

## Spermogram of Rabbit Bucks Fed Diets Supplemented with *Allium sativum* (garlic)

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**Abstract:** The study was designed to evaluate the effects of crude *Allium sativum* in diets of rabbit bucks on Semen characteristics. Twenty one (21) 10.0 ± 2.1 months old, 1.80 ± 0.2 kg average body weight rabbit bucks were used for the study. Bucks were randomly divided into 3 groups of 7 bucks each. Group A served as control, group B and C received 2.5% and 5.0% garlic in diets respectively. The bucks were allowed to acclimatise for 49 days during which semen samples were collected to establish a base line data. The feeding period lasted for 63 days during which semen samples were collected weekly between the hours of 8.00-10.00 am using artificial vagina for evaluation. Data collected were expressed as mean ± standard error of mean using graph pad prism version 5.0, repeated measure one way analysis of variance was used to test for differences between groups followed by Turkey's multiple comparism test, values of  $P < 0.05$  was considered significant. There were no significant differences in mean semen pH, motility, % live spermatozoa and % abnormal spermatozoa between control (A) and treatment groups (B and C). However, there were significant differences in mean semen volume at week 8 of the study between control and treatment groups. Also the semen concentrations were significantly higher in the treatment groups in a dose-dependent manner from week 3-9 of the study. In conclusion, *Allium sativum* at 2.5% & 5.0% inclusion rates improved sperm concentration in rabbit bucks.

**Key words:** Spermogram, Rabbit bucks, Diets, *Allium sativum*.

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### I. Introduction

Garlic belongs to the kingdom: Plantae; Phylum: Magnoliophyta; Class: Liliopsida; Order: Asparagales; Family: Alliaceae; Genus: *Allium*; Species: *sativum*. Other plants of the same genus as garlic include: Onions, Leaks and Chives (White and Zellner, 2008).

Garlic has been analysed for moisture, carbohydrates, protein, fat, minerals, energy, ash, pH, and essential oil contents (Haciseferogullari *et al.*, 2005). Protein content was found to be higher than that in other plants such as bean and pea (Cemeroglu and Acar, 1986), but crude oil content was considerably lower. Garlic is known to contain high levels of Potassium, Phosphorus, Magnesium, Sodium, Calcium and Iron, with the amount of these minerals in the bulb depending on the respective minerals in the soil where the bulb is grown. Vitamins like riboflavin, thiamine, nicotinic acid, vitamin C and vitamin E are important chemical constituents of garlic (Alejandra *et al.*, 2010).

Many of the *Allium* plants have been shown to reduce risks and modulate metabolism to favour the prevention of diseases. Garlic is considered to be the best disease preventive plant among the *Alliums* because of its potent and widespread effect (Harunabu *et al.*, 2001; Banerjee *et al.*, 2002). It was reported that consumption of garlic compromised some male reproductive functions, such as spermatogenesis and testosterone levels (Dixit and Joshi, 1982; Hammami *et al.*, 2008, 2009; Omotoso *et al.*, 2009). On the other hand, garlic was reported to increase sperm concentrations and improve male reproductive functions (Al-Bekairi *et al.*, 1990; Yuriko *et al.*, 2001; Salah *et al.*, 2014).

Rabbit (*Oryctolagus cuniculus*) production is a veritable means of alleviating animal protein deficiency in Nigeria (Abdulmalik, 1994; Hassan and Owolabi, 1996; Ajala and Balogun, 2004).

Rabbit production in Nigeria could be described as rudimentary or primitive as compared to countries such as France, Hungary, China and the United State. This is evident from the small rabbit keeping population, weak inventory of rabbit keeping, infrastructure, low consumption rate of rabbit meat, absence of an organized or thriving market for rabbit meat products and lack of governmental and institutional support limits the expansion of rabbit production (Onifade *et al.*, 1999).

Ironkwe and Amaefule (2007), reported between 83% and 140% returns on capital investment in rabbit farming but despite this attractive prospect, Nigerians are yet to appreciate rabbit production. Rabbit production in Nigeria like other aspect of livestock production is confronted by many challenges among which includes: diseases outbreaks, reproduction related problems especially infertility. To boost the production of healthy animal protein (low in cholesterol and sodium) in Nigeria which rabbit meat is reputed for, there is a compelling

need to incorporate in rabbit diet/feed botanicals known for its reputation in prevention of diseases, high nutritive values and above all, which may exert a positive effect on fertility in rabbit bucks.

There is a global campaign towards organic livestock production to prevent the deleterious effects of drug residues and hormones from edible animal tissues to humans. Drugs and synthetic hormones have being the mainstay for fertility boosting and reproductive related diseases treatment in farm animals before now. There is also, paucity of information on the effect of garlic on reproduction in animals particularly rabbits. Although a lot of work has been done to verify the medicinal properties of this plant, to the best of our knowledge, very little has been said or done concerning its effect on reproduction.

The objective of the study is to determine the semen characteristics of rabbit bucks fed diets supplemented with *Allium sativum* (garlic)

## II. Materials And Methods

### The study area

The study was carried out at the Animal house of the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

### Experimental animals

Twenty one (21) apparently healthy, domestic rabbit bucks (*Oryctolagus cuniculus*)  $10 \pm 2.0$  months old with average body weight of  $1.74 \pm 0.1$  kg were used for the study. The experiment was carried out under controlled ambient temperature. The bucks were screened and treated with broadspectrum medication (Kepromec®) against endoparasites and helminthes infestation before the commencement of the experiment, while water and feed were provided *ad libitum*. The bucks were housed in standard rabbit cages, one buck per cage.

### Plant sample

*Allium sativum* (Garlic) was obtained in June, 2014 from Sokoto main market, Sokoto State, Nigeria. The sample was identified, confirmed with a voucher Number 423 at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria. The fresh bulbs were peeled and dried under shade. The dried bulbs were then weighed accordingly and incorporated into the experimental diets.

### Experimental design

The rabbit bucks were randomly divided into three groups of seven each, designated as group A, B, and C. After 49 days of acclimatization, all rabbits were fed diets corresponding to their group as indicated in Table 3.1. The diets were of isonitrogenous and isocaloric values, consisted of maize, soyabean meal, rice offals, crude *Allium sativum*, vitamin premix, palm oil, bone meal, methionine and salt. Group A, B and C diets consisted of 0, 2.5% and 5.0% *Allium sativum*, respectively. The chemical composition of the feed is presented in Table 3.2, the period of feeding lasted for 63 days, semen samples were collected during the feeding period, on weekly bases for evaluation.

**Table 1 Composition of the experimental diets (%)**

Ingredients(kg)	A	B	C
Maize	30.16	29.29	28.57
Soyabean meal	28.12	27.40	26.64
Rice offals	35.32	34.41	33.46
Crude <i>Allium sativum</i>	0.0	2.5	5.0
Vitamin premix	0.5	0.5	0.5
Palm oil	1.0	1.0	1.0
Bone meal	4.0	4.0	4.0
Methionine	0.4	0.4	0.4
Salt	0.5	0.5	0.5
Total (%)	100	100	100

**Table 2 Chemical composition of experimental diets**

Item (%)	A	B	C
Dry Matter	93.6	91.77	91.68
Crude Protein	15.13	14.75	14.68
Ether Extract	11.51	13.23	9.86
Crude Fibre	19.65	19.39	17.77
Nitrogenfree extract	4.37	4.32	4.31
Ash	27.31	27.00	26.94

### Semen collection and evaluation

The bucks were trained for semen collection during the acclimatization period and semen collection was carried out with the aid of artificial vagina (AV adapted from IMV Technologies model) for rabbits.

The ejaculate obtained was evaluated as described by Zemjanis (1970). This included the visual or gross evaluation of the ejaculate soon after collection for volume, pH, as well as microscopic examination for motility, semen concentration, percentage live spermatozoa and percentage sperm abnormalities.

Volume: volume of semen was measured directly from the calibrated tube attached to the AV used for the collection.

Gross motility: was examined as quickly as possible after collection, a drop of the semen sample was placed on a pre-warmed glass slide, cover slipped and examined at  $\times 10$  magnification.

Semen concentration: was determined using Neubauer haemocytometer as described by Azawi and Ismaeel (2012). Micropipette was used to aspirate 25  $\mu$ l of semen and diluted with 5 ml of 3 % NaCl in a test tube to have a dilution factor of 5000. It was then examined using a microscope at  $\times 40$  magnification and the sperm cells were counted in five Thoma squares of the chamber (ie four corner and the centre squares) of the haemocytometer. The semen concentration was calculated as follows:

Concentration (sperm cells/mL) = Number of sperm cells counted in the twenty five small squares  $\times$  dilution factor  $\times 10^4$

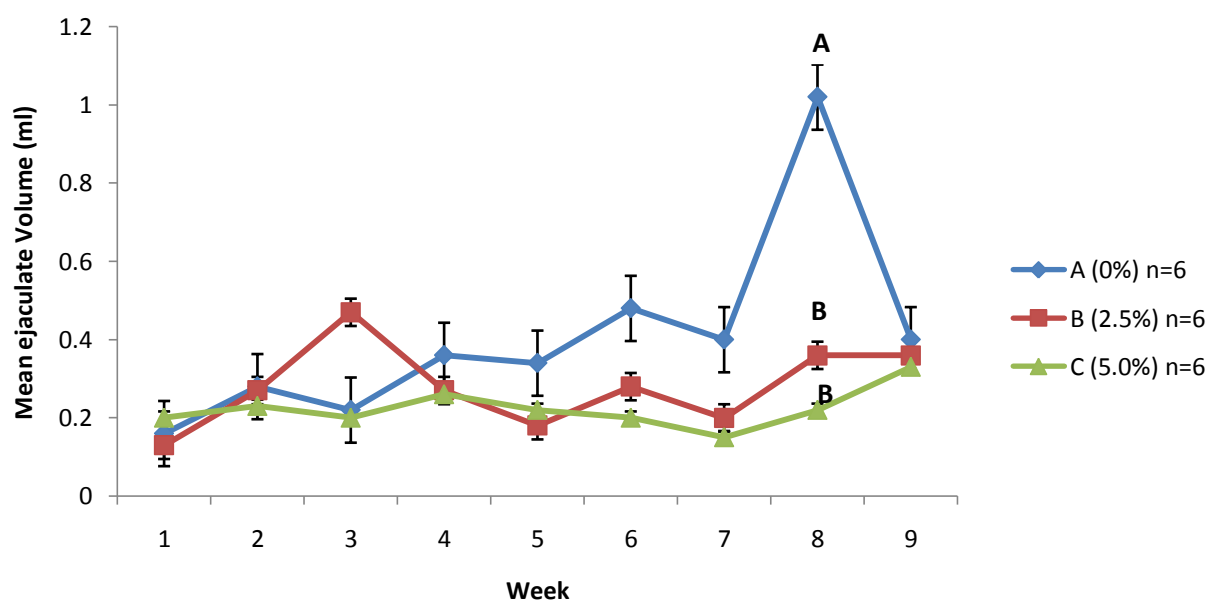
Percentage live sperm cells: this was determined as described by Estes *et al.* (2006). A thin smear of the semen was made on a clean grease free slide and stained with eosin-nigrosin stain.

Percentage sperm abnormalities: was determined by making a thin smear of the semen sample on a clean grease-free glass slide and stained with eosin-nigrosin. One hundred sperm cells were counted per slide using hand counter under light microscopy at  $\times 40$  magnification. Five cell types were recorded: normal cell, detached head, free tail, coiled tail and bent tail..

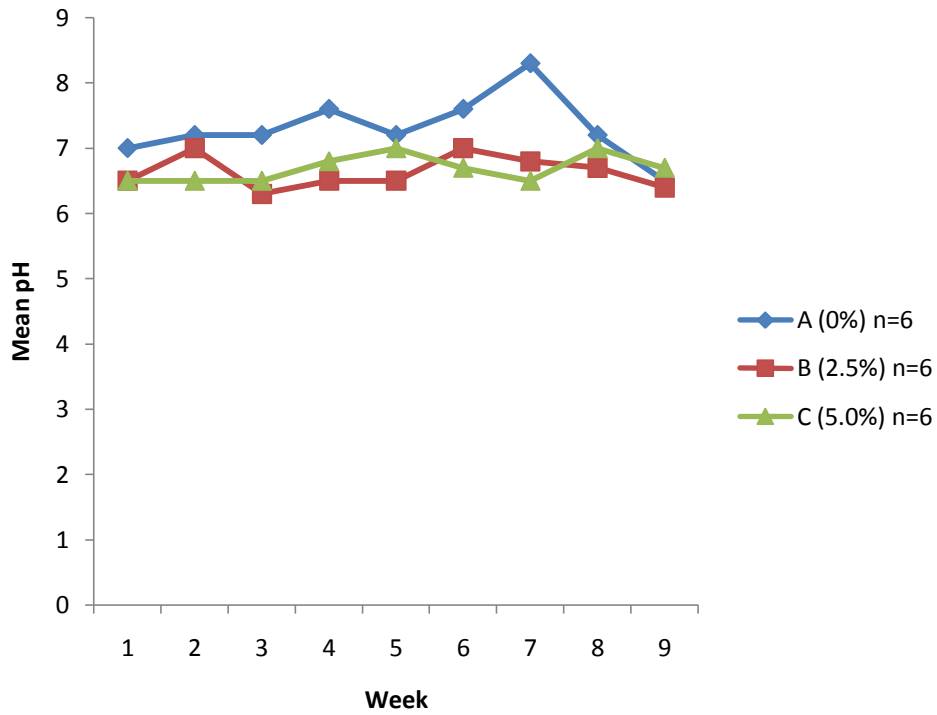
### Data analysis

Data collected were expressed as mean  $\pm$  standard error of mean (SEM) using Graphpad prism version 5.0. Repeated measure one-way analysis of variance (ANOVA) was used to test for differences between groups, followed by Tukey's multiple comparison Test. Values of  $P < 0.05$  was considered significant.

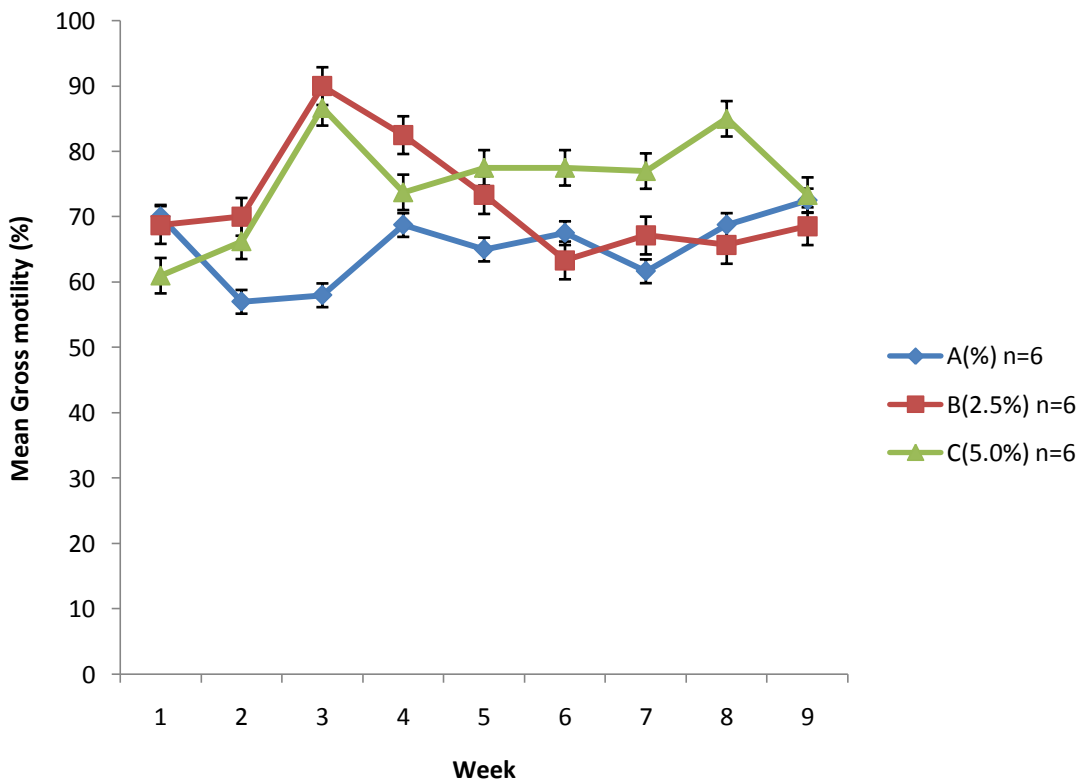
## III. Results And Discussion



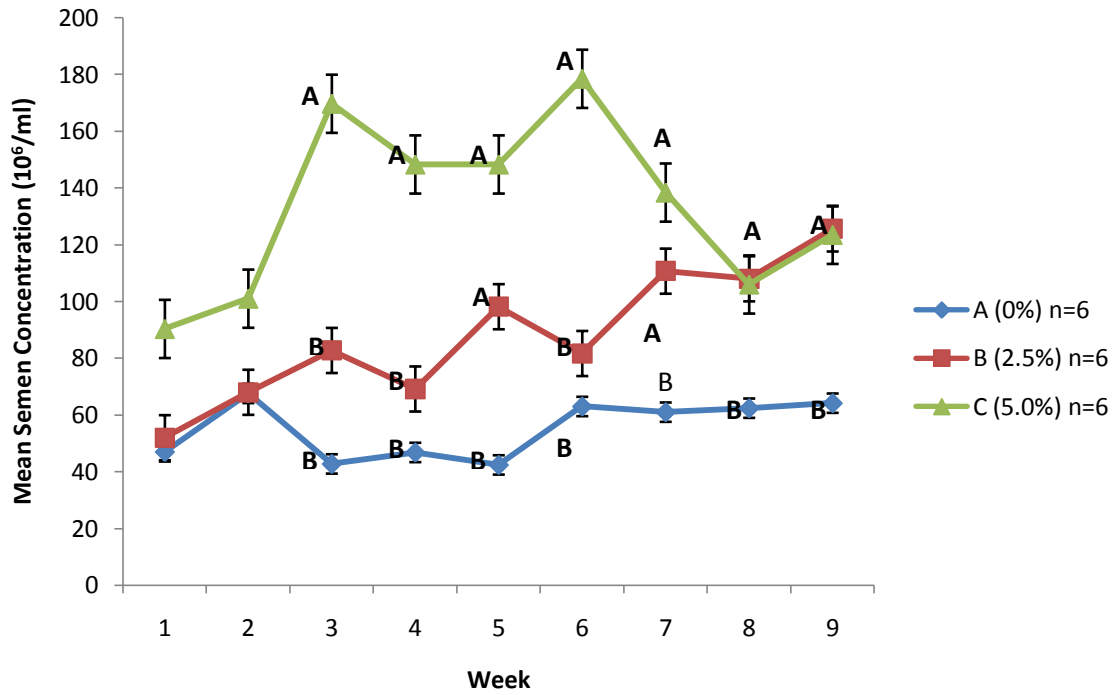
**Figure 1** Mean ejaculate volume of rabbit bucks fed diets containing 0, 2.5 and 5% inclusion of garlic (*A. sativum*).



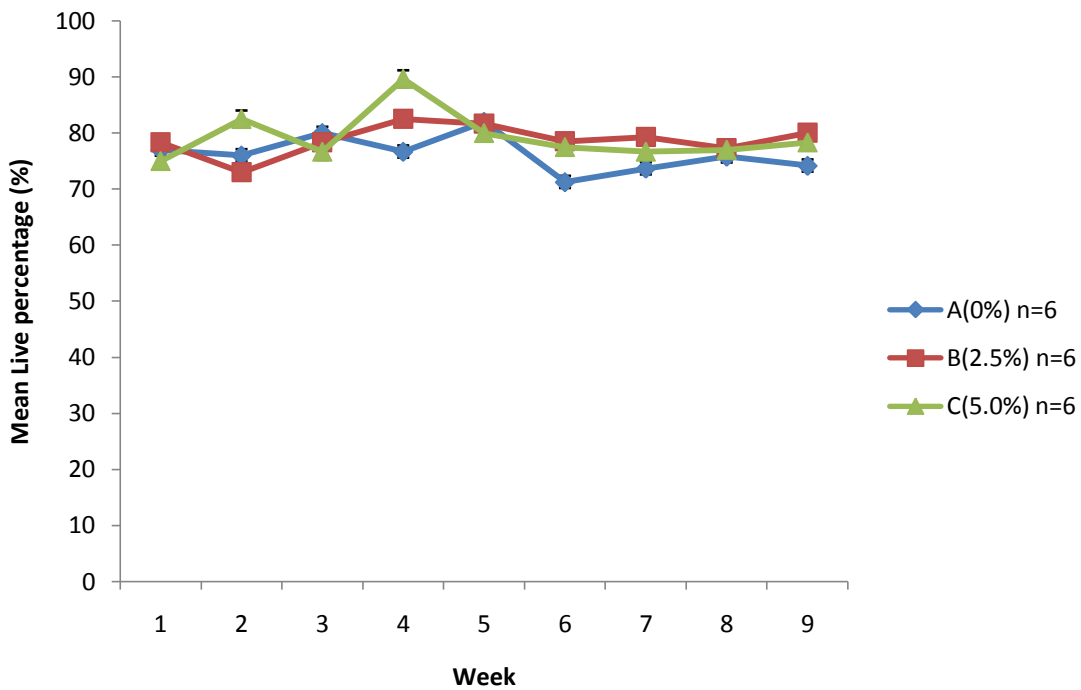
**Figure 2** Mean semen pH values of rabbit bucks semen fed diets containing 0, 2.5 and 5.0% of garlic (*A. sativum*).



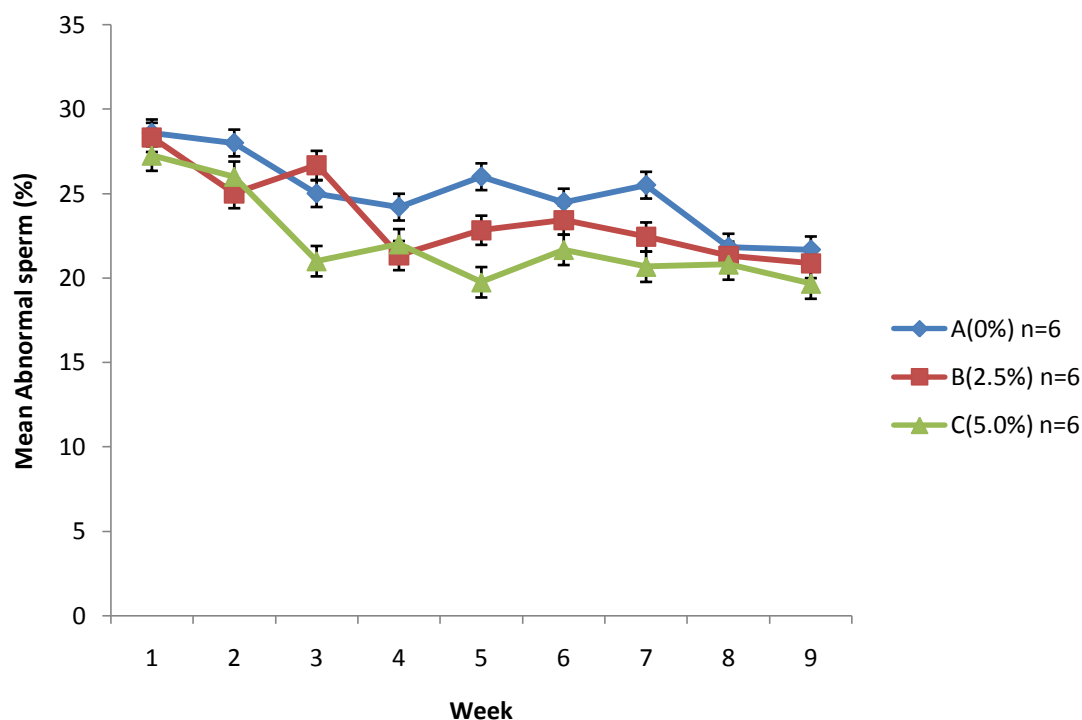
**Figure 3** Mean semen gross motility of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic (*A. sativum*).



**Figure 4** Mean semen concentration ( $\times 10^6/\text{ml}$ ) of rabbit bucks semen fed diets containing 0, 2.5 and 5.0% of garlic (*A. sativum*).



**Figure 5** Mean percentage live spermatozoa of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic (*A. sativum*).



**Figure 6** Mean percentage sperm abnormality of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic.

The observation of increased mean ejaculate volume of the control compared to experimental groups at week 8 of the study could be due to possibility of contamination of the semen by urine as highlighted by Jordi *et al.* (2005) who reported a 13% chance of contamination of rabbits' semen by urine during collection with artificial vagina. The normal ejaculate volume for rabbit bucks as reported by Campos *et al.* (2014) ranges between 0.3-0.6 ml, this is far below the abrupt increase we observed at this week of the study.

The absence of a significant difference in the mean gross sperm motility between the control and experimental groups, contradicts the findings of Omotoso *et al.* (2012), who observed a decrease in percentage spermatozoa motility in wistar rats treated with graded doses of *A. sativum*. This difference may be due to variety of garlic used, dose administered and also species differences.

Observation on the effect of garlic on sperm concentration has been varied in the present study. There were gradual increases in mean sperm concentration in the treatment groups (B and C), with a significant difference between group A and group C from week 3 to 9 of the study. This corroborates the study of Al-Bekairi *et al.* (1990), who evaluated the effect of 100 mg/kg/day of aqueous extract of *A. sativum* on epididymal spermatozoa in mice treated for 3 months and found out that the sperm count was significantly increased in the treatment groups compared to the control. Salah *et al.* (2014) reported a significant increase in sperm concentration in mice treated with aqueous and alcoholic extracts of *A. sativum*. However, this contradicts the findings of Hammami *et al.* (2008), who found out that after the administration of crude garlic at inclusion rate of 5.0%, 10%, 15% and 30% to wistar rats for 30 days, observed a reduction in sperm concentration at 10%, 15% and 30% doses. Omotoso *et al.* (2009) reported a decreased sperm concentration in wistar rat treated with 500 mg/kg/day and 1000 mg/kg/day of aqueous garlic extract for 30 days in a dose dependent manner. Saponin which is one of the constituents of garlic was reported to have some positive effects on libido and spermatogenesis (Francis *et al.*, 2002) this probably may have had an effect on the sperm concentration in the treated groups. It is possible to attribute the differences of other studies with our finding to higher inclusion rates/doses of the *A. sativum* used.

The absence of significant differences in percentage live spermatozoa among the groups, contradicts the findings of Omotoso *et al.* (2012), who observed a decreased percentage live spermatozoa in wistar rats, treated with higher doses of garlic.

The observation on percentage abnormal spermatozoa between the groups also disagrees with the findings of Omotoso *et al.* (2012), who observed a marked increase in percentage abnormal spermatozoa in a dose dependent manner in wistar rat treated with garlic. The several reasons adduced for differences in observations by different investigators could necessitate more intensive studies to determine the optimal

inclusion rates, varieties of the plant and other physicochemical properties necessary to advance the use of garlic in a more beneficial manner.

#### IV. Conclusion

*A sativum* inclusion at 2.5 and 5.0 % in the diets of rabbit bucks improved sperm concentration. Therefore, further studies should be carried out to advance its usage as a raw material in livestock feed formulation.

#### References:

- [1]. Abdulmalik, M. E. (1994). Rabbit production. In: Okaiyeto, P. O., Ndubuisi, A. H. and Okoh, A. E. (editors). *Advanced Animal Husbandry Practices for Subject Matter Specialists in the ADPs*. Training Manual for FACU/NAPRI, Workshop, Zaria, 13-17 December, 1994.
- [2]. Ajala, M. K. and Balogun, J. K. (2004). Economics of rabbit production in Zaria, Kaduna State. *Tropical Journal of Animal Science*, 7(1): 1-10.
- [3]. Al-Bekairi, A. M., Shah, A. H. and Qureshi, S. (1990). Effects of *Allium sativum* on epididymal spermatozoa, estradiol-treated mice and general toxicity. *Journal of Ethnopharmacology*, 29(2): 117-125.
- [4]. Alejandra, C. C., Ana, C. S., Marta, C. M. and Mar, V. (2010). A comprehensive survey of garlic functionality. In: Garlic consumption and health. (Pacurar, M. and Krejei, G. editors). 1-60.
- [5]. Azawi, O. I. and Ismaeel, M. A. (2012). Effects of seasons on some semen parameters and bacterial contamination of Awassi ram semen. *Reproduction in Domestic Animals*, 47: 402-406.
- [6]. Banerjee, S. K., Maulik, M., Mancahanda, Dinda, A. K., Gupta, S. K. and Maulik, S. K. (2002). Dose-dependent induction of endogenous antioxidants in rat heart by chronic administration of garlic. *Life Sciences*, 70: 1509-1518.
- [7]. Cemeroglu, B. and Acar, J. (1986). Fruit and Vegetable Processing Technology. *Turkish Association of Food Technologists, Ankara*, 6: 508-510.
- [8]. Dixit, V. P. and Joshi, S. (1982). Effects of chronic administration of garlic (*Allium sativum*) on testicular function. *Indian Journal of Experimental Biology*, 20: 534-536.
- [9]. Estes, M. C., Soler, A., Fernandez-santos, M., Quinteromoreno, A. A. and Garde, J. J. (2006). Functional significance of the sperm head morphometric size and shape for determining freezability in Iberian Red Deer (*Cervuselaphushispanicus*) epididymal sperm samples. *Journal of Andrology*, 27 (5): 662-670.
- [10]. Francis, G., Kareem, Z., Mekkar, H. P. and Becker, K. (2002). The Biological Action of Saponin in Animal System: a review. *British Journal of Nutrition*, 88(6): 587-605.
- [11]. Haciseferogullari, H., Ozcan, M., Demir, F. and Calisir, S. (2005). Some nutritional and technological properties of garlic (*Allium sativum* L.). *Journal of Food Engineering*, 68:463-469.
- [12]. Hammami, I., Amara, S., Benahmed, M., El-May, M. V. and Mauduit, C. (2009). Chronic crude garlic feeding modified adult male rat testicular markers: mechanisms of action. *Reproductive Biology and Endocrinology*, 7: 65-70
- [13]. Hammami, I., Nahdi, A. and Mauduit, C. (2008). The inhibitory effects on adult male reproductive functions of crude garlic (*Allium sativum*) feeding. *Asian Journal of Andrology*, 10: 593-601.
- [14]. Harunabu, A., Brenda, L. P., Hiromichi, M., Shigeo, K. and Yoichi, I. (2001). Recent advances on the nutritional effects associated with the use of garlic as a supplement. *Journal of Nutrition*, 131: 955-962.
- [15]. Hassan, W. A. and Owolabi, R. O. (1996). Production performance of domestic rabbits in semi-arid zone of Nigeria. In: Recent advances in Rabbit Sciences. *Proceedings, 6th World Rabbit Congress*. (Francois, L., editor). July 9<sup>th</sup>-12<sup>th</sup>. Toulouse, France, 3: 359-363.
- [16]. Ironkwe, M. O. and Amaefule, K. U. (2007). Economics of rabbit production in Rivers State, Nigeria. In: Agiang, E. A., Agwunobi, L. N. and Olawoyin, O. O. (editors). Sustainability of the Livestock Industry in an Oil Economy. *Proceedings of the 32nd Annual Conference of the Nigerian Society for Animal Production (NSAP)*, University of Calabar, Cross River State, Nigeria, 18-21 March, 2007.
- [17]. Jordi, R., Silvia, M., Juan, O., Inma, P., Juan, M. and Emilio, A. M. (2005). Influence of Constant Long Days on Ejaculate Parameters of Rabbits Reared Under Natural Environmental conditions of Mediterranean Area. *Livestock Production Science*, 94: 169-177.
- [18]. Omotoso, G., Oyewopo, A., Kadir, A., Olawuyi, S. and Jimoh, A. (2009). Effects Of Aqueous Extract Of *Allium Sativum* (Garlic) On Semen Parameters in Wistar Rats.. *The Internet Journal of Urology*, 7 (2): 1-3.
- [19]. Omotoso, G. O., Oyewopo, A., Onanuga, I. O. and Enaibe, B. U. (2012). Garlic consumption alters testicular histology and antioxidant status in wistar rats. *The Tropical Journal of Health Sciences*, 19(2): 31-33.
- [20]. Onifade, A. A., Abu, O. A., Obiyan, R. J. and Abanikannda, O. T. F. (1999). Rabbit Production in Nigeria: Some aspects of current status and promotional strategies. *World Rabbit Science*, 7 (2): 51-58.
- [21]. Salah, M. M. A., Rasha, F. A. and Dalia, A. S. (2014). Physiological and histological effect of aqueous and alcoholic extract of garlic (*Allium sativum*) on testicular function of albino male mice treated with lead acetate. *Journal of Biotechnology Research Centre* (Special edition), 8(2): 41-48.
- [22]. White, K. and Zellner, J. (2008). Hamilton College Seminar 235 Food for thought: The Science, Culture and Politics of Food Spring. Clinton New York. United States.
- [23]. Yuriko, O. I., Mika, I., Chiaki, S., Yutaka, K., Syoji, N. and Kazuo, I. (2001). Garlic Supplementation Increases Testicular Testosterone and Decreases Plasma Corticosterone in Rats Fed a High Protein Diet. *The Journal of Nutrition*, 131(8): 2150-2156.
- [24]. Zemjanis, R. (1970). Collection and evaluation of semen. In: *Diagnostic and Therapeutic techniques in Animal Reproduction*. 2nd Edition, Williams and Wilkins Co. Baltimore MD . 139-156.