INFLUENCE OF STORAGE LENGTH AND DILUENT COMPOSITION ON THE POTENTIAL VIABILITY OF CHILLED TURKEY SEMEN

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ABSTRACT

The complete dependence in commercial turkey production, on the use of artificial insemination led to the quest for an ideal diluent that can preserve turkey semen for a long period of time. This study compares the performance of four different diluents in preserving turkey semen for a period of 72 hours at 5°C. Ten (10) turkey stags averaging 15 kg body weight, 48 weeks of age, provided water ad libitum and breeders ration of 10 % CP with 2800 kcal/kg metabolizable energy and housed in individual pens were used for the study. Semen which was collected weekly, pooled, dispensed into four different diluents, labelled A, B, C and D, with spermatozoa concentration of 2 x 10^9 m/ml was subjected to semen viability analysis at 0 hour, 24 hours, 48 hours and 72 hours, respectively. In each instance viability was determined through the determination of spermatozoa Forward progressive movement (FPM), occurrence of morphological defects and dead cells. Data was generated over a period of 12 weeks. Forward progressive movement in all the diluents decreased significantly (P≥ 0.05) from 0 hours to 72 hours while the occurrence of morphological defects and dead cells increased significantly (P ≥ 0.05) throughout the storage period. The best performance was observed in diluent D with a percentage Forward progressive movement of 65 %, percentage Abnormal Spermatozoa (AS) of 4 % and percentage Dead Spermatozoa (DS) of 12 % at the end of the storage period. The least performance was that of the diluent C with percentage forward progressive movement of 55 %. Percentage abnormal spermatozoa of 5 % and percentage dead spermatozoa of 20 %. For optimum viability, it is recommended that semen diluted in all the diluents be utilized within 24 hours.

Key words: Storage, Diluent, Semen, Turkey

INTRODUCTION

The need to improve the current production of animal based protein is becoming imperative as communal clashes between the traditional suppliers of beef (the Fulani herdsmen) and arable farmers intensifies in Nigeria (Weekly Trust, 2014a). The implications are not farfetched, cattle production is becoming impossible in areas where it existed before, while areas that are favourable are either not accessible to the outside markets or products cannot be moved since cattle routes are being blocked. This situation restricts supplies to the southern markets. Another critical factor militating against cattle production and distribution within Northern Nigeria is terrorism (a deliberate act of violence against civilians aimed at gaining political, social, military or religious objectives and creating a climate of fear among the targeted geographical region). As lives and cattle are being wasted, production is on the decrease and alongside these current realities, human population is on the increase consequently, an era where livestock production will be restricted almost entirely to poultry, pigs, rabbits, small ruminants and any other livestock but not cattle is being approached. Worthy of note is the additional problem of cattle rustling involving the brutal extermination of lives which also limits production in cattle producing areas of the north and north central (Weekly Trust, 2014b). As we face these realities and the search for a solution continues, animal producers must look inwards to existing livestock. This study considers turkey production as being among these viable options.

The demand for turkey meat is enormous, it is used for festive occasions; especially the ‘‘Christian thanksgiving’’ and government policy on importation further ensures a market without international competition (NCS, 2013). In order to fully utilize these opportunities, local producers will have to increase production, which may come in two forms; Increase the current production of the local turkey, which has a slow growth rate and an eventual low matured weight or introduce the foreign heavy breasted breed that is fast growing and has a high mature weight.

The second option proffers a faster solution that is achievable within a short period. This option comes with its own problems, the challenge of the necessary use of Artificial Insemination (AI) to aid reproduction. The heavy breasted turkey stag is incapable of mating (mounting) because of its heavy weight (Donoghue and Wishart, 2000). The major problem of artificial insemination in turkey production is the fact that turkey semen do not store for long period of time (Donoghue and Wishart, 2000; Long and Bakst, 2008) and cryopreservation techniques have not provided acceptable levels of semen performance, neither do short time liquid storage lasting for more than 24 hours (Slaning *et al*, 2012).

According to Donoghue and Wishart (2000), this may be as a result of the morphology of the avian spermatozoa where the head is cylindrical and not much wider than the tail in diameter (approximately 0.5µm) hence less cytoplasmic volume resulting in lesser ability to move cryoprotectants into the sperm head, compared to bull, ram or boar spermatozoa. Furthermore, the avian sperm tail is quite long, about eight times the length of the head (90 -100µm). Semen diluents generally use for semen preservation and increasing of semen volume are of different types, and do exert their influences on semen quality (Kotlowska *et al*., 2007). They vary from those that the farmer can easily constitute, those readily available in the country in commercial quantities, to those that require importation.

Hocking (2009) asserts that the core or fundamental principle used in the preservation of chicken or turkey semen is the use of diluents that are produced based on the ionic environment of the male reproductive tract. This may not be that favourable to spermatozoa as its journey to initiate fertilization is carried out in the female reproductive tract. Thus, mimicking the environment for storage of spermatozoa within the hen would profoundly alter current systems for storing semen for extended periods of time in-vitro as observed by Donoghue and Wishart (2000).

Although most extenders provide the necessary requirements for both energy metabolism and buffering capacity (Hocking, 2009), the type of diluent used in storing semen will most certainly affect its keeping quality. Hence, to ease the farmers plight will be to detect the most suitable diluent that can maintain the semen quality for some time (approximately 72 hours). This will reduce the frequency of handling stags for semen collection and make it possible to use the semen far from the collection site.

Intense labour due to frequent handling of stags for semen collection, limitation of the use of semen far from collection sites and inability to utilize semen outside the optimum reproductive period of the male, are the consequences of the incapability of turkey semen to store for long period of time. However, the need to introduce a fast growing and heavy weight turkey and the quest for a suitable diluent, among the readily available ones, in order to narrow the gap between supply and demand of animal protein will reduce intense labour associated with frequent handling of stags for semen collection and enhance maximum utilization of the reproductive age of the stag. Therefore, this study is designed to identify the most suitable diluent among four different diluents, capable of sustaining optimum turkey semen quality within a storage period of 72 hours at 5^0C.

MATERIALS AND METHOD

The research was carried out in the Livestock Investigation Division of the National Veterinary Research Institute, Vom, Plateau State. Vom, Plateau State is located in North Central Nigeria and lies between latitude 9°44'N and 9°74'E and longitude 8°49'N and 8°80'E with an altitude of 1,222 meters above sea level. It has a temperature range of between 18 and 22°C with mean annual rainfall ranging from 135cm to 146cm (http://www.distanceto.com/coordinates/ng/vom-latitude-longitude/history/46294.html).

Ten (10) Turkey stags aged 48 weeks with mean body weight of 15 kg were used for this study. They were provided with breeders diet of 10 % CP and 2800 Kcal/kg metabolizable energy, All necessary medications were administered throughout the period of study and water was given *ad libitum*.

Semen was collected using the abdominal massage method as described by Donoghue and Wishart (2000) and Yahaya *et al*. (2013). On collection, the semen was pooled and dispensed into four different containers containing four different diluents marked A, B, C and D. Concentration of spermatozoa in each diluent was adjusted to 2×10^9 m/ml (Dumpala *et al,* 2006). Diluent composition are shown in Tables 1 to 4 below. The resulting dilutions maintained at 5°C were subjected to semen analysis at the following intervals; 0 hour, 24 hours, 48 hours and 72 hours. This weekly procedure was repeated over a period of 12 weeks, and in each instance spermatozoa characteristics studied were progressive motility, percentage live spermatozoa and percentage abnormal spermatozoa (Dumpala *et al.,* 2006 and NVRI, 2012).

Spermatozoa concentration was determined using the haemocytometer while live and abnormal cells were determined using a mixture of stains as described by Yahaya *et al*. (2013)

RESULTS AND DISCUSSION

The results are presented and discussed in two parts. The first part highlights the reaction of semen in each of the diluents (A, B, C and D), while the second part compares these reactions. Thus Tables 5 to 8 depicts the first part and Tables 9 to 11, the second part. In each instance semen quality assessments were based on forward progressive movement of spermatozoa, occurrence of morphological defects of sperm cells and their death as storage time increases from 0 hours to 72 hours at a constant temperature of 5°C.

Table 1: Composition of Diluent A

Composition	%
NaHPO4.12H2O	8.54
Tris (hydroxymethyl) aminomethane	50.90
Fructose	17.76
Citrate	1.35
NaOH	19.93
Mg acetate	1.33

Table 2: Composition of Diluent B

Composition	%
Glucose	18.24
Na glutamate	30.29
K citrate	1.59
NaHPO4	25.23
Na2H2PO4	6.39
Inositol	18.27

Table 3: Composition of Diluent C

Composition	%
Fructose	14.47
Citrate	1.10
Na acetate	24.19
Na glutamate	24.19
$Mgcl2.6H_2O$	0.94
$K_2HPO_4.3H_2O$	32.39
KH_2PO_4	2.72

Table 4: Composition of Diluent D

Composition	%
Na glutamate	13.18
Glucose	16.84
K- citrate	1.96
BES	40.70
Na acetate	5.72
Mg acetate.$4H_2O$	1.52
NaOH	17.00
Na_2PO_4	3.00
Streptomycin	0.08

BES- N,N-bis(2-hydroxyethyl)-2 amino ethane acid

Forward progressive movement in all the diluents (A, B, C and D), as shown in Tables 5, 6, 7 and 8 respectively, does not only decreased steadily but significantly ($P<0.05$). These significant decreases are a portrayal of the sperm cell lost of fertilizing ability as storage length increases. Thus with regards to forward progressive movement, it will be best to utilize the semen at 0 hour, followed by 24 hours, 48 hours and finally 72 hours with the exception of diluent D which showed no difference between 0 hours and 24 hours. The decrease in forward progressive movement with storage length which can be attributed to energy depletion among other factors coincides with the findings of Donoghue and Wishart (2000), Iaffaldano *et al.* (2005) and Dumpala *et al.* (2006).

Morphological defects in all the diluents as shown in Tables 5 to 8, increased significantly ($P<0.05$) with increases in storage lenght. Diluents A and D bear no differences with both their initial values on dilution (0 hour) and after 24 hours. The same applies to diluent B but diluent C on the other hand, had a significant ($P<0.05$) increases in the percentage of abnormal spermatozoa from 0 hour to 24 hours. At the end of the storage period, significant ($P<0.05$) differences exist between 0 hour and 72 hours in all diluents. The implications, with regards to percentages abnormal spermatozoa, is that, optimum semen quality is best achieved when the semen is utilized within 24 hours for diluents A,B and D while for diluent C the period lies between 0 hour and 24 hours. Increases in morphological defects during dilution and/or storage attributed to fluctuations in osmotic pressure have been reported by Donoghue and Wishart (2000) and Iattaldano *et al.*(2005).

Spermatozoa mortality increases significantly ($P<0.05$) with increase in storage length in all the diluents (Table 5 to 8). Diluents C and D maintained constant values between 0 hour and 24 hours which are 6 % and 9 %, respectively. Diluents A and B show a significant increase from 0 hour to 24 hours of 6 % to 9 % and 8 % to 11 % respectively. Thus, once more, optimum fertilizing ability of semen with regards to percentage dead spermatozoa is best achieved within 24 hours for diluents C and D and before 24 hours for diluents A and B. similar findings indicating increase in spermatozoa death during liquid storage as storage lenght increase have been reported by Donogue and Wishart (2000) and Dumpala *et al.*(2006).

Before proceeding to the second part of this discussion where the performance of the diluents are compared with the view of identifying the most suitable for maintaining semen viability within the storage period of 72 hours at 5^oC, it is pertinent to note that all the diluents maintain potential semen quality necessary for achieving acceptable level of fertilization. They have maintained forward progressive movement of spermatozoa at/and above 50 %, abnormal spermatozoa less than 20 % and dead spermatozoa not greater than 20 %.These according to Sarah (2002), Malecki and Martin (2002), Zahraddeen *et al.* (2005) and Yahaya *et al.* (2013), are all it takes for acceptable level of fertility.

The highest value for spermatozoa forward progressive movement after the storage period of 72 hours at 5^0C, was achieved in diluent D (65 %-Table 9), followed by diluent B (60 %), then

diluent C (55 %) and finally diluent A (50 %). The least value of abnormal spermatozoa within the study period, occurred in diluents D and B (4 % each) followed by diluents A and C (5 % each- Table 10). The least value of dead spermatozoa occurred in diluent B (11 %- Table 11) followed by diluents A and D (12 %) and finally diluent C (20 %).

In adherence to the criteria outlined earlier of acceptable levels of potential semen quality which are forward progressive movement being 50 % and above; Abnormal spermatozoa not more than 20 % and dead spermatozoa also, not more than 20 %, diluent D is ranked the highest and thus the most suitable for maintaining semen viability within the storage period of 72 hours at 5^0C; followed by diluent B, then diluent A and finally diluent C.

The precise reason(s) for the performance of diluent D above the other diluents is (are) subject for further studies but it would not be out of place to acknowledge the fact that it is the only diluent that contains an antibiotic – streptomycin. Thus microbial activities must have been inhibited (Donoghue and Wishart, 2000; Iaffaldano *et al*, 2005; Dumpala *et al*, 2006).

Table 5: Effects of Diluent A on Semen viability within the storage period of 72hours at 5°C.

	Times (hours)				
	0	24	48	72	SEM
Semen Characteristics (%)					
Forward Progressive Movement	80^a	75^b	60^c	50^d	0.633
Abnormal Spermatozoa	1^c	1^c	2^b	5^a	0.548
Dead Spermatozoa	6^c	9^c	10^b	12^a	0.145

^{a,b,c,d}: Means with different superscripts are significant at 0.05 level

Table 6: Effects of Diluent B on semen viability within the storage period of 72 hours at 5°C

	Times (hours)				
	0	24	48	72	SEM
Semen Characteristics (%)					
FPM	85^a	80^b	70^c	60^d	0.742
AS	2^b	2^b	3^b	4^a	0.500
DS	8^b	11^a	11^a	11^a	0.387

^{a,b,c,d}: Means with different superscripts are significant at 0.05 level

Table 7: Effects of Diluent C on Semen viability within the storage period of 72hours at 5^oC

	Times (hours)				
	0	24	48	72	SEM
Semen Characteristics (%)					
FPM	85^a	75^b	60^c	55^d	0.837
AS	2^c	3^b	5^a	5^a	0.447
DS	10^c	10^c	16^b	20^a	0.592

^{a,b,c,d}: Means with different superscripts are significant at 0.05 level

Table 8 Effects of Diluent D on semen viability within the storage period of 72hours at 5^oC

	Times (hours)				
	0	24	48	72	SEM
Semen Characteristics (%)					
FPM	80^a	80^a	70^b	65^c	0.592
AS	1^b	1^b	4^a	4^a	0.227
DS	6^c	6^c	11^b	12^a	0.447

^{a,b,c}: Means with different superscripts are significant at 0.05 level

Table 9: Comparison of forward progressive movement of spermatozoa(%) as affected by diluents A, B, C and D

	Forward Progressive Movement (%)			
	Times (hours)			
	0	24	48	72
Diluents				
A	80^b	75^b	60^b	50^a
B	85^a	80^a	70^a	60^b
C	85^a	75^b	60^b	55^c
D	80^b	80^a	70^a	65^a
SEM	0.671	0.633	0.806	0.707

^{a,b,c}: Means with different superscripts are significant at 0.05 level

Table 10: Comparison of Abnormal Spermatozoa as generated by diluents A, B, C and D

	Abnormal spermatozoa (%)			
	Times (hours)			
	0	24	48	72
Diluents				
A	1^b	1^c	2^d	5^a
B	2^a	2^b	3^c	4^b
C	2^a	3^a	5^a	5^a
D	1^b	1^c	4^b	4^b
SEM	0.025	0.450	0.387	0.317

^{a,b,c,d}: Means with different superscripts are significant at 0.05 level

Table 11: Comparison of the occurrence of dead spermatozoa occasioned by diluents A, B, C and D

	Dead spermatozoa (%)			
	Times (hours)			
	0	24	48	72
Diluents				
A	6^{a}	9^{b}	10^{c}	12^{d}
B	8^{a}	11^{b}	11^{b}	11^{b}
C	10^{a}	10^{a}	16^{b}	20^{c}
D	6^{a}	6^{a}	10^{b}	12^{c}
SEM	0.592	0.387	0.500	0.387

^{a,b,c,d}: Means with different superscripts are significant at 0.05 level

CONCLUSION AND RECOMMENDATION

The study has shown the possibility of holding turkey semen for up to 72 hours at 5°C. This possibility may open frontiers for marketing turkey semen nationwide and providing the opportunity for the optimum utilization of scarce technical expertise in the field of poultry artificial insemination. It has also been shown that the potential viability of the stored semen is within acceptable limits but the study did not evaluate the actual viability of the semen which can only be determined after insemination in the hen. Thus, it is concluded, based on the findings of this study, that, as far as turkey semen potential viability is concerned, turkey semen can be stored for as long as 72 hours. However, it is recommended that semen stored in all the diluents be utilized within 24 hours if preserved at 5°C, but caution their use after 72 hours because it is only the potential viability of the semen that was studied and not the actual viability. Thus the study of the actual viability is also recommended.

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