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Research Article

Effect of Gonadotrophin (Diclair®) on Kidney Function, Body Conformation and Sperm Reserves 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 kidney function, body conformation and sperm reserves. 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 6.75i.u Diclair®; T_3, administered with 13.50i.u Diclair® and T_4, administered with 20.25i.u Diclair®, with one cock per rplicate in a completely Randomized Design (CRD). The injections were divided into three doses each and administered intramuscularly in the thigh for three consecutive days. Blood was collected one week after Diclair® administration from 5 cocks in each treatment group by wing vein puncture of the cocks using needle and syringe for kidney function test. Four weeks after Diclair® injections, body size measurement was done using a measuring tape. The five cocks that were bled in each treatment group were slaughtered and the testicles collected and testiclular and epididymal sperm reserves were estimated following the homogenized count using a haemocytometer and a microscope. The results showed that there were significant differences ($P<0.05$) among the treatment groups in sodium, potassium, chloride, bicarbonate and creatinine values. The results further showed that there were significant differences ($P<0.05$) among the treatment groups in final body weight, body girth, body width, body length, shank length, drumstick length, keel length, and wing length. Similarly the result showed that there were significant differences ($P<0.05$) among the treatment groups in testis and epididymal sperm reserves. The result of this study suggest that Diclair® treatment enhanced sperm production and reserves and was not detrimental to kidney function and body conformation of the cocks.

Key words: Harco cocks kidney function, body conformation sperm reserves Diclair®

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 traits in subsequent 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 profitability in poultry enterprise (Iheukwumere *et al.* (2008).

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 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

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spermatogenesis would induce any side effects on kidney function and body formation of the cocks. This study was therefore carried out to determine the effect of Diclair® administration on kidney function, body conformation and sperm reserves or 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 (2 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 injection was administered as follows:Diclair® was supplied in 2 vials each containing FSH 75i.u and LH 75i.u per ml.

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

All treatments were administered intramuscularly on the thigh of each cock using a one ml syringe with 0.01ml graduation. Seven days after Diclair® injection, blood collection and haematological and serum biochemical evaluation were carried out.

Blood collection and analysis

The cocks were bled after one weekafter Diclair® injectionsbetween9am and 10.30am from wing veins using needle and syringe and aspirated about 3mls of blood from each cock. The blood samples werepoured into plain bottles (without ethylene diamine tetra-acetic acid i.e EDTA) to allow them coagulate. The bottles of coagulated blood were subjected to standard methods of serum separation and the harvested sera were used for evaluation of serum biochemical parameters. The standard flamephotometry using Gallenkamp analysis was used to determine serum sodium (Na^+) ion and potassium (K^+) ion. While creatinine, bicarbonate and chloride ions were assayed according to the methods of Baker and Silverton (1986).

Body conformation

The body weights of the cocks were measured in kg using a 50kg weighing balance. The body length, shank length, drumstick length, keel length, body girth, body width and wing length were measured in cm using a measuring tape.

Sperm collection and evaluation

Twenty eight (28) days after Diclair® injections, five cocks from each treatment group were slaughtered and the testicles collected and daily serum output, testicular and epididymal sperm reserves were estimated following the homogenized count using a haemocytometer and a microscopic (Bittoand Egbunike, 2006).

Data analysis

Data collected on kidney function, body conformation and testicular and epididymal sperm reserves were subjected to analysis of variance (ANOVA) using the technique of steel and Torrie (1980).Significant treatment means were separated using Duncan's New Multiple Range Test as outlined in Obi (1990).

RESULTSAND DISCUSSION

The result of Diclair® administration on kidney function of mature Harco cocks is shown on Table 1. There were significant differences ($P<0.05$) among the treatment group in serum sodium, potassium, chloride, bicarbonate and creatinine values. Cocks on T_4 recorded the highest value of 104.00 (mmol/l) in serum sodium and this differed significantly ($P<0.05$) from cocks on T_1,T_2 and T_3 which were similar ($P>0.05$) to each otherinsodium values. The sodium values obtained in this study, were lower than the range of 148-163 (mmol/l) recorded by Jain (1993) and lower than the range of 131.30 - 135.14 (mmol/l) reported by Iheukwumere *et al*.(2006) in Nigerian indigenous chickens, but higher than the range of 56-59 (mmol/l)reported by Iheukwumere *et al*.(2002) in broiler chickens. Serum electrolytes play important roles in physiological processes involved in homeostasis.

Cocks on T_4 recorded the highest value of 4.50 (mmol/l) in serum potassium and this differed significantly ($P<0.05$) from cocks on T_1, T_2 and T_3 which were similar ($P>0.05$) to each other in potassium values. The lowest value in serum potassium was observed in cocks on the control treatment (T_1) (3.80mmol/l).Semen potassium values obtained in this study were lower than the range of 4.6-6.5 (mmol/l) reported by Jain (1993), but were higher than the range of 1.43±0.02-1.74±0.15 (mmol/l) reported by Iheukwumere *et al*.(2006) in Nigerian indigenous chickens and higher than the range of 1.55-1.80 (mmol/l) reported by Iheukwumere *et al*.(2002) in broiler chickens. Potassium is excreted in the kidney

and elevations of plasma potassium is indicative of low excretion suggesting kidney impairment. When plasma potassium is low, the level of sodium in plasma is elevated. Thus, they help in depolarization and repolarization in the nerve cells and muscle cells, and in the transmission of impulses in the nerve cells, intracellular and extracellular fluid.

Cocks on T_4 recorded the highest value of 98.40(mmol/l) in serum chloride and this differed significantly (P<0.05) from cocks onT_1 and T_2 which were similar (P>0.05) to each other and similar (P<0.05) to cocks on T_3.There was no significant difference (P>0.05) between cocks on T_4 and T_3 in serum chloride values. The lowest value in serum chloride was observed in cocks on T_1 (94.00mmol/l).The serum chloride values obtained in this study where higher than the range of 33.0-34.10(mmol/l) reported by Iheukwumere *et al*.(2002) in broiler chickens, but lower than the range of 114-120(mmol/l) reported in Thai chickens by Simaraks *et al*.(2004) and lower than the range of 130.38±0.17-132.30±1.27(mmol/l) reported by Iheukwumere *et al*.(2006) in Nigerian indigenous chickens.

Cocks on T_4 recorded the highest value of 28.00(mmol/l) in serum bicarbonate and this differed significantly (P>0.05) from cocks on T_1 and T_2 which were also significantly different (P<0.05) from each other. There was no significant difference (P>0.05) between cocks on T_4 and T_3.Cocks on T_2 and T_3 were similar (P>0.05) to each other in serum bicarbonate values. The lowest value in serum bicarbonate was observed on cocks on the control treatment (T_1) (22.60mmol/l).The bicarbonate values obtained in this study were within the values stated by Durotoye *et al*. (2000) in chickens, but higher than the range of 13.44±0.38-15.60±0.22 (mmol/l) reported by Iheukwumere *et al*. (2006) in Nigerian indigenous chickens and higher than the range of 14.80-15.60 (mmol/l) reported by Iheukwumere *et al.* (2002) in broiler chickens. Bicarbinate is used in the buffering system in the blood, extracellular fluid and kidney.

Cocks on T_4 recorded the highest value of 6.50 (mg/µ) in serum creatinine and this differed significantly (P<0.05) from cocks on T_1, T_2 and T_3 which were significantly different (P<0.05) from each other. The lowest value in serum creatinine was observed in cocks on T_1 (2.50mg/µ).The serum creatinine values obtained in this study were higher than the range of 1-2mg/dl reported for birds (Reece and Swenson, 2004; Benerjee, 2005), but lower than the range of 18.00-18.50(mg/100ml) reported by Iheukwumere *et al*. (2002) in broiler chickens. Creatinine measurement is used exclusively in the assessment of kidney function. The rate of production of creatinine is constant and elevations of plasma creatinine are indicative of under excretion, suggesting kidney impairment.

Table 2 shows the results of Diclair® administration on body conformation and weight of Mature Harco cocks. There were no significant differences (P>0.05) among the treatment groups in initial body weight. The values obtained for initial body weight ranges from 2.66 kg in T_2 and T_3 to 2.67kg inT_1.

There were significant differences (P<0.05) among the treatment groups in body weight. Cocks on T_3 recorded the highest value in final body weight (3.25kg). The lowest value in final body weight was observed in cocks on T_4 (2.88kg).There were significant differences (P<0.05) among the treatment groups in weight gain. Cocks on T_3 recorded the highest value in weight gain (0.60kg). The lowest value in weight gain was observed in cocks on T_4 (0.22kg). The observation in this study that the group that received the highest dose of Diclair® recorded the lowest value in final body weight suggests that 20.25i.u/cock within three days given in this study could have reduced efficient utilization of nutrients that resulted in decreased final body weight.

Table 1: Effect of Gonadotrophin (Diclair®) on Kidney Functions of Mature Harco Cocks.

Parameters	Treatment (Diclair®i.u)				
	T_1 0.00i.u	T_2 6.75i.u	T_3 13.25i.u	T_4 20.25i.u	SEM
Sodium (mmol/l)	98.00^{b}	99.40^{b}	100.00^{b}	104.00^{a}	0.86
Potassium (mmol/l)	3.80^{b}	4.00^{b}	4.00^{b}	4.50^{a}	0.07
Chloride (mmol/l)	94.00^{b}	95.60^{b}	97.00^{ab}	98.40^{a}	0.71
Bicarbonate (mmol/l)	22.60^{c}	25.00^{b}	26.40^{ab}	28.00^{a}	0.60
Creatinine (mmol/l)	2.50^{d}	3.00^{c}	3.20^{b}	6.50^{a}	0.07

^{abc}: Means within row having the different superscripts are significantly (P<0.05) different.SEM: Standard Error of Mean.

Table 2: Effect of Gonadotrophin (Diclair®) on Body Conformation and Weight Gain of Mature Harco Cocks

Parameters	Treatment (Diclair®)				
	T_1 0.00i.u	T_2 6.75i.u	T_3 13.25i.u	T_4 20.25i.u	SEM
Initial body weight (kg)	2.67	2.65	2.65	2.66	0.02
Final body weight (kg)	3.17^{ab}	3.10^{b}	3.25^{a}	2.88^{c}	0.08
Weight gain (kg)	0.50^{b}	0.55^{ab}	0.60^{a}	0.22^{c}	0.08
Shank length (cm)	17.75^{a}	17.53^{a}	17.73^{a}	16.38^{b}	0.33
Drumsticklength (cm)	9.75^{a}	10.18^{a}	9.50^{a}	9.20^{b}	0.27
Keel length (cm)	14.05^{a}	13.93^{ab}	13.50^{b}	13.13^{b}	0.15
Body girth (cm)	40.13^{a}	38.63^{b}	40.50^{a}	38.38^{b}	0.34
Body width (cm)	31.50^{a}	31.50^{a}	31.00^{a}	28.13^{b}	0.56
Body length(cm)	36.50a	32.75b	31.25b	31.63b	0.88
Wing length(cm)	21.75a	20.63b	20.63b	20.38b	0.24

^{abc}: Means in the same row having different superscripts are significantly (P<0.05) different. SEM: Standard Error of Mean.

Table 3: Effect of gonadotrophin (Diclair®) on daily sperm output and sperm reserve of mature harco cocks

Parameter	Treatment (Diclair®i.u)				
	T_1	T_2	T_3	T_4	
	0.00i.u	6.75i.u	13.25i.u	20.25i.u	SEM
Daily sperm Output ($\times10^9$)	0.55^b	0.87^{ab}	1.09^{ab}	1.43^a	0.19
Testes sperm reserve ($\times10^9$)	2.00^b	3.20^b	4.00^{ab}	5.25^a	0.68
Caput sperm reserve ($\times10^8$)	1.05^b	2.05^b	6.05^{ab}	10.05^a	2.06
Corpus sperm reserve ($\times10^8$)	4.05^b	5.05^b	6.05^{ab}	7.55^a	0.75
Cauda sperm reserve ($\times10^8$)	10.05^b	11.05^b	17.05^{ab}	20.05^a	2.40
Vas deferens sperm reserve ($\times10^8$)	2.05^b	2.21^b	2.70^{ab}	3.55^a	0.34
Relative epididymal sperm distribution					
Caput 19.18					
Corpus 22.68					
Cauda 58.14					

[ab]: Means in the same row having different superscripts are significantly (P<0.05) different. SEM: Standard Error of Mean.

There were significant differences (P<0.05) among the treatment groups in shank length. Cocks on T_1 recorded the highest value in shank length (17.75cm). The lowest value in shank length was observed in cocks on T_4 (16.38cm).

Drumstick length for the treatment groups T_1, T_2,T_3 and T_4 were 9.75cm, 10.18cm, 9.50cm and 9.20 cmrespectively.T_1, T_2 and T_3 did not differ significantly (P>0.05), but differed significantly (P<0.05) from T_4 which recorded the lowest value for drumstick length.

There were significant differences (P<0.05) among the treatment groups in keel length. Cocks on T_1 recorded the highest value of 14.05cm in keel length. The lowest value of 13.13cm in keel length was observed in cocks on T_4.

Body girth for the treatment groups T_1, T_2, T_3 and T_4 were 40.13cm, 38.63cm, 40.50cm and 38.38cm respectively. T_1 and T_3 did not differ significantly (P>0.05) but they differed significantly (P<0.05) from T_2 and T_4 which were also similar (P>0.05) to each other in body girth values.

The values for body width for the treatment groups T_1, T_2 T_3 and T_4 were 31.50cm, 31.50cm, 31.00cm and 28.13cm respectively. T_1, T_2 and T_3 did not differ significantly (P<0.05) but differed significantly (P<0.05) from T_4 which recorded the lowest value in body width.

Body length for the treatment groups T_1, T_2, T_3 and T_4 were 36.50cm, 32.75cm, 31.25cm and 31.63cm respectively. T_2, T_3 and T_4 were similar (P>0.05) to each other in body length, but differed significantly (P<0.05) from T_1 which recorded the highest value in body length. Wing length followed the same pattern as body length. The values for wing length for the treatment groups T_1, T_2, T_3 and T_4 were 21.75cm, 20.63cm, 20.63cm and 20.38cm respectively. T_2, T_3 and T_4 did not differ significantly (P>0.05, but they differed significantly (P<0.05) from T_1 which recorded the highest value in wing length.

The observation in this study that the group which received the highest dose of Diclair® recorded the lowest values for all the parameters for body conformation except body length suggests that 20.25i.u/cock within three days given in this study could have reduced efficient utilization of nutrients that resulted in decreased values for shank length, drumstick length, keel length, body girth, body width and wing length. The results of Diclair® administration on sperm reserves of Mature Harco cocks are shown in Table 3.

There were significant differences (P<0.05) among the treatment groups in daily sperm output, testes sperm reserve, caput, corpus, cauda and vas deferens sperm reserves. Cocks on T_4 recorded the highest value in daily sperm output (1.43×10^9) 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), but differed significantly (P<0.05) from cocks on T_1. The daily sperm output values obtained in Diclair® treated cocks were higher than 0.60±0.01 ($\times10^9$) reported by Ahemen and Bitto (2007) in WAD rams. This could be attributed to drugs administration (Herbert *et al*., 2002). However, the daily sperm output in Diclair® treated cocks were lower than the mean value of 5.29 ($\times10^9$) reported by Maritinez *et al*. (1994) in temperate breeds of rams.

Cocks on T_4recorded the highest value of 5.25($\times10^9$) in the testes sperm reserve and this differed significantly (P<0.05) from cocks on T_1 and T_2 which were similar (P>0.05) to each other and similar (P>0.05) to cocks on T_3in testes sperm reserve values. There was no significant difference (P>0.05) between cocks on T_4 and T_3 in testes sperm reserve values. The lowest value in testicular sperm reserve was observed in cocks on T_1(2.00×10^9).The values for testicular sperm reserve of the Diclair® treated groups were higher than 2.15($\times10^6$) ± 0.05/ml for gonadal sperm reserve reported by Ahemen and Bitto (2007) in West African Dwarf rams. This is suggestive of induction of spermatogenesis by the Diclair® treatment.

Cocks on T_4 recorded the highest value of 10.05 ($\times10^8$) incaput sperm reserve and this differed significantly (P<0.05) from cocks on T_1 and T_2 which were similar (P>0.05) and similar (P>0.05) to cocks on T_3 in caput sperm reserve values. There was no significant difference (P>0.05) between cocks on T_4 and T_3 in caput sperm reserve values. The lowest value in caput sperm reserve was observed in cocks on the control treatment (T_1) (1.05×10^8).The values for caput sperm reserve of the Diclair® treated groups were higher than the value of $2.92\times10^8$for caput sperm reserve reported by Rajiand Njidda, 2014 in red sokoto goats and higher than the value of 2.25±0.03 for caput sperm reserve reported by Iheukwumere *et al.* (2008) in Yankasa rams. This could be attributed to high capacity for induction of spermatogenesis by Diclair® injection.

Cocks onT_4 recorded the highest value of 7.55($\times10^8$) in corpus sperm reserve and this differed significantly (P<0.05) from cocks on T_1 and T_2 which were similar

(P>0.05) to each other and similar (P>0.05) to cocks on T_3 in corpus sperm reserve values. There was no significant difference (P>0.05) between cocks on T_4 andT_3 in corpus sperm reserve values. The lowest value in corpus sperm reserve was observed in cocks on T_1 (4.05×10^8).The values for corpus sperm reserve of the Diclair® treated groups (T_3 and T_4) were higher than the highest value of 5.10±0.08 ($\times10^8$) for corpus sperm reserve reported by Iheukwumere *et al*. (2008) in Yankasa rams. This could be attributed to drug administration (Herbert *et al*., 2002).

Cocks on T_4 recorded the highest value of 20.05($\times10^8$) in cauda sperm reserve and this differed significantly (P<0.05) from cocks on T_1 and T_2 which were similar (P>0.05) to each other and similar (P>0.05) to cocks onT_3. There was no significant difference between cocks on T_4 and T_3 in cauda sperm reserve values. The lowest value in cauda sperm reserve was observed in cocks on T_1 (10.05×10^8).The cauda sperm reserve valus obtained in this study were lower than the mean value of 17.33×10^8 for cauda sperm reserve reported by Raji and Njidda, 2014 in red sokoto goats except cocks on T_4 whose cauda sperm reserve value was higher than the value reported for red sokoto goats.

Cocks on T_4 recorded the highest value of 3.55($\times10^8$) in vas deferens sperm reserve and this differed significantly (P<0.05) from cocks on T_1 and T_2 which were similar (P>0.05) to each other and similar (P>0.05) to cocks on T_3. There was no significant difference (P>0.05) between cocks on T_4 and T_3 in vas deferens sperm reserve values. The lowest value in vas deferens spem reserve was observed in cocks on T_1 (2.05×10^8). The values for vas deferens sperm reserve obtained in this study were higher than the highest value of $0.65\pm0.04\times10^8$ for vas deferens sperm reserve reported by Iheukwumere *et al*. (2008) in Yankasa rams. This could be attributed to technique of estimation (Ahemen and Bitto, 2007) and drug administration (Herbert *et al.,* 2002).

The sperm reserve of the caput epididymis represented 19.18% of the total sperm reserve of the organ, while the corpus and cauda accounted for 22.68% and 58.14% respectively. The distribution of epididymal sperm reserves in this study is similar to what has been reported for other livestock: Balami rams (Kwari and Waziri, 2001), WAD rams (Osinowo, 2006; Ahemen and Bitto, 2007) and Yankasa rams (Iheukwumere *et al.*, 2008).It is generally agreed that the cauda epididymis contains most of epididymal sperm reserves and hence it is the major site for sperm storage (Kwari and Waziri, 2001). In this study, it was observed that Diclair® induced spermatogenesis in the treated cocks. It is common knowledge that LH as interstitial cell stimulating hormone (ICSH) stimulates the interstitial cell of leydig to produce testosterone which facilitates the process of spermatogenesis (Herbert *et al*., 2002).

Conclusion

From the result of this study, it can be concluded that Gonadotrophin (Diclair®) improved daily sperm output, testicular and epididymal sperm reserves of Harco cocks at the level of 20.25i.u without any deleterious effects on kidney function and body conformation of the cocks.

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