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EFFECT OF *BORASSUS AETHIOPUM* EXTRACT AS A BIOLOGICAL EXTENDER ON SEMEN CHARACTERISTICS, FERTILITY AND HATCHABILITY IN CHICKENS

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Abstract: *Borassus aethiopum* extract as a biological extender was evaluated on semen quality, fertility and hatchability in chickens under conditions of warm preservation at 37^{o}C. The extenders were prepared in 1:5 and 1:10 ratios (extract to normal saline) and preserved for 0, 1, 2, 3 and 4 hours respectively in a factorial design layout. There was no significant ($p>0.05$) effect of the extender on semen characteristics within 2 hours of preservation. However, motility (%) was significantly ($p<0.05$) reduced when the semen was preserved for more than 2 hours. Interactions between the extender ratios and the period of preservation were significant ($p<0.05$) for motility (%), dead and abnormal cells. Motility (%) was insignificantly ($p>0.05$) higher in the extender of 1:10 than in the extender of 1:5 and it was above 70%. Using the extender of 1:10 in the fertility and hatchability trial in a completely randomized design layout showed that fertility decreased, 95% in the 0-hour treatment to 73% in the 4-hour treatment while hatchability decreased from 71.4% in the 0-hour treatment to 61.5% in the 4-hour treatment. It may be concluded that the *Borassus aethiopum* extract could be used to extend and preserve the semen of chickens for 3 hours providing good fertility and hatchability of eggs.

Key words: chicken, semen, *Borassus aethiopum* extract, fertility, hatchability.

Introduction

Artificial insemination (AI) has been considered as a valuable technique in the poultry industry (Benoff et al., 1981). It has the advantage of efficient use of male over natural mating. However, AI can be enhanced with the improvement of diluents (Mian et al., 1990) in the extender. The advantages of the semen extender include the maximum use of semen in short supply and reduction in the male to

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female ratio. The cock produces about 0.28±0.14 mL of semen per ejaculate (Bah et al., 2001) which may be difficult to handle as undiluted semen because of its viscous nature (Singh, 2005). Diluent enhances the spread of semen over many hens (Mian et al., 1990). It is known that sperm motility and fertility capacity in undiluted raw fowl semen *in vitro* usually decrease within one hour after collection (Dumpala et al., 2006).

Assessment of diluted and undiluted stored cock semen has revealed that the application of extender is essential to sustain semen quality (Bootwalla and Miles, 1992) which could be preserved up to 24 hours without impairing the viability and fertilizing ability of the semen (Lukaszewicz, et al., 2008). Semen extenders have the ability to support the biochemical composition of semen, otherwise the semen could coagulate with a loss of cell integrity.

The addition of various components to semen maintains motility, fertility capacity and preserves sperm membrane integrity (Riha et al., 2006). Egg yolk is generally accepted to be an effective agent in semen extenders (Aboagla and Terada, 2004), but it provides substrates for hydrogen peroxide production to the detriment of live spermatozoa (Singh, 2005). Generally, an extender will facilitate semen handling by maintaining sperm viability and inhibit the pathways that are detrimental to semen survival. Extenders are currently being used for both short-term and long-term storage of domestic fowl semen, and the most common practice for short-term storage (hours to days) requires the suspension of sperm cells *in vitro* in a suitable medium that maintains viability. Several plant sources such as coconut, tomato and carrot have been screened for their semen extending potentials (Banerjee, 2011). *Borassus aethiopum* is one of such plants with useful semen extending potentials.

Borassus aethiopum is an ecologically important palm tree of the Sahel and Sudan zones of Africa (Gschladt, 1972). The plant produces fruits which contain sap in the mesocarp. The sap is rich in soluble carbohydrates which contain minimum essential medium (MEM) for extending semen. According to Ahmed et al. (2010), palmyra palm contains about 5.83 g of soluble sugars making it a potential energy source for sperm survival. There is a growing concern for more knowledge regarding reproductive improvement with the use of semen extender. However, the utilization of natural plants to enhance semen viability and fertility in successful application of AI necessitated this investigation of the effects of *Borassus aethiopum* extract as a biological extender on semen characteristics and its usefulness in fertility and hatchability of eggs in chickens.

Materials and Methods

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

Preparation of the extract

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

Eight matured cocks (average weight 2.2±0.05 kg) aged 8 months of Isa brown strain were obtained from the Teaching and Research Farm. These birds were milked to obtain semen according to the procedure describe by Adeyina et al. (2016). The undiluted semen was pooled into a test tube and analyzed for semen quality and characteristics according to Adeyina et al. (2016). From the pooled semen, 0.5 mL was taken into each of eight test tubes in the water bath maintained at 37^{o}C and diluted with the prepared extender in the ratio of 1:5 and 1:10 respectively. The extender of 1:5 was added to four test tubes while the extender of 1:10 was added to the remaining four test tubes. All the semen solutions were maintained at the same temperature in the water bath. Samples in triplicate from the semen solutions were taken on a slide and observed under a microscope (Olympus model x40) on an hourly basis for 4 hours for semen characteristics according to the method described by Wishart (1995).

In the phase 2 of the research, the ratio of the 1:10 semen extender which best supported sperm characteristics was used in AI of laying hens to assess its effect on fertility and hatchability of eggs. Sixty actively laying birds weighing 1.8±0.02 kg were selected and inseminated using the 1:10 extender. The birds were divided into four treatments of 15 birds per treatment and three replicates (five birds per replicate). Each treatment represented an hourly interval of insemination (1hr, 2 hr, 3 hr and 4 hr). The birds were kept separately in a battery cage system and were fed the commercial layers mash of 17.5% CP and 2700 kcal/kg M.E. Water was offered *ad libitum*.

Then, 0.1 mL of the extended semen as recommended by Gordon (2005) was taken to inseminate each bird according to the methods of Adeyina et al. (2016). Eggs from these birds were collected 24 hours after insemination for four days before the insemination was repeated. A total of 300 eggs, 75 eggs/treatment were set in the incubator.

The experiment was conducted in line with the University's guidelines for the ethical treatment of experimental animals in accordance with the best practices within Institutional Animal Care and Use Committee (IACUC) guidelines.

Statistical analysis

Data obtained from an investigation in the phase one was subjected to analysis of variance (ANOVA) of a 2x4 factorial design while data from an investigation in the phase two was subjected to analysis of variance following a completely randomized design. Significant means were separated using Duncan multiple range test (Steel and Torrie, 1980).

Results and Discussion

Table 1 shows the semen characteristics of undiluted cock semen.

Table 1. Characteristics of undiluted semen.

Parameters	Values
Volume (mL)	0.45 ± 0.05
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
pH	6.90 ± 0.30

The average volume recorded for the cocks was within the range of 0.43 ± 0.002 mL and 0.73 ± 0.01 mL reported by Peters et al. (2008), while the motility of the sperm was above 60% indicating that the sperm had more active sperm cells that possessed good fertility potential (Wheeler and Andrew, 1943). The overall evaluation of the sperm suggests that the cocks were sexually matured and active. The undiluted semen could not survive for more than 1 hour. Table 2 shows the composition of the *Borassus aethiopum* extract.

Table 2. The composition of the *Borassus aethiopum* extract.

Parameters	Composition
Moisture content (%)	78.64 ± 6.34
Ash content (%)	1.88 ± 0.92
Crude protein (%)	0.99 ± 0.48
Crude fibre (%)	1.24 ± 0.81
Fat and oil content (%)	0.32 ± 0.50
Carbohydrate 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

The values reveal that the extract was rich in total sugar, soluble sugar and vitamin C. These values corroborate those reported for the *Borassus aethiopum* extract analyzed by Ahmed et al. (2010). Table 3 shows the effect of the *Borassus aethiopum* extract dilution and preservation period on semen quality.

Table 3. The effect of *Borassus aethiopum* extract dilution and preservation period on semen quality.

Parameter	Motility (%)	Sluggishness (%)	Dead (%)	Abnormal (%)
Dilution				
1:5	81.60	6.10	11.40	2.90
1:10	81.60	4.70	10.90	1.40
SE	0.45	1.50	1.60	0.59
Period (hour)				
0	90.50^{c}	4.25	1.75^{a}	3.50^{b}
1	90.25^{c}	2.75	5.75^{a}	1.50^{ab}
2	82.75^{c}	4.50	21.75^{b}	1.75^{ab}
3	72.00^{b}	5.50	8.00^{a}	3.75^{b}
4	71.25^{b}	10.00	18.50^{b}	0.25^{a}
Dilution*period	S	NS	S	S
SE	1.01	3.29	3.67	1.31

$^{a, b, c}$ – Means with different superscripts along the row differ significantly ($p<0.05$), S = Significant, NS = Not significant, SE = Standard error.

There was no significant effect ($p>0.05$) of the extender on semen characteristics from 0 to 2 hours. However, the motility (%) was significantly ($p<0.05$) reduced with an increase in preservation time above 2 hours. Interactions between the diluent ratios and the period of preservation were significant ($p<0.05$) in percentage motility, dead and abnormal cells. These suggest that the *Borassus aethiopum* extract as the extender improved the motility of the spermatozoa because of its minimum essential medium (MEM). According to Singh (2005), MEM for semen improvement and survivability includes soluble sugars (fructose, glucose) and antioxidants. The *Borassus aethiopum* extract contains soluble sugars, 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 an antioxidant for semen (Cutler et al., 2008) supporting preservation in stored semen and improved motility of spermatozoa (Raza et al., 2011). The presence of soluble sugars in the fruit also contributes to the improvement in the sperm motility (Fukuhara and Nishikawa, 1993).

Table 4 shows the effect of the *Borassus aethiopum* extract and time of preservation on sperm cell motility. Sperm motility was significantly ($P<0.05$) high when preserved within 0 to 2 hours compared to when preserved within 3 to 4

hours using the two diluent ratios. The motility (%) in the two ratios was still above 70% signifying that the extracts supported good fertilizing ability. However, the insignificantly ($p>0.05$) low motility in the 1:5 ratio compared with the 1:10 ratio could be due to dissolved solutes or osmolality. According to Singh (2005), high osmolality and salinity reduce the movement of sperm cells. Sperm cells are very sensitive in the diluent medium, so the salinity could eventually result in low fertility (Singh, 2005). The semen characteristics in the 1:5 ratio and the 1:10 ratio were of importance to the overall usefulness of *Borassus aethiopum* as an extender.

Table 4. The effect of the *Borassus aethiopum* extract (1:5 and 1:10) and time of preservation on sperm cell motility.

	Motility (%)	
Treatment/period (hours)	1:5	1:10
0	90.00^{a}	91.00^{a}
1	91.00^{a}	89.50^{a}
2	81.00^{b}	84.50^{b}
3	71.50^{c}	72.50^{c}
4	71.00^{c}	71.50^{c}
SEM	1.04	0.97

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

Table 5 shows the effect of the *Borassus aethiopum* extract (1:5 and 1:10) and time of preservation on sperm cell abnormality. The values observed for abnormal sperm cells were within the range of semen analyses for successful AI (Tselusi et al., 1999). Sperm cell abnormality did not significantly ($p>0.05$) change with the period of preservation in the 1:5 diluent but significantly ($p<0.05$) decreased in the 1:10 diluent. The reduction in sperm abnormality in 1:10 could be a result of available MEM sufficient enough to resuscitate the weak sperm cells in solution. This proves that semen characteristics could be maintained better in the 1:10 diluent than in the 1:5 diluent.

Table 5. The effect of the *Borassus aethiopum* extract (1:5 and 1:10) and time of preservation on sperm cell abnormality.

	Abnormality (%)	
Treatment/period (hours)	1:5	1:10
0	2.50	6.50^{a}
1	1.00	4.50^{ab}
2	2.50	2.00^{b}
3	1.00	1.00^{b}
4	0.00	0.50^{b}
SEM	1.44	1.16

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

Table 6 shows the effect of extended (1:10) and preserved semen on fertility and hatchability of eggs. Fertility decreased from 95.0% to 73.0% while hatchability also decreased from 71.4% to 61.5%. Fertility and hatchability of eggs decreased with an increase in duration of semen preservation. This could be due to loss of sperm cell integrity in the extender as a result of increased metabolic activity and the depletion of an energy source in the extender. Storage of semen above 5^{o}C requires mobilization of the metabolic process (Singh, 2005) and an increase in nutrient utilization. High fertility with corresponding low hatchability for successive hours could have resulted from sperm cells that were modified in the solution in which the fertilization could not result in hatchability due to embryo death.

Table 6.The effect of the *Borassus aethiopum* extract on fertility and hatchability of eggs.

Treatment/period (hours)	Fertility (%)	Hatchability (%)
0	95.0	71.4
1	88.5	70.0
2	87.5	65.4
3	75.0	63.5
4	73.0	61.5

Conclusion

The *Borassus aethiopum* extract has the capacity to be used as a potential extender and it is supportive of semen preservation. It has the advantage of maintaining viability of semen above 3 hours. It can therefore be concluded that the *Borassus aethiopum* extract can be used in short-term preservation of semen. Preserving semen in the extract for 3 hours gives considerably high fertility and hatchability of eggs and could be used in storage of cock semen for AI over a short distance.

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UTICAJ EKSTRAKTA *BORASSUS AETHIOPUM* KAO BIOLOŠKOG RAZREĐIVAČA NA KARAKTERISTIKE SPERME, PLODNOST I IZVODLJIVOST PILIĆA KOD KOKOŠAKA

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R e z i m e

Ekstrakt *Borassus aethiopum* kao biološki razređivač bio je evaluiran imajući u vidu kvalitet sperme, plodnost i izvodljivost kod kokošaka u uslovima toplog čuvanja na 37^{o}C. Razređivači su pripremljeni u odnosima 1:5 i 1:10 (ekstrakt do normalnog slanog) i čuvani su tokom 0, 1, 2, 3 odnosno 4 sata u faktorijalnom dizajn rasporedu. Nije bilo značajnog ($p>0,05$) uticaja razređivača na karakteristike sperme u roku od 2 sata čuvanja. Međutim, pokretljivost (%) je bila značajno ($p<0,05$) smanjena kada je sperma čuvana više od 2 sata. Interakcije između odnosa razređivača i perioda čuvanja su značajne ($p<0,05$) za pokretljivost (%), mrtve i abnormalne ćelije. Pokretljivost (%) je bila neznačajno ($p>0,05$) veća u razređivačima 1:10 nego u razređivačima 1:5 i iznosila je preko 70%. Korišćenje razređivača 1:10 u ogledu u vezi sa plodnošću i izvodljivošću u potpuno slučajnom dizajn rasporedu pokazalo je da se plodnost smanjivala, 95% u kontrolnom tretmanu do 73% u četvoročasovnom tretmanu, dok se izvodljivost smanjivala od 71,4% u kontrolnom tretmanu do 61,5% u četvoročasovnom tretmanu. Može se zaključiti da je ekstrakt *Borassus aethiopum* moguće koristiti za razređivanje i čuvanje sperme petlova tokom tri sata obezbeđujući dobru plodnost i izvodljivost.

Ključne reči: kokoška, sperma, ekstrakt *Borassus aethiopum*, plodnost, izvodljivost.

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