported by Sturkie (1965). Comparing the PCV of broilers to those of fighting cocks and other avian species, the PCV of the former is lower than those of canaries, budgerigars, Greater Indian Hill Mynah and Amazon parrots (Rosskopf and Woerpel, 1982). The PCVs of broilers in this experiment are also lower

than those of Japanese quails (Albarece, 1963) or to the mallard ducks during the dry and wet season (Hernandez, 1991 cited by Maala *et al.*, 2002).

A very high PCV could be associated with the struggling behavior of other birds during blood collection. According to Svenson (1977), excitement causes the release of epinephrine which in turn results in splenic contraction, thus releasing erythrocytes into the bloodstream. Another factor that could have influenced a higher PCV in other birds such as imported cocks was the high altitude where they were raised and trained since animals raised at higher altitudes have higher PCV and RBC values than those raised in cages or in low altitudes (Coles, 1986).

Red cell indices

Red cell indices are also called mean corpuscular values. These values are of particular importance in determining the morphological type of anemia and may also be of assistance in selecting therapy and guiding the continuation of an established therapeutic procedure (Coles, 1974). Results of this present experiment show that the interaction effect of L-carnitine and photoperiod on the different red cell indices (i.e., mean cell hemoglobin, mean cell hemoglobin concentration and mean corpuscular volume) are all insignificant ($p>0.05$). Results for red cell indices are presented in Table 3 below.

Mean cell hemoglobin (MCH) is the measure of the average amount of hemoglobin present in erythrocytes (Svenson, 1977). Therefore, MCH is influenced by the size of the erythrocytes and the hemoglobin content (Maala *et al.*, 2002). In the present work, MCH ranged from 32.53 to 41.40 picogram where T2 was 41.40±6.22 pg, followed by T4, T3 and T1 with 37.13±6.75, 34.00±8.84 and 32.53±8.42 pg, respectively. These results are lower (except T2) than that reported by Zinki (1986) in domestic chickens but closely similar with the MCH of Philippine fighting cocks, which ranges from 36.51 to 44. 83 pg (Maala *et al.*, 2002).

Table 4. White Blood Cells (Heterophil, Lymphocytes and H:L Ratio (Mean ± SEM) of chickens reared in two photoperiod conditions with and without L-carnitine (n= 60).

Photo-period	Treatments (w/ & w/o L-carnitine)	White blood cell differential (%)		
		Heterophil	Lymphocyte	H:L Ratio
A_1 (16 L: 8 D)				
	T_1 - No L-Carnitine	17.00±7.31[a]	81.20±7.66 [a]	0.22±0.14[a]
	T_2 - 0.02% (200 mg/L) L-carnitine	29.13±4.72[b]	70.00±4.71 [b,c]	0.42±0.10[b]
A_2 (8 L: 16 D)				
	T_3 - 0.02% (200 mg/L) L-carnitine	28.00±11.2[b]	68.53±13.9 [b]	0.47±0.36[b]
	T_4 - No L-Carnitine	19.13±6.95[a]	78.60±8.98 [a,c]	0.26±0.12[a,b]
Interaction effect ($p<0.05$)	L-carnitine vs. Photoperiod	ns*	ns	ns

Different letter superscripts within columns indicate significant difference ($p< 0.05$).
H: L Ratio – heterophil to lymphocyte ratio.

According to Svenson (1977), MCV, on the other hand, expresses the average cell size of the RBC in cubic microns or femtoliters (fl). In this experiment, the following mean MCV values were recorded: T2 with 134.67±21.3 fl, closely followed by T4, T3 and T1 with 121.20±22.7, 112.40±28.4 and 106.80±27.8 fl, respectively.

Finally, MCHC expresses the average percentage of MCV which the hemoglobin occupies (Samour, 2006). It is indeed valuable in the diagnosis of certain anemic conditions and reflects the capacity of the bone marrow to produce erythrocytes of normal size, metabolic capacity and hemoglobin content (Sturkie, 1965). The MCHC of the 4 treatment groups were

insignificantly different from one another (p>0.05), where values are closer to each other (30.27 to 30.49%). MCHC was highest at T2 with 30.49±0.46%. Expectedly, T2 was also highest in hemoglobin concentration (7.27 g/dL); thus, it is also the highest in MCHC.

All the four treatments in the three different red cell indices were within the normal range/reference range in broilers. This goes to show that, on average, all experimental animals (n=60) were clinically free from anemia or red cell-related diseases. In the event of anemic conditions (below the reference range), alterations in the average size of red cells (MCV) are paralleled by similar changes in MCH and, oftentimes, MCHC.

WBC differentials

The next set of blood profiles is the WBC differentials. The first set of WBC considered is the heterophils and lymphocytes (%) and H:L ratio, as reflected in Table 4. It can be observed that the highest % in terms of heterophil in treatment T2 (29.13±4.72) and the lowest being T1 (17.00±7.31). On the contrary, the highest lymphocyte was found in T1 (81.20±7.66), while the lowest was found to be in T3 (68.53+13.9). All values for heterophil were still on the reference range of Jain (1993) where heterophils in clinically healthy birds are within 15.0-40.0%, but in the case of lymphocytes, only T2 (70.00±4.71) and T3 (68.53±13.9) are within the reference range (45.0-70.0%).

The results below tell us that only those treatments (T2 & T3) which were supplemented with L-carnitine showed a "normal" % of heterophil and lymphocyte count in comparison with the standard reference range of Jain (1993). Despite the introduced stress (i.e., catching and blood sample collection) in the experimental animals, still, the % of heterophil remains within the reference point. In most cases, an increase in heterophil is accounted for with increased adrenalin along with fear and excitement, during exercise or in the onset of anemia (Coles, 1974). Leukocytic responses can be used as an indicator of stress status in poultry. In another study, Maxwell *et al.,* (1992) suggested that heterophilia may be a response to moderate stress but that heterophoria may develop during extreme stress conditions.

Heterophilia (decrease in circulating heterophils) occurs in systemic infections such as salmonellosis or septicemias and the degree of heterophilia is usually much greater with localized infection than systemic diseases (Coles, 1974). Tissue destruction (i.e., surgical procedure) irrespective of its cause will also be relevant in increasing the number of circulating heterophils. Hemorrhage is also often followed by an increase in heterophils, particularly if there has been hemorrhage into one of the serious cavities in the body (Coles, 1986).

Since the white blood cells in the avian species, in general, serve to phagocytic function similar to their mammalian counterparts (Campbell and Coles, 1986) and differential leukocytes count as well as H/L ratio were used as indicators of stress response and sensitive biomarkers crucial to immune function, a low leukogram of genetically developed new strains may be the reason behind their high susceptibility to avian pathogenic agents when compared with indigenous chickens which are relatively resistant to poultry diseases (Shini, 2003).

With regards to lymphocytes, observations from many studies discussed that stress reduces the number of lymphocytes in the blood, suggesting that there may be an adrenocortical regulating mechanism that appears to affect most of the short-lived cells (Coles, 1976). The principal function of the lymphocyte is in relationship to its immunological activity. As observed from the data in this recent experiment, lymphocyte % in T1 (81.20±7.66) and T4 (78.60±8.98) was above the reference value (45.0-70.0). This lymphocytosis (increased in lymphocytes) could be probably caused by either one or a combination of the following; (1) lymphocytic leukemia, (2) all conditions associated with heterophilia (increased in heterophils), (3) during the recovery stage of certain infections, adrenocortical

efficiency or hyperthyroidism. (Coles, 1974). In other circumstances, lymphopenia (decreased in lymphocyte is observed) and this could be attributed to the following; viral diseases, in response to stress condition as a result of secretion of adrenocortical substances that may cause dissolution of the cells (Coles, 1974). Similarly, stressors including food or water deprivation, temperature extremes, constant light and exposure to novel social situations elevate the number of heterophils and depress the number of lymphocytes (Gross and Siegel, 1986; Gross, 1989). In Table 4, the heterophil to lymphocyte ratio tells a partial view of the status of the animal. This is an index of stress that could be obtained in avian blood (Gross and Siegel; 1983; Maxwell, 1993). Table 4 also shows that the lowest H:L ratio is T1 (0.22±0.0.14) and the highest was T3 (0.47±0.36). There is no reference range provided by Jain (1993) for H:L ratio, but Gross and Siegel (1983), as cited by Altan *et al.*, (2005), reported that H/L ratios of about 0.2, 0.5 and 0.8 characterized low, optimum and high levels of stress, respectively. In addition, a lower H:L ratio is an indication of a lower stress level (Vleck *et al.*, 2000). Thus, it could be deduced that all H/L ratios from the four treatments were all in low-stress conditions.

Table 5. White Blood Cells Differential– monocyte, eosinophil and basophil (%), (Mean±SEM) of chickens reared in two photoperiod conditions with and without L-carnitine (n= 60).

Photo-period	Treatments (w/ & w/o L-carnitine)	White blood cell differential (%)		
		Monocyte	Eosinophil	Basophil
A_1 (16 L: 8 D)				
	T_1 - No L-Carnitine	1.13±1.6[a]	0.33±0.49[a]	0.26±0.46[a]
	T_2 - 0.02% (200 mg/L) L-carnitine	0.33±0.49[a]	0.13±0.35[a]	0.4±0.64[a]
A_2 (8 L: 16 D)				
	T_3 - 0.02% (200 mg/L) L-carnitine	2.26±3.6[a]	0.4±0.51[a]	0.8±0.74[a]
	T_4 - No L-Carnitine	1.73±2.7[a]	0.2±0.73[a]	0.33±0.49[a]

Different letter superscripts within columns indicate significant difference ($p< 0.05$).

The highest H:L ratio was found in T3 (0.47±0.36) followed by T2 (0.42±0.10). Siegel *et al.*, (1995) noted that increased H:L ratio is an accepted indicator of stress in chickens and broilers reared under continuous light (24L:0D), has a higher H:L ratio and experienced greater fear response than birds under a shorter photoperiod (Zulkifli *et al.*, 1998). Their result was the contrary in this experiment whereby T2 (16L:8D) was lower in H:L than T3 (8L:16D), although the difference was insignificant at $p<0.05$ level. Shini (2003) also added that H:L ratio quantifies the balance between the non-specific, fast-acting defenses of heterophils and the antigen-specific slower-acting defenses of lymphocytes. Thus, H:L ratio is considered as a sensitive hematological indicator of stress response among the chicken population (Graczyk *et al.*, 2003) and as a general biomarker relevant to immune function in poultry (Shini, 2003).

Broilers in T1 and T2 (both under 16L:8D photoperiod regime) should have a higher H:L ratio than T3 and T4 if the photoperiod alone was the basis for the number of heterophils and lymphocytes. This is because broilers reared under a longer photoperiod (e.g., 16 hours light or more) experienced greater fear response as indicated by increased tonic immobility time than birds reared under a 12L period. Such increase in tonic immobility is directly proportional to an increase in high H:L ratio, an indicator of stress in poultry (Siegel, 1995 and Zulkifli *et al.*, 1998). Restricted photoperiods (16L:8D) have an impaired immune response to disease challenges. The improved immune response may be due to the rest period that is provided during dark periods and the production of melatonin or to a combination of both

(Apeldoorn *et al.*, 1991). Analyzing the data gathered, data on H:L ratio indicates that the L-carnitine supplementation in T2 and T3 did not significantly reduce the stress condition of the experimental birds in this recent experiment. The next Table 5 shows the % monocytes, % basophils, % eosinophils taken from the differential count. The result tabulated below shows the means of each individual WBC (monocyte, eosinophil, and basophil), respectively. In all the treatments, all the monocytes were found below the range specified by Jain (1993), which is 5 -10%. This is also similar in terms of eosinophil count (reference range is between1.5 - 6.0). Although Jain (1993) identified in a series of clinical trials the presence of basophil, he mentioned that it is rare in normal birds and did not identify a reference range. The table also shows that monocyte, eosinophil and basophil values within each treatment did not vary significantly ($p<0.05$).

In terms of function, monocyte becomes macrophages that phagocytize pathogen and cell debris while eosinophils phagocytize antigen-antibody complexes and allergens. On the other hand, basophils release histamine during allergic reactions and promote blood flow to injured tissues (Mader, 2005).

Table 6. Platelet count, clotting time and bleeding time (Mean ± SEM) of chickens reared in two photoperiod conditions with and without L-carnitine (n= 60).

Photo-period	Treatments (w/ & w/o L-carnitine)	Hematological profile		
		Platelet Count (platelets/ µL)	Bleeding Time (second)	Clotting Time (second)
A_1 (16 L: 8 D)				
	T_1 - No L-Carnitine	5.87±1.13[a]	63.20±14.3[a]	68.007±11.2[a]
	T_2 - 0.02% (200 mg/L) L-carnitine	6.00±1.13[a]	46.67±8.04[b]	52.00±11.0[b]
A_2 (8 L: 16 D)				
	T_3 - 0.02% (200 mg/L) L-carnitine	5.80±1.08[a]	52.20±10.3[a,b]	54.73±9.99[b]
	T_4 - No L-Carnitine	5.73±1.10[a]	61.33±15.9[a]	62.87±16.5[a,b]
Interaction effect ($p<0.05$)	L-carnitine vs. Photoperiod	ns*	ns	ns

Different letter superscripts within columns indicate significant difference ($p< 0.05$).
ns* - not significant.

Monocytosis (an abnormal increase of monocyte) could occur in conditions like a chronic disease such as fungal infections where a considerable amount of tissue debris should be removed, prompting the monocyte to increase in number (Coles, 1974). It could be noticed from the percentage of eosinophils that all the treatments did not reach 1.5%, an indication of eosinophilia. This decrease in the number of circulating eosinophils may be seen in any stress condition or hyperactivity of the adrenal gland, occurring as a consequence of hyperplasia or neoplasia (Harrison and Lightfoot, 2006). Finally, it is noteworthy that basophilia (increase in basophils) occurs as a consequence of eosinophilia or basophilia granulocytic leukemia. Similarly, in the event of an increase in eosinophils, this would reflect hypersensitivity to parasitism and allergic reactions and adrenocortical insufficiency (Coles, 1974).

Platelet count, bleeding time and clotting time

Table 6 below shows the last set of blood values that include the platelet count, clotting time and bleeding time of broiler chickens from the four treatments. Platelet counts were comparable in all the 4 treatments with an insignificant *p*-value, although the highest thrombocyte (blood platelet) count was found in T2 (6.00±1.13), while the lowest count was identified in T4 (5.73±1.10). In all animals, platelets

are essential for the clotting of blood. When platelets are low, it may take longer for the blood to clot and when it is too high, unnecessary blood clots may occur instead (Campoto, 2005).

Still from the same table, data on bleeding time shows that T1 (63.20±14.3) has the highest bleeding time, followed by T4 (61.33±15.9), T3 (52.20±10.3) and T2 (46.67±8.04). The bleeding time of T1, T3 and T4 are statistically insignificant when compared to each other, while T2 vs. T3 is also insignificant at $p<0.05$. Coles (1974) stated that bleeding time is a useful technique for evaluating the efficiency of the capillary-platelet aspect of homeostasis. He also added that prolonged bleeding time might be associated with the following conditions: (1) defect in the blood vessel wall, (2) platelet defects as a consequence of the presence of abnormal platelets (3) severe liver disease and (4) uremia. Pairwise comparison under LSD showed that the interaction effect between L-carnitine and photoperiod on bleeding time was insignificant at $p>0.05$.

Finally, clotting time (seconds) could also be seen in Table 6. It shows that the highest clotting time was observed in T1 (68.00±11.2), whereas the lowest clotting time was recorded in T2 (52.00±11.0). T1 is statistically insignificant when compared to T4 (62.87±16.5) but not to T2 and T3 (54.73±9.99), respectively. Coles (1974) emphasized that the interval between the appearance of the blood and the appearance of a fibrin strand is the coagulation time. A long clotting time is seen in a variety of coagulopathies in animals which might include the following conditions: (1) defect in the intrinsic system (hemophilia), (2) severe liver disease (3) thrombocyte disease (4) circulating anticoagulants and (5) anemia.

As a general observation on the same table, it can be deduced that T2 conveys consistent and logical data since its high platelet count justifies the shorter bleeding and clotting time. This goes to show that more prothrombin was converted to thrombin, a protein necessary in changing fibrinogen to fibrin (Guyton, 1992).

Conclusion

Based on the findings of the study, the following conclusions were drawn: (1) photoperiod of 16L:8D was the better regime than 8L:16D as evidenced by hematological values within the normal reference range, and (2) L-carnitine works better at a photoperiod of 16L:8D by improving RBC count, hemoglobin and packed cell volume (PCV). Based on the conclusions made, this study recommends that farmers may synchronize the supplementation of 200 ppm L-carnitine in feeds and exposure of broilers to 16 h light:8 h dark photoperiod to achieve optimum productivity and normal blood values in broiler chickens. For future research, similar hypotheses may be proposed, but this time, to consider other economically important poultry animals such as quails, ducks and mallards, as well as turkey.

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