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

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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, pulverised, 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 & Oluyemi, 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 accessed 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), T_2 (200 mg/kg/bird ACSLE), T_4 (400 mg/kg/bird ACSLE) and T_8 (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_2 =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_4 (74±3.7 %), T_8 (78.4±3.9%) and the control (Figure 1). The sperm viability values (Figure 2) showed non-significant decrease in T_2 (82.5±3.3%) but significant decrease in T_4 (77.0±6.6%) and T_8 (79.6±5.8%) when compared to the control group (84±4.4%).

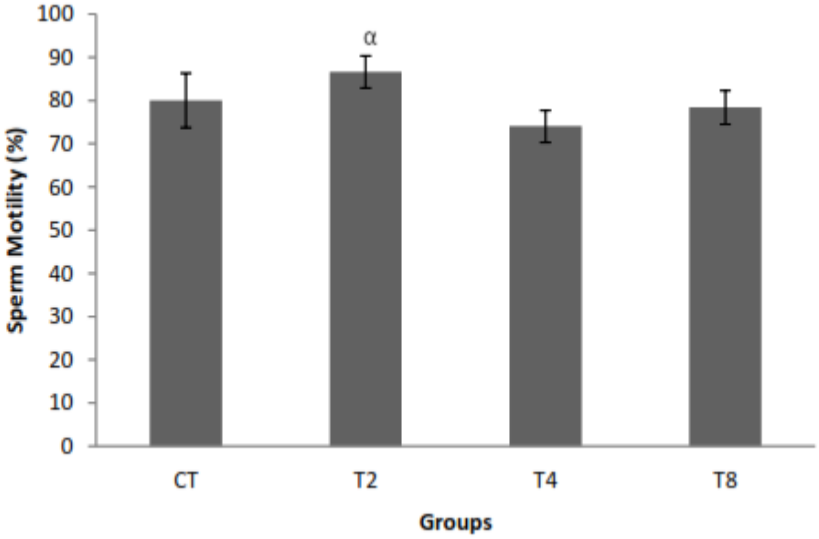

Figure 1: Effect of ACSLE on sperm motility of control and test birds in %, n = 5, $^{\alpha}P<0.05$ from CT. CT=Control, T_2=200 mg /kg ACSLE, T_4=400 mg /kg ACSLE, T_8=800 mg /kg ACSLE

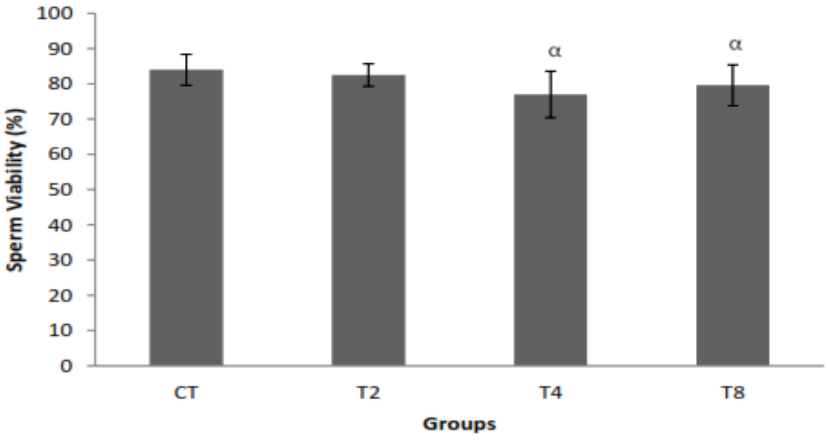

Figure 2: Effect of ACSLE on sperm viability of control and test birds in %, n = 5, $^{\alpha}P<0.05$ from CT. CT=Control, T_2=200 mg /kg ACSLE, T_4=400 mg /kg ACSLE, T_8=800 mg /kg ACSLE

For sperm morphology (Figure 3), there was a significant increase in T_2 (95.2±1.0%) and T_4 (97.2±0.7%) with a non-significant decrease in T_8 (76.8±1.0%) when compared with the control (90.6±4%).

Sperm concentration (Figure 4) was significantly lower in T_8 (1.55±0.9 x 10^9 mL^{-1}), but the values of T_2 (2.35± 0.7 x 10^9 mL^{-1}) and T_4 (2.61±0.9 x 10^9 mL^{-1}) were not significantly different when compared to the control (2.75±0.6 x 10^9 mL^{-1}).

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_2, 2.06 ± 2.0 ng/ml in T_4 and 1.06 ± 1.0 ng/ml in T_8 (Table 1). There were significant increases in T_2 and T_4 compared to CT but T_8 showed no significant difference.

LH concentration (Table 1) was significantly higher in T_2 (2.90 ± 1.4 ng/ml) and T_4 (15.06 ±13.6 ng/ml) but the value of T_8 (0.84±0.4 ng/ml) was not significantly different when compared to the control

(0.80 ± 0.5 ng/ml) at the end of the study.

Testosterone concentration (Table 1) was 4.94 ± 0.3 ng/ml in CT, 4.38 ± 1.1 ng/ml in T_2, 4.56 ± 0.1 T_4 and 4.98 ± 0.3 in T_8. There was no significant difference when the test groups were compared to the control group.

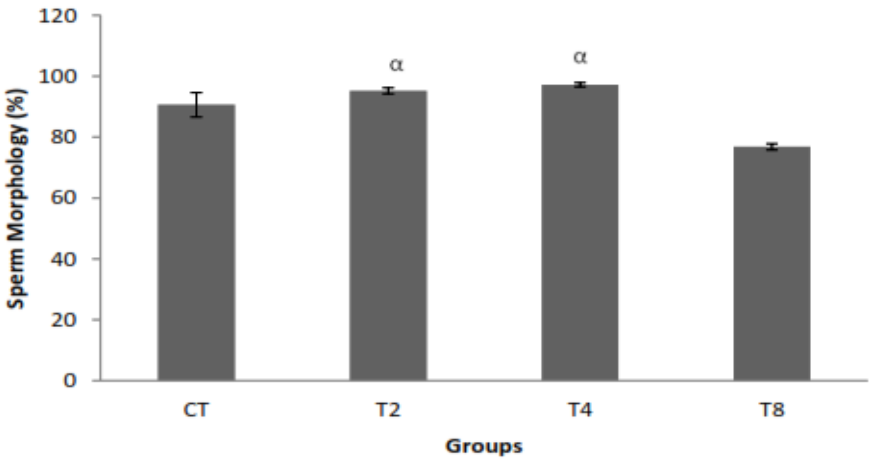

Figure 3: Effect of ACSLE on sperm morphology of control and test birds in %, n = 5, $^{\alpha}P<0.05$ from CT. CT=Control, T_2=200 mg /kg ACSLE, T_4=400 mg /kg ACSLE, T_8=800 mg /kg ACSLE

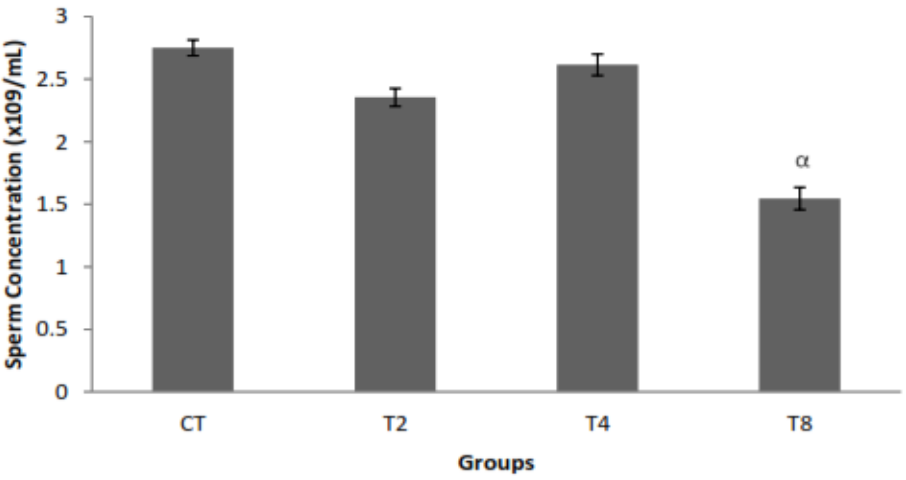

Figure 4: Effect of ACSLE on sperm concentration of control and test birds in $x10^9$/mL, n = 5, $^{\alpha}P<0.05$ from CT. CT=Control, T_2=200 mg /kg ACSLE, T_4=400 mg /kg ACSLE, T_8=800 mg /kg ACSLE

Discussion

In this study, results showed that oral administration of ACSLE significantly increased the sperm motility (T_2) and sperm morphology (T_2 and T_4) 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 T_4 and T_8 (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 T_2 (Figures 1 & 3) clearly indicates that administration of ACSLE has a positive effect on spermatogenesis in 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; Jedlinska-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 (Jedlinska-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

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

Hormones (ng/mL)	CT	T_2	T_4	T_8
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, T_2=200 mg /kg ACSLE, T_4=400 mg /kg ACSLE, T_8=800 mg /kg ACSLE

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 T_8.

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 T_8 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 (Hammani *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 T_4 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.

References

Agarwal A, Mulgund A, Hamada A & Chyatte MR (2015). A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology*, 10.1186/s12958-015-0032-1.

Alagawany M, Ali AE & Reda FM (2016). Effect of dietary supplementation of garlic (*Allium sativum*) and turmeric (*Curcuma longa*) on growth performance, carcass traits, blood profile and oxidative status in growing rabbit. *Annals of Animal Science*, **16**(2): 489–505.

Alkan S, Baran A, Ozdas OB & Evecen M (2002). Morphological defects in turkey semen. *Turkish Journal of Veterinary & Animal Science*, **26**(5): 1087-1092.

Ashby J, Tinwell H, Lefevre PA, Joiner R & Haseman J (2003). The effect on sperm production in adult Sprague–Dawley rats exposed by gavage to bisfenol A between postnatal days 91– 97. *Toxicological Science*, **74**(1): 129–38.

Augusti KT (1996). Therapeutic values of onion and garlic. *Indian Journal of Experimental Biology*, **34**(7): 634–640.

Babu SR, Sadhnani MD, Swarna M, Padmavathi P & Reddy PP (2004). Evaluation of FSH, LH and Testosterone Levels in different subgroups of Infertile Males. *Indian Journal of Clinical Biochemistry,* **19** (1): 45-49.

Barkhordari A, Hetmatimoghaddam S, Ajebali MA, Khalili TA & Noorani M (2013). Effect of Zinc Oxide nanoparticle on viability of human spermatozoa. *Iran Journal of Reproductive Medicine*, **11**(9): 767-771.

Banihani SA (2019). Testosterone in Males as Enhanced by Onion (*Allium Cepa* L.). *Biomolecules,* **9** (2): 75.

Bansal AK & Cheema RS (2014). Analysis of sperm and relationship between conventional sperm parameters and hypo-osmotic swelling test/acrylamide penetration assay – crossbred cattle bulls. *Advances in Applied Science Research*, **6** (1): 39–44.

Cheng FP, Guo TJ, Wu TJ, Lin TE, Ursem PJF, Colenbrander B & Fung HP (2002). Annual variation in semen characteristics of pigeons (*Columba livia*). *Poultry Science,* **81**(7): 1050-1056.

Creasy DM & Chapin RE (2013). Structure, Function and Cell Biology: In: *Haschek and Rousseaux's Handbook of Toxicologic Pathology,* Third edition. Pp 2522.

Cummings JH & Bingham SA (1998). Fortnightly review— diet and the prevention of cancer. *British Medical Journal*, **317**(7173): 1636–1640.

de Reviers M (1968). Determination de la durée des processus spermatogenetiques chez le coq à l'aide de thymidine tritiée. Proceedings of 6th International Congress on Reproduction and Artificial Insemination, Paris. Pp 183-185.

Egbunike CN & Oluyemi JA (1979). Comparative studies of the reproductive capacity of the Nigerian and exotic poultry breeds. *Nigerian Journal of Animal Production,* **6**(1): 47-51.

Ezzat W, Shoerib MS, Mousa SMM, Bealish AMA & Ibrahiem A (2011). Impact of Betaine, Vitamin C and Folic acid supplimentations to the diet on productive and reproductive performance of Matrouch poultry strain under Egyptian Summer condition. *Egyptian Poultry Science*, **31**(2): 521-523.

Fukushima S, Takada N, Hori T & Wanibuchi H (1997). Cancer prevention by organosulfur compounds from garlic and onion. *Journal of Cell Biochemistry*, **67**(S27): 100–105.

Griffiths G, Trueman L, Crowther T & Thomas B (2002) Onion a global benefit of health. *Phytotherapy Research*, **16**(7): 603-615.

Hafez ESE (1987) Techniques for improving reproductive efficiency. In: *Reproduction in Farm Animals,* fifth edition. Philadelphia, PA, USA, Lea and Febiger. Pp 455-497.

Hammani I, Amara S, Benahmed M, El May MV & Mauduit C (2009). Chronic crude garlic-feeding modified adult male rat testicular markers: mechanisms of action. *Reproductive Biology and Endocrinology,* **7**(1): 65.

Jedlinska-Krakowska M, Bomba G, Jakubowski K, Rotkiewicz T, Jana B & Penkowski A (2006). Impact of oxidative stress and supplementation with vitamins E and C on testes morphology in rats. *Journal of Reproduction and Development*, **52**(2): 203-209.

Jequier AM (2010). Semen analysis: a new manual and its application to the understanding of semen and its pathology. *Asian Journal of Andrology*, **12**(1): 11–13.

Kabir M, Oni OO & Akpa GN (2007). Osborne selection index and semen traits interrelationships in Rhode Island Red and White breeder cocks.

International Journal of Poultry Science, **6**(12): 999-1002.

Khaki A, Fathiazad F, Nour M & Hamadeh DVM (2008). Evaluation of androgenic activity of *Allium cepa* on spermatogenesis in the rat. *Folia Morphologica,* **68**(1): 44-45.

Khaki A, Fathiazad F, Nouri M, Khaki AA, Khamenehi HJ & Hamadeh M (2009). Evaluation of androgenic activity of *Allium cepa* on spermatogenesis in the rat. *Folia Morphologica,* **68**(1): 45-51.

Khaki A, Farnam A, Davatgarbadil A & Nikniaz H (2012). Treatment effects of onion (*Allium cepa*) and ginger (*Zingiber officinale*) on sexual behavior of rat after inducing an antiepileptic drug (lamotrigine). *Balkan Medical Journal,* **29**(3): 236-242.

Kumud K, Biju CM & Augusti KT (1990). Anti-diabetic and hypolipidemic effects of SMCS isolated from *Allium cepa* Linn*. Indian Journal of Biochemistry and Biophysics,* **32**(1): 49.

Lee SU, Lee JH, Choi SH, Lee JS, Ohnisi-Kameyama M, Kozukue N, Levin CE & Friedman M (2008). Flavonoid content in fresh, home-processed, and light-exposed onions and in dehydrated commercial onion products. *Journal of Agricultural Food Chemistry*, **56**(18): 8541–8548.

Lee B, Jung J & Kim H (2012). Assessment of red onion on antioxidant activity in rat. *Food and Chemical Toxicology,* **50**(11): 3912-3919.

Mosher WD & Pratt WF (1991). Fecundity and infertility in the United States: incidence and trends. *Journal of Fertility and Sterility*, **56**(2): 192-193.

Okoro VMO, Mbajiorgu CA & Mbajiorgu EF (2016). Semen quality characteristics of Koekoek breeder cocks influenced by supplemental inclusion levels of onion and garlic mixture at 35-41 weeks of age. *Revista Brasileira de Zootecnia*, **45**(8): 433-440.

Oyeyemi MO, Akusu MO & Ola-Davies OE (2000). Effect of successive ejaculations on the spermiogram of West African Dwarf goats (*Capra hircus*). *Veterinari Arhiv*, **70**(4): 215-222.

Oyeyemi MO & Ubiogoro O (2005). Spermiogram and morphological characteristics in testicular and epididymal spermatozoa of Large White Boar in Nigeria. *International Journal of Morpholology*, **23**(3): 235-239.

Riaz A, Aleem M, Ijaz A, Saeed MA & Latiff A (2004). Effect of collection frequency on the semen quality of broiler breeders. *British Poultry Sciences*, **45**(6): 823-827.

Saeed JM & Al-Soudi KA (1975). Semen quality traits of seven strains of chickens raised in the humid tropics. *Animal Veterinary Advances*, **7**(10): 848-849.

Salehi P, Shahrokhi SZ, Kamran T, Ajami A, Taghiyar S & Deemeh MR (2019). Effect of antioxidant therapy on the sperm DNA integrity improvement; a longitudinal cohort study, *International Journal Reproduction and BioMedicine*, **17**(2): 99–106.

Sejian V, Singh AK, Sahoo A & Naqvi SMK (2014). Effect of mineral mixture and antioxidant supplementation on growth, reproductive performance and adaptive capability of Malpura ewes subjected to heat stress. *Journal of Animal Physiology and Animal Nutrition,* **98**(1):72–83.

Slimestad R, Fossen T & Vagen LM (2007). Onions: A source of unique dietary flavonoids. *Journal of Agriculture and Food Chemistry*, **55**(25): 10067-10080.

Walker WH & Cheng J (2005). FSH and testosterone signaling in Sertoli cells. *Reproduction*, **130**(1): 15–28.

Yama OE, Duru FI, Oremosu AA, Osinubi AA, Norohna CC & Okanlawon AO (2011). Sperm quotient in Sprague-Dawley rats fed graded doses of seed extract of *Momordica charantia*. *Middle East Fertility Society Journal,* **16**(2):154-158.

Zhang C & Qiao H (2004). Effect of polychlorinated biphenyls on spermatogenesis and testosterone secretion in adult cocks. *Journal of Zhejiang University Science*, **5**(2): 193-197.