COCONUT WATER REDUCED ADIPOSITY MARKERS IN OVERFED-MALE BROILER BREEDERS

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ABSTRACT
Overfeeding of male broiler breeders causes adiposity and this leads to impairment in mating and reproductive capacities. As a result of this, broiler breeders are placed on feed restriction which is often associated with stress. There are published and anecdotal reports on anti-adiposity and weight-losing property of coconut water. This study evaluated the effect of coconut water (CW) supplementation on the level of reproductive hormones, stress and adiposity markers in overfed male broiler breeders. Thirty (12 weeks old) male broiler breeders were divided into three dietary treatment groups-A, B and C and reared for 12 weeks. Group A was placed on standard feeding regimen while groups B and C received double of the standard feeding regimen. Groups A and B were given ordinary water while birds in group C received 100 ml of CW/1L of water. Blood samples and semen were collected at the end of 12 weeks of rearing to evaluate sperm characteristics, reproductive hormones, leptin and cortisol levels. Mean ± SD values were calculated and compared for significant differences with one-way ANOVA at $p<0.05$. Serum cortisol level was significantly elevated in group A relative to group B and C. Sperm characteristics and the concentrations of testosterone were similar across the groups. Estrogen level decreased significantly in groups A and C when compared with group B. The concentration of leptin, though not significant, was elevated in group B when compared to other groups. These results revealed that high feed ration reduced stress and coconut water decreased adiposity markers in overfed male broiler breeders.
Key words: Male broiler breeder, High feed ration, Coconut water, Reproductive Hormones, Leptin, Cortisol

Introduction

The intense genetic selection of modern breeds of broilers for high breast-meat yield and feed conversion rate (Arnould and Leterrier, 2007) causes change in their appetite and associated poor reproductive performance (Julian, 2000). High predisposition of modern broilers to obesity due to voracious appetite and rapid growth has been reported (Julian, 2000). Overfeeding has been shown to accelerate sexual maturity [5] and as well increased adiposity and abdominal fat pad [6]. Meanwhile, both overfeeding and fat mass accumulation correlates with elevated synthesis and secretion of leptin (Liuzzi *et al*., 1999; Chen *et al*., 2006). Fertility of male broiler breeders declines in the last phase of their reproductive life (McGary *et al*., 2002) and this is linked to various factors including obesity or overweight of the birds. Obesity impairs fertility by causing reduction in mating capacity (Hocking and Bernard, 2000), poor semen quality (MacDonald *et al*., 2010) and alteration in reproductive hormones (Vermeulen, 1993). Paragraph

Feed restriction is a method used by farmers to control body weight of broiler breeder. This method is not perfect and negatively impact health of birds. Welfare issues associated with feed restriction include chronic hunger (Mench, 2002) and stress as demonstrated by increased plasma corticosterone (De Jong *et al*., 2002). Stress or affects reproduction by decreasing synthesis and secretion of gonadotropin-releasing hormone (Oakley *et al*., 2002).

Coconut water possesses lipid-lowering activity (Muhammed *et al*., 2013). Evidence from rat and human studies have also demonstrated anti-adiposity (Imaga *et al*., 2016) and weight loosing properties of the fluid. The adverse reproductive implication of overfeeding and welfare issues associated with feed restriction call for the need to find a management protocol that will guarantee both optimal reproduction and welfare of the birds. This study was therefore carried out to assess level of the reproductive hormones, stress and adiposity markers of male broiler breeders on standard feeding regimen, overfeeding and overfeeding with coconut water supplementation

Materials and Methods

Experimental Location and Source of raw materials ~~Coconut Water~~

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of the Oyo State College of Agriculture and Technology, Igboora, Nigeria. Ripe coconut fruits were bought at a ~~the~~ local market in Igboora and the coconut water from the fruit was manually extracted for this study. Thirty male broiler breeders (twelve weeks old) were obtained from a commercial farm in Ibadan, Nigeria.

Experimental Design

Thirty male broiler breeders (twelve weeks old) ~~were obtained from a commercial farm in Ibadan, Nigeria. The birds~~ were randomly assigned to three groups (A-C) of ten birds per group. The birds in group A (control) were placed on standard feeding regimen which was increased at one week interval-87 g/bird (13^{th} week), 92 g/bird (14^{th} week), 97 g/bird (15^{th} week), 102 g/bird (16^{th} week), 107 g/bird (17^{th} week), 112 g/bird (18^{th} week), 117 g/bird (19^{th} week), 122 g/bird (20^{th} week). The birds in group B and C were given double quantity of the standard feeding regimen. Groups A and B were placed on ordinary water whereas group C received water containing coconut water (100 ml of CW/L of water).

Collection of Blood and Semen Samples

At the end of the 20^{th} week, all the birds in each group were bled via wing vein puncture into plain bottles and the blood samples were allowed to clot at room temperature in order to get sera. Serum was separated by centrifugation at 2000-3000 Revolutions per minute for 20 minutes and then taken to the laboratory for analysis of cortisol, luteinizing hormone (LH), follicle stimulating hormone (FSH), estrogen and testosterone levels. Semen was collected manually by abdominal massage technique which involved massaging of the cloacal region to achieve phallic tumescence and cloacal stroke (Shinde *et al*., 2012).

Analysis of Hormones

Serum concentrations of leptin, testosterone and estrogen were analysed using ELISA-TE187S (Bioassay Technology Laboratory, USA) and ELISA-ES180S (Calbiotech) respectively. Serum concentrations of follicle stimulating hormone, luteinizing hormone and cortisol were determined using ELISA kit-FS232F (Calbiotech), ELISA kit-LH231F (Calbiotech) and ELISA kit-respectively.

Sperm Analysis

Semen collected was put unto slides and sperm count was determined using the improved Neuber counting chamber in the haemocytometer. To assess Sperm motility, a 10 L diluted sample (1:20 in 2.9% sodium citrate) was placed on warm slide (37^0C) and then covered with a coverslid. In five microscopic fields, the percentage of sperm motility was determined using light microscope. The viability was assessed by staining of sperm smear with eosin-nigrosin staining technique.

Data Analysis

Quantitative data collected from the experiment were statistically analyzed with SPSS (Version 16.0, 2007). Differences between the means were tested with independent one-way ANOVA. The results from each group were expressed as mean ± standard deviation (SD) and means were compared for significant differences at $p < 0.05$.

Results

Serum Reproductive Hormone, Leptin and Cortisol Level

The serum cortisol concentration in group A was significantly higher when compared with groups B and C, and the lowest cortisol level was observed in group C. The concentration of serum testosterone followed opposite trend with group A having the lowest value but no significant difference was observed in the testosterone level of the three groups. Although groups A and C had statistically similar estrogen concentration, estrogen level increased significantly in group B when compared with group A. In a similar trend, estrogen level in group C decreased significant relative to group B. There were no significant differences in the serum FSH and LH level of groups B and C when compared with group A, and when groups B and C were compared with each other. Although group B had the highest leptin concentration, leptin levels in groups B and C were significantly elevated when compared with group A. The sperm motility, sperm vitality and sperm count in groups B and C were not significantly different from value recorded in group A. Likewise, sperm motility, sperm vitality and sperm count in groups B and C were not significantly different when compared.

Table 1. Serum Levels of Reproductive Hormones and Leptin of Overfed

Parameter	Group A	Group B	Group C

Leptin (ng/ml)	5.40 ± 0.31	8.04 ± 0.27*	6.82 ± 0.28$^{**\#}$
Cortisol (ng/ml)	6.84 ± 0.22	5.17 ± 0.70*	4.06 ± 0.15$^{\#}$
Testosterone (ng/ml)	3.14 ± 0.21	3.32 ± 0.38	3.25 ± 0.24
Estrogen (ng/ml)	6.33 ± 3.51	16.90 ± 5.88*	6.40 ± 1.58
LH (miu/ml)	0.45 ± 0.02	0.40 ± 0.01	0.36 ± 0.03
FSH (miu/ml)	0.40 ± 0.06	0.42 ± 0.07	0.41 ± 0.02
Sperm Count (10^6/mm^3)	80.00 ± 2.74	81.00 ± 3.67	82.00 ± 1.22
Sperm Motility (%)	76.00 ± 1.87	77.00 ± 3.39	79.00± 1.87
Sperm Viability (%)	80.00 ± 2.74	81.00 ± 3.67	82.00 ± 1.22

Key: * and ** indicate significant difference when group B an C are compared with group A respectively

 # indicates significant difference when group C is compared with group B

Discussion

In this study, the low cortisol level in groups B and C suggested lesser stress level probably due to accessibility of birds in this the groups to high quantity of feed. Also, the lowest cortisol level in group C could be linked to exposure of the birds in this the group to extra energy sources especially glucose from coconut water. The observation in this present study agrees with the report of previous research which indicated reduced corticosterone level and low stress level in *ad libitum* fed broiler breeders (De Jong *et al*.,2003). In addition, birds with low body weight resulting from feed restriction has been shown to exhibit signs of stress (Bowling *et al*., 2018).

The substantial reduction in estrogen and leptin levels in group A and C could be due to low adiposity resulting from feed restriction and coconut water supplementation in group A and C respectively. Adipose tissue is one of the main sites of extra-gonadal synthesis of estrogen (Lecke *et al*., 2011; Byeon and Lee 2016) and intensity of adiposity is positively associated with feed intake. Also, there evidences that adiposity level correlates with the amount of leptin synthesized and secreted (Liuzzi *et al*., 1999). Studies have shown weight loss (Badmanaban *et al*., 2018) and anti-adiposity property of coconut water (Imaga *et al*., 2016) and their results corroborate low adiposity marker (leptin) observed in coconut water supplemented group in this present study

The absence of significant difference in the levels of follicle stimulating hormone and luteinizing hormone in the three groups suggests that gonadotropin production is dependent on availability of basic amount of energy and cannot be influenced by presence of extra energy. The similarity in the level of testosterone in the three groups is plausible as this may due to non-significant difference in level of gonadotropin in the three groups. In line with our observations was the report showing absence of increased secretion of FSH in dairy heifers when overfed (Spicer *et al*., 1984).

The three groups showed no differences in their sperm count, motility and viability and this could be attributed to the absence of significant alteration in the concentration of testosterone and gonadotropins observed in the groups. In contradiction to this finding is the report that showed improved sperm quality and quantity in overfed male broiler breeders (Cerolini *et al*., 1995).

In conclusion, overfeeding of male broiler breeders was associated with reduction in stress marker. Also, coconut water decreased adiposity markers in male broiler breeders placed on high feed ration.

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