APRW -38

Effect of Dietary Levels of *Zingiber officinale* and *Allium sativum* on Seminal Lipid Peroxidation and its Correlation with Semen Indices in Rabbit Bucks

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Abstract
To assess the effect of dietary level of *Zingiber officinale* (ZO) and *Allium sativum* (AS) on seminal lipid peroxidation in rabbit bucks, twenty-eight (28) bucks aged 9 – 10 months old were fed diets containing 0, 5, 10 and 15 g ZO and AS /Kg diet respectively for seven (7) weeks with four bucks per treatment. Semen was collected from the bucks weekly using an artificial vagina. Samples of fresh semen were assessed for semen volume (ml), spermatozoa motility (%) and live sperm cells (%).Seminal plasma was separated from the semen by centrifugation and lipid peroxidation was determined as Malondialdehyde (MDA) activity using standard procedures. All data obtained were subjected to statistical analysis. The result showed that semen volume and spermatozoa motility was significantly ($p<0.05$) influenced by the levels of the ZO and AS fed to the bucks. The values obtained for percentage live sperm cells were not significantly ($p>0.05$) different among the treatments. Lipid peroxidation was not significantly influenced by the dietary levels of the ZO and AS fed to the rabbit bucks. The values obtained for lipid peroxidation ranged from 0.36 – 0.41 nmol/ml in the *Zingiber officinale* group, while 0.29 – 0.35 nmol/ml was recorded in the *Allium sativum* group. A significant negative correlation exists between seminal lipid peroxidation and spermatozoa motility ($r = -0.525$; $p<0.05$) in the ZO group. It was concluded that for improved semen quality and quantity, 5 to 15 g/kg dietary level of *Zingiber officinale* can be fed to mature rabbit bucks to enhanced reproductive efficiency.

Keywords: Seminal plasma, ginger, garlic, malondialdehyde, reactive oxygen species

Introduction

Good quality semen is a major requirement for efficient reproduction in rabbit bucks. Nutrition, stress, management among others has been identified as non-genetic factors that influence semen quality and thus fertility in bucks. The attempt to improve semen quality in farm animals has resulted in the use of some herbs with antioxidant potentials to prevent peroxidation in semen. The semen is made up of mature sperm cells and several other fractions such as seminal plasma, droplets and vesicles. These other fractions account for 49.8% of the phospholids in semen (Castellini *et al*., 2006) which are important for the survival of the sperm cells. Phospholipid loss, membrane damage and loss of motility in mammalian spermatozoa are resultant effects of lipid peroxidation (Mann and Lutwak-Mann, 1981). Lipid peroxidation can be described generally as a process by which oxidants such as free radicals attack lipids especially polyunsaturated fatty acids. Like most cells in the body, spermatozoa are rich in polyunsaturated fatty acids which are capable of undergoing various changes that makes them susceptible to degradation by reactive oxygen species (Hseih *et al*., 2006).

Reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical, can cause damages to sperm cells (Mylonas and Kourestas, 1999). The presence of sufficient amount of antioxidant in the body suppresses the adverse effects of ROS. Antioxidant defense against ROS appears to be highly influenced by nutrition (Eid, 2008). Malondialdehyde (MDA) has been identified as probably the most mutagenic product of lipid peroxidation. The level of MDA in body fluid depicts the extent of lipid peroxidation. The effects of antioxidants on MDA levels have been reported (Yousef *et al*., 2003; Eid 2008). *Zingiber officinale* and *Allium sativum* have been used for their beneficial effects on health in livestock (Saeid *et al*., 2011; Alagawany *et al*., 2015). They are generally grouped as spices. Both plants possess antioxidant properties with androgenic activities (Morakinyo *et al*., 2008; Ogbuewu *et al*. 2013; Ewuola *et al*., 2017) which can enhance the quality of semen for improve rabbit production. The effect of these spices on weight gain, haematology and some semen indices in some farm animals have been reported by various researchers.

This study therefore aimed at assessing the effect of dietary levels of *Zingiber officinale* and A*llium sativum* on lipid peroxidation and its correlation with semen indices in rabbit bucks.

Materials and Methods

The study was conducted at the rabbitry unit of the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria.

Fresh *Zingiber officinale* (ginger) and A*llium sativum* (garlic) rhizome were bought from open market in Ile-Ife, Osun State, Nigeria. The spices were washed with clean water, cut into small sizes, air dried, ground and thereafter incorporated into concentrate diet at different levels (0, 5, 10 and 15 g) to obtain seven experimental diets (control 0g D1; *Zingiber officinale* group D2 – D4; and A*llium sativum* D5- D7) used for this study. Twenty-eight (28) bucks aged 9 – 10 months old were used for the experiment. The bucks were kept individually in metal cages raised from the floor and fed the experimental diets for seven (7) weeks. Semen was collected from the bucks once weekly using an artificial vagina (Ola, 2016). Samples of fresh semen were assessed for semen volume; spermatozoa motility and percentage live sperm cells as described in Adeyemi *et al*. (2014). Seminal plasma was separated from the semen by centrifugation at 4000 rpm for 15 minutes and assessed for lipid peroxidation. Lipid peroxidation was determined as Malondialdehyde (MDA) activity using the method developed by Nichans and Samualson, 1968 and adopted by Mokogwu *et al*. (2016).

All data obtained were subjected to statistical analysis of variance procedure using Statistical Analytical System (SAS, 2009). Treatment means were compared using Duncan Multiple Range Test of the same software. The results were expressed as mean ± SEM (standard error of means).

Results and Discussion

The results on seminal lipid peroxidation and some semen indices in rabbit bucks fed *Zingiber officinale* (ZO) and *Allium sativum* (AS) are presented in Table 1. Dietary levels of ZO and AS had no significant effect on seminal lipid peroxidation in bucks among the treatments. Bucks on the control (0g) had 0.43nmol/ml lipid peroxidation value while 0.36 to 0.41 nmol/ml was recorded in the *Zingiber officinale* group and 0.29 – 0.35 nmol/ml in the *Allium sativum* group. The result from this study is at variance with the findings of Saied *et al*. (2011) who reported a significant reduction in antioxidant malonhydialdehyde with the administration of aqueous extract of ginger to broiler breeder male for twenty (20) weeks. This data show that dietary levels of ZO and AS fed in this study did not significantly reduce the level of MDA in the seminal plasma of the bucks. Its role as a free radical scavenging natural antioxidant may be more effective if fed to the animals for a longer period.

Semen volume and spermatozoa motility were significantly ($p<0.05$) influenced by the levels of ZO and AS fed to the bucks among the treatments. The dietary levels ZO and AS fed to the bucks had no significant effects on percentage live sperm cells among the treatments. The semen volume of bucks fed 15 g/kg ZO (0.91ml) was significantly ($p<0.05$) different from that of those fed the control diet (0.53ml). This shows that feeding rabbit buck ZO up to 15g/kg can result in increased semen volume compared with those fed the control, 5 and 15g/kg AS. Inclusion of natural antioxidant sources in the diet of rabbit buck may improve their semen quality and quantity for efficient reproduction. The result from this study corroborate the findings of Eid *et al*. (2006) who reported that higher antioxidant intake was associated with higher sperm motility. Ogbuewu *et al*. (2013) observed a deleterious effect of dietary ginger at 15g/kg feed on sperm motility and % live sperm cells of pubertal rabbit bucks which is at variance with the findings from this study. Seminal plasma may play a potential role in sperm viability with regard to its lipid peroxidation level and antioxidant content (Castellini *et al*., 2000). Hence, the improved semen quality observed in this study may be attributed to the presence of bioactive substances such as flavonoids, alkaloids, (Ali *et al*., 2008; Ameh *et al*., 2013) in the herbs that possess androgenic properties.

The values obtained for lipid peroxidation (LP) in the seminal plasma showed low correlation with semen indices (Table 2 and 3). A significant negative correlation exist between LP levels and spermatozoa motility ($r = -0.525$; $p<0.05$) while it had anon-significant negative and positive correlation respectively exist with semen volume ($r = -0.386$) and percentage live sperm cells ($r = 0.186$) for bucks fed *Zingiber officinale* diets. In bucks fed *Allium sativum* diets, LP level negatively correlated with semen volume ($r = -0.082$) and percentage live sperm cells ($r = -0.083$) while a positively correlation exist with sperm motility ($r = 0.012$). This shows that the relationship between seminal lipid peroxidation and semen indices of bucks administered ZO and AS is relatively low. The significant negative correlation between LP and spermatozoa motility in the ZO group implies that a decrease in LP results in increased spermatozoa motility which is a major indicator of the fertilizing potentials of sperm cells. Reactive oxygen species (ROS) production can be used as a marker of oxidative stress in seminal fluid and is correlated with male infertility (Saeid *et al*., 2011). The positive correlation between seminal lipid peroxidation and percentage live sperm cells in the ZO group was expected, since the role of antioxidants in the survival of sperm cells is well established (Castellini *et al*., 2000).

Table 1: Seminal lipid peroxidation and semen indices of rabbit bucks fed dietary levels of *Zingiber officinale* and *Allium sativum*

Parameters	Control	*Zingiber officinale* (g/kg diet)			*Allium sativum* (g/kg diet)			±SEM
	0	5	10	15	5	10	15	
Lipid peroxidation (nmol/ml)	0.43	0.36	0.39	0.41	0.35	0.33	0.29	0.02
Semen Volume (ml)	0.53^{c}	0.67^{bc}	0.71^{abc}	0.91^{a}	0.56^{c}	0.82^{ab}	0.58^{c}	0.05
Spermatozoa Motility (%)	88.00^{ab}	95.11^{a}	92.88^{ab}	88.78^{ab}	86.12^{b}	88.00^{ab}	93.11^{ab}	1.27
Live sperm cells (%)	94.78	95.11	96.39	96.48	95.75	95.38	95.78	0.24

a, b, c -Means in the same row with different superscript are significantly ($p< 0.05$) different, SEM – standard error of means

Table 2: Correlation between seminal lipid peroxidation and semen indices in rabbit bucks fed dietary levels of *Zingiber officinale*

Characteristics	Mean	Correlation coefficient (r)	p value
Semen Volume (ml)	0.76	-0.386^{NS}	0.13
Spermatozoa Motility (%)	92.26	-0.525*	0.03
Live sperm cells (%)	95.93	0.186 ^{NS}	0.48

p – probability,*: significant $p< 0.05$, NS: non-significant $p> 0.05$

Table 3: Correlation between seminal lipid peroxidation and semen indices in rabbit bucks fed dietary levels of *Allium sativum*

Characteristics	Mean	Correlation coefficient (r)	p value
Semen Volume (ml)	0.65	-0.082 ^{NS}	0.78
Spermatozoa Motility (%)	89.19	0.012 ^{NS}	0.97
Live sperm cells (%)	95.64	-0.083 ^{NS}	0.78

p – probability,*: significant $p< 0.05$, NS: non-significant $p> 0.05$

Conclusion

Zingiber officinale and *Allium sativum* possess antioxidant potentials hence its use in rabbit bucks diet may reduce oxidative stress in seminal plasma. However, dietary levels of *Allium sativum* may not result in improved semen indices hence its use in other forms such as extract may be adopted. The relationship between seminal lipid peroxidation and semen indices of bucks administered ZO and AS is relatively low. For improved semen quality and quantity, 5 – 15 g/kg dietary level of *Zingiber officinale* may be recommended for efficient reproduction in mature rabbit bucks.

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