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EFFECTS OF *Borassus aethiopum* EXTRACT ON SEMEN CHARACTERISTICS, FERTILITY AND HATCHABILITY IN CHICKEN

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ABSTRACT

The study was conducted to investigate potential diluents for short term usage (0, 30, 60 minutes) of chicken semen in a complete randomized design model. Semen samples were collected and diluted with normal saline (NS) and *Borassus aethiopum* (BE) extract while undiluted semen (US) served as control at 37°C for short term usage in artificial insemination (A.I.). There was significant ($p < 0.05$) increase in sperm motility for US and B.E diluted semen compared with NS diluted semen. Sperm dead cell was significantly higher ($p < 0.05$) in NS and US compared with BE at 30 and 60 minutes respectively. There was a significant increase ($p < 0.05$) in fertility for BE at 0 minute. Fertility and hatchability significantly ($p < 0.05$) decreased in US and NS. There was no fertility noticed for US and semen in NS at 60 minutes. It can be concluded that BE diluents proved better than NS and US.

Key words: Chicken semen, Diluents, *Borassus aethiopum*, Fertility, Hatchability.

INTRODUCTION

Diluents are solutions used to extend semen and increase its volume while maintaining viability of spermatozoa in vitro and maximize the number of hens that can be inseminated. Addition of various compounds to semen maintains motility, fertilizing capacity and preservation of sperm membrane integrity (Riha *et al.*, 2006). Semen diluents have improved the application of artificial insemination techniques and storage of semen in poultry. Diluents are currently being used for both short and long term storage of domestic fowl semen to improve the general reproductive effectiveness of cockerels and to lower the cost of AI (Singh, 2005). It is difficult to handle the desired very small volume of undiluted chicken semen because of its viscous nature (Mian *et al.*, 1990) hence the use of diluents becomes very necessary.

The advantages of semen diluents include a maximum use of good quality semen in short supply and reduction in the ratio of males to females. Assessment of diluted and undiluted stored semen of cockerels revealed that application of diluents is essential to sustain sperm quality (Bootwalla and Miles, 1992). It was established that diluted fowl semen could be stored for up to 24 hours without impairing the viability and fertilizing capacity of the sperm cells (Siudzinska and Lukaszewicz, 2008). Several researchers have investigated different diluents for chicken and normal saline which is usually employed for diluting the avian semen on routine basis (Mohan *et al.*, 2015). Al-Daraji (2012) supplemented roosters' semen with orange juice. Addition of tomato juice to semen extender led to an increase in quality of

semen stored *in vitro* (Al-Daraji, 2014). Daramola *et al.*, (2016) reported an improvement in sperm motility with 10% orange and pineapple juice supplementation in extender. However,

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these plant sources are more demanded in human nutrition. Diluents from normal saline require some reagent which may not be easily accessible especially on the field. A careful development of semen diluents using novel fruit based ingredient is indispensable. *Borassus aethiopum* belongs to the palm family and it grows throughout the semi arid to sub humid region of Africa (Cretenet *et al.*, 2002). The tree produces 15 to 40 tons of fruit per hectare (Djibrilla, 2004) which are presently unutilized in Nigeria. *Borassus aethiopum* fruit contains sugar, carotene and vitamins (Ahmed *et al.*, 2010) essential for semen improvement. This study was therefore designed to compare *Borassus aethiopum* extract as diluent from plant source with normal saline as useful source of diluent for chicken semen.

MATERIAL AND METHODS

The research was conducted at the Poultry unit of the Teaching and Research Farm, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria.

Preparation of the Extract

Fresh *Borassus aethiopum* fruit was obtained at the premises of Living Faith Church, Gaa Fresh Imam, Ilorin. The fruit was washed and wipe dry using a sterilized damper and the fleshy mesocarp was cut opened with a sharp knife and sterilized spatula was used to scope out the pulp into a clean Petri -dish, from which 2g was taken and analyzed for proximate composition according to AOAC (2005). Another 2 g was taken into a test-tube and 10ml of distilled water was added to the pulp and was homogenized. The resultant solution was centrifuged at 3000rpm for one minute and the supernatant was carefully separated into another test- tube as the stock. This stock was further diluted with normal saline (NS) (8.5g sodium chloride/ l. distilled water) solution in the ratio 1:5 and placed in the water bath at 37°C.

Experimental Animals

Eight matured cocks (average wt 2.2 ±0.05kg) of 8 months of age of Isa Brown strain were obtained from the Teaching and Research Farm. These birds were milked to obtain semen according to the procedure described by Adeyina *et al.*, (2016). The undiluted semen were pooled into a test tube and analyzed for semen quality and characteristic according to Adeyina *et al.*, (2016). From the pooled semen, 0.5ml was taken into each of six test tubes in the water bath maintained at 37°C. Two of these test-tubes each was diluted with the prepared BE in the ratio 1:5 and NS in the ratio 1:5 while the remaining two were undiluted. All the semen solutions were maintained at 37°C in the water bath. Samples in triplicate from the semen solutions and undiluted semen were taken on a slide and observed under a microscope (Olympus model x40) at 0, 30 and 60min for semen characteristics according to the method described by Wishart, (1995).

Table 1: Characteristics of Undiluted Chicken Semen

Parameters	Values
Semen volume (ml)	0.45 ± 0.05
Spermatozoa concentration (x10 ⁶ /ml)	148.00 ± 42.29
Motility (%)	91.00± 8.55
Immobile sperm (%)	9.00± 2.65
Normal morphology (%)	86.00± 7.44
Abnormal morphology (%)	14.00± 3.32

Values are means of triplicate determinations

Effects of *Borassus aethiopum* Extract on Semen

Fifty four actively laying hens of Isa Brown strain weighing 1.8 ± 0.02 kg were divided into three groups of 18 birds per group. Group 1, 2 and 3 of hens were inseminated with US, NS diluted (1:5) and BE diluted (1:5) at 0, 30 and 60 minutes respectively. The hens were kept separately in a battery cage system and were offered 120g /hen/ day of commercial layers mash containing 17.5% CP and 2700kcal/kg M.E. Water was offered *ad-libitum*.

In accordance with recommendation by Gordon (2005), 0.1ml of the diluted and undiluted semen was then taken using 1ml syringe to inseminate each bird according to the methods of Adeyina *et al.*, (2016). Eggs from these hens were collected 24hours after insemination for four days before the insemination is repeated. A total of 360 eggs, 120eggs/group and 40 per period were set in the incubator according to the procedure of Mohan *et al.*, (2015). The experiment was conducted in line with the University's guidelines for ethical treatment of experimental animals in accordance with best practices within Institutional Animal Care and Use Committee (IACUC) guidelines.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) in a completely randomized design model. Significant means were separated using Duncan multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Table 1 shows the semen characteristics of undiluted semen of cock. The average volume recorded for the cocks is within the range of 0.43 ± 0.002 and 0.73 ± 0.01 reported by Peter *et al* (2008), while the motility of the sperm was above 60% indicating that the sperm is having more active sperm cells that possess good fertility potential (Wheeler and Andria 1943). The overall evaluation of the sperm suggests that the cocks are sexually matured and active. The undiluted semen could not survive more than 1 hour.

Table 2: Composition of *Borassus aethiopium* extract

Parameters	Composition
Moisture content (%)	78.64 ± 6.34
Ash content (%)	1.88 ± 0.92
Crude protein (%)	0.99 ± 0.48
Crude fibre (%)	1.25 ± 0.81
Ether extract (%)	0.32 ± 0.50
Total carbohydrates content (%)	16.92 ± 2.38
Total sugar (%)	5.55 ± 1.25
Vitamin C (mg/100)	132.80 ± 12.34
Soluble sugar (%)	4.51 ± 1.32

Values are means of triplicate determinations

Table 2 shows the composition of *Borassus aethiopum* extract. The values revealed that the extract is rich in total sugar, soluble sugar and vitamin C. These values corroborate those reported for *Borassus aethiopum* extract analyzed by Ahmed *et al* (2010).

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Table 3: Effect of different diluents on sperm cell motility (%)

Period(minutes)	Undiluted semen(US)	Normal saline(NS)	<i>Borassus aethiopum</i> (BE)	SEM
0	91.00 ^a	82.5 ^b	94.50 ^a	1.58
30	1.50 ^b	3.00 ^b	89.00 ^a	0.86
60	0.00 ^b	0.00 ^b	71.00 ^a	0.57

^{a, b, c} – means with different superscripts along the row differ significantly ($p < 0.05$)

Table 3 shows the effect of different diluents on sperm cell motility. There was a significant increase ($p < 0.05$) in sperm motility in BE diluents compared with NS at 0 minute. At 30 minutes, a significant ($p < 0.05$) reduction was found in sperm motility in NS and undiluted semen. Subsequently at 60 minutes, there was no sperm motility recorded in undiluted and NS diluted semen. It is known that sperm motility and the fertilizing capacity of undiluted raw fowl semen stored *in vitro* usually decreases within one hour after collection (Dumpala *et al.*, 2006) resulting from loss of viability. Normal saline (NS) is lacking in energy (sugar) and constituents for maintaining osmotic pressure of avian semen (Mohan *et al.*, 2015). Recorded motility in BE is suggesting that the *Borassus aethiopum* extract as extender improved the motility of the spermatozoa because of its minimum essential medium (MEM). According to Singh (2005) MEM for semen improvement and survivability include soluble sugars (fructose, glucose) and antioxidant among others. *Borassus aethiopum* extract contains glucose and fructose which are the main source of semen plasma (Singh, 2005). The improvement in motility could also be attributed to the presence of vitamins and phenolic compounds which function as antioxidant for semen (Culter *et al.*, 2008) supporting preservation in stored semen and improved motility of spermatozoa (Reza *et al.*, 2011). The presence of soluble sugar in the fruit also contributes to the improvement in the sperm motility Fukuhara and Nishikawa (1993).

Table 4: Effect of different diluents on dead sperm cells (%)

Period (minutes)	Undiluted semen(US)	Normal saline(NS)	BE	SEM
0	5.50	5.00	2.00	0.86
30	84.50 ^b	88.50 ^b	4.50 ^a	1.26
60	99.00 ^b	100.00 ^b	24.50 ^a	0.81

^{a, b, c} – means with different superscripts along the row differ significantly ($p < 0.05$)

Table 4 shows the effect of different diluents on percentage dead sperm cells. The number of dead cells significantly ($p < 0.05$) increased in undiluted semen and NS diluted semen at 30 and 60 minutes respectively, because of insufficient MEM to sustain metabolism.

Table 5: Effect of diluents on sperm cell abnormality (%)

Period (minutes)	Undiluted semen	Normal saline	B.F	SEM
0	0.50 ^b	4.50 ^a	3.00 ^{ab}	0.70
30	0.50	0.50	1.00	0.70
60	1.50	2.50	3.50	0.96

a, b, c -- means with different superscripts along the row differ significantly ($p < 0.05$)

Effects of *Borassus aethiopum* Extract on Semen

Table 5 showed the effect of different diluents on sperm cell abnormality. Sperm cells abnormality did not significantly ($p > 0.05$) change in different diluents, suggesting that the structural integrity of the cells are maintained.

Table 6: Effect of different semen diluents on fertilizing ability (%)

Period (minutes)	Undiluted semen	Normal saline	B.E	SEM
0	85.00 ^b	77.30 ^b	95.00 ^a	1.56
30	1.00 ^b	2.50 ^b	88.50 ^a	0.70
60	0.00 ^b	0.00 ^b	87.50 ^a	0.76

a, b, c -- means with different superscripts along the row differ significantly ($p < 0.05$)

Table 7: Effect of different semen diluents on hatchability (%)

Period (minutes)	Undiluted semen	Normal saline	B.E	SEM
0	70.00 ^a	68.10 ^a	71.40 ^a	0.70
30	0.00 ^b	0.00 ^b	70.00 ^a	0.70
60	0.00 ^b	0.00 ^b	65.40 ^a	0.96

a, b, c -- means with different superscripts along the row differ significantly ($p < 0.05$)

Tables 6 and 7 showed the effect of different semen diluents on fertilizing ability and hatchability of eggs respectively. There was a significant increase ($p < 0.05$) in fertility recorded for BE compared with NS and undiluted semen at 0 minute while the values of hatchability had no significant difference. Fertility and hatchability of eggs decreased significantly ($p < 0.05$) in undiluted semen and NS due to reduction in sperm motility. Reduction of motility with the advancement of storage period of spermatozoa in vitro may be the reason for decline in fertility (Mohan *et al.*, 2015).

It can therefore be concluded that *Borassus aethiopum* extract as semen diluent proved better than NS and undiluted semen. This implies that the composition of BE are comparatively more suitable for the efficiency of spermatozoa of chicken in vitro at 37°C and could easily be used to replace normal saline.

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