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Effect of aqueous tamarind pulp extract on semen quality and testicular morphometry of Noiler Cocks in a hot dry environment

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Target Audience: *Breeders, Farmers, Poultry Nutritionists, Researchers*

Abstract

The effect of aqueous tamarind pulp extract on semen quality and testicular morphometry of Noiler cocks in a hot dry environment was studied. Tamarind fruit was soaked at concentrations of 20, 30 and 40 g per litre of water for 24 hours and then sieved to obtain the tamarind pulp extract (TPE). Twelve Noiler cocks, 28 weeks of age and of similar initial body weight were divided into four dietary treatments in a completely randomized design with three replicates per treatment. Drinking water containing 0, 20, 30, and 40 g TPE/L was offered ad-libitum and designated as T_1, T_2, T_3 and T_4 respectively. A total of 144 semen samples were collected by abdominal massage technique and semen volume, colour, pH, motility and concentration were determined. Data on testicular morphometry and gonadal sperm reserve were also collected on all the cocks. Result showed that the semen volume was 0.22, 0.28, 0.42 and 0.40ml for control, 20, 30 and 40g/L TPE respectively. Cocks given 30 g/L TPE had the highest ($P<0.05$) mass activity (4.39) and motility (87.11%). Percentage of abnormal sperm cells was lowered by TPE. Means for testicular parameters were all similar across the dietary treatments. Paired testicular sperm reserves were 5.07, 5.08, 4.88 and 4.63 ($\times 10^9$) for 0, 20, 30 and 40g/L TPE respectively. The study concluded that aqueous tamarind pulp extract does not influence the gross testicular morphometry of cocks but exerts its effect on semen volume and sperm motility. Noiler cocks reared under a hot dry environment and given 30 g/L TPE will have improved semen quality.

Key Words: *Motility, sperm, sperm reserve, tamarind, testis*

Description of Problem

Currently, the potential of utilizing plant materials for improving reproduction in farm animals has attracted a lot of attention [1, 2, 3, 4]. Previous studies have proven the beneficial properties of onion and garlic [5], ginger [6], basil [7], cinnamon, orange peels [8], as rich sources of vitamins, flavonoids, and minerals. It was further noted by Kanaze *et al*.[8] that orange peel extract can improve sperm motility and cinnamon affects testosterone levels. Similarly, Alizadeh *et al.* [9] reported that red watermelon seed extract and orange peel extract reduced the amount of malondialdehyde (MDA) and increase the amount of superoxide dismutase (SOD). Other workers have reported that offering an aqueous solution of *Thymus vulgaris* or *Zingiber officinale* (5 or 10%) significantly increased ejaculate volume, sperm concentration, counts, movements and testes weight. They also reported a significant decrease in sperm motility and abnormalities [10]. Similarly, an aqueous extract of ginger caused an increase in the weight of the testes, ejaculate volume, sperm concentration, sperm counts and sperm movement [11].

Tamarind or *Tamarindus indica* L. of the family Fabaceae, sub-family Caesalpinioideae, is an important food in the tropics. It is a

multipurpose tree of which almost every part finds at least some use [12], either nutritionally or medicinally. Tamarind is indigenous to tropical Africa but it has been introduced and naturalized worldwide in over 50 countries [13]. According to Shindi *et al.* [14], tamarind pod contains high amount of edible pulp (55 %). Aqueous tamarind pulp extract contains sterol, terpene, saponin, citric acid, tartaric acid and malic acid [15]. Tamarind pulp possesses antimicrobial, antioxidant, and hypocholesterolemic properties, which boost growth, feed conversion efficiency and layer performance in chicks, and lowers serum cholesterol level [16]. It has also been reported that tamarind pulp has appetizing and stimulatory effect on the digestive process [17]. According to Rai *et al*. [18], *Tamarindus indica* is an ingredient in the traditional aphrodisiac formulations in Africa and India. In a six-month chronic toxicity study of aqueous tamarind pulp extract, it was concluded that tamarind pulp extract was generally safe and well tolerated.

In the tropics, a major influence on flock fertility is heat stress. A lot of reproductive changes are associated with heat stress in chickens. The reproductive ability of breeder chickens is reduced and the quality of semen produced is affected by heat stress [19]. In an *in vitro* study, it was demonstrated that *Tamarindus indica* fruit pulp had significant amount of phenolic (244.9610.1 mg GAE/extract) and flavonoid (93.962.6 mg RE/g extract) content and possessed antioxidant activities [20]. It was also reported that polyphenolic compounds in tamarind pulp extracts could reduce heat stress in broiler chickens [21]. In a study to evaluate the aphrodisiac potential and reproductive safety profile of aqueous extract of *Tamarindus indica* in male Wistar rats, it was concluded that aqueous extract of *Tamarindus indica* possessed aphrodisiac activity together with Spermatogenic potential [18].

However, there is paucity of research information on the effect of tamarind pulp extract on semen production and quality of cocks. Hence, this study was designed to determine the effect of aqueous tamarind pulp extract on semen quality and testicular morphometry of Noiler cocks in a hot dry environment.

Materials and Methods

The study was conducted at the Poultry Unit of the Teaching and Research Farm, Department of Animal Science, University of Maiduguri, Borno state, Nigeria. Maiduguri is located between latitude 11º 15^{I} and 12º north, longitude 30º 05 ^{I} and 14º east and at an altitude of 345m above sea level. It is characterized by hot and dry climate and short duration of erratic rainfall (3 – 4) months per annum and a long period of dry season. Ambient temperatures are low in December to January ranging from 15 – 19°C and high in March to June, ranging from 33 – 44^{0}C and low relative humidity ranging from 5 – 43.5% [22].

Dried tamarind fruit was purchased from a local market in Maiduguri metropolis. Tamarind fruits without the shells (pods) were soaked at concentrations of 20, 30 and 40 g per litre of water for 24 hours then sieved through cheesecloth into separate containers to obtain the tamarind pulp extract (TPE) and used for the study.

Twelve Noiler cocks, 28 weeks of age were individually weighed and divided into 4 treatment groups of similar weight in a Completely Randomized Design with three cocks per treatment, each of which served as a replicate. Drinking water containing 0, 20, 30, and 40g TPE/L was offered *ad-libitum* to groups T_1 (control) T_2, T_3, and T_4 respectively. A commercial diet (15% CP and 2800kcal/kg) was fed *ad libitum* throughout the twelve weeks experimental period. The experiment was carried during the hot dry season (March –

May).

A total of 144 semen samples (36/treatment) were collected weekly by abdominal massage technique into pre-weighed 2ml Eppendorf tubes® (Eppendorf India)**.** Semen volume was obtained as the difference between weight of eppendorf tube and sample and weight of empty tube, assuming the density of semen to be 1 g/ml as recommended by [23]. Semen colour was visually assessed immediately after collection. Semen pH was determined using a pH paper strip with a 1 - 14 calibration. Mass activity (gross motility) was determined by placing a drop of raw undiluted semen on a pre-warmed slide and covered with a slip to spread the semen into uniform thickness and viewed under microscope at 100× magnification. Motility was estimated by subjectively assessing the wave pattern and ranked according to standard method [42]. Individual sperm motility was examined at a magnification of 400×. Several fields were examined and an estimate to the nearest 10 % of motile sperm was made. Percentage live/dead and normal/abnormal spermatozoa were evaluated using the eosin/nigrosin staining procedure [23]. The morphologically abnormal spermatozoa were also estimated on the same smears by counting 200 spermatozoa in different microscope fields. Semen concentration was determined with the improved Neubauer haemocytometer using the direct cell count method [23].

At the end of the experiment, all the cocks were slaughtered and testicles carefully removed for gross morphological studies. Testes collected were trimmed of all adhering fat and tissues and weighed to the nearest 0.01g using an electronic scale. Testis length and width were measured using a digital vernier calliper to the nearest 0.01 mm. Volume was obtained using Archimedes principle [24]. Testicular sperm reserve was determined as described by [25].

Analysis of variance (ANOVA) was carried out on data using the General Linear Model of Statistix 9.0. Significant means were separated using the Least Significant Difference (LSD) method of the same statistical software.

Results and Discussion

Table 1 shows the effect of aqueous tamarind pulp extract on semen quality of Noiler cocks. Semen volume was 0.22, 0.28, 0.42 and 0.40 ml for 0, 20, 30 and 40 g/L TPE respectively. Cocks in 30 and 40g/L TPE groups had higher semen volume than those in the control group and T2 (20 g/L TPE). The result showed that the more the quantity of tamarind pulp extract, the more the semen volume. This is an indication that tamarind pulp extract tends to improve semen volume in cocks under a hot dry environment. This may be due to the antioxidant activities of tamarind pulp [20] in alleviating the effect of heat stress. Semen volume was lower than 0.61 – 0.68 ±0.005 ml reported [26] for Noiler cocks in the guinea region of Nigeria. The difference may be attributed to environmental temperature since the semi-arid region is characterized by high temperature. It has been reported that in heat stressed cocks, the synthesis of testosterone is defective which lowers semen production [19].

Table 1: Effect of aqueous tamarind pulp extract on semen quality of Noiler cocks in a hot dry environment

Parameter	T_1 0g/L TPE	T_2 20g/L TPE	T_3 30g/L TPE	T_4 40g/L TPE	SEM
Volume (ml)	0.22^{b}	0.28^{b}	0.42^{a}	0.40^{a}	0.05
pH	6.91^{a}	6.57^{b}	6.74^{ab}	6.91^{a}	0.08
Colour	White	Cream	Cream	Cream	NA
Sperm motility (%)	72.61^{b}	65.34^{b}	87.11^{a}	64.78^{b}	6.61
Mass motility	3.61^{b}	3.14^{b}	4.39^{a}	3.36^{b}	0.37
Total motile sperm/ml ($\times 10^9$)	1.89^{a}	1.67^{ab}	1.50^{ab}	1.28^{b}	0.30
Concentration/ml ($\times 10^9$)	2.60^{a}	2.45^{ab}	1.70^{c}	1.94^{bc}	0.33
Concentration/ejaculate ($\times 10^9$)	0.56	0.80	0.76	0.76	0.17
Live sperm cells (%)	82.67^{ab}	73.67^{bc}	84.94^{a}	72.50^{c}	5.06
Total Live Sperm/ml ($\times 10^9$)	2.23^{a}	1.76^{ab}	1.16^{b}	1.39^{b}	0.31
Abnormal sperm cell (%)	1.75^{ab}	2.06^{a}	1.17^{b}	1.11^{b}	0.41

[a, b, c]Means within the same row with different superscripts differ significantly ($P<0.05$); SEM: standard error of mean; NA – Not analysed; TPE: Tamarind Pulp Extract

In a similar study, Nuhu *et al*. [4] reported an increase in semen volume when aloe vera gel was offered to Noiler cocks. In a similar study in Egypt, Ezzat *et al.* [6] reported that feeding cock dried ginger rhizome increased semen volume. Semen volume for all the groups was however, within the normal range of 0.2 – 0.5 ml [27], although the control group was on the lower limit.

The semen of cocks in 20g/l TPE group was more acidic ($P<0.05$) compared to the other groups. The pH in this study was lower than the range 7.17 -7.30 reported by Amur *et al*. [26]. However, rooster sperm cells can have a pH range of 6.0 - 8.0 [28]. Low pH reduces motility, lactic acid production and oxygen whereas high pH increases metabolic rates *in vitro* [29]. Cocks offered tamarind pulp extract had cream coloured semen compared to the control which was white. The colour of semen is generally an indication of density of ejaculate. This means tamarind extract tends to support high sperm density. Tamarind pulp extract significantly ($P<0.05$) influenced mass activity and sperm motility. Cocks on 30g/L tamarind pulp extract had the highest mass activity (4.39) and motility (87.11%) compared to the other treatment groups which had similar means. Birds on 40g/L TPE had significantly ($P<0.05$) lower total motile sperm than those in the control group. While cocks given 20 g/L and 30g/L TPE had means that were similar to both control and 40g/L TPE. This confirms the observation of Rai *et al*. [18] who reported a significant increase in sperm motility for rats that received tamarind pulp extract at 250 mg/kg compared to normal control. The improved motility of birds offered 30g/L TPE may be related to the high antioxidative properties of TPE. The sperm plasma membranes contain a high amount of unsaturated fatty acids. Therefore, it is particularly susceptible to peroxidative damage, the lipid peroxidation destroys the structure of the lipid matrix in the membranes of spermatozoa, and it is associated with a loss of motility and membrane integrity [30]. The use of other phytogenic substances with potential antioxidant properties has been shown to improve motility both *in vivo* and *in vitro* [2, 4, 5, 31- 33].

Sperm concentration was lowest for cocks on 30g/L TPE although, it was not different from 40g/L TPE. Sperm concentration per

ejaculate was however similar among all the treatment groups although the tamarind groups had higher actual counts. Cocks in the control group had more ($P<0.05$) total live sperm than those given 30g/L TPE and 40g/L TPE while the group offered 20g/L TPE was similar to all the other groups.

Percentage live sperm cells were similar for the control group and those on 30g/l TPE but lowest in the group given 40g/L TPE. Percentage of abnormal sperm cells was lowered by TPE with cocks in the 30 g/L and 40g/L TPE having the lowest means. This corroborates reports of lowered abnormalities in sperm cells when ginger [11; 6], aloe vera gel [34] and dried tomato pomace [35] were fed to cocks.

The effect of aqueous tamarind pulp extract on live weight and testicular morphometry of Noiler cocks in a hot dry environment is presented in Table 2. Live weight of the cocks was significantly ($P<0.05$) influenced by dietary treatments. Cocks offered 40g/L TPE had higher body weight than all the other treatment groups. This is similar to observations in broiler chickens where TPE improved body weight [36, 37]. The live weight values in this study were similar to those (3.43 – 3.83kg) reported by Amur *et al*. [38] for Noiler cocks.

Table 2: Effect of aqueous tamarind pulp extract on Testicular morphology of Noiler cocks in a hot dry environment

Parameter	T_1 0g/L TPE	T_2 20g/L TPE	T_3 30g/L TPE	T_4 40g/L TPE	SEM
Live weight (g)	3183.00^b	3033.00^b	3233.00^b	3633.00^a	157.23
Paired testicular weight (g)	39.06	39.75	46.31	42.32	8.25
Left Testis weight (g)	22.10	20.62	22.95	21.35	3.94
Right testis weight (g)	17.02	19.22	23.45	21.02	5.58
Left testis length (mm)	52.27	49.95	54.81	51.48	3.63
Right testis length (mm)	48.46	51.99	52.40	56.06	7.57
Left testis width (mm)	27.34	26.51	27.71	29.50	1.61
Right testis width (mm)	24.54	26.83	29.65	27.44	2.59
Left testis volume (ml)	21.33	20.67	21.00	20.67	4.26
Right testis volume (ml)	15.00	18.33	22.33	20.67	5.95
Paired testis volume (ml)	36.33	39.00	43.33	41.33	8.24
Gonadosomatic Index (%)	1.24	1.31	1.42	1.17	0.25
Paired testis density (g/ml)	1.08	1.03	1.06	1.03	0.05
Left testis density (g/ml)	1.03	1.02	1.10	1.03	0.07
Right testis density (g/ml)	1.19	1.05	1.05	1.03	0.11

$^{a, b, c}$Means within the same row with different superscripts differ significantly ($P<0.05$); SEM: standard error of mean; TPE: Tamarind Pulp Extract

According to Olarotimi and Adu [25], testicular parameters such as weight and length, are usually used in assessing their normality, thus, improving the detection of any deviation from normal that might result during the experimental process. The testicular weight, length, width, volume, gonadosomatic index and testis density were all similar across dietary treatments. This is an indication that TPE does not affect the gross morphometry of chicken testes. This is similar to the observation by Shinkut [39] that no significant differences existed in mean gonadal/testicular weight and length of rabbit bucks fed graded levels of garlic. Most of the testicular morphological parameters measured in this

study were higher than those reported (paired testicular weight 32.61- 37.00g, paired testicular volume 29.95 41.07ml, right testicular length 39.81 – 42.10mm) by Amur *et al*.[38] despite the similarity in body weight of the cocks except for testicular width and volume which were similar. The gonadosomatic indices obtained in this study were slightly higher than the 1.1 % reported by Chidozie *et al.* [40] for mature Nigerian local cocks. The higher values may be because the Noiler cock is an improved strain of the local cock. Gonadosomatic index is an expression of gonad weight as a percentage of total body weight; used as an indication of sperm production efficiency [24, 41]. The high gonadosomatic index recorded in all the treatments is a clear indication the Noiler cocks are efficient in spermatogenesis.

Table 3 shows the effect of Tamarind pulp extract on gonadal sperm reserve of Noiler cocks. Tamarind pulp extract significantly (P<005) influenced the right and paired testicular reserves but had no significant influence on the left testicular reserve. Sperm reserve in the right testis of the control was higher than that of cocks given 30 and 40g/L TPE but it was similar to those offered 20g/L TPE. While the paired sperm reserve was lowest for cocks on the highest level of tamarind pulp extract, although it was not significantly different from the means in the group offered 30g/L TPE. The trend observed seem to show a dose dependent reduction in sperm reserve as TPE level increases. This is contrary to the observation of [43] who noted an increase in sperm reserve of the left testis of rabbits with increase in garlic dose. The difference in observation may be due to species difference.

The range (4.63 – 5.08 $\times 10^{9)}$ of sperm reserve recorded in this study is higher than 2.11 x 10^9 reported by [44] for local cocks in Maiduguri but lower than 76.47 - 82.18 x 10^9/g testis reported by [45] for Anak 2000 broiler breeder cocks. The differences observed may be because the Noiler cocks are hybrids of the local and improved chickens.

Table 3: Effect of aqueous tamarind pulp extract on Testicular sperm reserve of Noiler cocks in a hot dry environment

Parameter	T_1 0g/L TPE	T_2 20g/L TPE	T_3 30g/L TPE	T_4 40g/L TPE	SEM
Left testis sperm reserve/testis (×10^9)	2.04	1.92	1.77	1.64	0.24
Right testis sperm reserve/testis (× 10^9)	2.17^a	1.86^{ab}	1.81^b	1.64^b	0.16
Paired testis sperm reserve (× 10^9)	5.07^a	5.08^a	4.88^{ab}	4.63^b	0.14

$^{a, b, c}$Means within the same row with different superscripts differ significantly (P<0.05); SEM: standard error of mean; TPE: Tamarind Pulp Extract

Conclusion and Applications

1. Aqueous tamarind pulp extract (TPE) does not influence the gross testicular morphometry of cocks.
2. However, its effect influenced semen volume, sperm motility and gonadal sperm reserve.
3. Noiler cocks reared under a hot dry environment and given up to 30g/L Tamarind pulp extract (TPE) will have improved semen quality.

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