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Full Length Research

Semen and haematological responses of rabbit bucks administered oral folic acid

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ABSTRACT: Some researchers have inferred that folic acid is necessary for reproduction and could enhance blood formation. Thus, a Completely Randomized Design Experiment (CRD) was conducted to evaluate the impact of oral administration of folic acid on the semen and haematological characteristics of New Zealand White rabbit bucks. The treatments designated treatment 1 (T₁), treatment 2 (T₂) and treatment 3 (T₃) having 12 rabbits each were replicated 3 times with 4 rabbits per replicate. The ages of the 36 pre-pubertal rabbit bucks were between 2 to 3 months, and weighed approximately 2.56 kg. Three experimental diets were formulated to meet the nutrient requirements of rabbit bucks. Each rabbit buck on T₁ were orally administered folic acid at 0.0 mg, T₂ 2.5mg folic acid and T₃ 5.0 mg folic, respectively. Data were collected for semen characteristics and haematology from the rabbit bucks. Data collected on different parameters were subjected to analysis of variance (ANOVA). Results showed that significant increases (p<0.05) were observed on libido, semen pH, spermatozoa progressive motility (67.40-80.20%), spermatozoa live proportion (83.01-94.12%), sperm concentration (112.24-133.80 x10⁶/ml), total number of sperm per ejaculate (50.65-67.66 x10⁶/ml), total viable sperm (291.58-496.69 x10⁹/ml), normal sperm proportion (85.16-91.64%). Also, significant reductions (p<0.05) were observed on the percentage head abnormality of the spermatozoa (3.74-3.18), total abnormality (2.13-0.93), mid-piece abnormality (2.35-0.79), cytoplasmic abnormality (7.17-2.89), and total abnormality (14.84-8.35); while the haematological parameters such as haemoglobin (13.53-14.20g/dl), packed cell volume (33.00-34.96%), white blood cell (6.81-7.80 x103mm3) and the differential white blood cells improved significantly (p<0.05) following the oral administration of folic acids to the rabbit bucks. Thus, the oral administrations of folic acid at 5.0 mg per rabbit buck most significantly improved the semen characteristics, enhanced the overall spermatozoa morphology, reduced sperm cells abnormalities and also improved some haematological parameters of the rabbit bucks.

Keywords: Folic acid, haematology, immuno-modulatory, semen, rabbit bucks.

INTRODUCTION

In the face of the rising unemployment, many Nigerians have ventured into animal agriculture as an anchor for sustainable economy, human development and as a means of employment. As such, it has become imperative to enhance animal physiology and production potentials of farm animals so as to sustain the present interest of those venturing into animal agriculture. One of the primary purposes of animal agriculture is reproduction. Semen

characteristics and blood profile is very essential for effective reproduction in rabbits. Reproductive failure has been a major source of economic loss in animal agriculture. Poor physiological conditions and malnutrition has contributed to the decline in reproductive performance of farm animals (Frandson et al., 2000; Lotti and Maggi, 2015; Onunkwo et al., 2018; Uchewa et al., 2018). Inefficient reproductive system, poor management, and

decline in blood profiles and poor semen constituents have contributed to this failure (Brugh and Lipshultz, 2004; Barratt, 2007; Fisch, 2008; Butt and Akram, 2013; Amaduruonye et al., 2018). The general physiology of rabbits have been reported to be largely affected by several factors such as nutrition, drugs, hormones, environmental and other physiological factors (Addass et al., 2012; Sabra and Al-Harbi, 2014; Amaduruonye, et al., 2017).

Folic acid (Vitamin B-9) is a water soluble vitamin belonging to the group of vitamin B complexes. Folic acid (Folacin) is converted into folate by the body and used as a dietary supplement. Folic acid helps the cells to build and maintain new cells; and also help to prevent changes in the DNA that may lead to cancer. Folic acid is used to treat and correct folic acid deficiency and certain other types of anemia (Foster et al., 2009; Baykan et al., 2011). On reproduction, folic acid helps to prevent major birth defects, such as the defects of the Central Nervous System (CNS) and the spines of the developing fetus (Czeizel, 2000; Coll et al., 2004; Unusan, 2004; Nawapun and Phupong, 2007). Folic acid increases sperm production in men, boosts fertility in males and females, and could be used as a fertility supplements for males and females (Morin et al., 2002; Ajrouche et al., 2014; Hodgetts et al., 2015). Folic acid is an antioxidant, anti-aging and moisturizing, helping to maintain the skin natural beauty (Josh et al., 2001, Debowska et al., 2005).

The retardation of male reproductive performance is one of the major indicators of reduction in reproductive capacity in farm animals. To solve these problems, efforts should be geared toward enhancing the efficacy of rabbit reproduction through improvements in semen and blood profile. Therefore, there is a need to improve the overall physiology of rabbit bucks so as to enhance rabbit reproduction. This study was aimed to examine the impact of oral administration of folic acid on semen and haematological characteristics of rabbit bucks.

MATERIALS AND METHODS

This research was conducted in the Rabbitry Unit of the Teaching and Research Farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Umudike is located in Abia State, Nigeria. The location is situated at latitude 05°29¹ North and longitude 07°31¹ East; and at an altitude of 122 meters above sea level (NRCRI, 2004). It lies within the tropical rainforest zone of South Eastern Nigeria. The location is characterized by average annual rainfall of 2,177 mm in 148 to 155 rain days (NRCRI, 2004). The average ambient temperature is 25.5°C with minimum and maximum temperature of 22 and 29°C, respectively. Relative humidity ranged from 57 to 91%. The metrological data were collected from the Meteorological Center of National Root Crop Research

Institute, Umudike, Abia State (NRCRI, 2004).

Thirty-six (36) pre-pubertal New Zealand white rabbit bucks aged 2 to 3 months sourced from the Teaching and Research farm of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, were used for the study. A quarantine period of 2 weeks pre-experimental period was allowed during which the animals were vaccinated against ecto and endo-parasite using Ivomectin and Levamisole (0.1 ml/kg body weight), respectively. The experimental animals were housed singly in pens of colony hutches for ease of identification throughout the experimental period. They were fed the experimental diet and administered oral folic acid. Feed and clean drinking water were provided ad libitum. Routine management practices were also carried out appropriately. The compositions of the experimental diets are presented in Table 1.

Experimental design

The study was a Completely Randomized Design (CRD) trial with three treatments consisting of T₁, T₂, and T₃. The T₁ served as the control. Twelve (12) rabbit bucks were randomly assigned to each treatment, balanced for weights and replicated 3 times, with 4 rabbits per replicate. The Folic acid were administered orally to the rabbit bucks between 7.00 and 8.00 am Nigeria local time after feeding, on each day of administration at two days interval throughout the experimental period. The folic acid was dissolved in 5 ml of water and drenched orally to the rabbit bucks using a 10 ml string on each day of administration to ensure efficient and uniform consumption. Each rabbit buck on T₁ were orally administered folic acid at 0.0 mg, T_2 2.5 mg folic acid and T_3 5.0 mg folic acid, respectively. The field work lasted for 16 weeks. The experimental model is as follows:

$$Y_{ij} = U + T_{i} + e_{ij}$$

Where: $Y = \text{individual observation on the rabbit characteristics; } \mu = \text{overall mean; } T_i = \text{treatment effect; } e_{ij} = \text{random error assumed to be independently, identically and normally distributed with zero means and constant variances.}}$

Data collection and evaluation

A matured doe (teaser) was introduced to the buck prior to semen collection to monitor their sex drive. The time in seconds it took for the rabbit bucks to sniff, groom and mount the female was recorded with a stop watch. Libido (reaction time) were determined by observing the time taken (seconds) from exposure of the buck to the doe and the first copulation as recommended by Herbert and Acha (1995).

Table 1. Feed composition and calculated nutrients of experimental diets.

Ingredients	Percentage composition
Maize	44.94
Soya beans	17.31
Rice husk	32.00
Fishmeal	2.00
Bone meal	1.00
Limestone	2.00
Vit/min Premix*	0.25
Salt	0.50
Total	100.00
Calculated nutrients	
	17.00
Crude Protein (%)	
Metabolizable Energy (ME) (Kcal/kg diet)	2505.42
Crude fiber (%)	11.36
Lysine (%)	0.514
Methionine (%)	0.199

*Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3, 2500 IU; vitamin E, 50.00mg; vitamin K3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotin, 0.08mg; vitamin B12, 0.25mg; folic acid, 1.00mg; chlorine chloride,300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; iodine, 1.55mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg.

During semen collection, Artificial Vagina (AV) with a calibrated glass collection tube constructed by Herbert and Adejumo (1995) was used for semen collection. Two weeks prior to semen collection, the bucks were trained to serve an artificial vagina using a teaser rabbit doe. The semen was collected between 9.00 am and 10.00 am local Nigeria time on the day of semen collection. During semen collection, the rabbit doe was taken to the buck's cage and held in position for service. The AV was lubricated using glycerol and its temperature adjusted to 36 to 40°C. The artificial vagina was strategically placed under the belly of the doe such that the penis of the buck was introduced into the artificial vagina.

Estimation of semen characteristics

Semen evaluation involved the estimation of both the macroscopic and microscopic indices. Semen volume was determined in milliliter directly from the calibrated glass collection tube attached to the Artificial Vagina (AV). Semen pH was determined using a pH meter. Semen color and consistency were determined subjectively using the scoring pattern as described by Chibundu (2005). Spermatozoa concentration was determined using a haemocytometer as described by Jequier, (2010). The total number of spermatozoa was determined by multiplying semen volume by the spermatozoa concentration.

Sperm motility was determined subjectively with a drop of fresh semen on a glass slide covered with slip and examined using a microscope as described by Oguike et al. (2019). Sperm morphology was determined by performing differential counts of the morphologically normal and abnormal structures of the spermatozoa using eosin-nigrosin stain. Spermatozoa live proportion, total number of spermatozoa per ejaculate, percentage relative gel formation and relative clumping of spermatozoa were determined using the methods of Brazil (2010).

Haematology

Blood samples for hematological examination were collected from the rabbit bucks. A 5 ml syringe fitted with a sterile needle was used to collect 2 ml of blood and quickly transferred to ethylene diamine tetraacetic acid (EDTA) sample bottles. The EDTA sample bottles were shaken gently to prevent clotting. The following hematological indices were determined: hemoglobin, packed cell volume, red blood cell, white blood cell and its differentials, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration. The packed cell volume (PCV) was determined by the micro-hematocrit method as described by Kahn et al. (2010). Haemoglobin (Hb) concentration was determined using a spectrophotometer through the cyanomethaemoglobin method as described by Putwain (2008). Red blood cell (RBC), white blood cell (WBC) counts and its 'differentials' were determined using Neubauer hemocytometer method as described by Feldman et al. (2000). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Bain (2006).

Statistical analysis

Data collected on different parameters were subjected to analysis of variance (ANOVA) in accordance with the methods of Steel and Torie (1980). Significance means were separated according to Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

The semen characteristics of rabbit bucks on oral administration of folic acid is presented in Table 2. From the results in Table 2, no significant differences (p>0.05) were observed on the semen volume, semen color and spermatozoa motility on the oral administration of folic acid to the rabbit bucks. They are statistically similar with the control group. Furthermore, significant improvements (p<0.05) were observed on the libido, semen pH, semen consistency, spermatozoa progressive motility, spermatozoa live proportion, semen concentration, total number of spermatozoa per ejaculate, total viable spermatozoa and

Table 2. Semen characteristics of rabbit bucks on oral administration of folic acid.

Parameter	T ₁	T ₂	Т3	SEM
Libido/Reaction time (sec.)	12.82ª	10.47 ^b	9.00 ^b	1.12
Semen volume (ml)	0.45	0.45	0.50	0.08
Semen color (1-2)	1.00	1.00	2.00	0.23
Semen consistency (1-4)	2.00 ^b	3.20 ^a	3.66a	0.45
Semen pH (1-14)	7.47 ^a	6.89 ^b	6.70 ^b	0.54
Spermatozoa motility (1-4)	3.00	3.00	3.00	0.05
Spermatozoa progressive motility (%)	67.40 ^c	74.65 ^b	80.20 ^a	2.41
Spermatozoa live proportion (%)	83.01 ^c	88.60 ^b	94.12 ^a	1.33
Spermatozoa concentration (x10 ⁶ /ml)	112.24 ^c	125.78 ^b	133.80 ^a	2.54
Total number spermatozoa per ejaculate (x106/ml)	50.65 ^b	56.66 ^{ab}	67.66a	2.13
Total viable spermatozoa (x109/ml)	291.58 ^c	381.39 ^b	496.69 ^a	10.98
Normal spermatozoa proportion (%)	85.16 ^b	90.02 ^a	91.64ª	1.07

abc: Means with different superscripts along rows are significantly different (p <0.05). SEM= Standard error of means.

normal spermatozoa proportion on the oral administration of folic acid on the rabbit bucks. The oral administration of the folic acid might have stimulated the secretion of testosterone for spermatogenesis which increases the libido of the rabbit bucks and reduced the reaction time. The semen volume and semen concentration observed on this study agrees with the findings of Herbert and Acha (1995) and Ekuma et al. (2017) who reported that rabbit semen volume ranges between 0.4 to 0.71 ml and semen concentration range of 50 to 350 x10⁶ /mm³, respectively.

The pH of the collected semen sample ranges between 7.47 in T_1 and 6.70 in T_3 . The decrease on semen pH as observed in this study were as a result of increase in spermatozoa concentration of the collected semen, increase on the total number of spermatozoa per ejaculate and as well as the increase in spermatozoa metabolic activity as the level of administration of the folic acid increased from 2.5 to 5.0 mg; which in turn increased the rate of fructolysis and fructolytic index of the collected semen. Fructolysis index is the rate of fructose utilization in a semen sample by spermatozoa in one hour at 37°C for their metabolic activities (Harvey and Ferrier, 2011; Sun and Empie, 2012; Sabra and Al-Harbi, 2014). The rate of fructolysis is higher in good quality semen. The higher the semen concentration, the higher the metabolic activities in the semen sample, the more fructose utilization in a given semen sample. Semen quality can be assessed by measuring the rate of fructose utilization and fructolytic index in a semen sample. Fructose utilization by sperm cells produced lactic acid in the semen, which in turn increased the semen pH (Molina et al., 2010; Misro and Ramya, 2012; Toragall, et al., 2019). Therefore, increased in semen concentration and increased in sperm cell number as observed in Table 2 increased the rate of metabolic activity in the collected semen, increased the accumulation of lactic acid in the semen, and also increased the fructolysis index; thereby increasing the pH of the collected semen sample. Moreover, the tail and mid piece of a spermatozoa are responsible for spermatozoa motility (Björndahl, 2010; Mukhopadhyay et al., 2010; Naina and Sing, 2015; Meri and Anu, 2017). Therefore, the significantly improvements (p<0.05) observed on the Spermatozoa progressive motility is attributable to the significant reduction in the clumping of the spermatozoa, reduction on the tail and mid-piece abnormalities of the rabbit bucks administered oral folic acid (Table 3). The lower the tail and mid-piece abnormalities, the higher the spermatozoa progressive motility. Also, the significant improvement on the spermatozoa live proportion, total viable spermatozoa and normal spermatozoa proportion is attributed to the significant reduction on the total abnormalities and cytoplasmic abnormalities of the sperm cells in the semen sample collected from the rabbit bucks administered oral folic acid (Table 3). From these observations, it could be inferred that the oral administration of folic acid at 2.5 and 5.0 mg improved the semen characteristics of the rabbit bucks.

The differential spermatozoa morphology of rabbit bucks on oral administration of folic acid is presented in Table 3. The result of the differential spermatozoa morphology of rabbit bucks showed that significant reductions (p<0.05) were observed on the abnormalities of the head, tail, midpiece, cytoplasmic, total abnormalities, gel formation and clumping of spermatozoa following the oral administration of folic acid at 2.5 and 5.0 mg compared to the control. The significant reduction (p<0.05)observed on abnormalities of the tail, mid-piece and clumping of the spermatozoa following the oral administration of folic acid must have been responsible for the observed significant increase on the progressive motility of the spermatozoa (Table 2). Abnormalities in spermatozoa structures and morphologies (teratospermia) are sometimes caused or influenced by genetic, nutrition, drugs, age, chemicals, and environmental factors (Brugh and Lipshultz, 2004; Mukhopadhyay et al., 2010; Harris et al., 2011; Naina and Singh, 2015). Also, the significant reductions (p<0.05)

Table 3. Differential spermatozoa morphology of rabbit bucks on oral administration of folic acid.

Parameter (%)	T ₁	T ₂	T ₃	SEM
Head abnormality	3.74 ^a	3.64 ^a	3.18 ^b	0.09
Tail abnormality	2.13 ^a	1.49 ^b	0.93 ^c	0.12
Mid-piece abnormality	2.35 ^a	1.98ª	0.79 ^b	0.34
Cytoplasmic abnormality	7.17 ^a	2.86 ^b	2.89 ^b	1.26
Total abnormality	14.84 ^a	9.98 ^b	8.35 ^c	1.32
Relative gel formation	5.66 ^a	2.54 ^b	1.00°	0.43
Relative clumping of spermatozoa	4.66 ^a	2.05 ^b	1.10 ^b	0.61

abc: Means with different superscripts along rows are significantly different (p <0.05). SEM= Standard error of means.

Table 4. Haematology of rabbit bucks on oral administration of folic acid.

Parameters	T ₁	T ₂	T ₃	SEM
Haemoglobin (g/dl)	13.53 ^b	13.96 ^{ab}	14.20 ^a	0.21
Packed Cell Volume (%)	33.00 ^b	33.66 ^b	34.96 ^a	0.31
Red Blood Cell (x10 ⁶ mm ³)	5.41	5.62	5.76	0.32
White Blood Cell (x10 ³ mm ³)	6.81°	7.20 ^b	7.80 ^a	0.07
Mean Corpuscular Volume (fl)	60.99	59.86	60.84	1.34
MCH (pg)	25.01	24.83	24.65	1.65
MCHC (g/dl)	13.53	13.96	14.20	1.06

abc: Means with different superscripts along rows are significantly different (p <0.05). SEM= Standard error of means. MCH= Mean Corpuscular Hemoglobin; MCHC= Mean Corpuscular Hemoglobin Concentration.

Table 5. Differential white blood cells of rabbit bucks on oral administration of folic acid.

Parameters (%)	T ₁	T ₂	T ₃	SEM
Lymphocyte	56.00 ^c	65.00 ^a	60.00 ^b	1.20
Neutrophil	35.00 ^a	32.00 ^b	30.00 ^c	0.80
Monocyte	6.00 ^a	5.00 ^{ab}	3.66 ^b	0.76
Eosinophil	1.00 ^c	1.66 ^b	3.00 ^a	0.02
Basophil	0.00	0.00	0.00	0.00

abc: Means with different superscripts along rows are significantly different (p<0.05). SEM= Standard error of means.

observed on the cytoplasmic abnormalities and total abnormality must have contributed to the improvements observed on the normal spermatozoa, total spermatozoa and live sperm proportions of the rabbit bucks administered oral folic acid as observed in Table 2. From these observations, it showed that the oral administration of folic acid improved the spermatozoa morphology of the New Zealand rabbit bucks.

The haematology of rabbit bucks on oral administration of folic acid is presented in Table 4. The results of the haematological analysis of the rabbit bucks showed that red blood cells, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations were not significantly affected. They are statistically similar (p>0.05) with the control group. Furthermore, significant improvements (p<0.05) were observed on the haemoglobin, packed cell

volume and white blood cells of the rabbits administered oral folic acid compared to the control group. This showed that folic acid stimulated the production of the red blood cells from the bone marrow, improved the oxygen carrying capacity of the haemoglobin, which could enhanced the muscular activities of the rabbit bucks. Moreover, the haemoglobin concentration, packed cell volume and white blood cell are within the normal range (10.4 – 17.4g/dl) for haemoglobin, (25.0 – 50.0%) for PCV, and white blood cell (2.71-12.23x10³ mm³) as recommendation by Jenkins (2006), Moore et al. (2015) and Leineweber et al. (2018), respectively, for healthy rabbit bucks. These results showed that oral administration of folic acids at 2.5 and 5.0 mg improved the haemoglobin, packed cell volume and the white blood cells of the rabbit bucks.

The differential white blood cells of rabbit bucks on oral administration of folic acid is presented in Table 5. The

result of the differential white blood cells showed that there were significant increases (p<0.05) on the lymphocyte and eosinophil, while the neutrophils and monocytes of the rabbit bucks administered folic acid significantly decreased (p<0.05) compared to the rabbit bucks on the control group. The functions of these differential white blood cells were to protect and defend the body against different forms of infections and diseases by stimulating the immune system response on defense against disease infestations (McCarrel et al., 2012; Cross et al., 2015; Riboh et al., 2016; King et al., 2018). The physiological actions impacted by the administered folic acid on the white blood cells and its 'differentials' of the rabbit bucks is an indication that folic acid has the potency to stimulate the immune system response and possessed an immunemodulatory properties.

Conclusion

Based on the results and observations from this study, it is concluded that oral administration of folic acids at 5.0 mg per rabbit buck most significantly improved the semen characteristics, enhanced the spermatozoa morphology, reduced sperm cell abnormalities and improved some haematological parameters of the rabbit bucks.

Recommendation

From the results obtained, it is recommend that up to 5.0 mg of folic acid per rabbit buck could be administered orally to improve the semen quality and some haematological parameters of New Zealand white rabbit bucks.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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