ANTIOXIDANT ACTIVITIES OF BUCK SEMEN EXTENDED WITH NORMAL SALINE-WATER MELON FRUIT JUICE STORED AT ROOM TEMPERATURE

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ABSTRACT
This study aimed at evaluating the antioxidant activity of normal saline-watermelon fruit juice in buck semen at room temperature over a period of time. Semen was collected from 17 WAD bucks aged 1-3 years old with an average body weight of 12.15±1.51kg, divided into 6 portions, which indicated the treatments. The treatment groups comprised 100% skimmed milk-glucose (T1) which served as negative control,100% normal saline (T2) which served as positive control, 95.00% NS + 5% WMFJ (T3), 90.00 % NS + 10 % WMFJ (T4), 85.00% NS + 15% WMFJ (T5) and 80.00 % NS + 20 % WMFJ (T6) in a Completely Randomized Design. Seminal plasma was separated from the ejaculate by centrifugation at 4000 rpm for 15 minutes; the treatments were then assessed for antioxidant activities at 2 hours interval using standard procedures. Data were subjected to descriptive statistics and one-way analysis of variance procedure of SAS and means were compared using Duncan's multiple range test of the same software. Results showed that total antioxidant capacity in T6 was significantly (P<0.05) higher than other treatments at 2 and 4 hours. At 6 hours, total antioxidant capacity in T5 was significantly (P<0.05) higher than other treatments. At 8 hours, total antioxidant capacity in T6 was significantly (P<0.05) higher than other treatments and at 10 hours, antioxidant capacity in T4 and T6 were significantly (P<0.05) higher than other treatments while T1 and T2 recorded the least value. At 0 hour, lipid peroxidation in T4, T5 and T6 were significantly (P<0.05) lower than T1, T2 and T3. At 2 and 4 hours, lipid peroxidation in T1 and T2 were significantly (P<0.05) higher than T3, T4, T5 and T6 and at 10 hours, lipid peroxidation in T1, T2 and T3 were significantly (P<0.05) higher than other treatments. At 0 and 2 hours, catalase activity in T5 was significantly (P<0.05) higher than other treatments. At 4 hours, catalase activity in T5 was significantly (P<0.05) higher than other treatments, although similar to T3 while T2 recorded the least value. At 6 hours, catalase activity in T3 was significantly (P<0.05) higher than other treatments. This suggest that incorporation of extender with watermelon fruit juice up to 20% increased total antioxidant capacity, enzyme activities and inhibited lipid peroxidation.
Keywords: Lipid peroxidation, Catalase, Goat semen preservation, Antioxidant enzymes, West African Dwarf Buck.

INTRODUCTION

Oxidative stress is a major contributory factor affecting fertility in animals (Makkar *et al*., 2009). An imbalance between the ROS and antioxidants in the body can lead to spermatozoa damage, deformity and eventually male infertility. Antioxidants are advocated for inclusion in semen extenders due to their ability to lower plasma lipid peroxidation (Parfitt *et al*., 1994), hinder the production of reactive oxygen species (ROS) and neutralise the adverse effect of free radicals. Free radicals are types of reactive oxygen species (ROS) which include all highly reactive oxygen-containing molecules such as the hydroxyl radical, the superoxide anion radical and hydrogen peroxide (Kohen and Gati, 2000). Protection against ROS in fresh goat ejaculate is provided by secretions of the reproductive tract in the form of antioxidant enzymes and low molecular weight antioxidants. However, these naturally protective antioxidants in the seminal plasma are diluted when the ejaculate is extended. Therefore, it is plausible that deterioration of semen quality could be due to the action of ROS through propagation of the lipid peroxidation cascade during the storage period post-collection in the extended semen. When ROS production is in excess, they result in oxidative stress on the seminal plasma that is rich in polyunsaturated fatty acids and this eventually damage the sperm integrity leading to infertility. Consequently, research investigating the factors that affect the presence of ROS in fresh and extended buck ejaculate is warranted. Watermelon as a natural fruit contains different compounds (e.g. lycopene, carotenoids, vitamins A, B, C, E, flavonoids and some specific amino acids (arginine, citrulline etc.) that may be responsible for its antioxidant properties. Therefore, this study aimed at evaluating the antioxidant activity of normal saline-watermelon fruit juice in buck semen at room temperature over a period of time.

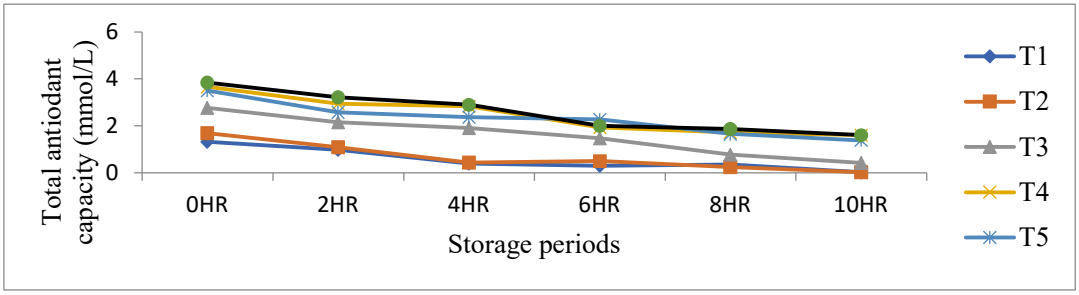
MATERIALS AND METHODS

The experiment was conducted at the Small Ruminant Unit of Teaching and Research Farm, University of Ibadan, while the analysis of seminal plasma was carried out at the Physiology Laboratory of Department of Animal Science of the same institution. Seventeen adult bucks aged 1-3 years old with an average body weight of 12.15±1.51kg were used for the study. Semen was collected using electro ejaculator according to Tingari *et al*. (1986). The semen was harvested into collection tubes in a warm flask and temperature was maintained at 37℃, divided into 6 portions, and then the various extenders were added to the plain bottles according to the treatments. The treatments comprised

100% skimmed milk-glucose (T1) which served as negative control,100% normal saline (T2) which served as positive control, 95.00% NS + 5% WMFJ (T3), 90.00 % NS + 10 % WMFJ (T4), 85.00% NS + 15% WMFJ (T5) and 80.00 % NS + 20 % WMFJ (T6) in a completely randomized design. Seminal plasma was separated from the ejaculate by centrifugation at 4000 rpm for 15 minutes, the treatments were then assessed for antioxidant activities at 2 hours interval at 24-29℃ using standard procedures. Total antioxidant capacity was according to Koracevic *et al*. (2001), Lipid peroxidation was according to Yagi (1984) and superoxide dismutase was according to Ewuola and Olaleye (2015). Data were analysed using descriptive statistics and one -way analysis of variance procedure of SAS (2011) and means were compared using Duncan's multiple range test of the same software.

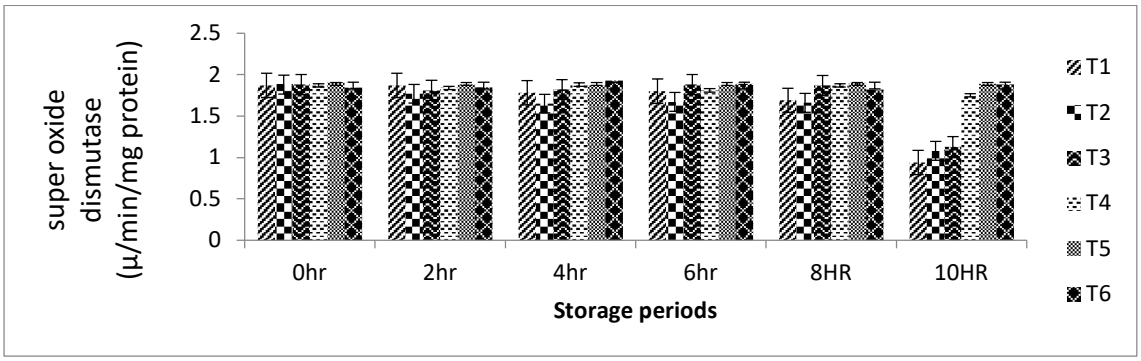
RESULTS

At 2 hours, total antioxidant capacity in T6 (3.22±0.01 mmol/L) was significantly (P<0.05) higher than other treatments while T1 (0.98±0.03 mmol/L) recorded the least value. Total antioxidant capacity followed a similar trend among the treatments at 4 hours as observed at 2 hours. Total antioxidant capacity was significantly (P<0.05) higher in T6 (2.90±0.02 mmol/L) than other treatments while T1 and T2 (0.44±0.02 mmol/L) recorded the least value. At 6 hours, total antioxidant capacity in T5 (2.27±0.01 mmol/L) was significantly (P<0.05) higher than other treatments while T1 (0.30±0.02 mmol/L) recorded the least value. At 8 hours, total antioxidant capacity in T6 (1.87±0.04 mmol/L) was significantly (P<0.05) higher than other treatments while T2 (0.25±0.01 mmol/L) recorded the least value and at 10 hours, antioxidant capacity in T4 andT6 were significantly (P<0.05) higher than other treatments while T1 and T2 recorded the least value. Superoxide dismutase activity in WAD goat semen extended with normal saline incorporated with watermelon fruit juice at room temperature is shown in Figure 2 At 0 hour, superoxide dismutase activity in T2 was significantly (P<0.05) lower than T1, T3, T4 and T5, although not significantly different from T6. At 2 hours, T2 was significantly (P<0.05) lower than T1, T4, T5 and T6, but not significantly different from T3. At 4 hours, T6 was significantly (P<0.05) higher than T1, however it was not significantly different from T2, T3, T4 and T5 and At 8 hours, T1 and T2 were significantly (P<0.05) lower than T3, T4, T5 and T6.



T1: 100% Skimmed milk–glucose, T2: 100% NS + 0 % WMFJ, T3: 95 .00% NS + 5% WMFJ, T4: 90.00 % NS + 10 % WMFJ, T5: 85 .00% NS + 15% WMFJ, T6: 80.00 % NS + 20 % WMFJ

Figure 1: Total antioxidant capacity (mmol/L) of WAD goat semen extended with normal saline incorporated with watermelon fruit juice at room temperature (24 -29℃)



T1: 100% Skimmed milk–glucose, T2: 100% NS + 0 % WMFJ, T3: 95 .00% NS + 5% WMFJ, T4: 90.00 % NS + 10 % WMFJ, T5: 85 .00% NS + 15% WMFJ, T6: 80.00 % NS + 20 % WMFJ

Figure 2: Superoxide dismutase activity (u/min/mg protein) of WAD goat semen extended with normal saline incorporated with watermelon fruit juice at room temperature (24-29℃)

Lipid peroxidation assay of WAD goat semen extended with normal saline incorporated with watermelon fruit juice at room temperature is shown in Table 1. At 0 hour, lipid peroxidation in T4, T5 and T6 were significantly ($P<0.05$) lower than treatments 1, 2 and 3. At 2 and 4 hours, lipid peroxidation in T1 and T2 were significantly ($P<0.05$) higher than treatments 3, 4, 5 and 6. At 6 and 8 hours, lipid peroxidation were not significantly ($P>0.05$) different across the treatments while at 10 hours, lipid peroxidation in treatments 1, 2 and 3 were significantly ($P<0.05$) higher than other treatments.

Table 1: Lipid Peroxidation (x10^{3}MDA/mg protein) of WAD goat semen extended with normal saline incorporated with watermelon fruit juice at room temperature (24 - 29℃)

Hours	T1 100% SMG	T2 100%NS+ 0%WMFJ	T3 95%NS+ 5%WMFJ	T4 90%NS+ 10%WMFJ	T5 85%NS+ 15%WMFJ	T6 80%NS+ 20%WMFJ
0	0.07±0.01^a	0.07±0.02^a	0.07±0.01^a	0.06±0.00 ^{b}	0.06±0.01^b	0.06±0.01^b
2	0.07±0.00^a	0.08±0.001^a	0.06±0.01^b	0.06±0.01^b	0.06±0.01^b	0.06±0.02^b
4	0.08±0.01^a	0.08±0.02^a	0.07±0.01^b	0.07±0.01^b	0.06±0.01^b	0.06±0.02^b
6	0.08±0.02	0.08±0.02	0.07±0.01	0.08±0.02	0.08±0.003	0.07±0.01
8	0.07±0.3	0.07±0.03	0.07±0.01	0.07±0.001	0.08±0.01	0.08±0.01
10	0.08±0.01^a	0.08±0.01^a	0.08±0.03^a	0.07±0.00^b	0.07±0.01^b	0.07±0.01^b

At 5% level of significance; a,b,c : Means along the same row with different superscripts are significantly ($P< 0.05$) different SMG: Skimmed milk-glucose extender, NS: Normal saline, WMFJ: Water melon fruit juice

DISCUSSION

At 2, 4, 8 and 10 hours, treatment incorporated with 20% of watermelon fruit juice had significantly higher total antioxidant capacity than other treatments while treatments 1 and 2 without water melon fruit juice had significantly lower value. Incorporation of normal saline diluent with watermelon fruit juice as a source of natural antioxidant (lycopene) resulted in higher antioxidant activities thereby scavenging free radicals that could induce oxidative stress in the medium. This showed that the antioxidant defense system is high and intact therefore oxidative damage in the cells may not occur. This is in agreement with the findings of Lasso (2000). Natural antioxidant system has been described as a defense functioning mechanism against lipid peroxidation in semen. Therefore, inclusion with watermelon fruit juice could reduce the impact of oxidative stress during sperm storage process. Lipid peroxidation significantly decreased with addition of watermelon fruit juice suggesting that the antioxidant property of watermelon protected spermatozoa from lipid peroxidation. This finding corroborates the result of Zhu *et al*. (2015) who reported that the addition of vitamin E (Trolox) to extender in rabbit decreased lipid peroxidation significantly. Lipid peroxidation results showed that at collection time, treatments 1, 2 and 3 were significantly higher than treatments 4, 5 and 6. At 2 and 4 hours, treatments having no (0%) watermelon fruit juice as source of antioxidants had significantly higher value than the treatments incorporated with watermelon juice. This was also observed in the antioxidant level of the treatments.

Treatments that had high antioxidant capacity was observed to have lower lipid peroxidation, thus inhibiting lipid peroxidation cascade formation that could lead to oxidative stress. Watermelon incorporation had significant reduction of malondialdehyde production and protection of antioxidant profiles of goat semen stored *in vitro* for up to 6 hours at room temperature. This is in line with the findings of Perumal (2014) who reported a significant reduction in malondialdehyde production upon supplementation of superoxide dismutase in Mithun (*Bos frontalis*) semen. Normally, there is a balance between radical generating and scavenging system. However, high generation of reactive oxygen species by sperm processing, accompanied by low scavenging and antioxidant levels in seminal plasma induce a state of oxidative stress (Anghel *et al.,* 2010). Presence of high concentration of polyunsaturated fatty acids within the lipid fractions necessitates the presence of an efficient antioxidant system to protect against peroxidative damage and possible associated sperm dysfunction (Aitken *et al.*, 2004).

Superoxide dismutase activity in treatments incorporated with watermelon was higher than treatments without watermelon fruit juice at collection time and at 8 hours. Thus superoxide dismutase protected the spermatozoa against oxygen toxicity and lipid peroxidation (Sikka, 1996). It also played a major role in decreasing lipid peroxidation and protecting spermatozoa against oxidative damage. High levels of superoxide dismutase activity

will cause increase in scavenging of extracellular and intracellular superoxide anion and prevent peroxidation of cell membrane (Sikka, 1996).

CONCLUSION AND RECOMMENDATION

Incorporation of extender with watermelon juice up to 20% increased total antioxidant capacity, superoxide dismutase activity and inhibited lipid peroxidation. Lipid peroxidation was inhibited by water melon fruit juice inclusion thereby sustaining the lifespan of spermatozoa for a longer time as well as protecting the sperm cells from oxidative stress.

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