Oxidative stress can reduce semen quality, thereby resulting in poor reproductive performance of male rabbits. This study was conducted to investigate the antioxidant potentials of dietary Zingiber officinale (Z) and Allium sativum (A) on rabbit semen quality. Twenty-eight male rabbits aged 9-10 months old were randomly allotted to seven diets containing 0 (control), 5, 10, and 15 g of Z, and 5, 10, and 15 g of A per kg diet respectively, and fed the experimental diet for seven weeks. Semen was collected weekly using an artificial vagina and assessed for semen volume (ml), spermatozoa motility (%), sperm concentration (cells/ml), and sperm viability (%). Seminal plasma was separated from the semen by centrifugation for Seminal Total Antioxidant Capacity (STAC) concentration. All data obtained were subjected to Analysis of Variance. The result showed that semen volume was significantly (p<0.05) higher in bucks fed Z and A than those fed the control diet. Spermatozoa motility was higher in Z group. Dietary levels of Z and A had a significant (p<0.05) effect on STAC; the highest value was recorded in bucks fed diet A (5.31 mol/L). No correlation exists between seminal total antioxidant capacity and semen parameters in the Z and A groups of bucks. The study suggests that supplementation of male rabbits' diet with Zingiber officinale or Allium sativum at the levels used in this study may not result in an optimum semen quality improvement as parameters assessed varied across the groups. However, dietary Allium sativum at 10g/kg can boost sperm production and antioxidant level in rabbit semen.

**Keywords:** dietary supplement, ginger, garlic, reactive oxygen species, sperm quality, seminal plasma.


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**El estrés oxidativo puede reducir la calidad del semen, lo que resulta en un pobre desempeño reproductivo en conejos. Este estudio se realizó para investigar el potencial antioxidante del Zingiber officinale (Z) y Allium sativum (A) en la calidad del semen de conejo. Se asignaron al azar veintiocho conejos machos de 9 a 10 meses de edad a siete dietas que contenían 0 (control), 5, 10 y 15 g de Z, y 5, 10 y 15 g de A por kg de dieta, respectivamente, y se alimentaron con las dietas experimentales durante siete semanas. El semen se colectó semanalmente utilizando una vagina artificial y se evaluó el volumen seminal (ml), la motilidad espermática (%), la concentración de espermatozoides (células/ml) y la viabilidad espermática (%). El plasma seminal se separó del semen mediante centrifugación para la determinación de la capacidad antioxidante total del plasma seminal (STAC). Todos los datos obtenidos se sometieron a análisis de varianza. El volumen seminal fue significativamente (p <0.05) mayor en los machos alimentados con Z y A que aquellos alimentados con la dieta control. La motilidad fue mayor en el grupo Z. La incorporación de Z y A en la dieta tuvo un efecto significativo (p <0.05) sobre STAC; el valor más alto se registró con la dieta A (5,31 mol/L). No existe correlación entre la capacidad antioxidante total del plasma seminal y los parámetros seminales en los grupos Z y A. Este estudio sugiere que la suplementación de la dieta de conejos con Zingiber officinale o Allium sativum en los niveles usados puede no resultar en una mejora óptima de la calidad del semen ya que los parámetros evaluados variaron entre los grupos. Sin embargo, Allium sativum a 10 g/kg puede aumentar la producción de esperma y el nivel de antioxidantes en el semen de conejo.

**Palabras clave:** suplemento dietético, jengibre, ajo, especies oxigeno reactivas, calidad espermática, plasma seminal.


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Inconsistent productivity has been a major challenge of rabbit production in the tropics, and this is often attributed to depressed fertility in males or females. Infertility in males is associated with low sperm production and quality, which is influenced by environmental, physiological, and genetic factors. Non-genetic factors such as stress, nutrition, age, and management are believed to influence semen characteristics and, subsequently, buck fertility (Eid, 2008). Oxidative stress has also been identified as a significant factor that affects the fertility status and physiology of spermatozoa (Agarwal et al., 2008). Oxidative stress results in excessive ROS production - free radicals and peroxides (Bansal & Bilaspuri, 2011), leading to low sperm motility and viability. Some infertility signs in male rabbits include low testicular testosterone concentration, reduction in sperm concentration, and the percentage of morphologically abnormal spermatozoa.

Antioxidant defense against ROS appears to be highly influenced by nutrition (Eid, 2008). Spice herbs are rich sources of antioxidants (Embascado 2015) due to their phytochemical content. Zingiber officinalis (ginger) and Allium sativum (garlic) both possess antioxidant properties with androgenic activities (Morakinyo et al., 2008), which can enhance the quality of semen for improved rabbit production. Latona et al. (2012) reported that Zingiber officinalis (ginger) is rich in phytochemicals such as gingerol, shogaols, gingerdial, gingerdione, and phenolic ketone derivatives that possess antioxidant activity. Yuriko et al. (2001) observed that garlic supplementation increases testicular testosterone concentration in rats. Shinkut et al. (2016) reported that to enhance the supply of healthy animal protein from rabbits in Nigeria, it may be necessary to incorporate plants with health benefits that may positively affect semen production in the rabbit diet. Little is known about their dietary effect on semen quality in male rabbits. This study, therefore aimed at assessing the effect of dietary levels of Zingiber officinalis and Allium sativum on seminal total antioxidant and semen quality of rabbit bucks.

**MATERIALS AND METHODS**

**Preparation of test ingredient and experimental diet:**
Fresh Zingiber officinalis (ginger) and Allium sativum (garlic) rhizome were bought from an open market in Ile-Ife, Nigeria. The spices were washed with clean water, cut into small sizes, air-dried, ground, and after that incorporated into concentrate diet at different levels 0 (control), 5, 10, and 15 g Zingiber officinalis (Z) and 5, 10, and 15 g Allium sativum (A) /Kg diet to obtain seven experimental diets (control group, Zingiber officinalis group Z5, Z10, Z15, and Allium sativum A5, A10, A15) used for this study. Ingredients for the concentrate diet were sourced from a reputable commercial sales outlet in Ile-Ife, Nigeria. The experimental diet contained 17% crude protein, 11% crude fiber, and 2450kcal/Kg digestible energy.

**Experimental location and Animal Management:**
The twenty-eight (28) bucks aged 9 - 10 months old used for this study were managed at the Rabbitry unit of the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria. They were housed individually in cages and fed the experimental diets for seven weeks during the late rainy season (late September to early November).

**Semen collection and Evaluation:**
Semen was collected from the bucks once weekly during the last five weeks of the study using an artificial vagina. Samples of fresh semen were assessed for semen volume, spermatozoa motility, sperm concentration, and percentage of live sperm cells using the methods described in Adeyemi et al. (2014). Semen volume was measured using the graduated collection tube. Spermatozoa motility was assessed by placing a drop of undiluted semen mixed with a drop of slightly warmed sodium citrate on a sterile glass slide, covered with a coverslip and observed under the microscope at ×400 magnification and scored within a rating of 0 - 100%. Sperm concentration was evaluated by visual count under the microscope using haemocytometric method. Percent of live sperm cells was estimated by placing a drop of semen on a glass slide, mixed with one drop of eosin nigrosin, smeared, and observed under the microscope. The unstained cells represented the live cells, while the stained cells are the dead ones. The values obtained were recorded like a percentage. Seminal plasma was separated from the semen by centrifugation at 4000rpm for 15minutes and assessed for seminal total antioxidant activity using the method described by Koracevic et al. (2001). This method measures TBARS production in body fluids. The reagents used for the evaluation are Sodium phosphate buffer (100mmol/liter, pH 7.4), Sodium benzoate (10mmol/liter), Sodium Hydroxide (50mmol/liter) EDTA (2 mmol/liter in phosphate buffer), Fe(NH4)2SO4(2 mmol/liter), Fe-E-DTA complex, H2O2 ((10mmol/liter) acetic acid (20%), thiobarbituric acid (TBA - 0.8% (wt/vol) in 50mmol/liter NaOH) and uric acid (1 mmol/liter in 5mmol/litreNaOH); some of these were freshly prepared prior to use. The sample and reagents were dispensed into the tubes as presented below (Table 1).
Seminal Total Antioxidant activity was calculated as:

\[ AOA (\text{mmol/l}) = \frac{(CUA)(K - A)}{K - UA} \]

where \( K \) = absorbance of control \((K1 - K0)\); \( A \) = absorbance of sample \((A1 - A0)\); \( UA \) = absorbance of uric acid solution \((UA1 - UA0)\); \( CUA \) = concentration of uric acid (in mmol/liter)

### RESULTS

**Effect of *Zingiber officinale* and *Allium sativum* on semen indices in rabbits**

Table 2 shows the effect of *Zingiber officinale* (Z) and *Allium sativum* (A) on semen parameters in rabbits. Among the semen parameters assessed, semen volume and spermatozoa motility were significantly \((p<0.05)\) influenced by Z and A levels. Semen volume increased by 41.8% and 35.4% in Z15 and A10 groups, respectively, compared to the control group. Spermatozoa motility was higher in buck fed diets with 5g of Z (95.1%) but not significantly different from that of the control group (88%). No effects of diets on sperm concentration and livability were observed \((p>0.05)\). However, spermatozoa concentration increased by 19.7% in the A10 group compared with that of bucks fed the control diet.

### Table 2. Semen indices of rabbit bucks fed dietary levels of *Zingiber officinale* and *Allium sativum* (g/kg diet)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (0)</th>
<th>Zingiber officinale (Z)</th>
<th>Allium sativum (A)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen Volume (ml)</td>
<td>0.53c</td>
<td>0.67bc</td>
<td>0.71bc</td>
<td>0.05</td>
</tr>
<tr>
<td>Spermatozoa Motility (%)</td>
<td>88.0ab</td>
<td>95.1b</td>
<td>92.9bc</td>
<td>1.27</td>
</tr>
<tr>
<td>Sperm Concentration (x10^7 cells/ml)</td>
<td>28.9</td>
<td>27.7</td>
<td>29.4</td>
<td>1.49</td>
</tr>
<tr>
<td>Sperm Livability (%)</td>
<td>94.8</td>
<td>95.1</td>
<td>96.4</td>
<td>0.24</td>
</tr>
</tbody>
</table>

**Seminal Total Antioxidant Capacity of rabbit bucks**

The values obtained for Seminal Total Antioxidant Capacity (STAC) in rabbit fed *Zingiber officinale*, and *Allium sativum* are presented in Figure 1. The inclusion of Z and A in the diet of rabbits significantly influenced STAC \((p<0.05)\). The values obtained for STAC in bucks fed A10
(5.31mol/L) was 48.2% higher than those fed with A₅ (1.73mol/L). Seminal total antioxidant capacity in rabbit fed diets supplemented with Z, and A showed no correlation with semen indices (Table 3).

**DISCUSSION**

The inclusion of natural antioxidant sources in rabbit bucks diet may improve their semen quality and quantity, improving reproductive performance. *Zingiber officinale* at 15g/kg and *Allium sativum* at 10g/kg resulted in increased semen volume. The estimated number of spermatozoa per ejaculate (product of semen volume and sperm concentration) was higher in Z₁₅ and A₁₀ groups (24.0 x 10⁷ and 29.5 x 10⁷) compared to those in other groups (13.9 - 20.9 x 10⁷). The improved semen quality observed Z and A diets used in this study might be attributed to bioactive substances such as flavonoids and alkaloids (Ali et al., 2008; Ameh et al., 2013) which possess androgenic properties. Ogbuewu et al. (2013) observed a deleterious effect of dietary ginger at 15g/kg feed on sperm motility and percentage live sperm cells of pubertal rabbits, which differs from the findings from this study.

Seminal plasma may play a potential role in sperm viability concerning its lipid peroxidation level and antioxidant content (Castellini et al., 2000). Higher STAC (5.31mol/l) in A₁₀ group did not result in increased sperm motility in this study, which contrasts with Eid et al. (2006), who reported that higher antioxidant intake is associated with higher sperm motility. The result from this study on seminal antioxidant is at variance with the findings of Saied et al. (2011), who reported a significant reduction in antioxidant malondialdehyde with the administration of aqueous extract of ginger to broiler breeder male for twenty (20) weeks. Ginger extract preserved testicular structural integrity and increased the total antioxidant in serum of rats (Al-Shathly et al. 2020). Incorporation of Z and A in the diet did not significantly reduce MDA

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**Table 3. Total Seminal Antioxidant and its correlation with Semen Indices in rabbit bucks fed Zingiber officinale and Allium sativum**

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Zingiber officinale</th>
<th>Allium sativum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (Correlation coefficient (r))</td>
<td>Mean (Correlation coefficient (r))</td>
</tr>
<tr>
<td>Semen Volume (ml)</td>
<td>0.76 (0.056&lt;sup&gt;NS&lt;/sup&gt;)</td>
<td>0.65 (0.189&lt;sup&gt;NS&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Sperm Motility (%)</td>
<td>92.3 (-0.088&lt;sup&gt;NS&lt;/sup&gt;)</td>
<td>89.2 (-0.056&lt;sup&gt;NS&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Sperm Concentration (cells/ml)</td>
<td>28.1 (-0.080&lt;sup&gt;NS&lt;/sup&gt;)</td>
<td>28.1 (-0.055&lt;sup&gt;NS&lt;/sup&gt;)</td>
</tr>
<tr>
<td>Sperm Livability (%)</td>
<td>95.9 (0.288&lt;sup&gt;NS&lt;/sup&gt;)</td>
<td>95.6 (0.048&lt;sup&gt;NS&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

p — probability, NS: non-significant p> 0.05
levels in the seminal plasma. The role of *Zingiber officinale* and *Allium sativum* as a source of free radical scavenging natural antioxidant may be more effective if fed to the animals for a more extended period or in a different medium. Also, the effect Z and A on semen in rabbits may be more if fed during the dry season when heat stress is generally high.

**CONCLUSIONS**

In conclusion, our findings confirmed that *Zingiber officinale* and *Allium sativum* possess antioxidant potentials that may reduce oxidative stress in seminal plasma. Supplementing diet to rabbits with 10mg of *Allium sativum* per kg can improve semen quality. *Allium sativum* may function as a better antioxidant scavenger in rabbit semen.

**REFERENCES**


