NIGERIAN VETERINARY JOURNAL
ISSN 0331-3026
Nig. Vet. J., September 2019 Vol 40 (3): 201 - 210.
https://dx.doi.org/10.4314/nvj.v40i3.4
ORIGINAL ARTICLE

Optimal nutrition, a key to addressing Reproductive Performance in Nigerian Local Turkey Toms

Yahaya, M. S.[1*]; Nwannenna, A. I.[2]; Fadason, S.T.[3] and Rekwot, P.I.[4]

[1]Department of Theriogenology and Animal Production, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.
[3]Department Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria.
[4]National Animal Production Research Institute, Ahmadu Bello University, Zaria, Nigeria
*Corresponding author: Email: amsyzur@yahoo.co.uk, Tel No: +234 8069796802

SUMMARY

The Nigerian local turkey has the potential to augment the supply of poultry protein in the country and across the region. However, the fecundity of the breed is low due to neglect and lack of improvement. This work is therefore aimed at shedding some light in some reproductive indices of the local turkey under optimum nutrition. A group of fifteen toms and nine hens were used in this study. The males were grouped into three groups of five and placed on varying levels of protein, 12% CP, 16% CP and 20% CP for groups 1, 2 and 3 respectively. Semen samples were collected and analysed twice weekly for thirteen weeks. Ejaculate volume, semen concentration, semen PH, gross and individual motilities, live and dead sperm and sperm morphology were investigated and recorded. Data were summarized as mean ± SEM (Standard Error of the Mean). The toms in groups 3 had significantly ($P < 0.05$) higher ejaculate volume 0.29 ± 0.03 mls and semen concentration7.766 ± 0.612 $x10^9$ than groups 1 and 2. The fertilizing ability, which was assessed through *in vivo* and *in vitro* sperm penetration assays revealed significantly higher number of sperm penetration holes ($P < 0.05$) in Groups 2 and 3, 160.97 ± 8.084 and 172.83 ± 7.647 (*in vivo)*; 187.96 ± 8.121 and 189.16 ± 6.446 (*in vitro)* respectively. The local turkey toms could parallel their exotic counterpart under optimum environment, without the need for genetic hybridization and that 20% CP had more positive influence on the semen quality and fertilizing ability of indigenous Nigerian turkey toms followed by 16% CP with 12% CP exerting the least positive influence.

Key words: Artificial Insemination, Crude protein, Reproduction, Semen, Turkey toms

INTRODUCTION

Animal protein supply and intake is still grossly inadequate in the developing countries, especially the sub-Saharan Africa (Tacher *et al*., 2000; FAO, 2011). The situation is usually attributed inferior genetic makeup of the local breeds or non-

selection (Bindari *et al*., 2013), although harsh environment and poor husbandry practices among other factors, may be more important bottlenecks (Ladokun *et al*., 2006). To address these problems some breeders usually resort to anthropogenic hybridization between local and exotic breeds, which are thought to be of superior genetic makeup, the result is not always favourable as it leads to loss of local genetic resources (van Wyk *et al*., 2017) usually due to production of maladaptive hybrids (Todesco *et al*., 2016) or even sterile F1 offspring, which would not be able to reproduce (Mideksa, 2017).

Turkeys, like other poultry species, have short generation interval and so the integration of the local turkeys into the conventional commercial poultry production is likely to play a role in supplementing the provision of animal protein (Ben Sassi, Averós, & Estevez, 2016) ().

The contrast in the size of turkey toms and hens and consequent low fertility of the males after natural mating has resulted in the integration of Artificial Insemination (AI) in commercial production. AI has therefore been a critical component of reproduction in turkeys since the 1960s and is used almost exclusively for commercial flock production in some areas of the world(Bakst and Dymond, 2013). Reproductive performance is critical to efficient production in poultry(McDaniel *et al*., 1998). Success in AI requires quality semen which has the capacity to reach the site of fertilization, fertilize the egg and activate embryonic development. The local turkey is kept as a scavenger and thus may not be able to compare well with its exotic counterparts in terms of reproductive capacity. Optimum nutrition, which is a strong environmental factor affecting animal's productivity both in terms reproductive capacity and production traits(Cordova Izquierdo, 2015; Rekwot *et al*., 1994; Papazyan *et al*., 2006; Jibril *et al*., 2011), and which is usually in short supply for the breed may be used to investigate its performance under conducive environmental condition. The present study therefore intended to determine the effect of varying crude protein levels on semen quality.

MATERIAL AND METHODS

Study Area

The study was conducted in the Department of Theriogenology and Production, Ahmadu Bello University, Zaria, situated in the Northern Guinea Savannah, between latitudes 11º 1573' N and between longitude 7º 64989' E at an elevation of 646 m above sea level. The mean annual rainfall in the area is 1100 mm lasting from May to October (816mm/month). Mean daily temperatures during the wet season are 25ºC and mean relative humidity of 72%. The dry season lasts from November to April,the mean daily temperature ranges from 14 to 36ºC and the relative humidity 20-30% (Anon,. 2014).

Animal feed and its composition

The feed used for this study was formulated in collaboration with the Department of Animal Science, Faculty of Agriculture, Usmanu Danfodiyo University Sokoto, while compounding and proximate analysis were done at the National Animal Production Research Institute (NAPRI) Shika, Ahmadu Bello University, Zaria.

Experimental Design

Using G power version 3.1 and Altman's Normogram, at power of 85% and effect size of 0.8 a minimum of 15 animals was arrived at. Therefore fifteen (n = 5) apparently healthy turkey toms (age = 30-32 weeks, and live weight of 3.5 – 4.0 kg) and nine (n = 9) apparently healthy (age 24-26 weeks) were used in the study. The turkey toms were randomly placed in three groups (Group1, Goup2 and Group3) of five and were tagged appropriately. They toms received 400g/bird /day of feed containing different levels of crude protein, viz: 12% CP, 16% CP and 20% CP for Groups 1, 2 and 3 respectively. The hens were placed in three groups of three and were placed on conventional chicken layer mash, 16% CP.

The live weights of all the turkey toms were determined weekly throughout the period of the study. They were caged individually and acclamatised for a period of two weeks during which the toms were trained to produce semen on abdominal massage.

Semen Collection and Evaluation

Semen samples were collected twice weekly (two ejaculates per collection), by a modification of the method of Baskt and Long (2010), from the toms in each group and evaluated for; volume, colour, motility (Gross and Individual), concentration, percentage live and dead cells and morphology.

Volume

The semen was collected into a 1 mL graduated tube and the volume was recorded for each tom.

Mass Motility

Microscopic examination for wave pattern (gross sperm motility) was determined as described by Baskt and Long (2010), by placing a drop of raw undiluted semen on a pre-warmed slide and cover-slipped, it was viewed using a microscope at x4 and x10 objectives and mass motility was estimated.

Individual motility; This was determined using diluted semen samples. Semen samples were diluted to ensure single layer of sperm cells for ease of counting. This was achieved by adding 3 drops of Dulbecco's Modified medium to one drop of raw semen. A drop of diluted semen was then placed on a grease free microscope slide and viewed at x40 objective. Sperm cells progressively moving forward were counted as percent motile cells.

Concentration; Semen dilution: Neat semen was pre-diluted 1:2 with Egg-yolk-Citrate extender. Ten (10) µL of the extended semen was diluted in 10 mL of 3% NaCl, ten (10) µL of diluted semen was used to fill one chamber of haemocytometer and the spermatozoa were counted in five Thoma squares of the chamber (i.e. four corners and the center squares). The concentration was determined by the following equation;

Concentration (sperm cells/mL) = Number of sperm cells counted in the twenty-five small squares x dilution factor x 10^4.

Live sperm count; this was determined as described by Esteso *et al*., (2006) with modification. A drop of semen sample was placed at the edge of a clean grease free glass slide and three drops of eosin-nigrosin stain were mixed with the semen. A smear was made from the mixture and allowed to dry. Four different counts were obtained using light microscope at X100 objective (oil immersion). The nigrosine-eosin solution used in this work contained potassium citrate 1.280g, sodium glutamate 1.7351g, sodium acetate 0.851g, magnesium chloride 0.686g, eosin 1.0008g and nigrosine 5.0002g (Łukaszewicz *et al*., 2008).

Spermatozoa morphology: Morphology was determined by making a thin smear from a mixture of the semen sample and eosin-nigrosin on clean grease free glass slide. Four hundred sperm cells were counted per slide using light microscope at X100 magnification (oil immersion) (Esteso *et al*., 2006).

Sperm Penetration (SP) assay (in vivo)

Inseminating Hens

All hens were inseminated weekly according to the method described by Sotirov, (2002). The hens were turned upside down, pressure applied to the right side of the abdomen until the vent everted (venting) and an insulin syringe containing the fresh diluted semen which was obtained from the toms was inserted to a depth of about 1.5-2 cm into the cloaca, and semen containing 100 $x10^6$ spermatozoa was deposited in the vagina.

Number of sperm cells inseminated: A the start of the insemination the average semen concentrations was $5x10^9$ for Group 1, and $6x10^9$ for Groups 2 and 3 respectively.

In Group 1 100 µl was diluted in 2.5ml of Dulbecco's Modified Eagles Medium (DMEM), each hen was thereafter inseminated with 0.5ml of the diluted semen. In Group 2 and 3, 83µls were used instead.

In Vivo Assay

Sperm penetration assay was carried out according to the method described by Baskt and Long (2010). The oviposited eggs (from inseminated hens) were broken and the albumen separated from the yolk. The yolk was then placed in a pan with the blastoderm positioned upwards. Excess albumen was removed by blotting with Kim wipe and 2% NaCl solution was added; an area of 3.0 mm^2 of the perivitelline layer over the blastoderm was carefully cut and immediately rinsed in phosphate buffered saline to remove excess yolk material. The perivitelline layer was then placed on a glass slide and 4 drops of 3% formalin were added to fix the membrane and immediately decanted. The perivitelline layer was finally stained with Schiff's reagent. The holes were then counted under Datyson Biological microscope at a magnification of X 40.

Sperm Penetration (SP) Assay (in vitro)

This is an *in vitro* sperm quality assay in which spermatozoa were co-incubated in a test tube with inner perivitelline layer (IPL) from fresh unfertilised eggs. The number of sperm holes per unit area of the IPL were then viewed under dark-field optics and quantified as a measure of sperm activity. The IPL samples for the assay were obtained from fresh eggs sourced from poultry layer unit of the National Animal Production Research Institute (NAPRI). They were separated from the outer perivitelline layer (OPL) by acid hydrolysis as described by Baskt and Long (2010). Briefly the procedure is described below.

Separation of IPL from OPL:

This was done as follows:

The eggs were cracked open over a waste beaker retaining the yolk in one half of the shell.

The albumen was thoroughly decanted and the yolk isolated.

The isolated yolk was placed in a small dish and washed several times with 1% NaCl to remove any excess albumen. The adherent pieces of albumen were removed using blunt-ended forceps.

The yolk was placed the yolk in a 100-mL beaker containing 75 mL of 0.01 M HCI and the beaker placed in a 37 ºC incubator for 1 hour.

After 1 hour, the beaker was removed from the incubator and the HCI was decanted taking care not to burst the yolk. The yolk was then transferred to a small bowl and the perivitelline layer (PL) bursted from the side with the aid of sharp forceps and the PL isolated.

The isolated PL was then placed in a petri dish containing 1 % NaCI and washed in several changes, until all yolk particles were removed; and the PL was then spread out in a petri dish, containing fresh 1% NaCI. The IPL was distinguished from the OPL by its thinner, more transparent, appearance. The two layers were then separated using sharp forceps and the isolated IPL was stored in a petri dish containing NaCI-TES at 5°C for the sperm egg assay (Bakst and Long, 2010).

Co-incubation of Sperm and IPL

1) Ten (10) µl of fresh semen were diluted in 1000 µl of NaCI-TES for Group 1 and 8µl parts of fresh semen was diluted in 1000 µl parts of NaCI-TES for Groups 2 and 3; the aliquots were stored at 40 ºC in a shaking water bath for 45 min before assay.

2) A fragment of the isolated IPL approximately 0.5 cm x 0.5 cm square was cut and added to a vial containing 100 µl of NaCl-TES diluted semen in 1 ml Dulbecco's Modified Eagle Medium (DMEM). The mixture was incubated at 40°C for 5 min in a shaking water bath.

3) The incubated IPL was removed from the vial and washed in 1% NaCI.

4) The IPL was carefully spread onto a microscope slide using forceps avoiding wrinkles and then cover-sliped .

5) The slide was then viewed immediately using dark phase microscopy at X 10 and X 40 objectives.

6) The number of holes per 3 mm^2 were counted according to the method described by Baskt and Long (2010) with modifications.

RESULTS

Semen analysis

The results (mean ± SEM) of the semen analysis (semen volume, concentration, motility, live sperm, pH and morphology) for the three groups of toms are presented in table 2. Table 2 shows the preliminary results before treatment began.

Semen volume

The value (mean ± SEM) of the semen (ejaculate) volumes of the three groups are shown on TABLE II Significant difference was observed between Group 1 and Group 2 ($P < 0.05$), there was also a significant difference between Group 1 and Group 3 ($P < 0.05$) but no significant difference was observed between Group 2 and Group 3 ($P > 0.05$).

Semen Concentration

TABLE II presents the results (mean ± SEM) of the semen concentrations of the three groups. There was significant difference between Group 1 and Group 2 ($P < 0.05$). There was also a significant difference between Group 1 and Group 3 ($P < 0.05$) but no significant difference was observed between Group 2 and Group 3 ($P > 0.05$).

Sperm motility

The results (mean ± SEM) of the mass and individual motilities are presented on table 2. For both parameters, significant difference was observed between Group 1 and Group 2 and also between Group 1 and Group 3 ($P < 0.05$). There was no significant different between Group 2 and Group 3 for both parameters ($P > 0.05$).

Live sperm

TABLE II presents the results (mean ± SEM) of the live sperm. There was significant difference between Group 1 and Group 2 in both the number (percentage) of live and that of the dead cells ($P < 0.05$), there was also a significant difference between Group 1 and Group 3 ($P < 0.05$) but no significant difference was observed between Group 2 and Group 3 in both collections ($P > 0.05$).

TABLE I. Chemical composition of feeds

(%)	12% CP	16% CP	20% CP
Energy(kcal/kg)	2802.69	2837.01	2825.57
Protein (%)	12.10	15.99	20.01
Lysine (%)	0.60	0.62	0.79
Methionine (%)	0.21	0.25	0.28
Calcium (%)	1.37	1.39	1.41
Phosphorous (%)	0.38	0.38	0.39
Fibre (%)	3.61	3.81	4.22
Ether Extract (%)	3.80	4.05	4.28

Semen PH and Sperm Morphology

The results (mean ± SEM) of the PH and sperm morphology are shown on TABLE II. In both parameters, there was no significant difference between all the three groups ($P > 0.05$).

Sperm Penetration Assay

The numbers of holes (mean ± SEM) of the *in vivo* and *in vitro* sperm penetration assay are presented in TABLE IV Significant ($P < 0.05$) differences were observed between groups 1 and 2; and between groups 1 and 3, but the results showed no significant ($P > 0.05$) difference between groups 2 and 3. The number of holes (mean ± SEM) of the *in vitro* sperm penetration assay are $148.88^{a} \pm 9.16$, $187.96^{b} \pm 8.121$ and $189.16^{b} \pm 6.46$ for groups 1, 2 and 3 respectively. The results revealed significant ($P < 0.05$) difference between groups 1 and 2; and, groups 1 and 3, but the difference between groups 2 and 3 was not significant.

The weekly trend observed for the semen volumes and semen concentration of the three groups is presented in figures 1 and 2 respectively. The trends revealed significant ($P < 0.05$) difference between groups 1 and 2, and between groups 1 and 3 but there was no significant ($P > 0.05$) difference between groups 2 and 3.

TABLE II. Preliminary results of semen parameters before treatment

Parameters	Group 1 (12% CP) n = 5	Group 2 (16% CP) n = 5	Group 3 (20% CP) n = 5
Live weight (kg)	4.38 ± 0.24	4.43 ± 0.18	4.32 ± 0.23
Semen Volume (mLs)	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Ph	6.88 ± 0.37	7.08 ± 0.19	7.25 ± 0.20
Mass motility (%)	47.20 ± 2.39	52..50 ± 3.68	55.31 ± 3.99
Individual motility (%)	54.23 ± 3.73	59.48 ± 5.03	59.38 ± 2.99
Semen Concentration (x10^9)	2.99 ± 0.49	3.23 ± 0.78	3.03 ± 0.86
% Live sperm (%)	55.50 ± 5.75	60.31 ± 5.16	61.25 ± 5.23
Total Defects (%)	35.43 ± 2.57	30.57 ± 1.85	32.49 ± 2.01

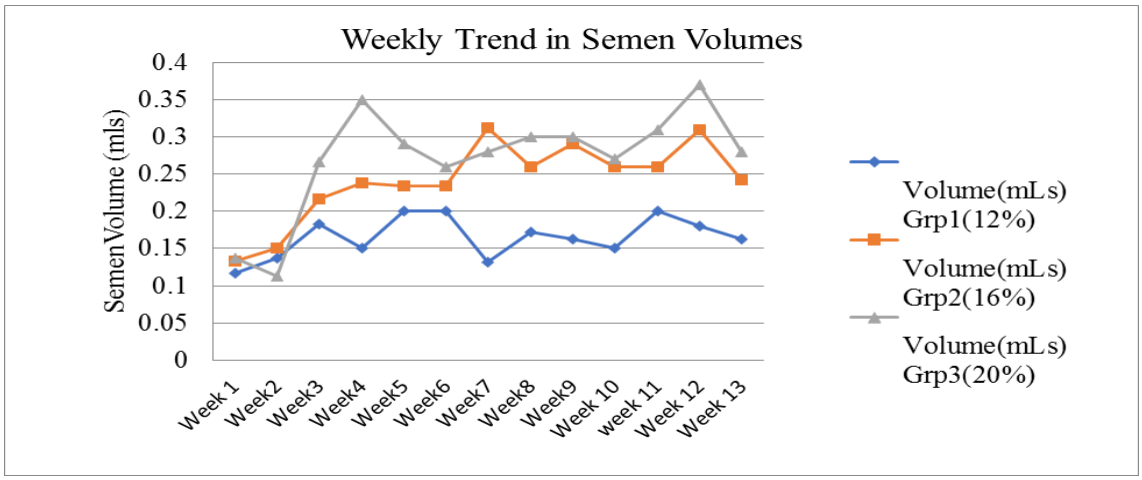

Figure I. The weekly trend in semen volumes of turkey toms fed varying levels of protein diets.

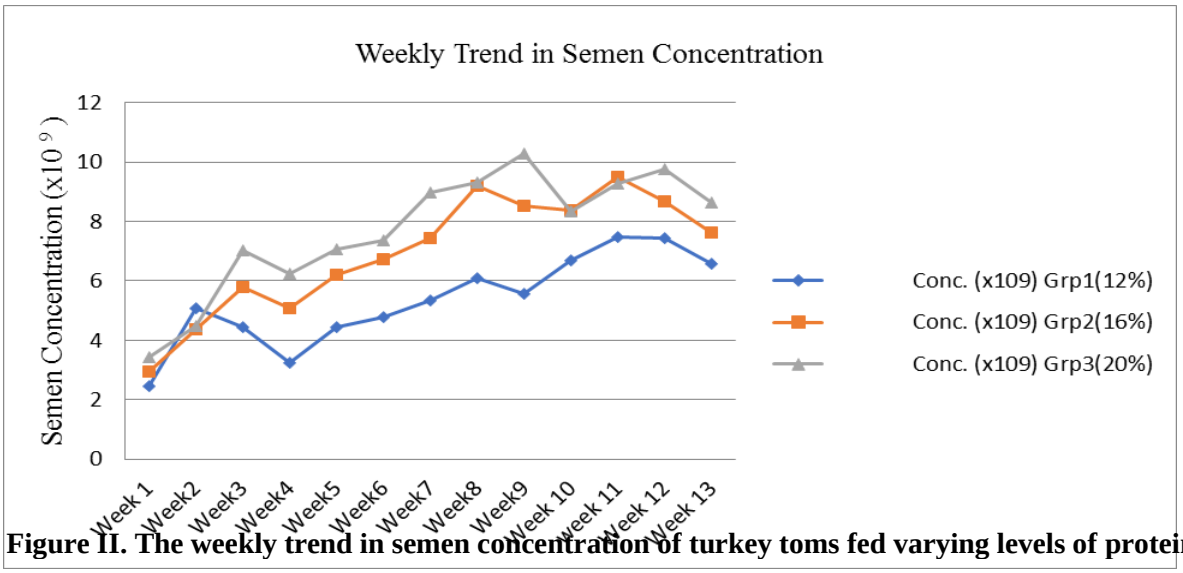

Figure II. The weekly trend in semen concentration of turkey toms fed varying levels of protein

DISCUSSION

Three different quality tests were employed to assess the suitability of the local turkey tom's semen for AI at different CP levels; crush side semen evaluation, *in vitro and in vivo* sperm penetration assays. The results from the crush side tests show that semen volume, concentration, motility, morphology and live-dead proportions, both increased by between 57% and 163% when compared with the preliminary results. When compared within the treated groups the response was higher in group 3 (20% CP) followed by group 2 (16% CP) with group one (12% CP) having the least scores. We have not found significant difference between Group 2 (16% CP) and Group 3 (20% CP), perhaps because the optimal protein level lies between the two group. However, significant difference was found between group1 and Group 2, and between Group 1 and Group 3. This is so perhaps because the treatment for group 1 has fallen short of the optimal, some past, works have shown similar protein effects on these parameters. (Jibril *et al*., 2011) in rams, has reported higher reproductive performance in lower CP level, which is thought to be the optimum, than in higher CP levels. The results are similar to the reports of various authors, TABLE III. Semen parameters and live weights of turkey toms fed varying level of protein diets.

P < 0.005 Values with different superscripts (across rows) differ significantly working with various animals; (Rekwot *et al*., 1987 and Rekwot *et al*., 1988 in bulls; (Louis *et al*., (1994) in boars; (Ladokun *et al*., (2006) in pubertal rabbit bucks; Ghonim *et al.*, (2010) in Drakes who reported better performance in animals fed higher CP levels than those fed lower CP levels. It also agrees with the work of Sotirov *et al*., (2002) who reported higher ejaculate volume in turkeys fed 17% CP than in their counterparts fed 14% CP. These therefore support the fact that for optimal performances, animals require optimal environmental conditions which include nutrition. Although parameters like the ejaculate volumes obtained in Group 1(12% CP) was the similar as

TABLE III. Semen parameters and live weights of turkey toms fed varying level of protein diets.

Parameters	Group 1(12% CP) n = 15	Group 2(16% CP) n = 15	Group 3(20% CP) n = 15
Live weight (kg)	5.29 ± 0.25 (0.559)	5.39 ± 0.20(0.447)	5.63 ± 0.22(0.449)
Semen Volume (mls)	0.17^{a} ± 0.01(0.022)	0.22^{b} ± 0.02(0.045)	0.29^{c} ± 0.03(0.067)
pH	6.86 ± 0.06(0.134)	7.03 ± 0.06(0.134)	6.85 ± 0.07(0.157)
Mass motility (%)	73.01^{a} ± 1.34(2.996)	79.09^{b} ± 1.36(3.041)	80.00^{b} ± 1.19(2.661)
Individual motility (%)	80.18^{a} ± 1.29(2.884)	85.58^{b} ± 1.26(2.817)	85.39^{b} ± 1.12(2,504)
Semen Concentration ($x10^{9}$)	5.33^{a} ± 0.43(0.961)	6.90^{b} ± 0.56(1.252)	7.77^{c} ± 0.61(1.363)
% Live sperm (%)	76.81 ^{a}± 1.36(3.040)	81.14^{b} ± 1.32(2.951)	82.80^{b} ± 1.12(2.504)
Total Defects (%)	18.43 ± 1.07(2.392)	17.31 ± 0.99(2.213)	16.89 ± 0.80(1.788)

P < 0.005 Values with different superscripts (across rows) differ significantly

TABLE IV. Mean number of sperm penetration holes for *in vivo* and *in vitro* assays using semen from turkey toms fed varying levels of protein diets.

Sperm Penetration Holes (/3 mm^2)			
	Group 1(12% CP)	Group 2 (16% CP)	Group 3 (20% CP)
No. of Animals	(n=5)	(n=5)	(n=5)
In vivo	136.80^{a} ± 4.150(9.28)	160.97 ^{b}± 3.613(8.08)	172.83^{b} ±3.421(7.65)
In vitro	148.88^{a} ± 4.096(9.16)	187.96 ^{b} ± 3.631(8.12)	189.16^{b} ± 2.884(6.45)

those reported by Zahraddeen *et al*., (2005) in local in the same breed, which were also fed suboptimal (10%) crude protein, they were much lower the ranges reported by (Dukes and Swenson (1984), Wishart, (2007) and Christensen, (2005) for exotic breeds. However, the volumes obtained in Group 2 (16% CP) and Group 3 (20% CP) were within the range given for the exotic breeds and was higher than the 0.17 ± 0.02 mls reported by Zahraddeen *et al*., (2005) in local breeds. The differences observed between the values in this work and the values reported by Zahraddeen *et al*., (2005) in the same breeds indicates clearly that the local turkey's reproductive performance improves with increased CP up to an optimum.

Sperm penetration assay shows significant result for samples from groups 2 and 3 than those in group 1. This has indicated that increase in the amount of protein in the diet to an optimum, improved the quality of sperm cells produced by group 2 and 3. Although the SP assay is an indirect test of fertility, it correlates positively with fertility and hatchability of eggs following insemination. Al-Daraji, (2000) reported superiority of roosters based on their higher SP score than others.

Weekly trends show net increase in semen volume and concentration, from the start to finish of the experiment, they also reveal the consistency in the measurements employed for all the groups. For each group and at the CP level measured, the confidence interval can be seen to be narrow, as indicated by the various standard errors (SE).

Because protein was the only item varied in the feeds given to the toms, this is suggestive of the fact the higher the level of CP in the diet the better the fertility up to an optimum. This is in line several other reports (Zambrano *et al*., 2005; Begum, *et al*., 2009; Wu *et al*., 2009). Because of the results obtained in this work we believe that the local turkey can be improved not necessarily by hybridization with exotic breeds but through the provision of optimum nutrition and good husbandry and recommend a more robust research to determine the actual optimum level of other feed ingredients for the local turkey tom. Before then we recommend the feeding of 16% - 20% CP based diet.

REFERENCES

Al-Daraji, H. J. (2000). Sperm-egg penetration in laying breeder flock: a technique for the perdiction of fertility. *British Poultry Science*, *42*(1), 266–270.

Bakst M. R. and Dymond, J. S. (2013). Artificial Insemination in Poultry. *Success in Artificial Insemination - Quality of Semen and Diagnostics Employed*, 722–723. https://doi.org/10.1079/WPS19560016

Begum, H., Moniruddin, A.B.M. and Nahar, K. (2009). Environmental and nutritional aspect in male infertility. *Journal of Medicine*, *10*(1), 16–19.

Ben Sassi, N., Averós, X., & Estevez, I. (2016). Technology and Poultry Welfare. *Animals : An Open Access Journal from MDPI*, *6*(10). https://doi.org/10.3390/ani6100062

Bindari, Y. R., Shrestha, S., Shrestha, N., & Nath Gaire, T. (2013). Effects of nutrition on reproduction-A review. *Advances in Applied Science Research*, *4*(1), 421–429. Retrieved from www.pelagiaresearchlibrary.com

Christensen, V. L. (2005). Artificial insemination (poultry). In C.M Kalm and S. Line (Ed.), *Mark Veterinary Manual* (9th ed.). New York, NY: Merk Inc.

Cordova Izquierdo, A. (2015). Best Practices in Animal Reproduction: Impact of Nutrition on Reproductive Performance Livestock. *Advances in Dairy Research*, *04*(01), 1–4. https://doi.org/10.4172/2329-888X.1000152

Dukes, H. H. (Henry H., & Swenson, M. J. (1984). *Dukes' Physiology of domestic animals*. Comstock Pub. Associates.

Esteso, M. C., Soler, A. J., Fernández-Santos, M. R., Quintero-Moreno, A. a, & Garde, J. J. (2006). Functional significance of the sperm head morphometric size and shape for determining freezability in iberian red deer (Cervus elaphus hispanicus) epididymal

sperm samples. *Journal of Andrology*, *27*(5), 662–670. https://doi.org/10.2164/jandrol.106.000489

FAO. (2011). *Mapping supply and demand for animal-source foods to 2030*. (T. P. R. & F. Pozzi, Ed.) (Vol. 14). https://doi.org/10.1287/opre.1080.0628

Jibril, A., Atte, I. U., Rekwot, P. I., and Osuhor, C. U. (2011). Effect of graded Levels and sources of protein on scrotal circumference and semen profile of Yankasa rams. *Sokoto Journal of Veterinary Sciences*, *9*(1), 22–27.

Ladokun, A. O., Egbunike, G. N., Adejumo, D. O. and Sokunbi, O. A. (2006). The effect of three dietary crude protein levels on digestibility and testis function in male pubertal rabbits. *Tropicultura*, *24*(1), 3–6.

Louis, G. F., Lewis, A. J., Weldon, W. C., Miller, P. S., Kittok, R. J. and S. W. W. (1994). The Effect of Protein Intake on Boar Libido, Semen Characteristics and Plasma Hormones Concentration. *Journal of Animal Science*, *72*(1), 2038–2050.

Łukaszewicz, E., Jerysz, A., Partyka, A., & Siudzińska, A. (2008). Efficacy of evaluation of rooster sperm morphology using different staining methods. *Research in Veterinary Science*, *85*(3), 583–588. https://doi.org/10.1016/j.rvsc.2008.03.010

McDaniel, C., Hannah, J., Parker, H., Smith, T., Schultz, C., & Zumwalt, C. (1998). Use of a sperm analyzer for evaluating broiler breeder males. 1. Effects of altering sperm quality and quantity on the sperm motility index. *Poultry Science*, *77*(6), 888–893. https://doi.org/10.1093/ps/77.6.888

Mideksa, B. A. (2017). Opportunities of preserving local animal genetic resources in Ethiopia, *5*(2), 369–376.

Papazyan, T. T., Lyons, M. P., Mezes, M., & Surai, P. F. (2006). Selenium in poultry nutrition - Effects on fertility and hatchability. *Praxis Veterinaria*, *54*(1–2), 85–102.

Rekwot, P. I, Oyedipe, E. O., Akerejola, O. O. and Kumi-Diaka, J. (1988). The effect of protein intake on body weight, scrotal circumference and semen production of Bunaji bulls and their Friesian crosses in Nigeria. *Animal Reproduction Science*, *16*(1), 1–9.

Rekwot, P. I., Oyedipe, E. O., Akerejola, O. O., Kumi-Diaka, J., & Umoh, J. E. (1987). The effect of protein intake on the onset of puberty in Bunaji and Friesian × Bunaji crossbred bulls in Nigeria. *Theriogenology*, *28*(4), 427–434. https://doi.org/10.1016/0093-691X(87)90247-0

Rekwot, P. I., Oyedipe, E. O., & Ehoche, O. W. (1994). The effects of feed restriction and realimentation on the growth and reproductive function of Bokoloji bulls. *Theriogenology*, *42*(2), 287–295. https://doi.org/10.1016/0093-691X(94)90273-9

Sotirov, L., Dimitrov, S., & Jeliazkov, E. (2002). Semen lysozyme levels and semen quality in Turkeys (Meleagris gallopavo) fed with various dietary protein levels. *Revue De Medecine Veterinaire*, *153*(12), 815–818. Retrieved from http://apps.isiknowledge.com.ezproxy.is.cuni.cz/full_record.do?product=WOS&search_mode=GeneralSearch&qid=5&SID=Q1o67F89Ek3b3APHp7F&page=1&doc=4

Tacher, G., Letenneur, L., & Camus, E. (2000). A perspective on animal protein production in sub-Saharan Africa. *Annals of the New York Academy of Sciences*, *916*, 41–49. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11193654

Todesco, M., Pascual, M. A., Owens, G. L., Ostevik, K. L., Moyers, B. T., Hübner, S., … Rieseberg, L. H. (2016). Hybridization and extinction. *Evolutionary Applications*, *9*(7), 892–908. https://doi.org/10.1111/eva.12367

van Wyk, A. M., Dalton, D. L., Hoban, S., Bruford, M. W., Russo, I. R. M., Birss, C., … Kotzé, A. (2017). Quantitative evaluation of

hybridization and the impact on biodiversity conservation. *Ecology and Evolution*, *7*(1), 320–330. https://doi.org/10.1002/ece3.2595

Wishart, G. J. (2007). Cryopreservation of avian spermatozoa. *Methods in Molecular Biology (Clifton, N.J.)*, *368*, 219–225. https://doi.org/10.1007/978-1-59745-362-2_15

Wu, G., Bazer, F.W., Davis, T.A., Kim, S.W., Li, P., Marc Rhoads, J., Satterfield, M.C., Smith, S.B., Spencer, T.E. and Yin, Y. (2009). Arginine metabolism and nutrition in growth, health and disease. *Amino Acids*, *37*(1), 153–168. https://doi.org/10.1196/annals.1320.017

Zahraddeen, D., Butswat, I. S. R., Kalla, D. J. U., Sir, S. M., & Bukar, M. T. (2005). Effect of frequency of ejaculation on semen characteristics in two breeds of Turkeys (Meleagris gallopavo) raised in a tropical environment. *International Journal of Poultry Science*, *4*(4), 217–221. https://doi.org/10.3923/ijps.2005.217.221

Zambrano, E., Rodríguez-González, G. L., Guzmán, C., García-Becerra, R., Boeck, L., Díaz, L., … Nathanielsz, P. W. (2005). A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. *The Journal of Physiology*, *563*(1), 275–284. https://doi.org/10.1113/jphysiol.2004.078543