



Research Article

## Effects of Graded Dose of Honey Administered Orally on Gonadal and Extra-Gonadal Sperm Reserves of Rabbit

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### ABSTRACT

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Honey is a nutritive natural by-product of bees obtained from nectar collected from flowering plants. It contains moisture, sugars, enzymes, essential elements and vitamins. This study is designed to determine the effects of graded dose of honey administered orally on gonadal and extra-gonadal sperm reserves of rabbit. Twelve apparently healthy male rabbit (buck) aged two months weighing  $4 \pm 1.2$  kg were purchased and housed in a cages at the Large Animal Clinic, Veterinary Teaching Hospital, University of Maiduguri. The rabbits were fed, grower mesh and supplemented with groundnut leaves, drinking water was provided *ad libitum*. The rabbits were acclimatized for 14 days. Thereafter, they were randomly divided into 4 groups A, B, C and D as treatment groups and control group, respectively, with 3 rabbits in each group. Groups A, B and C were administered orally graded dose of honey at the dose rate of (5, 10 and 15) ml respectively, between 9:00 - 10:00 am daily for 6 weeks. At the end of the experimental period the rabbits were aseptically prepared for surgical removal of their testes. The testes and epididymides were carefully sampled, weighed and processed. The results revealed that the right, left, and paired testicles weights were not significantly different among the treatment groups compared to the control (group D). However, the testicular sperm reserves were significantly ( $P < 0.05$ ) influenced by the treatments. Sperm reserves in both left and right testicles of rabbits treated with 5 ml of honey were not significantly different with the group that was administered with 10ml of water but, groups that were administered 10ml and 15ml of honey were significantly ( $P < 0.05$ ) higher than the group that was administered with 5 ml of honey compared to the group treated with 10 ml of water. The epididymal sperm reserves were significantly ( $P < 0.05$ ) higher than the groups treated with 10 ml and 15 ml of honey compared to the group treated with 5 ml of honey and the group treated with 10 ml of water. This study suggests that honey has the potential to influence gonadal and extragonadal sperm reserves of rabbits at dosage of 10-15 ml per day for optimum quantity and quality of semen

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## INTRODUCTION

The quality and bio-security of rabbit meat have been attracting increased attention and interest from both the scientific communities, developed and the developing countries, with it attributes of good quality animal products for human consumption (Abdelhafiz *et al.*, 2008). Therefore, there is a global trend to reduce the use of drugs agents and biological hormones as substances to improve semen quantity and quality in animal farms and food animals with alternatives that is more safe and healthy (Hashem *et al.*, 2013). In general, nutritional diets are considered as important factor that directly influence animal well-being and improve its reproductive potentials. Honey can be defined as a natural substance (nutriceutical) synthesized in the venom gland of honey bees (*Apis mellifera*) and has a unique properties that are rich of active components such as enzymes and peptides which have several pharmaceutical and medicinal properties (Raji *et al.*, 2014). This kind of alternative therapy as supplement has been used for centuries in developed and developing countries (Hashem *et al.*, 2013). Honey can be added in animal diet or administered orally to enhance the productivity and reproductive performance. Some of the medicinal values of honey are prevention or treatment of some disease (Apitherapy). Honey have been reported by several scholars as potential substance that has antimicrobial Estevinho *et al.* (2008), anti-inflammatory Prakash *et al.* (2008) and immunomodulatory properties Timm *et al.* (2008). Traditionally, honey is frequently consumed by human population as a nutrient, as well as a substance that can enhance fertility and vitality, as reported by Gheldof, *et al.* (2002). Recently, a higher sperm count was observed following the oral administration of honey at a dose rate of 1.2 g/kg body weight/day for 28 days in rats Mahaneem *et al.*, (2007). Hence, honey can also work as fertility regulatory agent since the

effective substance components of honey are fructose and glucose (80–85%); water (15–17%); ash (0.2%); proteins and amino acids (0.1–0.4%) and trace amounts of enzymes, vitamins and other substances, such as phenolic compounds (Raji *et al.*, 2017). However, honey composition varies depending on the types of plants from which the bee consumes nectar (Raji *et al.*, 2014). Nevertheless, nearly all honey worldwide contains similar types of phenolic acids, including caffeic, ellagic, ferulic and p-coumaric acids; flavonoids, such as apigenin, chrysin, galangin, hesperetin, kaempferol, pinocembrin and quercetin; and antiox- idants, such as tocopherols, ascorbic acid, superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) (Hashem *et al.*, 2013). However, caffeic, ellagic, ferulic, and p-coumaric acids, as well as the flavonoids apigenin, chrysin, galangin, kaempferol, pinocembrin, and quercetin, and the antioxidants tocopherols, ascorbic acid, superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), are present in nearly. Each component of honey offers distinct health benefits, and when combined, they work synergistically to make honey useful for a range of purposes (Hashem *et al.*, 2013).

The relationships between semen qualities and fertility have been studied in several animal species with the general result of predicting high fertility and percentage semen quality (Nesrein *et al.*, 2021). Moreover, understanding the sexual behavior such as libido of the animals is very important because they influence it sexual efficiency directly as reported by Nesrein *et al.* (2021). Generally, there is paucity of information on the effect of honey on gonadal and extragonadal sperm reserves (Raji *et al.*, 2017). Therefore, this study was carried out to investigate the effects of graded dose of honey administered orally on gonadal and extragonadal sperm reserves of male rabbits (buck).

## MATERIALS AND METHODS

### Study Area

This study was conducted in the Faculty of Veterinary Medicine University of Maiduguri. Borno State Situated in North Eastern part of Nigeria. It is Cosmopolitan in nature, located at an elevation of 354 meters above sea level located between latitudes 11<sup>0</sup> and 14<sup>0</sup>N and longitudes 10<sup>0</sup> and 14<sup>0</sup>E, within the Sahel zone and has a total land mass of 50,778 square kilometers (BMLS, 2007). It has a population density of 1,738 people per square kilometers, and a total population of 521,492 (NPC, 2006). The temperature ranges from 35-40 °C for most parts of the year with two distinct seasons, a rainy season with mean annual rainfall of 647mm from July to October and a prolonged dry season for the rest of the year (LCRI, 2007). The state due to its geographical features favors the production of honey even though not at colossal economy, and similarly, the state derives great economic activity from its rich livestock and fishery production (NPC, 2006).

### Experimental Animals

Twelve, healthy rabbits aged 1-8 months with an average body weight of 4.0 ± 1.2 kg were purchased from the market. The rabbits were examined to ensure that they were in good physical health. Thereafter, the rabbits were acclimatized for two weeks. The rabbits were kept under intensive management and fed on groundnut and salad leaves water was provided ad libitum.

### Sources of the Honey

Five litters of ripe honey in nectar were purchased from Monday market Maiduguri Metropolis, Borno State.

### Evaluation of Honey Quality

The honey was physically evaluated for colour, consistency, taste, flavor and mouth feel using 9-point hedonic scale as described by Narpinder and Parminder, (2011) for overall acceptability. A pure honey does not foam under any circumstance, is quite thick and only trickles in a

stream, soft texture thin filament-like and never separates into layers with a good aroma. Thereafter, the honey were extracted and filtered according to the method described by Narpinder and Parminder, (2011).

### Housing of the Experimental Animals

The experimental animals (Rabbit) were kept in laboratory animal research cages of the Large Animal Clinic Unit, Faculty of Veterinary Medicine University of Maiduguri. The rabbits were kept for 6 weeks before the commencement of the Experiment.

### Experimental Design

The rabbits were randomly selected into four groups (A, B, C and D) of three rabbits per group. Groups A, B and C were administered honey orally at a different dose 5, 10 and 15 ml respectively. Group D served as untreated control and was administered 10 ml of distilled water orally. The honey was administered in the morning between 9:00 - 10:00 am daily for 6 weeks. Body weight was recorded according to the method described by (Iliyasu *et al.*, 2020), once every week throughout the experimental period.

### Surgical Removal of the Testes of the Rabbit

At the end of the experiment, aseptically rabbit testes were removed surgically following administration of local anesthesia and disinfection of the scrotum. The buck was restrained and the scrotum was infiltrated with 1ml of lidocaine. The testes were examined grossly and evaluated for testicular weight, length, gonadal and extragonadal sperm reserves. The right and left testes as well as the right and left epididymis were evaluated for individual rabbit of each group. The epididymides were further divided into corpus, caput and cauda and used for the extragonadal sperm reserves analyses. A surgical blade was used to incise the scrotum and expose the testicle, the testicle was cut and separated from the blood vessels and spermatic cord, the major blood vessels and spermatic cord were legated

with haemostatic forceps. The fascia was further incised to reach the tunica albuginea, thereafter, the epididymis was also separated from the testes using sharp scissors, and the tunica albuginea was incised and separated from the testicular parenchyma using scalpel as described by McGlinn *et al.*, (1974).

#### **Determination of Gonadal and Epididymal Sperm Reserves**

Gonadal and epididymal sperm reserves were determined as described by Iliyasu *et al.* (2014). The testicular parenchyma was sliced and homogenized with a high-speed blender for two minutes with 50 ml of 0.9 % NaCl solution containing antibiotics (sodium penicillin G, 100 IU/ml and streptomycin sulphate 1 mg/ml) to prevent bacterial growth. For determining the epididymal sperm reserves, the caput, corpus and cauda epididymides were isolated, and minced with a pair of scissors separately in 20 ml of 0.9 % NaCl solution. All tissues were homogenised for 2-6 hours after minced. Testicular homogenates and epididymal samples were refrigerated overnight. After 24 hours, the samples were filtered through gauzed and the filtrate volumes were measured. The gonads was homogenized with 50 ml of normal saline and filtered. The filtrate was diluted at a ratio of 1: 2 one milliliter of filtrate was diluted separately with 2 ml of normal saline solution prior to examination as described by Rekwot *et al.*, (1987). Sperm concentration of the testes and epididymal sample were determined using a haemocytometer and light microscope, as described by (Iliyasu *et al.*, 2014).

#### **Measurement of Gonadal and Epididymis Organ Dimensions**

An Essae® digital weighing scale was used to weigh the testicular parenchyma, and a Venire caliper was used to measure its dimensions. In accordance with gross anatomy, the left and right epididymides were divided into the caput, corpus, and cauda sections. Using a Venire caliper and a digital weighing scale, respectively, each component was measured and weighed.

Thereafter they were placed in normal saline for onward estimation of epididymal sperm reserves using haemocytometer.

#### **Statistical Analyses**

Data were analyzed using GraphPad Prism version 5 (GraphPad, 2000). Data were subjected to one-way ANOVA and Dunnett Posthoc test. Values were presented as mean  $\pm$  SEM and the results were considered significant at  $p < 0.05$ .

#### **RESULTS**

The mean weights of the rabbit's testes at the end of the experiment showed no significant differences ( $P > 0.05$ ) in morphometric dimensions of the testicular and epididymal weight among treated groups compared to the group that was treated with 10 ml of distilled water. The right, left and paired testes weights of the rabbits were not significantly different among the treatments. However, the right and paired epididymis weights of the rabbits were not significantly different among the treatment groups while the right epididymis of the treatment groups showed significant increase ( $P < 0.05$ ) compared to the control (group treated with 10 ml of distilled water) as presented in (Table 1).

**Table 1:** Effects of graded dose of honey administered orally on morphometric dimension of testicular and epididymis organs of rabbits

Parameters/groups	A (Honey 5 ml)	B (Honey 10 ml)	C (Honey 15 ml)	D (Water 10 ml)
Testicular weight (g)				
Right testis	1.03 <sup>a</sup> ±0.08	0.90 <sup>a</sup> ±0.23	0.78 <sup>a</sup> ±0.00	1.03 <sup>a</sup> ±0.07
Left testis	1.04 <sup>a</sup> ±0.78	0.79 <sup>a</sup> ±0.20	1.19 <sup>b</sup> ±0.18	1.05 <sup>a</sup> ±0.13
Paired testes	2.07 <sup>b</sup> ±0.14	1.69 <sup>b</sup> ±0.10	1.97 <sup>b</sup> ±0.18	2.08 <sup>b</sup> ±0.13
Testicular length (cm)				
RTL	3.22 <sup>c</sup> ±0.00	3.20 <sup>c</sup> ±0.01	3.24 <sup>c</sup> ±0.05	3.25 <sup>c</sup> ±0.03
LTL	3.20 <sup>c</sup> ±0.00	3.19 <sup>c</sup> ±0.00	3.22 <sup>c</sup> ±0.00	3.24 <sup>c</sup> ±0.03
Epididymal weight (g)				
Right epididymis	0.57 <sup>d</sup> ±0.03	1.03 <sup>a</sup> ±0.36	0.64 <sup>d</sup> ±0.01	0.46 <sup>d</sup> ±0.06
Left epididymis	0.50 <sup>d</sup> ±0.04	0.36 <sup>d</sup> ±0.03	0.58 <sup>d</sup> ±0.03	0.46 <sup>d</sup> ±0.06
Paired epididymis	1.07 <sup>a</sup> ±0.05	1.39 <sup>a</sup> ±0.37	1.22 <sup>a</sup> ±0.05	0.92 <sup>d</sup> ±0.11

All values were expressed as mean ± SEM values with different superscripts <sup>a,b,c,d</sup> within the columns are significantly different at (P < 0.05).

**Key:** RTL= Right testicular length; LTL= Left testicular length

The gonadal and extragonadal sperm reserves among groups treated with graded dose of honey were significantly (P < 0.05) increase in a dose dependant manner compared to the control (group D). However, the testicular sperm reserves were significantly (P<0.05) influenced

by the treatments at dose dependent manner. Sperm reserves in both left and right testicles were significantly increase compared to the control group D. Similar findings were observed on extragonadal sperm reserves as shown in (Table 2).

**Table 2:** Effects of graded dose of honey administered orally on gonadal and extra gonadal sperm reserves of rabbits

Parameters/groups	A (Honey 5 ml)	B (Honey 10 ml)	C (Honey 15 ml)	D (Water 5 ml)
Gonadal sperm reserves (x10 <sup>6</sup> )				
RGSR	31.51±6.04 <sup>b</sup>	37.27±4.43 <sup>b</sup>	53.30±5.19 <sup>a</sup>	26.01±2.91 <sup>b</sup>
LGSR	28.45±2.09 <sup>a</sup>	26.70±2.13 <sup>b</sup>	26.30±2.04 <sup>b</sup>	25.05±2.57 <sup>b</sup>
Extra gonadal sperm reserves (x10 <sup>6</sup> )				
RESR	42.51±5.07 <sup>b</sup>	72.53±6.10 <sup>ab</sup>	81.40±9.26 <sup>a</sup>	69.14±12.51 <sup>ab</sup>
LESR	65.60±6.17 <sup>ab</sup>	78.82±7.37 <sup>a</sup>	86.15±8.16 <sup>a</sup>	41.36±4.14 <sup>b</sup>
Paired Caput	36.24±5.17 <sup>b</sup>	55.40±5.31 <sup>a</sup>	63.60±8.65 <sup>a</sup>	23.35±2.86 <sup>b</sup>
Paired Corpus	21.26±3.43 <sup>b</sup>	30.31±5.63 <sup>ab</sup>	31.51±2.58 <sup>a</sup>	24.93±3.97 <sup>b</sup>
Paired Cauda	50.43±9.13 <sup>b</sup>	65.61±8.63 <sup>ab</sup>	72.51±8.43 <sup>a</sup>	62.14±11.62 <sup>b</sup>

All values were expressed as mean ± SEM values with different superscripts <sup>a,b</sup> within the columns are significantly different at (P < 0.05).

**Key:** RESR= Right epididymal sperm reserves; LESR= Left epididymal sperm reserves; LGSR = Left gonadal sperm reserves

## DISCUSSION

Several studies have shown influence of honey on enhancement of reproductive activities and improvement of semen quality this is evidence to the findings in the current studies. It was also reported that honey with its unique structure can play multi-biological functions in animal body such as growth promoter Hashem *et al.*, 2021, anti-inflammatory Hashem *et al.*, 2013 and anti-microbial Maghsoudlou *et al.*, 2019 with no side effects detected; but only few available articles that have been interested to study the impact on rabbit semen characteristics and health Abdelhafiz *et al.*, 2008. Therefore, this current study aimed to investigate the effects of graded dose of honey on gonadal and extragonadal sperm reserves of rabbit bucks Attia *et al.*, 2011. However, the testicular sperm reserves were significantly influenced by the graded dose of honey in the treatments groups this agreed with findings reported by (Raji *et al.*, 2017) in goats. Sperm reserves in both left and right testicles of rabbits were significantly higher compared to the control group but they were significantly lower than what was reported by (Raji *et al.*, 2017) in goat, the differences might be attributed to differences in species of animal and doses used.

The extragonadal or epididymal sperm reserves of the rabbits were not significantly different among the treatment groups this agreed with the findings reported by Ogunlade *et al.* (2006). The sperm reserves in caput, corpus and caudal epididymis in both left and right followed the same trend. The sperm reserves were significantly higher in rabbits treated with 10 ml and 15 ml of honey than those treated with 5 ml and the control group. Conversely, it has been established that high correlation exists between organs weight, age and sperm reserves of animals as reported by Osinowo *et al.* (1981) and Oyedipe *et al.* (1982). However, onset of puberty is a function of body weight as well as the organs weight than age Biobaku and

Oguntona, 1997. Atangwho *et al.* (2020) suggest that increased organ weight caused by the use of growth-promoting agents may trigger the start of puberty, which will directly affect sperm production in the testis and sperm stores in the epididymides. Another theory is that honey is one of the foods that aid in the formation of the digestive mucosa, which increases the digestion of nutrients. Fuller, 1989; Mosenthin and Baller, 2000; Ewuola *et al.*, 2011 that could have provided the Sertoli cells with nourishment and increased the volume of seminal fluid that nourishes the germ cells in the testes for optimum function and sperm production.

The findings in the current study on sperm reserves in the testes and epididymis was similar to the findings reported on matured rabbits by Ogunlade *et al.* (2006) and Ewuola and Egbunike (2010) while the caudal epididymides accounting for about half of the total epididymal sperm reserves followed by the caput and corpus epididymides as also reported by (Iliyasu *et al.* (2014) in Yankasa rams and Egbunike and Elemo (1978) in boars. In the current study, the disparity observed between percentage of the total spermatozoa in each of the three-epididymal compartments in rabbits of the treatment in groups compared to the control group might be attributed to the potential of the ingredients available in the honey that had the capacity of improving physiological activities of the testes that assured optimum reproductive performance and efficiency in rabbits as also reported by Ewuola *et al.* (2011) in rabbits and (Raji *et al.*, 2017) in goats.

## CONCLUSION

Honey at a dose of (10 and 15) ml can be effective and safe to use in rabbit to enhance semen quality and promote sexual activities. The current study showed, the mean weight of rabbit testes at the end of the experiment was not significantly different in morphometric dimension of the testicular epididymal weight among the treatment groups. The gonadal and

extragonadal sperm reserves were increased in groups treated with graded dose of honey compared to the control. Therefore, the semen quality has tremendously improved at the end of the study among the treated rabbits.

### CONFLICT OF INTEREST

The authors declared no conflict of interest in respect to the manuscript

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