

EFFECT OF GRADED LEVELS OF *Cucurbita pepo* (Pumpkins) PULP POWDER ON RABBIT BUCKS SEMEN QUALITY, TESTICULAR GROSS MORPHOLOGY AND HISTOLOGY

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ABSTRACT

Twenty four (24) adult Composite breed Rabbit bucks were used in an experiment to evaluate the effect of *Cucurbita pepo* (pumpkins) pulp powder on rabbit bucks semen quality characteristics, gross testicular morphology and histology. The rabbits were allotted to four (4) dietary treatments T1, T2, T3, and T4, consisting of six (6) rabbits per group in three (3) replicate of two (2) rabbits per replicate in a Completely Randomized Design (CRD). Four (4) different diets were formulated using different inclusion levels of pumpkin pulp powder at 0%, 5%, 10%, and 15% to treatment 1, 2, 3 and 4 respectively. At the end of the experiment semen was collected from three (3) rabbits in each treatment via artificial vagina and they were then slaughtered and dissected to obtain testicular tissues for testicular morphology. The results revealed statistical significant ($P > 0.05$) differences in the findings for the semen quality characteristics of reaction time and semen concentration. Significant difference ($P < 0.05$) was also observed for semen morphology for live and dead cell ratios. For testicular morphology, only left testis length was significantly ($P < 0.05$) different. Testicular histology revealed T3 having larger and elongated seminiferous tubules that were very clustered compared to other treatments. For all findings T3 (10% pumpkin inclusion) performed better and it was thus concluded that addition of pumpkin to rabbit feed at 10% inclusion rate did have a beneficial effect on rabbit reproductive performance and as such it was recommended for use by rabbit farmers.

Key words: Rabbit Bucks, Pumpkin pulp powder, Semen quality, Testicular morphology, Testicular histology

Introduction

Rabbits are herbivores with post-gastric digestion and can effectively utilize fodders and serve as alternative sources of meat to the populace (Amao *et al.*, 2012). Being a monogastric herbivore, with digestive system that can cope with fibrous plant matter, such as grasses, legumes or other hays, rabbits can thrive on forage diets although potential weight gains are not attained because of the poor nutritive value of tropical forages (Adejumo *et al.*, 2013). Most African countries are bedeviled by food shortage especially protein. Most protein sources used in compounding animal feed are in competition by people thus reducing their availability and increasing cost (Ige *et al.*, 2006). This leads to the use of unconventional feed materials from plant sources as a supplement for protein. *Cucurbita pepo* is a some of the cheapest forages that could be used to feed both ruminants and monogastric animals such as rabbit (Christian, 2006). Rabbit production in Nigeria is faced with multiple failures due to unfavorable climatic condition, inbreeding regression, poor nutrition etc. These problems cause significant fertility failures which are characterized by poor libido, poor semen quality and morphological changes in gonadal and extra-gonal tissues. *Cucurbita pepo* (Pumpkin) is a popular vegetable in our country. The powder of pumpkin is rich in oil and nutrients. It is used as a potentially attractive source of lipid, protein and crude fibre (Agatemor, 2006). The powder has lactating properties and is consumed by the nursing mothers (Nworgu *et al.*, 2007) and this led to our investigation of the powder supplemented in diet as a possible booster for male reproductive indices to improve rabbit production in Nigeria.

Material and Methods

The study was carried out at the Micro-livestock unit of Prof. Lawal Abdu Saulawa, Livestock Teaching and Research Farm, Department of Animal Science, Federal University Dutsin-Ma, Katsina State. The site lies between latitude 12°27'18"N and 7°29'29"E and 605 meters above sea level with an annual average rainfall of 700mm.

Experimental Animals and Management

A total number of twenty four (24) Adult composite breed rabbit bucks were purchased from Dutsin-Ma market and adapted for two weeks prior to the start of the experiment. The experiment was lasted for a period of sixty days

Experimental Diet

Four different diets were formulated using four different level of Pumpkin of 0, 5, 10 and 15% of pumpkin powder respectively. The diet was based on the dietary recommendation for rabbits according to (NRC, 2002).

Table 1: Experimental Diet

Ingredient	T1	T2	T3	T4
G/ nut hay	10	10	10	10
Maize	20	20	20	20
Maize offal	35	35	35	35
Pumpkin	0	5	10	15
Wheat offal	20	20	20	20
Soy bean meal	15	10	5	0
Bone meal	0.5	0.5	0.5	0.5
Premix	0.5	0.5	0.5	0.5
Common salt	0.5	0.5	0.5	0.5
Total				100

Experimental Design

The rabbits were allotted into four (4) dietary treatments consisting of six (6) rabbits per group in three (3) replicate of two (2) rabbits per replicate in a Completely Randomized Design (CRD)

Data Collection

Semen Characteristics and Sperm Morphological Defect Determination

After semen collection from bucks, the semen color, volume and pH were taken immediately after collection and recorded according to the methods of Zemjanis, 1970; Kalamah *et al.*, 2002; and Kamar *et al.*, 1979 respectively. Semen smear was prepared on a glass slide to determine the motility of the semen using a field microscope at magnification of $\times 100$ according to the method described by (Nagae *et al.*, 1987) then stained with eosin nigrosin. This was air dried thereafter to determine the semen live and dead ratio along with the sperm morphological defects according to the methods of (Kalamah *et al.*, 2002). For the concentration evaluation, another smear was made on a different glass slide to obtain the semen concentration as described by Smith *et al.*, 1995.

Testicular Gross Morphology Determination

At the end of the experiment (8 weeks) 3 rabbit bucks were randomly selected from each treatment and slaughtered by severing the jugular veins. Immediately after slaughtering the rabbit were dissected to obtain the testes. The testicular morphological parameters were determined according to Procedure outline by Eme and Ebunike (2004).

Testicular Histology

The testicular histology were carried out accordingly

Result and Discussion

The results clearly indicated that there are no significant ($P < 0.05$) difference in semen volume, percentage mortality, and semen pH (Table 2). However, the result revealed a significant ($P < 0.05$) difference in reaction time and semen concentration where T2 had the fastest reaction time (3.50secs) which was a clear indicator of aggressive and libido nature of rabbits in this study probably due to the pumpkin content in feed. Higher semen concentrations were observed in T3 followed T1 (control) and then other groups (Table 2). The significantly lower semen concentration observed in T4 may be a direct link to the higher volume of semen observed in T4 and possibly to the lower protein content of the diet of T4. The reason for that may be precisely due to the fact watery semen volume has a less sperm cell concentrations, morphology and livability percentages.

Table 2: Effect of Pumpkin Powder Supplement on Composite Breed Rabbit Bucks Semen Quality Characteristics

Parameters	T1	T2	T3	T4	SEM	LOS
Semen volume (ml)	0.350 ^a	0.200 ^a	0.400 ^a	0.650 ^a	0.177	NS
Reaction time (sec)	11.000 ^a	3.500 ^b	4.500 ^b	5.500 ^{ab}	1.49	*
Mortality (%)	67.500 ^a	62.500 ^a	72.500 ^a	50.000 ^a	6.495	NS
pH	7.000 ^a	7.000 ^a	6.750 ^a	8.000 ^a	0.515	NS
Semen Con ($\times 10^9$)	118.50 ^a	96.00 ^b	120.50 ^a	54.50 ^b	17.144	*

The results showed that there was no significant ($P>0.05$) difference in the morphology, bent tail, coiled tail, detached head, full tailed, and normal cell although numerical variations exist. The result however revealed that the percentage live cell and dead cell have significant ($P<0.05$) difference, where T3 had the highest live cells percentages of 82.50% followed by T1 with 80% while lowest live cell percentages were observed in T4 with 45.00% live cells. Highest dead cells percentages were observed in T4 with 55% dead cells while lowest dead cells percentages were reported in T3 (17.50%). This may be correlated with a significantly higher watery semen volume and lower semen concentrations observed in T4 in this study and indicate that supplementing pumpkin powder at 15% affect the sperm production negatively while supplementing at 10% gave optimum semen production.

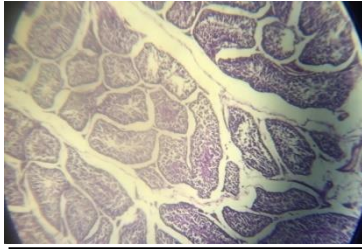
TABLE 3: Effect of Pumpkin Powder on Composite Breed Rabbit Bucks Semen Morphology

Parameter	T1	T2	T3	T4	SEM	LOS
Morphology	0 ^a	0 ^a	0 ^a	0 ^a	0	NS
Bent tail (%)	3.500 ^a	8.500 ^a	14.500 ^a	12.500 ^a	5.123	NS
Coiled tail (%)	1.500 ^a	2.500 ^a	4.000 ^a	4.500 ^a	1.299	NS
Detached head (%)	3.500 ^a	4.000 ^a	6.500 ^a	13.000 ^a	2.716	NS
Full tailed (%)	2.50 ^a	4.50 ^a	42.50 ^a	7.50 ^a	16.332	NS
Normal cell (%)	89.00 ^a	80.50 ^a	72.50 ^a	62.50 ^a	8.856	NS
Live cell (%)	80.00 ^{ab}	72.50 ^{ab}	82.50 ^a	45.00 ^b	9.186	*
Dead cell (%)	20.00 ^{ab}	27.59 ^{ab}	17.50 ^b	55.00 ^a	9.186	*

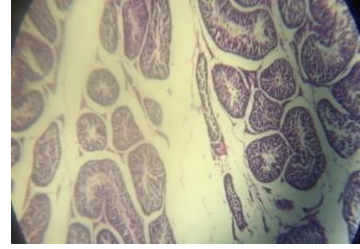
The result on testicular morphology indicated no significant ($P>0.05$) difference in any values except for left testis length which was significantly ($P<0.05$) longer in T1 and T3 had the highest left testis length with 7.250cm while lowest left testis length were observed in T2 with 6.200cm left testis length. The reason for that may be precisely due to the fact testes are not of equal size (Eme and Ebunike, 2004) and left testis are relatively longer and larger than the right testis. The higher protein content in feed and optimal inclusion level of pumpkin powder in T3 as observed in previous results could be attributed to this finding.

TABLE 4: Effect of Pumpkin Powder on Composite Breed Rabbit Bucks Testicular Morphology

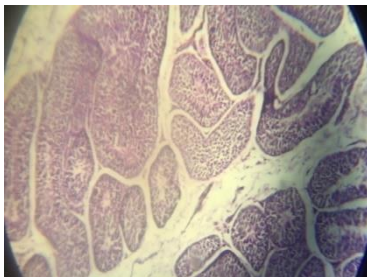
Parameter	T1	T2	T3	T4	SEM	LOS
Animal weight (kg)	1.450 ^a	1.300 ^a	1.250 ^a	1.250 ^a	0.106	NS
Right testis weight (g)	3.950 ^a	3.400 ^a	3.000 ^a	4.050 ^a	0.586	NS
Left testis weight (g)	4.050 ^a	4.050 ^a	3.350 ^a	3.800 ^a	0.549	NS
Paired testis weight (g)	8.000 ^a	7.450 ^a	6.350 ^a	7.900 ^a	1.001	NS
Relative testis weight (%)	554.30 ^a	577.00 ^a	509.00 ^a	621.50 ^a	53.983	NS
Right testis volume (ml)	4.000 ^a	4.000 ^a	4.000 ^a	4.500 ^a	0.250	NS
Left testis volume (ml)	4.500 ^a	4.000 ^a	4.000 ^a	4.500 ^a	0.612	NS
Testes volume (ml)	8.500 ^a	8.000 ^a	8.000 ^a	9.000 ^a	0.750	NS
Testes density	0.945 ^a	0.935 ^a	0.805 ^a	0.865 ^a	0.086	NS
Right testis length (cm)	6.350 ^a	6.500 ^a	6.550 ^a	7.650 ^a	0.411	NS
Left testis length (cm)	7.250 ^a	7.250 ^a	6.500 ^{ab}	6.200 ^b	0.256	*



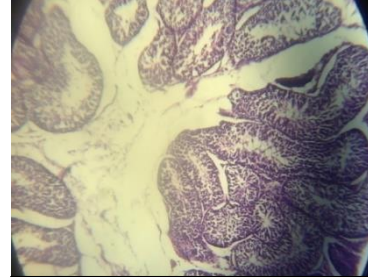
Microphotograph of T1 (0% Pumpkin) testicular tissue: there are dense and cluster semeniferous tubules with a visible leydigs cells. H&E Staining X10 magnification



Microphotograph of T2 (5% Pumpkin) testicular tissue: There are wider interstitial tubules and scanty semeniferous tubules. H&E staining x10 magnification



Microphotograph of T3 (10% Pumpkin) testicular tissue: there are larger and elongated semineferous tubules that appear very clustered. H&E staining x10 magnification



Microphotograph of T4 (15% Pumpkin) testicular tissue: there are less clustered seminiferous tubules and wider interstitial cells. H&E Staining x 10 magnification

Conclusion

It was concluded that inclusion pumpkin pulp powder in rabbit diets at 10% resulted in higher sperm cell concentration as well as sperm live to dead cell ratio and also normalized sperm cells morphological defect while showing the best testicular histological features indicating good reproductive performance in male rabbits and it is therefore recommended that inclusion of pumpkin into rabbit diet at 10% inclusion level will improve reproductive performance.

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