Effects of *Allium cepa* L. peels extract on gonadotropins, testosterone and sperm variables in Oba Marshal broiler cocks

OE Adeleye\(^1\)*, OM Oguntayo\(^1\), Al Adeleye\(^2\), AS Adetomiwa\(^3\) & OE Ariyo\(^4\)

1. Department of Veterinary Physiology and Pharmacology, Federal University of Agriculture, Abeokuta, Nigeria
2. Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria
3. Department of Public Health and Reproduction, Federal University of Agriculture, Abeokuta, Nigeria
4. Obasanjo Farms Nigeria, Lanlate, Oyo State, Nigeria

*Correspondence: Tel.: +2348037551972; E-mail: adeleyeoe@funaab.edu.ng

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Abstract

*Allium cepa* (onion), a natural seasoning agent that contains significant amounts of potent antioxidants in its scaly leaves is used in folkloric medicine to manage several diseases globally. Antioxidants have an essential effect on sperm health parameters; however, there is limited information on the effects of *Allium cepa* scaly leaf extract on reproductive functions in Oba Marshal breeder cocks. This study was conducted to investigate the effects of the aqueous extract of *Allium cepa* scaly leaf on reproductive functions in sexually matured Oba Marshal breeder cocks. *Allium cepa* bulbs were obtained from a market in Abeokuta, Ogun State. Dry scaly leaves were peeled, pulvemised, macerated in distilled water, filtered and concentrated. Twenty, 42 weeks old Oba Marshal breeder cocks (3.48 – 3.62 kg) were divided into 4 groups (n = 5) and treated daily for 2 weeks thus: CT (control, distilled water, 0.5 mL/kg), T\(_2\) (extract 200 mg/kg/bird), T\(_4\) (extract 400 mg/kg/bird), T\(_8\) (extract 800 mg/kg/bird). Sperm characteristics were assessed microscopically. Testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were analysed using ELISA. Data were analysed using ANOVA at \(\alpha = 0.05\). Treated birds had significantly (\(p < 0.05\)) higher sperm motility, morphology but non-significant changes in sperm viability and concentration compared with the controls. Also, serum FSH and LH significantly increased, while testosterone had no significant change in test groups compared to the control. Aqueous extract of *Allium cepa* scaly leaf improved testicular functions and morphology in the test cocks. The reproductive function enhancement of the extract may be due to its antioxidant effect.

Keywords: *Allium cepa*, Breeding, Broiler cocks, Spermatogenesis, Testosterone

Introduction

Infertility in farm animals is a major issue of breeding, with approximately 20 - 70% of the problems male related (Khaki *et al*., 2009; Lee *et al*., 2012; Barkhordari *et al*., 2013, Agarwal *et al*., 2015). Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Climate (Saeed...
& Al-Soudi, 1975), time of collection (Egbunike & Oyeyemi, 1979), frequency of collection (Riaz et al., 2004), and nutrition (Kabir et al., 2007) are some environmental factors that have influence on the quality of semen. Other factors such as drug chemotherapy, toxins, air pollution, and insufficient vitamin intake may have harmful effects on spermatogenesis and the normal production of sperm (Mosher & Pratt, 1991; Zhang & Qiao, 2004). Researchers have reported that using antioxidants and vitamins A, B, C, and E in the daily diet may protect sperm DNA from free radicals and increase the stability of blood-testis barrier (Jedlinska-Krakowska et al., 2006). A wide variety of plant derived pharmaceutical products are now being employed in trado-medicine as a result of their beneficial properties in managing infertility (Yama et al., 2011). The onion (Allium cepa) has been used for long in traditional medicine, and it is one important Allium species commonly used in our daily diet. It has been documented to have antithrombotic, hypolipidaemic, hypotensive, diaphoretic, antibiotic, antidiabetic, antiatherogenic, and anticancer medicinal properties (Augusti, 1996; Lee et al., 2008; Khaki et al., 2009; Khaki et al., 2012; Alagawany et al., 2016). Onion contains exogenous and endogenous antioxidants like selenium, glutathione, vitamins A, B, and C and flavonoids such as quercetin and isorhamnetin (Griffiths et al., 2002). These antioxidants protect DNA and other important molecules from peroxidation damage that can lead to apoptosis, and invariably improve sperm health parameters, and hence increase fertility (Khaki et al., 2008; Sejian et al., 2014; Salehi et al., 2019).

The biological action of Allium products is ascribed to its organo-sulphur and phenolic compounds (Kumud et al., 1990). The role of nutritional factors in reproduction and sub-fertility is important and it has been stated that sperm quality of breeder stock improves when their feeds are supplemented with vitamin C (Ezzat et al., 2011). Maintenance of fertile cocks in breeding poultry farms has been tedious in the tropics for quite some time, with high semen producing capacity cocks often few and quickly reduce in fecundity due to age, poor nutrition, unfavorable climatic conditions, and poor management (Okoro et al., 2016). A better understanding of the mechanisms responsible for sub-fertility or infertility with evaluation of biochemical and nutritional factors will help to improve diagnosis and treatment (Fukushima et al., 1997). Quality assurance of semen is expedient for good results in artificial insemination of chickens (Alkan et al., 2002).

Semen evaluation in poultry breeding for selection of breeding males or for routine monitoring of their reproductive performance is very important (Cheng et al., 2002). The fertilizing ability of the semen can be ascribed by its motility, viability, sperm concentration and morphological evaluations (Oyeyemi et al., 2000; Oyeyemi & Ubiogoro, 2005; Bansal & Cheema, 2014). When critical percentages (i.e. < 10%) of sperm cell abnormalities are present in the semen, the male subject is usually considered infertile (Cummings & Bingham, 1998). The aim of the present study was to evaluate the effects of different doses of onion peel extract on semen variables and reproductive hormones in male Oba Marshal breeder cocks.

Materials and Methods

Red onion (Allium cepa L.) bulbs were obtained from a local market in Abeokuta and were authenticated at the herbarium of Department of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture, Abeokuta (FUNAABH0029). The dry scaly leaves were taken off, extracted according to the methods of Khaki et al. (2009) and used for the studies. A test concentration of the Allium cepa scaly leaf extract (ACSLE) was prepared and given to the experimental birds at different doses thus: 200, 400 and 800 mg/kg/bird.

Twenty, 42 weeks old Oba Marshal broiler cocks weighing between 3.48 and 3.62 kg procured from Obasanjo Farms Nigeria®, Oyo State were used for the experiment. They were assigned into 4 groups of 5 birds each and treated as follows: CT (control, distilled water, 0.5 mL/kg), T1 (200 mg/kg/bird ACSLE), T2 (400 mg/kg/bird ACSLE) and T3 (800 mg/kg/bird ACSLE).

The extract was administered for 2 weeks [period equivalent to spermatogenesis duration in cock (de Reviers, 1968)] by oral gavage thereafter; semen and blood were collected from the birds and analyzed.

Semen collection and evaluation

Semen collection was done by the abdominal massage technique and the manipulation of cloaca as described by Hafez (1987). Semen was collected at the end of two weeks period and was immediately analysed. The abdominal massage technique involved massaging the cloacal region to achieve phallic tumescence, followed by a cloacal stroke and a squeeze of the region surrounding the sides of the cloaca to express the semen. The semen was then
milked down by firm finger pressure on either side of the vent into the labeled collecting tube. The semen was analysed to check for sperm motility, concentration, viability and morphology as described by Jequier (2010).

**Hormonal assay**

Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and testosterone levels were determined by analyzing the plasma using ELISA (Enzyme-linked immunosorbent assay) kit (Inteco™ UK). Statistical comparisons were made using the ANOVA test and Tukey as post hoc for comparison of data between the control and experimental groups. The results were expressed as mean ± SEM (Standard error of mean) with P<0.05 as significant.

**Results**

**Effects of ACSLE on sperm variables**

On administration of ACSLE for 14 days, cocks that received 200 mg/kg of extract had significantly (p < 0.05) higher sperm motility (T<sub>2</sub>=86.6±3.72%) than the control (80±6.3 5%) but there were no significant (p > 0.05) differences between the sperm motility of T<sub>4</sub> (74±3.7 %), T<sub>8</sub> (78.4±3.9%) and the control (Figure 1). The sperm viability values (Figure 2) showed non-significant decrease in T<sub>2</sub> (82.5±3.3%) but significant decrease in T<sub>4</sub> (77.0±6.6%) and T<sub>8</sub> (79.6±5.8%) when compared to the control group (84±4.4%). For sperm morphology (Figure 3), there was a significant increase in T<sub>2</sub> (95.2±1.0%) and T<sub>4</sub> (97.2±0.7%) with a non-significant decrease in T<sub>8</sub> (76.8±1.0%) when compared with the control (90.6±4%). Sperm concentration (Figure 4) was significantly lower in T<sub>8</sub> (1.55±0.9 x 10<sup>9</sup> mL<sup>-1</sup>), but the values of T<sub>2</sub> (2.35± 0.7 x 10<sup>9</sup> mL<sup>-1</sup>) and T<sub>4</sub> (2.61±0.9 x 10<sup>9</sup> mL<sup>-1</sup>) were not significantly different when compared to the control (2.75±0.6 x 10<sup>9</sup> mL<sup>-1</sup>).

**Effect of ACSLE on testosterone, FSH and LH**

FSH concentration was 1.04 ± 0.7 ng/ml in CT, 2.94 ± 1.8 ng/ml in T<sub>2</sub>, 2.06 ± 2.0 ng/ml in T<sub>4</sub> and 1.06 ± 1.0 ng/ml in T<sub>8</sub> (Table 1). There were significant increases in T<sub>2</sub> and T<sub>4</sub> compared to CT but T<sub>8</sub> showed no significant difference.

LH concentration (Table 1) was significantly higher in T<sub>2</sub> (2.90 ± 1.4 ng/ml) and T<sub>4</sub> (15.06 ± 13.6 ng/ml) but the value of T<sub>8</sub> (8.4±0.4 ng/ml) was not significantly different when compared to the control.
Testosterone concentration (Table 1) was 4.94 ± 0.3 ng/ml in CT, 4.38 ± 1.1 ng/ml in T2, 4.56 ± 0.1 T4 and 4.98 ± 0.3 in T8. There was no significant difference when the test groups were compared to the control group.

Discussion
In this study, results showed that oral administration of ACSLE significantly increased the sperm motility (T2) and sperm morphology (T2 and T4) when the mean values were compared with the control (Figures 1 & 3) which is in tandem with the findings of Khaki et al. (2009). Although sperm viability and concentration reduced significantly in T4 and T8 (Figures 2 & 4), which may be due to the high dose of ACSLE administered as recorded also by Okoro et al. (2016), the significant increase in sperm motility and morphology especially in T2 (Figures 1 & 3) clearly indicates that administration of ACSLE has a positive effect on spermatogenesis in Oba Marshall cocks. ACSLE contains exogenous and endogenous antioxidants (Griffiths et al., 2002) that protect DNA and other important molecules from peroxidation damage. The damage could arise from stress and other climatic factors (Riaz et al., 2004; Jedlińska-Krakowska et al., 2006; Kabir et al., 2007). That could lead to apoptosis. These antioxidants improve sperm health parameters, and invariably increase fertility (Khaki et al., 2008; Sejian et al., 2014; Salehi et al., 2019). ACSLE increased blood-testis barrier stability (Jedlińska-Krakowska et al., 2006) and improved sperm quality of breeder stocks that were fed with vitamin C supplemented feeds (Ezzat et al., 2011; Okoro et al., 2016). ACSLE administered to the birds supports this finding in that the sperm quality improved significantly compared to the controls. Khaki et al. (2008) documented that administration of onion juice (1 g/bird/day) for 20 days increased sperm count, viability, and motility in birds. ACSLE is an antioxidant in the category of vitamin C and E. Okoro et al. (2016) used a combination of garlic and onion inclusions in feed which gave similar results like this present study in that at higher inclusion rate (5 g/600 g feed), the actual live sperm count and motility were reduced significantly but at lower inclusion rates (2.5 g/600 g feed), these values improved significantly.

Testosterone and FSH are necessary for the attainment of full reproductive capabilities in males (Walker & Cheng, 2005). Khaki et al. (2009) showed that FSH, LH and testosterone levels are associated
with spermatogenesis. A decrease in testicular testosterone production negatively affects spermatogenesis (Ashby et al., 2003) and this could be the reason for reduction in sperm concentration seen in T4.

Khaki et al. (2009) showed that levels of FSH and LH are inversely associated with sperm concentration, motility and morphology. FSH, which is a gonadotropin produced and secreted by the anterior pituitary, acts on Sertoli cells in the seminiferous tubules to initiate spermatogenesis. Sertoli cells secrete inhibin-B, which is a protein hormone. The inverse associations of FSH, with inhibin-B and with sperm concentration may be due to the feedback effects exerted by inhibin-B on the anterior pituitary to inhibit FSH secretion. In this study, there was no higher concentration of FSH as against what Babu et al. (2004) documented. Babu et al. (2004) documented that higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage, which has been shown to be associated with azoospermia.

However, the result of this study showed physiologic mean of FSH, LH and testosterone levels (Table 1), sperm motility, viability and concentration but a significant decrease in T4 when compared with the control group. This may be attributed to the absence of epithelial damage and the anti-oxidative effect of onion peel extract.

In another study on male rats, plasma testosterone and luteinizing hormone significantly decreased while follicle stimulating hormone increased in response to treatment with Allium sativum extract (Hamman et al., 2009) but in the findings of this work, LH and FSH increased significantly in response to Allium cepa L. scaly leaf extract administration. The difference seen may be due to the effect of the difference in active principle and antioxidant composition (Slimestad et al., 2007) of the specie of the Allium.

Banihani (2019) documented that Allium cepa L. extract enhances testosterone production in males and the mechanisms is mainly by enhancing LH production, neutralizing the damaging effects of formed free radicals and enhancing the antioxidant defence mechanism in the testis, ameliorating insulin resistance, promoting nitric oxide production in Leydig cells, and altering the activity of 5’ AMP-activated protein kinase. The administered Allium cepa L. scaly leaf extract in this present study significantly increased LH and FSH but not testosterone levels and this might be due to interference with LH receptors within Leydig cells and hence the non-significance change in testosterone levels.

Stimulation of LH initiates the Leydig cells to produce testosterone, but the significant change in gonadotropin levels even with reduced testosterone concentration shows that the administration of ACSLE which led to this finding occurred downstream of the hypothalamic–pituitary axis and may be due to interference with LH receptors within Leydig cells. In dogs and primates, a pulse of LH is followed closely by a pulse of testosterone but in rats, several LH pulses may not elicit rise in testosterone, or there may be a significant delay before a testosterone pulse occurs (Creasy & Chapin, 2013). This may be the reason why there was no significant concurrent increase in testosterone concentrations of T4 with increase in LH concentrations (Table 1).

In conclusion, results from the findings of this work suggest that ACSLE has the ability to improve fertility of Oba Marshal breeder cocks. This was confirmed by the increased sperm motility and morphology and enhanced FSH and LH concentrations. The mechanism of action of ACSLE may be due to its antioxidant ability which protects DNA and other important molecules from peroxidation damage, which could arise from stress and other climatic factors.

The findings of this research show that ACSLE could be helpful in enhancing the reproductive efficiency of Oba Marshall breeder cocks. Further work may be conducted to check if the administration of ACSLE could elongate the fecundity rate of Oba Marshal breeder cocks.

Conflicts of Interest
The authors declare no conflict of interest.

**Table 1**: Effect of ACSLE on circulating LH, FSH and Testosterone in Oba Marshal broiler cocks

| Hormones (ng/mL) | CT (1) | T2 (2) | T4 (2) | T8 (2) |
|------------------|--------|--------|--------|--------|
| FSH              | 1.04 ± 0.7 | 2.94 ± 1.8\(\alpha\) | 2.06 ± 2.0\(\alpha\) | 1.06 ± 1.0 |
| LH               | 0.80 ± 0.5 | 2.90 ± 1.4\(\alpha\) | 15.06 ± 13.6\(\alpha\) | 0.84 ± 0.4 |
| TST              | 4.94 ± 0.3 | 4.38 ± 1.1 | 4.56 ± 0.1 | 4.98 ± 0.3 |

Columns represent mean ± SEM, \(\alpha\)P<0.05, comparison with control. CT=Control, T2=200 mg /kg ACSLE, T4=400 mg /kg ACSLE, T8=800 mg /kg ACSLE.
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