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Original Article

Physical and Semen Characteristics of Mexican Creole Roosters in Summer and Autumn, at 19° North Latitude with a Constant Photoperiod

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■Keywords

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ABSTRACT

The objective of this study was to evaluate the effect of the seasons (Summer and Autumn), on live weight, body condition, mass motility, percentage of live spermatozoa, and sperm-cell concentration of Creole roosters (*Gallus domesticus*) from Mexico. Semen from 35-week-old Creole roosters was collected weekly during 10 weeks in Summer and Autumn, through the dorso-abdominal massage technique. Roosters were individually kept under a constant photoperiod (16 hours light:8 hours dark). The average live weight was 4.5% higher ($p<0.05$) in Autumn (2.78 kg) than in Summer (2.66 kg), therefore this variable increased with age (r = 0.85, $p<0.05$). Category 2 of body condition occurred ($p<0.05$) with higher probability than the others (0, 1 and 3), being practically the same ($p>0.05$) in Autumn (99.96%) and in Summer (99.81%). On average (and in weeks 1 and 3-10), the percentage of live spermatozoa was higher in Summer than in Autumn. Accordingly, the percentage of live spermatozoa decreased with age (r = -0.82, $p<0.05$). However, on average, sperm-cell concentration did not change between seasons ($p>0.05$). In conclusion, Mexican Creole roosters showed higher percentage of live spermatozoa in Summer than in Autumn. Therefore, it is advisable to select these animals of about 2.7 kg and reproduce them in Summer.

INTRODUCTION

Animal backyard production is carried out by about 90% of rural families in Mexico, and this activity represents about 10% of national poultry production (Segura-Correa, 1998; Camacho-Escobar *et al.*, 2006). Reproductive performance of backyard poultry (*Gallus gallus domesticus*) can be described in terms of fertility, which in turn influences hatchability (Peters *et al.*, 2008). On the other hand, this reproductive performance characteristic depends on semen quality (Ajayi *et al.*, 2011). This quality can be assessed according to variables such as pH, appearance, volume, mass motility, viability, morphology, and sperm-cell concentration (Elagib *et al.*, 2012; Shanmugam, *et al.*, 2012; Adamu *et al.*, 2019). Furthermore, semen quality depends on bird genotype (Adamu *et al.*, 2019; Tabatabaei *et al.*, 2009), age (Juárez-Caratachea *et al.*, 2018), and season (Bah *et al.*, 2001; Obidi *et al.*, 2008).

Environmental temperature is considered to particularly influence rooster semen characteristics (Adamu *et al.*, 2019; Santiago-Moreno *et al.*, 2009a; Santiago-Moreno *et al.*, 2011). For optimal reproductive performance, the environmental temperature should range between 18 and 22 °C (Lin *et al.*, 2006). It has been observed that exposure of roosters to high environmental temperature (>31 °C) negatively affects the percentage of live spermatozoa and motility (McDaniel *et*

al., 2004). The effects of environmental temperature on reproductive characteristics are more pronounced in Summer (Bah *et al.*, 2001).

On the other hand, there is evidence that higher relative humidity improves the reproductive performance of local breeds (Obidi *et al.*, 2008). Therefore, environmental temperature and relative humidity are important factors influencing the reproductive performance of roosters; however, knowledge of the effects of these factors, especially in Mexican Creole roosters, is scarce and they need to be further investigated (Balnave, 2004).

The search for optimal reproductive performance of domestic animals begins with their characterization. For this genotype of poultry, studies have focused on the characterization of Mexican productive systems and phenotypic characteristics such as plumage colour, live weight, crest type, and egg production (Segura *et al.*, 2007; Zaragoza *et al.*, 2013; Cuca-García *et al.*, 2018). Rodríguez-Ortega *et al.* 2018 are probably the only ones to have addressed the reproductive issue of naked-necked Creole roosters with different type of crest in Mexico (Rodríguez-Ortega *et al.*, 2019). This information exists in other developing countries (Santiago-Moreno *et al.*, 2009b; Rakha *et al.*, 2017; Adamu *et al.*, 2019), but is scarce in Mexico.

Therefore, the objective of this study was to evaluate the effect of Summer and Autumn (environmental temperature, relative humidity, and age) on live weight, body condition, and semen characteristics of Mexican Creole roosters.

MATERIALS AND METHODS

Location

The study was conducted during the Summer and Autumn of 2019, at the poultry facilities of the Postgraduate College, Campus Montecillo, in Texcoco, State of Mexico, Mexico, located at 19° 29' north latitude, 98° 53' west longitude and 2,247 masl. Climate was classified as Cw, which corresponds to temperate with summer rainfall, a mean annual temperature of 14.6 °C and a mean precipitation of 558.5 mm (García, 2004).

Experimental birds

Seventeen 35-week-old Creole roosters (2.6579 ± 0.0378 kg live weight) were used in Summer, and the same roosters, 44-week-old (2.7814 ± 0.0381 kg live weight), were used in Autumn. No mortality occurred during the experimental period.

Birds were kept individually in cages (0.6×0.6×0.6 m) inside a poultry house with natural ventilation, regulated by side curtains, with a constant photoperiod (16 h light:8 h dark) during Summer and Autumn. Roosters were fed a diet containing 17% crude protein and 2800 kcal ME kg^{-1}. During the experimental period, 120 g of feed per animal per day were offered, and water was provided *ad libitum*. Birds were managed according to the standards of the Postgraduate College Animal Welfare Committee (COLPOS, 2016).

Temperature and relative humidity

Temperature and relative humidity inside the poultry house were recorded daily, three times a day, by three homogeneously distributed sensors (digital thermometer-hygrometer with a LCD probe, Veanic, USA). In addition, precipitation, temperature and relative humidity data were obtained from the Montecillo Campus Meteorological Station.

Physical characteristics of Creole roosters and semen collection

Live weight

Before the experimental period, birds were trained for ten days to extract semen through the dorso-abdominal massage technique (Burrows & Quinn, 1937). Before semen extraction and collection, the live weight and body condition of each rooster were recorded. Live weight of the fasted roosters was recorded using a digital scale with 5.0 kg capacity and 5 g accuracy (L-PCR, Torrey, Mexico).

Body condition

This variable was determined according to the Gregory & Robins (1998) methodology. The assessment was carried out by holding the rooster with the left hand, hugging it, and avoiding his flapping. The pectoral region was palpated with a hand, the volume of breast muscles was assessed and the protrusion of the keel was evaluated. Each rooster was categorized using a scale from 0 to 3: keel with prominent edge and limited breast muscle development (0); keel still prominent, but with more breast muscle development (1); keel less prominent and moderate breast muscle development (2); flat keel edge and well-developed breast muscles (3).

Semen collection

Once both live weight and body condition were recorded between 9:00 and 11:00 AM, semen was collected from each bird in graduated Eppendorf tubes

(5 cm^3) and was also pre-warmed at 41 °C to avoid a temperature shock of the spermatozoa. Semen was evaluated before 45 minutes post collection. During the waiting period, semen samples were kept in a water incubator at 41 °C.

Semen characteristics of Creole roosters

Semen evaluation consisted of an analysis of ejaculation time (s), semen volume (cm^3), mass motility (scale 0 to 5), percentage of live (%), dead (%) and abnormal (%) spermatozoa, and sperm-cell concentration (spermatozoa cm^{-3}). Each of them is described below.

Mass motility

This variable was assessed using the Evans & Maxwell (1987) technique based on a scale from 0 (the worst mass motility) to 5 (the best mass motility) to determine the vigour of sperm movement: category 1: 10% of spermatozoa show movement; category 2: no formation of waves, 20-40% of spermatozoa are active; category 3: 45-65% of spermatozoa are active; category 4: vigorous movement, 70-85% of spermatozoa are active and category 5: very vigorous movement with dense waves, 90% or more of spermatozoa are active.

Live, dead and abnormal spermatozoa percentages

Percentages of live, dead, and abnormal spermatozoa were determined by the eosin-nigrosine staining technique described by Bamba (1988). After the smear, 100 cells were selected and the number of spermatozoa with stained (dead) and intact (live) membrane, and with abnormalities in head or tail was counted (Alkan *et al.*, 2002).

Sperm-cell concentration

This variable was determined as described by Cortez & Gallegos (2014). Spermatozoa were counted using an enhanced Neubauer chamber and a red blood cell pipette. Spermatozoa were counted in 5 (0.02 mm^3=5×0.004 mm^3) of the 25 large squares (0.004 mm^3 each) in the chamber (0.1 mm^3), and the sperm-cell concentration per mm^3 was calculated using the following formula: SC=N×F×D, where SC=sperm-cell concentration (spermatozoa mm^{-3}), N=number of sperm-cells counted in 0.02 mm^3, F=multiplication factor: 50 mm^3 (because 0.02 mm^3 is 1/50th of 1 mm^3), D=dilution rate: 200 (because semen was diluted 1/200). Multiplying by 1000 (because 1 cm^3 equals 1000 mm^3), SC resulted in sperm-cells cm^{-3}.

Statistical analysis

The only factor studied was season of the year with two levels: Summer and Autumn. Variables live weight, semen volume, ejaculation time, spermatozoa percentages, and sperm-cell concentration were analysed by repeated measures over time using the GLMIXED procedure. A multinomial generalised linear mixed (MGLM) model with ordinal multinomial response was used for the ordinal variables: body condition and mass motility, which were reported as probability. The LSD test, $\alpha = 0.05$, was used to compare all means. A Pearson correlation coefficient ($p<0.05$) was obtained between each pair of physical and semen variables. The statistical package used was SAS (2011) version 9.4.

RESULTS

Temperature and relative humidity

Environmental data recorded during the study period (Table 1) indicated that both temperature and relative humidity inside the poultry house were, on average, higher in Summer than in Autumn (21 and 20 °C, and 75.5 and 59.5%, respectively). Also, environmental temperature outside the poultry house was higher in Summer than in Autumn (17 and 16 °C, respectively). The higher relative humidity in Summer was due to a higher rainfall precipitation in this season than in Autumn (2.3 and 1.5 mm, respectively).

Physical characteristics of Creole roosters

Live weight

In Autumn, live weight ranged from 2.69 to 2.91 kg with a mean value of 2.78 ± 0.038 kg, higher ($p<0.05$) than that observed in Summer, when live weight ranged from 2.50 to 2.76 kg with a mean value of 2.66 ± 0.038 kg (Figure 1).

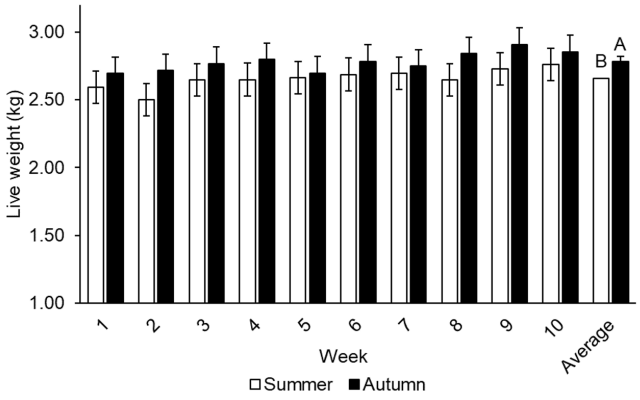

Figure 1 – Mean ± standard error of average and weekly live weight of Creole roosters during 10 weeks in Summer and Autumn, 2019 in Texcoco, State of Mexico, Mexico. Different letters above the bars indicate statistical differences ($p<0.05$).

Table 1 – Environmental data during Summer and Autumn of 2019 in Texcoco, State of Mexico, Mexico.

Weeks	Summer	Autumn	Summer	Autumn	Summer	Autumn	Summer	Autumn
	[1]Temperature, °C		[1]Precipitation, mm		[2]Temperature, °C		[2]RH, %	
1	17.1	17.0	0.2	3.4	22.4	21.6	66.8	61.3
2	16.0	16.9	4.2	2.0	20.1	20.9	79.4	60.2
3	16.7	17.1	3.5	1.2	20.8	21.0	77.1	61.3
4	17.4	17.5	6.9	1.0	21.4	20.5	79.8	64.8
5	18.1	16.4	3.4	4.9	21.9	21.3	80.3	61.3
6	17.2	15.9	0.9	0.4	20.4	20.1	76.8	66.7
7	17.5	15.3	0.4	2.2	21.8	20.6	66.3	60.9
8	17.4	15.7	0.4	0.0	21.1	20.5	72.0	58.1
9	16.6	13.1	2.2	0.0	20.7	19.6	79.0	45.7
10	17.1	13.2	1.2	0.0	21.1	18.1	77.7	54.5
Mean	17.0	16.0	2.3	1.5	21.0	20.0	75.5	59.5

[1]Meteorological station, [2]Poultry house, RH=relative humidity.

Body condition

Of the four body condition categories (0 to 3), only categories 1 and 2 were observed (Figure 2). The probability of observing category 2 in Creole roosters was the same (p>0.05) in Summer (99.81%) and in Autumn (99.96%).

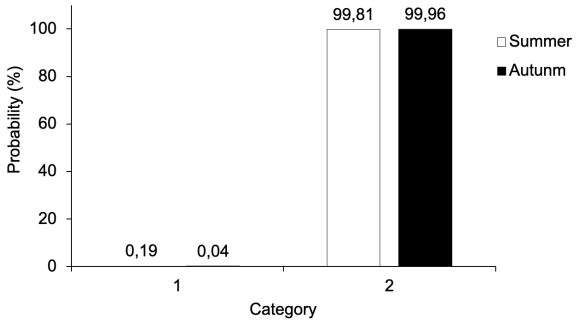

Figure 2 – Body condition of Creole roosters during Summer and Autumn, 2019 in Texcoco, State of Mexico, Mexico. 1: keel still prominent, but with more breast muscle development; 2: keel less prominent and moderate breast muscle development (Gregory & Robins, 1998). Over each category, the LSD test was used for comparison of means.

Semen characteristics of Creole roosters

Ejaculation time

On average, ejaculation time (Figure 3) was not different (p>0.05) between seasons: Summer (7.79 ± 0.2796 s), Autumn (7.54 ± 0.2719 s). However, in contrast with weeks 3, 6, and 8, on weeks 9 and 10 this variable was lower (ejaculation was faster) in Summer than in Autumn.

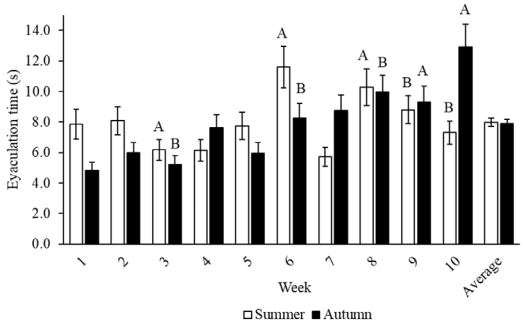

Figure 3 – Mean ± standard error of average and weekly ejaculation time of Creole roosters during 10 weeks in Summer and Autumn, 2019 in Texcoco, State of Mexico, Mexico. Different letters above the bars indicate statistical differences (p<0.05).

Semen volume

Except for weeks 5 (higher in Autumn) and 10 (higher in Summer), semen volume of Creole roosters (Figure 4) was similar (p> 0.05) between Summer (0.28 cm^3) and Autumn (0.26 cm^3) for the other weeks, as well as on average.

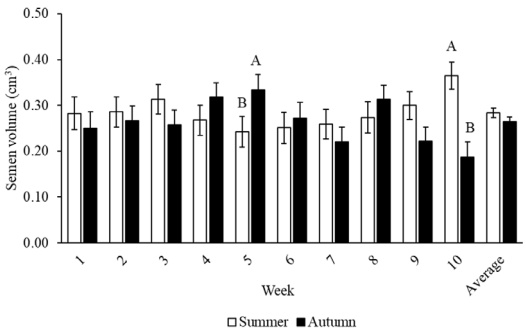

Figure 4 – Mean ± standard error of average and weekly semen volume of Creole roosters during 10 weeks in Summer and Autumn, 2019 in Texcoco, State of Mexico, Mexico. Different letters above the bars indicate statistical differences (p<0.05).

Mass motility

Probability values of semen mass motility of Creole roosters (Figure 5) were not different between seasons (p>0.05). With respect to the others, category 5 occurred with the highest probability: Summer (59.32%) and Autumn (56.42%), and category 2 occurred with the lowest value: Summer (6.37%) and Autumn (7.01%).

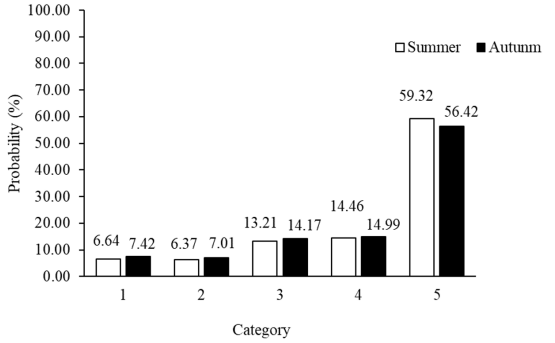

Figure 5 – Mass motility of Creole roosters during the Summer and Autumn, 2019 in Texcoco, State of Mexico, Mexico. Category 1: 10% of spermatozoa show movement; category 2: no formation of waves, 20-40% of spermatozoa are active; category 3: 45-65% of spermatozoa are active; category 4: vigorous movement, 70-85% of spermatozoa are active and category 5: dense waves with rapid movement and 90% or more of spermatozoa are active (Evans & Maxwell, 1987). Over each category, the LSD test was used for comparison of means.

Percentage of live, dead and abnormal spermatozoa

On average, as well as on most of the weeks, the percentage of live spermatozoa from semen of Creole roosters (Figure 6) was higher (p<0.05) in Summer (avg. 96%) than in Autumn (avg. 77%). In contrast, the average percentage of dead spermatozoa was lower (p<0.05) in Summer (2 ± 0.42%) than in Autumn (20 ± 3.26%), while the percentage of abnormal spermatozoa was the same (p>0.05): 1%, in both seasons.

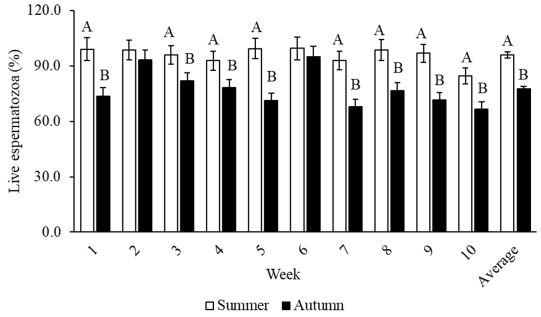

Figure 6 – Percentage of live spermatozoa (mean ± standard error) from Creole roosters during 10 weeks in Summer and Autumn, 2019 in Texcoco, State of Mexico, Mexico. Different letters above bars indicate statistical differences (p<0.05) (Bamba, 1988).

Sperm-cell concentration

On average, the sperm-cell concentration of Creole roosters did not differ between seasons. However, contrary to weeks 3 and 6, when it was higher in Autumn, this variable was higher in Summer than in Autumn from weeks 8 to 10 (Figure 7).

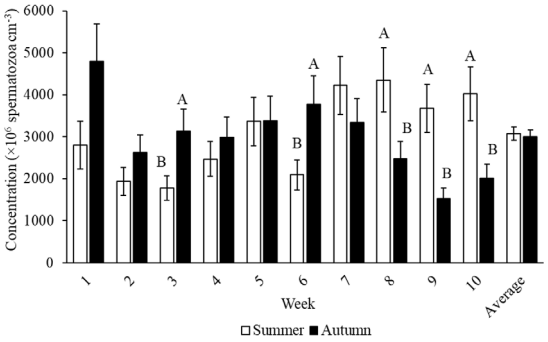

Figure 7 – Mean ± standard error of average and weekly sperm-cell concentration ($\times 10^6$ spermatozoa cm^{-3}) during 10 weeks in Summer and Autumn, 2019 in Texcoco, State of Mexico, Mexico. Different letters above bars indicate statistical differences (p<0.05) (Cortez & Gallegos, 2014).

Pairwise correlation of physical and semen characteristics

A correlation analysis was performed among all physical and semen variables, including the factors age, and environmental temperature, relative humidity and precipitation. These correlations are not reported in table form because only two were statistically significant (p<0.05): age vs. live weight (r = 0.85, p<0.05) and age vs. percentage of live spermatozoa (r = -0.82, p<0.05).

DISCUSSION

The effect of factors such as breed, age and season on semen characteristics of roosters in different regions of the world has been studied by Santiago-Moreno *et al.* (2009a), Elagib *et al.* (2012), and Adamu *et al.* (2019). However, for Mexican Creole roosters, little or no information was available.

The findings of this study indicate that the live weight of Creole roosters increased with age, results consistent with those of Juárez-Caratachea *et al.* (2018), using Rhode Island roosters from 6 to 18 months of age. The increase in live weight of broiler breeders is related to a decrease in fertility (Silveira *et al.*, 2014), so it is advisable to control feed intake to maintain body weight without affecting this reproductive characteristic (Romero-Sánchez *et al.*, 2007). After maturity, live weight increased slowly with age (Juárez-Caratachea *et al.*, 2018), which explains why roosters gain weight as the experimental period progressed in this study. Concordantly, Romero-Sánchez *et al.* (2007) found that broiler males still tend to gain live weight after sexual maturity.

In this study, category 2 of body condition (low prominent keel and moderate breast muscle development) occurred with higher probability than the other categories (0, 1 and 3), and it was practically the same (p>0.05) in Autumn (99.96%) as in Summer (99.81%). A higher category (3, for instance) with well-developed and broad breast could affect mating (McGary *et al.*, 2003), therefore, all birds had a good category to enter the mating season. However, more studies are required to assess a possible correlation between body condition and fertility.

To achieve ejaculation and obtain semen of good quality, an adequate stimulus is required (Gee & Temple, 1978). In commercial and local breeds no studies were found where ejaculation time was evaluated. In other species, ejaculation time was found to be positively correlated with semen volume (Oberlender *et al.*, 2012), however, no correlation of this type was found in the present study. Therefore, it is advisable to further investigate ejaculation time of the Mexican Creole rooster.

In this study, semen volume in Summer (0.28 cm^3) and Autumn (0.26 cm^3) was greater than that reported by Elagib *et al.* (2012) in one-year-old White Leghorn roosters (Summer: 0.21 cm^3 and Autumn: 0.23 cm^3). Using roosters of a local breed in Nigeria, Bah *et al.* (2001) obtained 0.28 cm^3 of semen, a similar value to the one found in this study. Such authors indicated that the month of the year impacts semen volume (0.24 to 0.32 cm^3); however, Jafari *et al.* (2013) mentioned that semen volume depends on genotype and latitude, recording values ranging from 0.50 to 0.52 cm^3 with Ross 308 roosters. At 33.7° N latitude, 44.8° E longitude, an Iraqi indigenous chicken of Abu-Ghraib produced the highest ejaculate volume in summer (30.6 °C) and the lowest one in winter (12.9 °C): 0.21 and 0.37 cm^3, respectively; a similar trend was observed with the White Leghorn and New Hampshire breeds, the lowest ejaculate volume was observed in the winter season (Saeid & Al-Soudi, 1975). These authors reported a positive correlation between semen volume and temperature (p<0.05), however, as in the present study, season (that is, relative humidity and temperature) did not affect (p>0.05) semen volume.

Probably due to the constant photoperiod, the mass motility in this study did not show significant differences between Summer and Autumn. A 16-hour light photoperiod acts as an environmental signal, stimulating the secretion of FSH, LH, and testosterone, very important hormones for spermatogenesis and semen production (Thurston & Korn, 2000). For native Spanish roosters under natural conditions, Santiago-Moreno *et al.* (2011) suggest that Spring is the best season to obtain good spermatozoa motility. Neither temperature nor relative humidity affected (p>0.05) mass motility (Saeid and Al-Soudi, 1975). The number 5 category of the present study (Summer: 59.3% and Autumn: 56.4%) was lower than that observed by Santiago-Moreno *et al.* (2009a) in Castellana roosters of one year old under natural photoperiod and temperature conditions in Spain (Summer: 72.2% and Autumn: 67.7%).

In this study, although the photoperiod was constant, the percentage of live spermatozoa from Creole roosters was higher (p<0.05) in Summer (higher temperature and relative humidity, and younger age of birds) than in Autumn. In the present study, the correlation analysis indicates that the percentage of live spermatozoa was negatively associated (p<0.05) with age, that is, this variable was higher in Summer (high temperature and relative humidity) than in Autumn. Bah *et al.* (2001) reported high numbers of spermatozoa in July and August, when the relative humidity is also high. Akhlaghi *et al.* (2014) found that in Cobb 500 roosters the percentage of live spermatozoa decreased with the aging of birds. Fragoso *et al.* (2013) reported that testes weight and testosterone levels decreased with age. In contrast, the results of this study differ from those reported by Shanmugam *et al.* (2014), who evaluated breeding roosters of the Indian Dahlem Red line and did not find a decrease in the percentage of live spermatozoa with age.

Shanmugam *et al.* (2014) reported that the percentage of dead spermatozoa in roosters of the Indian Dahlem Red line at 23, 42, and 65 weeks of age was less than 9%, and no differences (p>0.05) were observed among ages. These percentages of dead spermatozoa were between those obtained in the present study: 2% (Summer) and 20% (Autumn).

In the present study, the percentage of abnormal spermatozoa was very low, 1% in both seasons, and it was lower than that found by Machebe & Ezekwe (2002), who reported about 10% abnormalities in three genotypes of local breeds of roosters in the Spring of a tropical region in Nigeria. In another study, Obidi *et al.* (2008) reported 7.8% abnormal spermatozoa in Shikabrown line roosters evaluated during the Spring in Nigeria. Tabatabaei *et al.* (2009) reported a lower percentage of abnormal spermatozoa in local breeds (7%) than in Ross 308 roosters (10%). It is suggested that the maximum percentage of abnormal spermatozoa in the semen of local breeds should be 10% (Fattah *et al.*, 2016).

In the current study with constant photoperiod, the average sperm-cell concentration did not differ between Summer and Autumn (3076 and 3008×10^6 spermatozoa cm^{-3}). These results agree with Santiago-Moreno *et al.* (2009a) (Summer: 899×10^6 and Autumn: 714×10^6 spermatozoa cm^{-3}), who studied native Spanish roosters under natural temperature and photoperiod conditions (Summer: 18 to 24°C with 14 to 16 hours of daylight; and Autumn: 4 to 20°C with 12 to 8 hours of daylight). Similarly, Adamu *et al.* (2019), did not find a significant difference on sperm-cell concentration of one year old local roosters from a semi-arid region of Nigeria between Summer and Autumn [Early Dry (4090×10^6 cm^{-3}) and Late Rainy (4710×10^6 cm^{-3}) seasons]; these concentrations were higher than those found in the present study. Tyler *et al.* (2011) observed no significant difference on sperm-cell concentration in Ross 708 breeding roosters, from 40 to 60 weeks of age at different photoperiods (12, 14, 16, 18, 20 and 22 hours of light). The sperm-cell concentration reported by these authors was lower than that obtained in our study. Therefore, the results of these studies suggest that environmental factors did not influence this variable. Similarly, Saeid & Al-Soudi (1975) found no significant correlations between sperm-cell concentration with temperature, nor with relative humidity.

The correlation (p<0.05) age vs. live weight (r=0.85) indicates that roosters were still growing during the experimental period. On the other hand, the correlation (p<0.05) age vs. percentage of live spermatozoa (r=-0.82) indicates that as the roosters got older, the percentage of live spermatozoa decreased. Apparently, both relative humidity and environmental temperature must be optimal to maximize productive performance. For example, sperm-cell concentration, semen volume, and motility of local cocks from Nigeria were maximal between July and August, when the warmest temperature is low and the relative humidity is high (Bah *et al.*, 2001).

CONCLUSIONS

In Summer (July to August), as compared to Autumn (September to December), most Creole roosters had an adequate live weight and an appropriate reproductive age, most of them were in good body condition; they ejaculated faster in weeks 9 and 10, they had a higher semen volume in week 10, most of them produced a very vigorous semen with a higher percentage of live spermatozoa in most weeks of the study, and had higher sperm-cell concentration in weeks 8, 9 and 10. Therefore, it is advisable to select Creole roosters with these characteristics to reproduce them in the Summer.

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