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Research Article

Effect of Gonadotrophin (Diclair®) on Semen Characteristics, Testicular Morphometry and Hormonal Profile of Mature Harco Cocks

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ABSTRACT

Twenty sexually matured (24 weeks old) healthy Harco cocks were used to determine the effect of Gonadotrophin (Diclair®) on semen characteristics, testicular morphometry and hormonal profile. The cocks were divided into 4 treatment groups of 5 cocks per group identified as T_1 (control) administered with 1ml physiological saline, T_2, administered with 13.50i.u Diclair®, T_3 27.00i.u and T_4, administered with 40.50i.u Diclair®, with one cock per replicate in a Completely Randomized Design (CRD). The injections were divided into three doses each and administered intramuscularly in the thigh for three consecutive days. Semen was collected one week after Diclair® administration, twice a week for 4 weeks by the abdominal massage and manipulation of the cloaca method. Five cocks were randomly selected from each treatment group and bled one week after Diclair® injections to collect blood for hormonal profile evaluation. The results showed that there were significant differences ($P<0.05$) among the treatment groups in all the semen parameters; semen volume, semen pH, individual motility, sperm concentration, proportions of dead sperm cells and live sperm cells. The results further showed that there were significant differences ($P<0.05$) among the treatment groups in testicular, epididymal and vas deferens weights. Similarly, the results showed significant differences ($P<.05$) among the treatment groups in follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels. The results of this study suggest that Diclair® treatment enhanced semen quality, testicular development and was not detrimental to the hormonal profile of the cocks.

Key words: Diclair® semen quality, Testicular morphometry, Hormone, Cocks

INTRODUCTION

Harco chicken was developed in New England in the middle of the 19th century and was first exhibited as a breed in 1869. The Harco chicken is a dual-purpose, cold-hardy bird and therefore makes a great breed for the small farm or backyard flock owner. Harco cocks are large, long-lived chicken weighing between 2.5kg-2.8kg and are bred principally for meat. They possess a long broad back, a moderately deep, full breast, yellow skin and legs. The birds are dark barred in colour which means that the bars of dark colour are wider than the white colour.

In order to carry out any sustainable improvement in livestock, there should be methods of ensuring the repeatability and multiplication of desired trials in sub-sequent generations. Reproduction is a process by which an organism gives rise to a new member of its species. It is a vital factor in determining the efficiency of animal production and its performance is closely related to profit-ability in poultry enterprise (Iheukwumere *et al.* (2008).

Sperm producing potentials are evaluated by aspects of semen output: volume, motility of sperm cells, morphology of spermatozoa, proportion of live sperm cells and concentration in ejaculate. No single parameter has been proved to be an accurate predictor of the quality of individual ejaculates (Iheukwumere *et al.,* 2001). Sperm formation involves the use of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Iheukwumere *et al.,* 2004). Most of these preparations of FSH and LH are very expensive perhaps because of the brand names, some of them require cold chain storage and often deteriorate because of inadequate storage and handling (Herbert *et al.,* 2000).

Diclair®, also known as Humegon or Mentrophin and with similar constituents as plusset® is a gonadotrophin preparation lyophilized in vials containing a mixture of follicle stimulating hormone and luteinizing hormone in a ratio 1:1 (Dixon and Hopkins, 1996). Follicle stimulating hormone and LH in Diclair® play vital role in the initiation of spermatogenesis. The hormone preparation is

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cheap, readily available and does not require cold chain storage (Iheukwumere, 2005). It has not been determined if the administration of the hormone preparation for spermatogenesis and semen production would induce any side effects on the testicular morphometry and hormonal profile of the cocks. This study was therefore carried out to determine the effect of Diclair® administration on the semen quality, testicular morphometry and hormonal profile of mature Harco cocks.

MATERIALS AND METHODS

Experimental birds and their management

Twenty clinically sound and sexually matured (24 weeks old) Harco cocks purchased from Elgibbor farms in Isuikwuato Local Government Area, Abia State Nigeria, were used for this study. The birds were dewormed and vaccinated soon after purchase. A two-week pre-experimental period was allowed to enable the animals acclimatize. The birds were housed and raised on a deep-litter system. They were fed commercial Grower mash containing 20% CP and 2000 Kcal ME/kg diet twice daily (in the morning and evening). Water was provided *ad libitum.*

Experimental design

The twenty sexually matured (24 weeks old) Harco cocks were divided into 4 treatment groups, identified as T_1, T_2, T_3, and T_4. Each treatment group consisted of 5 cocks with one cock per replicate in a Completely Randomized Design (CRD), with four levels of Diclair® as treatment. The levels of Diclair® were 0.00ml, 0.09ml, 0.18ml, and 0.27ml represented as T_1, T_2, T_3 and T_4 respectively. T_1 (Treatment 1) which contained no Diclair® served as the control. Diclair® treatment was by intramuscular injection. The injections were administered as follows:

Diclair® was supplied in 3 vials each containing FSH 75i.u and LH 75i.u. The content of the vial was dissolved in 1ml of physiological saline solution provided immediately prior to use, resulting in a solution of DFSH 75i.u plus DLH 75i.u per ml. All treatments were administered intramuscularly on the thigh of each cock using a one ml syringe with 0.01ml graduation. The doses and concentration of Diclair® administered are shown in tables 1 and 2.

Semen collection and evaluation

The cocks were trained to ejaculate by abdominal massage and manipulation of the cloaca as described by Abu *et al*. (2006). By this method, ejaculates were collected from each cock after one week of Diclair® injections and continued at one week interval for 4 weeks.

This involved a gentle massaging of the abdomen while the opposite hand simultaneously stroked the lower back and tail feathers of a restrained cock. When phallic tumescence was achieved, the collector's hands were placed around the cloaca with a downward pressure while the lower hand exerted slight upward pressure. The semen which pools on the phallus after each squeeze of the cloaca, was collected into a clean dry test tube. To minimize the spread of pathogens, care was taken by the collector not to touch the cloaca structure.

Semen evaluation was done as promptly as possible post collection as described by Rodriguez-Martinez and Barth (2007) for qualitative and quantitative parameters such as semen volume, sperm concentration, sperm motility, semen colour, semen pH, dead sperm percentage and live sperm percentage.

Testicular morphometry

Three cocks in each treatment group were slaughtered and via cervical dislocation and dissection using surgical blade, forceps, scissors, organ weights were measure in grams using a sensitive weighing balance. The testicular organ parts that were measured include; the left and right testis, paired testes, left and right epididymis, paired epididymis, left and right vas deferens, paired vas deferens. At the end, the mean average results of the testicular organ weights from each treatment group was determined.

Hormonal assay

Blood samples (5ml each) were obtained by wing vein puncture of the 20 cocks on day 7 after the Diclair® injection, for testosterone, FSH and LH evaluation. It was cooled immediately in iced water and transferred to the laboratory, refrigerated at 4^0C for 1 hour and the serum separated by centrifugation at 5,000rpm for 10 minutes. The serum was stored immediately at – 20^0C until enzyme immune assayed (EIA) for testosterone, FSH and LH as described by Micallef *et al.* (1995).

Data analysis

Data collected on semen characteristics, testicular morphometry and hormonal profile of mature Harco cocks were subjected to one - way analysis of Variance (ANOVA) using the technique of steel and Torrie (1980). Significant treatment means were separated using Duncan's New Multiple Rande Test as described by Obi (1990).

RESULTS AND DISCUSSION

The results of Diclair® administration on semen characteristics of Harco cocks are shown in Table 3.

There were significant differences ($P<0.05$) among the treatment groups in semen volume, pH, individual motility, sperm concentration, proportions of dead and live sperm cells. Cocks on T_4 recorded the highest value of 1.00ml in semen volume and this differed significantly ($P<0.05$) from rams on T_2 and T_3 which were similar ($P>0.05$) to each other in semen volume. Values of semen volume obtained in this study were higher than the range of 0.24 $\pm$ 0.1 – 0.26 $\pm$ 0.3ml reported by Abu *et al*. (2006) in cocks and higher than the range 0.25 $\pm$ 0.2 – 0.31 $\pm$ 0.14 reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. Volume of semen varies with species, age, breed, season and frequency of ejaculation (Ozkan *et al.*, 1998).

Cocks on T_4 recorded the highest value of 8.00 in semen pH and this differed significantly ($P<0.05$) from cocks on T_1, T_2 and T_3. Cocks on T_1 and T_2 were similar ($P>0.05$) in PH but differed significantly ($P<0.05$) from cocks on T_3. The lowest value in pH (6.00) was observed in cocks on T_3. The pH values obtained in this study were

within the normal range of 7-8 reported by Meacham (2002) except cocks T_3 whose pH value (6.00) was lower than the normal range. The measured pH can depend on the length of time since ejaculation and it tends to increase shortly after ejaculation as a result of loss of CO_2 (Meacham, 2002).

Cocks on T_4 recorded the highest value of 82.60% in individual motility and this differed significantly ($P<0.05$) from cocks on T_1, T_2 and T_3 which were also significantly ($P<0.05$) different from each other in individual motility. The lowest value in individual motility was observed in cocks on the control treatment (T_1). The values for percentage of motile spermatozoa obtained in this studys were higher than the ranges of 36 – 52% and 48.3 – 57.3% reported by Abu *et al.* (2006) and Ameh (2004) respectively in Nigerian cocks. The differences observed in sperm motility may be attributed to breed (Oguike, 2000). Cold shock could not have been responsible for the relatively low motility observed when lower doses of the drug were administered, because estimation of the progressive motility was done soon after semen collection.

Cocks on T_4 recorded the highest value of 1.54 ($x10^9$/ml) in sperm concentration and this differed significantly ($P<0.05$) from cocks on T_1, T_2 and T_3. Cocks on T_2 and T_3 were similar ($P>0.05$) to each other, but they differed significantly ($P<0.05$) from cocks on T_1. The lowest value in sperm concentration was observed in cocks on T_2 and T_3 (0.52×10^9)/ml.

The highest value for sperm concentration obtained in this study (1.54×10^6)/ml compares favourably with the reports of Abu *et al.* (2006), who recorded 1.98 – 2.10 x 10^9/ml; Ezekwe *et al.* (2003) who recorded 1.25 – 2.13 x 10^9/ml and Oguike *et al*. (2000) who reported 1.18 – 2.13 x 10^6/ml, in Nigerian local cocks but lower than the value of $3.8x10^6$/ml reported by Chalov (1970) in cocks. This variation in sperm concentration of the individual cocks could be attributed to factors such as breed (Oguike *et al.*, 2000), plane of nutrition, ambient temperature, frequency of semen collection and drug administration (Abu *et al.*, 2006).

Cocks on T_1 recorded the highest value of 49.50% in dead sperm cells and this differed significantly ($P<0.05$) from cocks on T_2 T_3 and T_4 which were similar ($P>0.05$) to each other in proportion of dead sperm cells. The lowest value in dead sperm cells was observed in cocks on T_4 (35.00%). The percentages of dead sperm cells obtained in this study (35.00 – 49.50%) were higher than the range of 16.54 $\pm$ 0.7 – 20.05 $\pm$ 0.05% reported by Iheukwumere *et al.* (2008), the range of 21.0 – 22.5% reported by Abu *et al.* (2006) and the mean value of 14.25% reported by Ameh (2004) in Nigerian local cocks. Neck abnormalities were numerous, followed by tail and mid piece abnormalities. Iheukwumere *et al.* (2008) pointed out that the neck is the most fragile part of the spermatozoa and may be broken while handling semen when making smears.

Cocks on T_4 recorded the highest value of 65.00% in live sperm cells and this differed significantly ($P<0.05$) from cocks on T_1. There were no significant differences ($P>0.05$) among cocks on T_4, T_3 and T_2 in proportion of live sperm cells. The lowest percentage of live sperm cells (50.50%) was observed in cocks on the control treatment (T_1). The highest percentage of live sperm cells obtained in this study (65.00%) was higher than the range of 44.2 – 59.2% reported by Abu *et al.* (2006) and higher than the range of 51.3-4.4% reported by Iheukwumere *et al.* (2008), but lower than the average value of 79.82% reported by Oguike *et al.* (2000) in Nigerian local cocks. It is suggested that high live sperm cells are vital for high fertility (Abu *et al.*, 2006).

Table 1: Doses of Diclair® administered to mature Harco Cocks

Day	Treatment (Diclair® i.u)			
	T_1	T_2	T_3	T_4
1	0.00	0.03	0.06	0.09
2	0.00	0.03	0.06	0.09
3	0.00	0.03	0.06	0.09
Total	0.00	0.09	0.18	0.27

Table 2: Concentration of Diclair® on mature Harco Cocks

Day	Concentration of Diclair® (i.u)			
	T_1	T_2	T_3	T_4
1	0.00	4.50	9.00	13.50
2	0.00	4.50	9.00	13.50
3	0.00	4.50	9.00	13.50
Total	0.00	13.50	27.00	40.50

The observation in this study that the group that received higher dose of the test drug recorded the highest percentage of live sperm cells and lowest percentage of dead sperm cells suggests that a higher dose of the drug such as 40.50i.u/cock with 3 days could have high capacity for induction of spermatogenesis and have no deleterious effects on sperm cells. The results of Diclair® administration on testicular morphometry of mature Harco cocks are presented in Table 4.

There were significant differences ($P<0.05$) among the treatment groups in all the testicular parameters measured.

Cocks on T_4 recorded the highest values in right testis (13.20g) left testis (13.309) and paired testes (25.90g) weights. The lowest values in these parameters were observed in cocks on T_2; 10.15g for right testis, 11.95g for left testis and 22.10g for paired testis weights.

Cocks on T_4 recorded the highest values in right, left and paired epididymal weights, 0.90g, 0.80g and 1.60g respectively. The lowest values in right, left and paired epididymal weights; 0.40g, 0.40g and 0.90g were observed in cocks on T_1, T_2, T_1 and T_2 respectively.

Cocks on T_4 recorded the highest value in right. left and paired vas deferens weights; 0.80g, 0.70g, and 1.40g respectively. The lowest value in right, left and paired deferens weights; 0.50g, 0.50g, and 1.10g were observed in cocks on T_3, T_1, and T_3 respectively.

The observation in this study that cocks on T_4 recorded the highest values in testicular, epididymal and vas deferens weights suggests that 40.50i.u Diclair® treatment could have increased metabolism and efficient utilization of nutrient as well as improved the function of androgen (testosterone) responsible for the development of male reproductive tract (Brackett, 2005).

The results of Diclair® administration on hormonal profile of mature Harco cocks are presented in Table 5. There were significant differences ($P<0.05$) among the treatment groups in all the hormonal parameters measured; FSH, LH or ICSH, and testosterone.

Table 3: Effect of Diclair® on semen characteristics of mature Harca Cocks

Parameters	Treatment (Diclair® i.u)				
	T_1 0.00	T_2 13.50	T_3 27.00	T_4 40.50	SEM
Colour	Creamy-white	Creamy white	Creamy white	Creamy white	Creamy white
Semen volume (ml)	0.88^a	0.56^b	0.48^b	1.00^a	0.05
pH	7.00^b	7.00^b	6.00^c	8.00^a	0.22
Individual motility (%)	7.00^d	77.00^b	75.00^c	82.60^a	0.79
Sperm concentration ($x10^9$/ml)	1.14^b	0.52^c	0.52^c	1.54^a	0.06
Proportion of dead sperm cells (%)	49.50^a	39.60^b	38.00^b	35.00^b	3.14
Proportion of live sperm cells(%)	50.50^b	60.40^a	62.00^a	65.00^a	3.14

[abcd:] Means within row having different superscript are significantly (P<0.05) different. SEM = Standard error of means.

Table4: Effect of Diclair on Testicular Morphometry of Mature Harco Cocks

Parameters	Treatment (Diclair® i.u)				
	T_1 0.00	T_2 13,50	T_3 27.00	T_4 40.50	SEM
Right testis weight (g)	11.00^b	10.30^b	10.15^b	13.20^a	0.70
Left testis weight (g)	12.70^{ab}	12.40^b	11.95^b	13.30^a	0.28
Paired testis weight (g)	24.30^a	22.70^b	22.10^b	25.90^a	0.85
Right epididymis weight (g)	0.40^c	0.50^{bc}	0.80^{ab}	0.90^a	0.12
Left epididymis weight (g)	0.50^{bc}	0.40^c	0.70^{ab}	0.80^a	0.09
Paired epididymis weight (g)	0.90^b	0.90^b	1.60^a	1.60^a	0.20
Right vas deferens weight (g)	0.70^a	0.70^a	0.50^b	0.80^a	0.06
Left vas deferens weight (g)	0.50^b	0.60^{ab}	$.60^{ab}$	0.70^a	0.04
Paired vas deferens weight (g)	1.30^a	1.30^a	1.10^b	1.40^a	0.06

[abcd:] Means within row having different superscript are significantly (P<0.05) different. SEM = Standard error of means.

Table 5: Effect of Diclair® on hormonal profile of mature Harco Cocks

Parameters	Treatment (Diclair® i.u)				
	T_1 0.00	T_2 13.50	T_3 27.00	T_4 40.50	SEM
FSH (iu/L)	3.30^c	4.18^c	25.90^a	6.36^b	0.31
LH (iu/L)	4.26^c	5.16^c	37.50^a	14.46^b	0.71
Testosterone (mg/ml)	2.86^c	5.74^b	12.66^a	1.98^c	0.20

[ab:]Means within row having different superscript are significantly (P<0.05) different. SEM = Standard error of means.

Cocks on T_3 recorded the highest value of 25.90(i.u/L) in FSH and this differed significantly (P<0.05) from cocks on T_1, T_2 and T_4. Cocks on T_1 and T_2 were similar (P>0.05) to each other, but they differed significantly (P<0.05) from cocks on T_4 in FSH values. The FSH values obtained in this study were higher than the range of 0.08 $\pm$ 0.08i.u/L – 1.34 $\pm$ 0.01i.u/L reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. This may be attributed to differences in breed and drug administration (Culbnert *et al*., 1977; Herbert *et al*., 2002). Follicle stimulating hormone has been associated with the control of seminiferous tubule growth and differentiation (Culbnert *et al.*, 1977; Iheukwumere *et al.*, 2008).

Cocks on T_3 recorded the highest value of 37.50(iu/L) in luteinizing hormone (LH) and this differed significantly (P<0.05) from cocks on T_1, T_2 and T_4. Cocks on T_1 and T_2 were similar (P>0.050 to each other, but they differed significantly (P<0.05) from cocks on T_4 in LH values.

The LH values obtained in this study were higher than the range of 0.65 $\pm$ 0.04 i.u/L – 1.36 $\pm$ 0.04i.u/L reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. This may be attributed to differences in breed and drug administration (Culbnert *et al*., 1977; Herbert *et al.*, 2002). Luteinizing hormone (LH) or ICSH stimulates the interstitial cells of leydig to produce testosterone which facilitates the process of spermatogenesis (Herbert *et al.*, 2002).

Cocks on T_3 recorded the highest value of 12.66ng/ml in testosterone and this differed significantly (P<0.05) from cocks on T_1, T_2 and T_4. Cocks on T_1 and T_4 were similar (P>0.05) to each other, but they differed significantly (P<0.05). from cocks on T_2 in testosterone values. The testosterone values obtained in this study were higher than the range of 0.15 $\pm$ 0.01ng/ml – 0.21 $\pm$ 0.21ng/ml reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. This may be attributed to differences in breed and drug administration (Culbnert *et al.* 1977; Herbert *et al.*, 2002).

Conclusion

From the results of the study, it can be concluded that Diclair® improved semen characteristics and testicular, epididymal and vas deferens development of Harco cocks at the level of 40.50i.u, without any deleterious effects on hormonal profile of the cocks.

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