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Semen and microbial characteristics of two breeds of turkeys in an arid tropical environment of Bauchi State, Nigeria

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A study was conducted at the poultry unit of Abubakar Tafawa Balewa University, Bauchi, Teaching and Research Farm to investigate semen characteristics and the sensitivity of semen microbes to some antibiotics in exotic (large white – LW) and local (indigenous – I) breed of turkeys for a period of six months. Data were analysed using one way analysis of variance (ANOVA). Results show significant breed differences (P≤0.01) in live weight (15.24±0.88 kg and 6.53±0.53 kg) for LW and I breeds, respectively. Significant breed differences were also recorded for semen volume (0.35±0.05 and 0.18±0.02 ml), total sperm per ejaculation (7.43±1.22 and 2.77±0.29×10^8/ml), daily sperm output (1.02±0.17 and 0.39±0.05×10^8/ml), total live spermatozoa (5.68±1.12 × 10^8 and 1.99±0.27×10^8/ml), total live normal spermatozoa (4.57±0.99 ×10^8 and 1.76±0.24×10^8/ml) for LW and I breeds, respectively. In the LW breed also, sperm concentration correlated positively (r = 0.79) with semen volume, total live spermatozoa (r= 0.77) with total spermatozoa per ejaculation, daily sperm output (r=0.68) with total live spermatozoa, daily sperm output (r=0.52) with semen motility and live weight (r=0.54) with semen volume. In the I breed, there was significant and positive correlation between total live normal spermatozoa (r=0.76) with total spermatozoa per ejaculation, daily sperm output (r=0.91) and total spermatozoa per ejaculation, daily sperm output (r=0.84) and total live normal spermatozoa. *Enterobacter* spp. was the only microbe isolated from the semen of both LW and I breeds of turkey and was susceptible to Ciproxine (Cip) and Gentamycin (GN). There were turkey breed differences in semen characteristics in our environment, and they compare favourably with those obtained elsewhere in the tropics. The LW breed appears to have a higher reproductive potential than the I breed. Ciproxine and Gentamycin could be used in the control of bacteria in turkey semen in our environment.

Key words: Turkey semen, microbial characteristics, breeds, arid tropical environment.

INTRODUCTION

Artificial insemination (AI) is a very important practice by modern turkey breeding systems. It is one of the animal production technologies that augment production and returns from livestock at a faster rate and enhance cross Breeding programmes. The evaluation of the semen of any animal species gives an excellent indicator of its

quality and is sine-quanon to an effective artificial insemination programme, but there is paucity of information on this aspect for the Nigerian indigenous species of poultry and turkey in particular.

Rapid human population growth and low protein intake are some of the major problems facing developing countries like Nigeria. With a population of about 174 million (PRB, 2013) and with over 70% of the population living on less than a dollar a day (Watts, 2006), Nigeria is the most populous country in Africa. Nigerians own a variety of farm animals with poultry being the highest in number and 80 - 90% of these flocks owned by small scale farmers (Ebangi and Ibe, 1994). Poultry offers an avenue for rapid transformation in animal protein consumption. The average Nigerian consumes 9 grams of animal protein per capita per day as compared to over 50 g per capita per day in North America and Europe (Boland et al., 2013). The poultry population in Nigeria is estimated at 140 million (Adeleke et al., 2010), producing about 268,000 metric tons of poultry products annually (FAO, 2013). Indigenous poultry constitute more than 90% of total poultry in Nigeria (Gueye, 1998). Sonaiya (1999) confirmed that of the 82.4 million chickens in Nigeria, commercial holdings accounted for only 10 million chickens or 11%. Philips (1996) indicated that nearly every household keeps poultry, thus protein from poultry sources is still available to most families.

Research on the indigenous breeds of poultry is increasing in Nigeria. There are some reports on studies carried out on chickens (Oke and Ihemeson, 2010; Peters et al., 2010; Adeleke et al., 2011); guinea fowl (Butswat et al., 2001) and turkeys (Peters et al., 1997; Zahraddeen et al., 2005; Ironkwe and Akinola, 2010; Ilori et al., 2011; Yakubu et al., 2013). Turkey production is both an important and a profitable agricultural industry, with a rising global demand for its products (Anandhl et al., 2012). Indigenous turkey is the least produced among domesticated poultry species in Nigeria numbering about 1.05 million (Yakubu et al., 2013), despite its greater potential as a meat bird than the chicken (Shingari and Sapra, 1993; Ajayi et al., 2012).

At present, the production of exotic poultry species is unaffordable by many Nigerians. The indigenous breeds are numerous, better adapted and cheaper to raise (Fisinin and Zlochevskaya, 1989). These birds are natural foragers and scavengers and always range farther. Indeed, they thrive best where they can move about freely feeding on seeds, fresh grass, locusts, crickets, grasshoppers, worms, slugs and snails (Singh and Sharma, 2012). It is therefore, necessary that more research be conducted on them. There is scarcity of information on semen and microbial characteristics and the reproductive performance of indigenous turkeys in Nigeria. To improve local stock, knowledge of their reproductive potential is relevant, and to derive fullest benefits from breeding turkeys, proper knowledge of their sperm output is essential (Butswat, 1994). Semen collected is normally preserved in specially compounded diluents pending insemination (Niba et al., 2002). Antibiotics and sometimes antimycotics are often added for the diluents before conservation. This is done to protect the semen content from destruction before insemination. Quite often, antibacterial application is done without prior knowledge of the type of microorganisms prevalent in semen. Microorganisms have a deleterious effect on sperm function, both directly by altering the structure of the sperm, by affecting its motility (Depuydt et al., 1998) or by provoking a premature acrosome reaction (Kohn et al., 1998), the putrefaction of diluents components of biological origin, and the utilization of metabolic substrates (Lamming, 1984), and indirectly stimulating the production of antibodies that can be directed against the sperm glycocalyx complex (Kurpisz and Alexander, 1995). Most ejaculates collected from healthy animals are contaminated with bacteria to some extent. Some reports indicate that metabolic products such as endotoxins from some bacteria appear to have detrimental effects on the survival of sperm (Almond and Poolperm, 1990). Hence, semen quality and the quantity of viable sperm cells may be reduced with bacterial contamination.

The aim of this study is to improve breeding practices in turkeys, by establishing a baseline for assessing and comparing the semen characteristics of the two genotypes. The objective was therefore designed to evaluate the semen characteristics and microbial flora, and the sensitivity of semen microbes to antibiotics in the two genotypes.

MATERIALS AND METHODS

Climate and vegetation of the study site

Bauchi State is in north eastern Nigeria. It occupies a total land area of 49,259.01 km^2 (5.3% of Nigeria) and is situated between latitude 9° and 12°30' North, and between longitudes 8°50' and 11° East. According to the 1991 census, Bauchi State has a population of 2,826,444 inhabitants. The society is primarily agrarian and agriculture contributes about 75% to the State's economy. The climate is characterized by two well defined seasons: The rainy season (usually May to October) and the dry season (usually November to April). The vegetation is Sahel/Sudan in the north, guinea savannah in the central and western zones of the state (IAR/BSADP, 1996). The mean annual rainfall is 905.33 mm with an annual temperature range of 11-14°C. The annual rainfall is between 700 and 1250 mm in the north and south – south west zones, respectively. Mean monthly hours of sunshine are about 300

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h (highest) in December and about 150.1 h (lowest) in August. August records the highest relative humidity of 65.5% and February records the lowest, 16.5% (Kowal and Knabe, 1972).

Breed description and management of experimental birds

Two breeds of turkeys were used in the study. The word 'breed' is used loosely to distinguish the birds.

The indigenous breeds of turkeys were not well defined. Various color phases exist with black and white occurring as pure colors. Brown color also exists and has numerous pale barring and mottling of the feathers especially of the tail, primaries, secondaries and wing coverts; a metallic sheen of the plumage usually accompany the black and brown color phases. Yearling males weigh between 4.6 - 4.8 kg and females 2.4 - 3.2 kg (Malia, 1998).

The large white (LW) exotic breed are heavy, broad-breast, white in color and with a well developed snood, dewlap and caruncles, which are bright red in color. There is little amount of blue skin around the eyes. All birds are flightless, markedly tame and of a calm disposition thereby being easily approached. Beaks, shanks and feet are pink tan. Mature body weights for the toms range from 25 - 35 kg and for the hens 8 -12 kg.

The exotic (LW) breed was sourced from ZARTECH Ltd. Farms, Ibadan, Oyo State while the indigenous (I) breed was purchased from some local farmers in Bauchi. Four animals of each breed, age between 9 and 11 months old and weighing averagely 11 and 4.2 kg respectively were used in this study. All the toms were housed singly in a netted pen in well ventilated poultry houses and fed a standard breeder diet (17% crude protein, 2900 kcal of metabolizeble energy/kg). The experimental diets in mash form and fresh clean drinking water were provided *ad libitum*. The ambient relative humidity was 50-60%. All groups of toms were trained for semen ejaculation on breed basis over a period of three weeks.

Tetracolivit (an anti-stress containing Oxytetracycline, vitamin and minerals) was administered in water for one week following stocking. On the seventh day, the birds were dewormed using Piperazine Citrate. Deworming was repeated after one week. Birds were treated against external parasites (fowl lice) using *dichlovos* as active ingredient in a devlap spray and drops were sprayed in saw dust and pen walls. During the training period, *Trisulmycine Forte* (containing Trimethoprim and Sulphadiazine as active ingredients) was used to control attacks against respiratory diseases.

Semen collection and evaluation

Semen collection was carried out by two persons using the double hand lumbar massage method of Burrows and Quinn (1937) and Watson (1990).

Semen volume was obtained using a graduated conical test tube calibrated to the nearest 0.01 ml. Semen motility was evaluated using a haemocytometer and a light microscope with warm stage in accordance with the methods outlined by Sorensen (1976) and Sexton (1981).

Sperm count was carried out using a haemocytometer 450x magnification. The number of sperm counted in five large diagonal squares multiplied by 10^6 (N x 10^6) equalled sperm concentration/ml. Total spermatozoa per ejaculation were obtained by multiplying ejaculate volume and concentration.

Percent live/dead spermatozoa and percent normal/abnormal sperm cells were determined using the technique of Ernst and Ogasawara (1970). A mixture of Eosine/Nigrosine was used as dye/stain mixed with semen freshly collected. Thereafter, on microscopic examination, normal and abnormal morphology were observable. Cells that were dead or damaged were seen to be stained while live cells absorbed no dye colour.

Total live spermatozoa were obtained by multiplying total spermatozoa per ejaculation and percent live spermatozoa. Total live and normal spermatozoa were obtained by multiplying total live spermatozoa and percent normal sperm (Sexton, 1986).

Daily sperm output was measured by dividing total spermatozoa per ejaculation by the interval of semen collection (7 days) (Brillard and de Reviers, 1985). Weights of birds were determined using a weighing scale.

Semen microbial analysis

Isolation and identification of micro-organisms

A loopful of the test semen was aseptically streaked on nutrient agar (NA) plates (Oxoid Ltd.) in triplicates and incubated aerobically at 37°C for 24 h for bacteria and on already prepared potatoes dextrose agar (PDA) plates (Oxoid Ltd.) in triplicates and incubated at 25°C for 5 days for fungi. This was repeated for 10 collections. Bacterial colonies of discrete cultural characteristics were carefully picked and purified by repeated sub-cultures on nutrient agar plates and their morphology was studied. Pure cultures were then preserved on nutrient agar plates and used for Gram staining. Gram stained slides were identified after microscopy using Bergey's Manual of determinative bacteriology (Buchannan and Gibbons, 1974).

Sensitivity of semen microbes to antibiotics

Using a sterile wire loop, a loopful of the test organisms was picked up and streaked according to standard medical laboratory techniques, (SMLT) (Harrigan and McCance, 1976). A sterile bent glass rod spreader was used to make a fine lawn and the antibiotic discs (Poly-Tes-Multo-disks PS 003 gram negative) were used according to agar diffusion method and the results reported according to SMLT. When more than one level of a particular antibacterial was available, the maximum dosage was tested. The antibiotics used for the study were Ciproxine (Cip) – 5 mcg, Gentamycine (GN) -10 mcg, Tetracycline (TE) -50 mcg, Nalidixic acid (NA) -30 mcg, Ampicillin (AM) -25 mcg, Cefuroxine (CF) -20 mcg, Norbactin (NB) – 10 mcg and Cotrimoxazole (CO) -50 mcg. The effectiveness of each antibacterial tested was determined by measuring the minimum inhibitory zone (mm) surrounding each disc (Sexton et al., 1989b). The minimum inhibitory zone is the diameter surrounding the sensitivity disc in which no growth occurred and is measured from one side of the circular zone to the other side as seen from the underside of the agar plate.

Interpretation of susceptibility or resistance to antibacterial treatment was aided by data collected with test cultures provided by the manufacturers. A susceptible score indicated that the micro-organism in semen was most likely affected by antibacterial while a resistant score indicated that micro-organisms were not affected.

Data analysis

Breed effects on the various semen characteristics were determined using analysis of variance (ANOVA) (Ryan et al., 1985).

RESULTS

Live weight

The overall live weight for the two breed of turkeys was 10.89 ± 0.5 kg. Analysis of variance showed a significant difference ($P \leq 0.01$) in weights among the two breeds of turkey used for the study, being 15.24 ± 0.88 and 6.5 3 ± 0.53 kg for LW and I, breed respectively (Table 1).

Table 1. Mean semen characteristics by breed.
±

Semen parameter	Overall	Exotic (LW)	Indigenous (I)	SEm±	LOS
Live weight (kg)	10.89±0.51	15.24±0.88	6.53±0.53	0.73	**
Volume (ml)	0.26±0.03	0.35±0.05	0.18±0.02	0.04	**
Motility (%)	82.95±1.14	84.63±0.96	81.27±2.07	1.61	NS
Conc. x (10^8 sperm/ml)	1.99±0.16	2.27±0.26	1.73±0.18	0.22	**
Tot. Sperm/ejac x 10^8	5.19±0.16	7.43±1.22	2.77±0.29	0.95	*
Tot. live Spz x 10^8	3.84±0.64	5.68±1.12	1.99±0.27	0.90	*
Tot. live Nor Spz. x 10^8	3.16±0.56	4.57±0.99	1.76±0.24	0.79	**
Daily sperm output x 10^8	0.70±0.10	1.02±0.17	0.39±0.05	0.14	NS
Live Spz (%)	83.53±0.96±	84.91±1.38	82.43±1.34	1.36	NS
Dead Spz (%)	16.48±0.96	15.39±1.38	17.57±1.34	1.36	NS
Normal Spz (%)	86.71±0.81	88.29±1.08	85.13±1.18	1.14	NS
Abnormal Spz (%)	13.16±0.81	11.96±1.11	14.36±1.17	1.15	NS

Tot. = Total, ejac. = ejaculate, Spz = spermatozoa, Nor.= normal; *- P≤0.05; **-P≤0.01; NS- not significant; LOS- Level of significance.

Semen characteristics

The overall mean of the various semen characteristics studied in breed of turkeys are shown in Table 1. The effects of breed on semen volume, total sperm per ejaculation and daily sperm output were significantly different (P≤0.01). Total live spermatozoa and total live normal spermatozoa were also significantly different (P≤0.05), the values for the LW are 5.68 ± 1.12 and 4.57 ± 0.99 as compared to 1.99 ± 0.27 and 1.76 ± 0.24 (x10^8 sperm/ml) for I breed. The highest yield of 1.1 ml good quality semen was obtained among the LW breed while 0.1 ml was obtained in the I breed. The effects of breed on the other semen characteristics namely percent motility, sperm concentration per ml, percent live/dead and percent normal/abnormal sperm cells were however not significant. Semen volume for the LW was 0.35 ± 0.05 ml as against 0.18 ± 0.02 ml for the I breed. The overall motility percentage was 82.95 ± 1.14 (%). Similarly, the concentration of sperm cells was 2.27 ± 0.26 and 1.73 ± 0.18 (x10^8 sperm/ml) for LW and I breeds respectively. Total live spermatozoa, total live normal spermatozoa and daily sperm output were higher for LW than for I breeds (5.68 ± 1.12 vs 1.99 ± 0.27, 4.57 ± 0.99 vs 1.76 ± 0.24 and 1.02 ± 0.17 vs 0.39 ± 0.05 x 10^8/ml respectively. In the case of total sperm per ejaculation, LW had the highest value while I breed had the lowest (7.43 ± 1.22 and 2.77 ± 0.29 x 10^8/ml sperm cells, respectively.

The overall percent live and dead cells were 83.52 ± 0.96 and 16.48 ± 0.96%, respectively. On the other hand, the overall percentage normal as compared to abnormal sperm cells was 86.71 ± 0.81 and 13.16 ± 0.81%, respectively.

Relationship between semen characteristics

The correlation coefficients among the studied parameters are presented in Table 2 and showed that correlations between some parameters studied were weak and non significant (p≤0.05) while some parameters showed negative relationships. Highly significant (p≤0.01) and strong associations were observed between most parameters with r values ranging from 0.91 between daily sperm output (DSO) and total spermatozoa (TS), DSO and total live normal sperm (TLN), concentration (CON) and semen volume (SV), total live spermatozoa (TLS) and total spermatozoa (TS), total live normal spermatozoa (TLN) and total spermatozoa (TS) to 0.68 between DSO and TLS. However, correlations between daily sperm output and semen motility percentage (r = 0.05) and between body weight and semen volume (r = 0.54) were low but still significant (P≤0.05).

Semen microbiology

Data on sensitivity test of semen microbes to some antibiotics (Gram negative) are shown in Table 3. *Enterobacter* spp. was the only microbe isolated from the semen of both LW and I breeds of turkey. Among the antibiotics tested on bacterial isolates, only Ciproxine (Cip) and Gentamycin (GN) were found to be effective. The mean millimeter zone of inhibition for 3 replicates was 27 and 15.03 mm for Ciproxine and Gentamycin, respectively.

DISCUSSION

Live weight, semen characteristics and relationship between semen traits

Livestock breeders everywhere are interested in old and new factors that can affect the breeding capacity of the male of any species. Live weight obtained for the exotic breeds used in this study was lower than the 25.8 kg reported by Noirault and Brillard (1999). The ranking in live weights of the breeds studied agrees with those

Table 2. Correlation matrix between semen characteristics measured on genotypes.

Genotype		SV	SM	NS	AS	CON	TS	TLS	TLN	DSO	BDW	Li %
	SM	-0.210										
	NS	-0.328	0.181									
	AS	0.302	-0.192	-0.992**								
	CON	0.793**	-0.104	-0.229	0.219							
	TS	-0.183	0.426	0.042	-0.056	-0.227						
Exotic (LH)	TLS	-0.196	0.274	0.016	-0.034	-0.209	0.772**					
	TLN	-0.109	0.113	0.287	-0.280	-0.176	0.070	0.339				
	DSO	-0.177	0.052*	0.221	0.284	-0.274	0.444	0.679**	0.012			
	BDW	0.541*	0.007	-0.226	0.253	-0.109	0.180	0.098	-0.237	-0.121		
	Li (%)	-0.224	-0.241	-0.020	-0.004	-0.349	0.323	0.245	-0.012	0.149	0.233	
	DD (%)	0.224	-0.241	-0.020	0.004	-0.349	0.323	0.245	-0.012	0.149	0.233	-1.000**
	SM	-0.238										
	NS	-0.063	0.115									
	AS	0.062	-0.115	-1.000								
	CON	0.170	-0.489	0.163	-0.164							
	TS	0.347	0.005	0.275	0.275	0.245						
Local (I)	TLS	-0.263	0.260	0.174	0.174	0.388	0.165					
	TLN	-0.521*	0.065	0.350	-0.349	0.241	0.755**	0.192				
	DSO	-0.492	0.065	0.334	-0.333	0.160	0.905**	0.220	0.835**			
	BDW	-0.064	0.288	-0.310	0.309	0.003	-0.330	-0.235	-0.138	-0.213		
	Li (%)	-0.430	0.164	-0.065	-0.066	0.096	-0.045	0.033	0.092	0.193	0.264	
	DD (%)	0.430	0.164	0.065	0.066	0.096	0.045	-0.033	0.092	-0.193	-0.264	-1.000**

SV = Semen volume, SM = Semen motility, NS = Normal spermatozoa, AS = Abnormal spermatozoa, CON = Sperm concentration, TS = Total spermatozoa, TLS= Total live spermatozoa, TLN = Total live normal spermatozoa, DSO = Daily sperm output, BDW = Body weight, Li (%) = Live percentage, DD (%) = Dead percentage. * = $P \leq 0.05$, ** = $P \leq 0.01$.

of Oluyemi and Roberts (1988). The higher live weight obtained in the LW also suggests that the breed has a higher potential for use in breeding programmes in this environment since live weight has been linked to testes weight (Yakubu et al., 2012). Furthermore, earlier studies with crosses between indigenous hens and exotic cooks resulted in better progeny live weights (Ebangi and Ibe, 1994).

Quantitative evaluation of semen is an aspect of reproductive status assessment in males. Cecil and Bakst (1988), Egbunike and Nkanga (1999) and Etchu et al. (2013) have all reported that spermatozoa output, measured by volume and concentration; tend to vary with breed, nutrition and season. The overall results of the seminal traits for the two breeds showed that the LW breed was superior to the I breed. There was a significant difference between breeds in their yields of semen. The exotic toms (LW) yielded the highest volume of semen (0.35 ± 0.05 ml). This value was however lower than those reported (0.56 ml) by Sexton (1981) and obtained (0.43±0.12 ml) by Noirault and Brillard (1999) and Bonato et al., 2011 (1.16 ± 0.05 ml) in ostriches but higher than the value (0.31 ml) reported by Taras et al. (1997). The overall mean yield of semen in this study is lower than the 0.5ml reported by Hafez (1995) but within the range of 0.25 - 2ml given by Cerolini et al. (2003) for chickens.

Sperm concentration is influenced by breed, nutrition, season and even method of ejaculation (Butswat et al., 2001). The overall mean concentration of semen for all breeds in this study (1.99 ±

Table 3. Results of antibacterial disc survey on micro-organisms of Turkey semen.

Antibiotics	Level or quantity (mcg)	Kill zone (mm)[a]	Sensitivity[b]
Ciproxine (cip)	5	27	+++
Gentamycin (GN)	10	15.03	++
Tetracycline (TE)	50	0	+
Nalidixic acid (NA)	30	0	+
Ampicilln (AM)	25	0	+
Norbactin (NB)	10	0	+
Cotrimoxazol (Co)	50	0	+

[a] = Values are the mean mm for 3 replicates; [b] = Organisms were in the highly sensitive range, if kill zone > 16 mm (+++), intermediate or slightly sensitive if kill zone is between 12 - 16 mm (++) and resistant or non sensitive if kill zone < 12mm (+).

0.16 x 10^8/ml) is lower than the value for low concentration semen (3.8 x 10^9/ml) reported by Moss et al. (1979) and far lower than that obtained (3.74 ± 70.83 x 10^9/ml) by Zahraddeen et al. (2005) in turkeys. There was no significant difference in the sperm concentration in the two breeds used in this study. These results are at variance with those obtained by Zahraddeen et al. (2005) in indigenous and exotic turkeys. Idi (2000) also obtained significant deference in sperm concentration using indigenous and exotic breeds of cocks in Bauchi. However, an explanation for the absence of significant differences in sperm concentration between breeds in this study may be due to the expertise of the semen collector as expertise is linked to increase in the flow of accessory fluids and hence reduction in concentration. Because total spermatozoa per ejaculation is the product of concentration and ejaculate volume, LW breed which has the highest volume of semen also had the highest total sperm yield (7.43±1.22x 10^8/ml). This value is however lower than those obtained (4.32±0.74 x 10^9/ml) by Noirault and Brillard (1999). Total sperm value for I breed was 2.77 ± 0.29 x 10^8/ml, much lower than the values (1.23 ± 0.15 x 10^9ml) reported by Idi (2000) for indigenous cocks.

Motility is the semen evaluation parameter that is normally first used to indicate the presence of live spermatozoa in a semen sample. Sexton (1981) reported excellent motility scores of 80% and above. The overall mean motility scores of 82.95 ± 1.14, and individual breed scores of 84.63 ± 0.96 and 81.27 ± 2.07 (%) for LW and I were higher than the values of 67 and 45% obtained by Onuora (1982) for guinea fowls and those reported by Nwagu et al. (1996) in chickens. The motility percentage scores were also higher than those obtained by Butswat et al. (2001) for local and exotic guinea fowls (38.5±2.8 Vs 51.6 ± 1.7%) and Idi (2000) for local and exotic cocks (67.97 ± 1.40 vs. 67.58 ± 1.2%) but were similar to the values of 84.6% obtained by Taras et al. (1997). The values were however not significant confirming the findings of Zahraddeen et al. (2005) in turkeys but at variance with the findings of Butswat et al. (2001) using guinea fowls and Egbunike and Nganga (1999) and Etchu et al. (2013) using chickens.

The overall mean number of live sperm cells (3.84 ± 0.64 x 10^8/ml) which is equivalent to 83.52 ± 0.96 in ordinary percentages and number of dead cells which is 16.48 ± 0.96% is rather high, although percentage motility is comparable to the minimum score of 70-80% viable spermatozoa normally required in a semen sample for insemination. Both LW and I breeds had dead cells below the maximum 20% that results in poor fertility.

The overall mean normal sperm cells (86.71 ± 0.81%) and individual breed values of 88.29 ± 1.08 and 85.13 ± 1.18% for the LW and I breeds respectively, are lower than those reported by Idi (2000) and Etchu et al. (2013) but higher than those (78.52 – 79.58%) reported by Nwagu et al. (1996). Butswat et al. (2001) reported means of 33.4 ± 0.76 and 19.5 ± 0.001 for total abnormal sperms of local black variety and exotic pearl variety of guinea fowls, respectively. Gbadamosi and Egbunike (1999) also obtained means of 10.66 ± 0.24 and 12.80 ± 0.30% for total abnormal sperms of exotic and local cocks, which were higher than the values (3.05 ± 0.47 and 2.38 ± 0.55%) by Idi (2000) for Barred Plymouth Rock and indigenous cocks, respectively. Mean abnormal sperm number in this study for the LW and I breeds (11.96 ± 1.11 Vs 14.36 ± 1.17%) respectively, compares favourably with those of Zahraddeen et al. (2005) who obtained 11.19 ± 0.73 and 13.61 ± 0.73% for abnormal cells among exotic and local turkey semen in Bauchi. Herbert and Acha (1995) attributed spermatozoa abnormalities to deformities or accidents and that radiations could cause both deformities and dead cells. These workers further elucidated that abnormalities may also occur as artefacts caused by the staining procedure.

The levels of daily sperm output (DSO) obtained from males of the two breeds (LW and I) differed significantly. The overall mean DSO (0.70 ± 10.0 x 10^8) and individual breed values of 1.02 ± 0.17 and 0.39 ± 0.05 (x 10^8/ml) for LW and I respectively were lower than the values obtained (0.62 ± 0.11 x 10^9 sperm) by Noirault and Brillard (1999) for large white turkeys and 0.73 ± 0.21 x

10^9 obtained by Etchu et al. (2013) for broiler breeder cocks. Noirault and Brillard (1999) also observed that, following a rest period of 2 days or more, 3 days of daily semen collection are necessary for turkey males to reach their DSO base level of approximately 1.6 -1.9 x 10^9 spermatozoa per ejaculation. Their values were however well above those observed by Cecil et al. (1988) in large turkeys (0.52 x 10^9 sperm per male).

The genotypes showed a positive correlation between DSO and semen motility, total spermatozoa, total live spermatozoa and total live normal spermatozoa. This implies that as one trait increases, the other traits also increase. This corresponds with the findings of McDaniel et al. (1995) who noted that the evaluation of the male chicken for breeding soundness must be based on semen motility and concentration. Daily sperm output could therefore serve as a useful indicator of the quality and quantity of viable semen in turkeys. Positive and significant coefficients were also obtained between semen volume and concentration and between body weight and semen volume. However, there was a negative correlation between semen volume and total live normal spermatozoa. This is an indication that volume may not be a good indicator of semen quality. This finding is in agreement with the results of Oke and Ihemeson (2010), who obtained a negative correlation between semen concentration and semen volume in different chicken genotypes and concluded that volume may not represent an excellent indicator of semen viability and fertility.

Generally, the semen of LW showed better quality over that of I and it is therefore more capable of giving good fertility. Selection of genotype with large testicular size and better semen quality will be good for breeding programme. Foote (1980) and Herbert and Adejumo (1993) attributed these differences to genetic make-up of the breeds as well as pre and post natal growth and development.

Semen microbiology

Virtually all semen samples are contaminated at the time of collection (Almond and Poolperm, 1990). Poultry semen becomes heavily contaminated with bacteria as it issues from the papillae on the wall of the cloaca during collection. Sexton et al. (1980) reported that turkey semen collected by artificial ejaculation contains on the average 1300 x 10^6 bacteria/ml. The effects of bacterial contaminants in semen have been reported (Revell and Glyssop, 1989, Sone, 1982). Some reports indicated that metabolic products, such as endotoxins from some bacteria and fungi appear to have detrimental effects on the survival of sperm. Watson (1990) observed that not only pathogen but, other microflora can have adverse effects on the fertility of semen by the production of toxins and by utilisation of metabolic substrates. For example, *Aspergillus* spp. are known to be associated with the secretion of a toxic fungal metabolite, aflatoxin. Clarke et al. (1987) observed that ingestion of aflatoxin contaminated feed can lead to widespread reproductive abnormalities in male chicken including a reduction in circulating hormonal levels of testosterone. In another experiment, Clarke and Ottinger (1989) reported that the percentage free testosterone in the peripheral circulating blood was significantly higher in aflatoxin-treated male chickens than the control. They however, stressed that this was not due to direct inhibition of the ability of the testicular cells to produce testosterone but rather a change in the rate of clearance of testosterone from the blood. There is also a direct influence of bacteria on fertilisation (conception) especially if the number of bacteria reaching the site of fertilisation in the oviduct results in the step-wise decrease in sperm counts during transit to the oviduct. Regardless of whether or not bacterial contamination reduces semen quality, interferes with fertilisation or causes uterine infection, it is clear that “infected” semen reduces the overall success of an artificial insemination (AI) programme. Several micro-organisms identified in poultry semen include *Staphylococcus albus, Staphylococcus aureus*, *Escherichia coli*, *Proteus* spp., *Hemolytic streptococci* spp., *Diphtheroid bacilli* and *Bacillus* spp. (Sexton et al., 1980). *Enterobacter* spp. were the only bacteria isolated from the two breeds of turkeys used in this study. The use of antibiotics to prolong the survival of spermatozoa and the reduction of bacterial load has been extensively studied (Laing, 1970). *Pseudomonas aeruginosa*, *E. coli* and *Candida stellatoidea* were the isolates reported and of all the Gram positive and negative antibiotics tested on these microbes only Ciproxine, Nobactin, Nalidixic acid, Chloramphenicol, Ampicillin and Gentamycin were found to be effective on one or both microbes. Sexton et al. (1980) also reported that Gentamycin, Kanamyan, Neomycin and Tobramyan were the only antibacterial tested which controlled microbial growth in turkey semen without affecting sperm viability for up to 24 h of storage at 5°C. Sexton (1988a) found higher fertility in semen stored in Gentamycin + Minnesota Turkey Grower Association (MTGA) extender than in MTGA alone. He opined that the beneficial effect of Gentamycin is from a mechanism other than an antibacterial one. He further confirmed that antibiotics in semen extenders act by either chelating metallic cations to slow spermatozoa metabolism, removing toxic cations from solutions or maintaining the shelf life of the extender (Sexton, 1980).

The superior effect of Gentamycin was also confirmed in this study, where only Ciproxine and Gentamycin were susceptible among the Gram negative antibiotics tested on *Enterobacter* spp. It is therefore suggested that the tested antibiotics and many others could be explored further for their use and inclusion in diluents preparation for handling turkey semen in our environment.

Conclusion

From the result of this investigation on the reproductive potentials of the breed of turkeys studied, there is a clear

indication of breed differences in semen characteristics and thus reproductive potentials. The LW breed had better potentials than the indigenous breeds of turkeys in Bauchi, north east Nigeria. Irrespectively of the breed, all the turkey toms used were potentially fertile and as such their semen could be used for any AI programs in inseminating the hens. They will be effective with a guarantee of having fertile eggs which will subsequently hatch to healthy poults, all conditions being equal. The tested antibiotics could be used in the control of bacteria in semen samples of turkey within the study area.

Conflict of interest

The authors declare that they have no conflict of interest.

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