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Organ, Testicular and Epididymal Morphometrics of Male Rabbits fed Dietary Cerium Oxide

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Abstract

32 growing buck rabbits of about 12 weeks old were used to assess the effects of inclusion of different dietary concentrations of Cerium oxide (CeO), a Rare Earth Element, at 0, 50, 100, 150mg on organ and relative organ weights, testes and epididymal morphometrics of the animals. The animals were randomly allotted to the four dietary groups, with eight animals per group. The weights, lengths and widths of reproductive organs, other body organs (like liver, kidney, spleen, heart, adrenal gland, pancreas, lung and gastro-intestinal tract) were determined. The results showed no significant ($P<0.05$) influence of dietary cerium oxide on organs weights (except for relative liver and kidney weights) and the testicular volume but the testicular and the epididymal weights and lengths were significantly ($P>0.05$) influenced by dietary cerium oxide especially at 50 mg level of cerium oxide inclusion.

Keywords

Cerium Oxide, Rabbits, Organ Weights, Epididymal, Testes Morphometrics

1. Introduction

The application of Rare earth elements (REE), as feed additive for farm animals has been practiced in China for decades. However, it is not until 1999 that growth promoting effects of rare earth elements were recognized beyond China [1]. Within the scope of increasing population, animal production needs to be enhanced by 2% per year to assure adequate feed supply while keeping environmental loads as low as possible; efficient use of available resources is required which among other things may be achieved with feed additives [2]. With the ban of all antibiotic feed additives throughout Europe in 2006, due to public concerns about both development and dissemination of multi-resistant bacteria, strong growth hormone agents vanished from the feed market. This led to a strong demand for new, efficient, safe and inexpensive feed additive that may satisfy the needs provoked by these changes. Rare earth elements may therefore be a promoting approach.

Most Rare earth elements are not rare as the name implies [2]. Cerium the most abundant REE comprises more of the earth crust than copper or lead. Many REEs are more common than Tin and molybdenum, and all but promethium are more common than silver or mercury [3].The term rare earth elements comprise 15 lanthanide elements with atomic numbers 57 (lanthanum) through 71 (lutetium), which are in group III A of the periodic table.

In China, REEs have been in use for over 50 years as performance enhancers in crop production and remarkable results have been reported from Chinese agricultural operations [4]. In animal production, Chinese literature has reported amazing results achieved by supplying REE in animal diets [2]. It was reported that proper concentrations of REE in diet can improve animal growth performance without any form of interference with the quality of products [5].

Feeding experiments performed under European conditions showed that dietary supplementation of REE had positive effects on both animal growth and feed conversion of pigs and poultry ([1]; [6], [7], [8]).

The performance enhancement effects of REE supplemented diets could be achieved in a great variety of farm animals as well as in aquaculture. However, not only the effectiveness but also thesafety of REE application has been assessed in China, prior to its utilization. [2] also stated that adverse effects should not be expected from possible accumulation of REE in animal tissues as their concentration is generally reported to be low in animal products.

This study was therefore aimed at evaluating the effects of dietary rare earth element on the organs and relative organ weights, testes and epidididymal morphometrics of growing male rabbits.

2. Materials and Methods

2.1. Experimental Site and Animals

The study was carried out at the Rabbit unit of the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria.

Thirty-two growing male rabbits of about 3 months old (12 weeks) were sourced from reputable farm and individually housed in wire-meshed cages in an indoor pen and were fed *ad-libitum* at 0, 800, 1600h for two weeks of physiological adjustment period. During the period of acclimatization, the animals were served Kepromec oral® in their drinking water as recommended by the manufacturer.

2.2. Experimental Diet

Four experimental diets were formulated: control (diet 1) with non-inclusion of REE, diets 2, 3 and 4 with 50, 100 and 150 mg inclusion levels of cerium oxide, respectively as shown in Table 1. The diets were isocaloric, isonitrogenous and satisfied the nutrient requirements of the animals as recommended by [9]. The composition of the diets fed during this study is presented in Table 1.

Table 1. *Gross composition (%) of growing rabbit test diets*

	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**
Ingredients	**Control**	**50mg CeO**	**100 mg CeO**	**150 mg CeO**
Maize	32.10	32.10	32.10	32.10
Wheat offal	41.80	41.80	41.80	41.80
Ground nut cake	3.50	3.50	3.50	3.50
Palm kernel cake	20.00	20.00	20.00	20.00
Oyster shell	1.50	1.50	1.50	1.50
Bone meal	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15
Methionine	0.05	0.05	0.05	0.05
Vitamin Premix	0.20	0.20	0.20	0.20
Salt	0.45	0.45	0.45	0.45
Cerium oxide (mg)	-	50	100	150
Calculated Nutrient				
Crude Fibre (%)	10.83	10.83	10.83	10.83
Crude Protein (%)	10.38	10.38	10.38	10.38
ME* (MJ/kg)	2906	2906	2906	2906
Ether Extract	4.52	4.52	4.52	4.52

ME*: Metabolisable Energy

2.3. Experimental Details

The animals were randomly assigned into one of the four dietary treatments (eight per treatment) after a 2-week physiological adjustment period. They were provided with fresh, clean water and appropriate feed for eight weeks. After the eight-week feeding trial, all the experimental rabbits were slaughtered and their reproductive systems were carefully dissected, organs like the liver, kidney, spleen, heart, adrenal gland, pancreas, lung and gastro-intestinal tract were excised and the testes and epididymides carefully collected, trimming off adhering tissues and weighed using a sensitive electronic balance. Testicular and epididymal morphometric characteristics: testis length, testis width, testis volume and epididymal length were also measured. The testis length, testis width and epididymal length were measured with the aid of a pair of vernier calipers, while the testis volume was measured by water displacement according to Archimedes principle [10]. Paired and mean testicular and epididymal parameters were computed from data for left and right testes and epididymis.

2.4. Statistical Analyses

The design used for this experiment is Completely Randomization Design (CRD). Data collected were subjected to statistical analysis using ANOVA procedure of [11]. The Duncan's multiple range tests of [11] was used to separate all means at 5% probability level.

3. Results

3.1. Organs and Relative Organs Weights

The organ weights of bucks fed dietary cerium oxide are as presented in Table 2. The results showed that the weights of all the organs (Liver, heart, spleen, kidney, adrenal gland, GIT, pancreas and lungs) were not significantly ($P>0.05$) influenced by the varied levels of dietary cerium oxide.

Also, the relative weights of the organs showed no significant ($P>0.05$) difference except the relative weights of liver and kidney. The relative liver weight ranged between 1.97 and 2.05% for diets 2, 3 and 4 respectively as against 2.17% for control diet while that of kidney ranged from 0.30-0.31% across the dietary treatments compared with 0.34% in control diet. The relative weights of the heart, spleen, adrenal gland and pancreas could be regarded as constant across the dietary treatments with no specific pattern of increment or reduction.

3.2. Testis Morphometrics

Table 3 shows the testis morphometrics of bucks fed varied levels of dietary cerium oxide. The results showed that the varied levels of dietary cerium oxide significantly ($p<0.05$) increased the weights of the right and the paired testes except that of the left testis. The weight of the right testis ranged from 1.86-2.23g across the dietary treatments compared with 1.81g for control diet and for the left testis, 4.67, 4.01 and 4.00 g for diets 2, 3 and 4 respectively as against 3.89g for control diet. The level of significance was at 50 mg level of inclusion (diet 2) for both.

The length of the left, right and paired testes were significantly ($p<0.05$) influenced while the volumes were not significantly ($p>0.05$) different. The length of the left testis ranged from 4.13-5.11cm across the dietary treatments as against 4.30cm for control diet while the length of the right testis ranged from 3.94-4.88cm compared with 4.08cm for control diet and the level of significance was at 50 mg (diet 2) level of inclusion. The length of the paired testis were 9.99, 9.53 and 8.07cm for diets 2, 3 and 4 respectively as against 8.38cm for control diet and the length increased significantly at 50 mg (diet 2) and 100 mg (diet 3) levels of inclusion of dietary cerium oxide.

The results further revealed that the width of the right and paired testes were not significantly (p>0.05) influenced except the width of the left testis. The widths of the left testis ranged from 1.09-0.97cm across the dietary treatments and were all similar.

***Table 2.** Organs and Relative Organ Weights of Bucks fed Dietary Cerium Oxide*

	Diet 1	Diet 2	Diet 3	Diet 4	±SEM
Parameters	**Control**	**50 mg**	**100 mg**	**150 mg**	
Liver weight (g)	37.91	37.50	38.35	36.83	1.31
Relative liver weight (%)	2.17^{a}	1.98^{b}	1.97^{b}	2.05^{b}	0.07
Heart weight (g)	2.60	2.72	2.64	2.71	0.20
Relative heart weight (%)	0.15	0.14	0.14	0.15	0.01
Spleen weight (g)	0.54	0.53	0.61	0.54	0.07
Relative spleen weight (%)	0.03	0.03	0.03	0.03	0.00
Kidney weight (g)	5.98	5.75	6.03	5.59	0.35
Relative kidney weight (%)	0.34^{a}	0.30^{b}	0.31^{b}	0.31^{b}	0.01
Adrenal gland weight (g)	0.16	0.20	0.16	0.19	0.03
Relative adr. gland weight (%)	0.01	0.01	0.01	0.01	0.00
GIT weight (g)	270.10	283.23	277.60	271.18	31.50
Relative GIT weight (%)	15.43	14.99	14.27	15.10	1.70
Pancreas weight (g)	0.42	0.36	0.38	0.45	0.10
Relative pancreas weight (%)	0.02	0.02	0.02	0.03	0.006
Lung weight (g)	6.56	6.37	6.01	5.83	1.06
Relative lung weight (%)	0.37	0.35	0.31	0.33	0.07

ab: Means on same row with different superscripts differ significantly (P<0.05)
Relative Ad.gland weight: Relative Adrenal gland weight
SEM- Standard Error of Mean

***Table 3.** Testis Morphometrics of Bucks fed Dietary Cerium Oxide*

	Diet 1	Diet 2	Diet 3	Diet 4	±SEM
Parameters	**Control**	**50 mg**	**100 mg**	**150 mg**	
Weight (g)					
Left testis	2.09	2.44	2.12	2.14	0.22
Right testis	1.81^{b}	2.23^{a}	1.90^{ab}	1.86^{ab}	0.24
Paired testis	3.89^{b}	4.67^{a}	4.01^{ab}	4.00^{ab}	0.45
Length (cm)					
Left testis	4.30^{b}	5.11^{a}	4.90^{a}	4.13^{b}	0.37
Right testis	4.08^{bc}	4.88^{a}	4.63^{ab}	3.94^{c}	0.36
Paired testis	8.38^{b}	9.99^{a}	9.53^{a}	8.07^{b}	0.70
Width (cm)					
Left testis	1.01^{ab}	1.09^{a}	0.97^{b}	1.01^{ab}	0.07
Right testis	0.94	1.03	0.93	0.99	0.10
Paired testis	1.95	2.12	1.90	2.00	0.15
Volume (ml)					
Left testis	1.07	1.11	1.09	1.11	0.10
Right testis	1.05	1.09	1.08	1.11	0.08
Paired testis	2.13	2.21	2.17	2.22	0.17

abc: Means on same row with different superscripts differ significantly (P<0.05)
SEM - Standard Error of Mean

3.3. Epididymal Morphometrics

Table 4 shows the epididymal morphometrics of bucks fed varied levels of dietary cerium oxide. The result showed that the lengths and the widths of the left, right and paired epididymis were significantly (p<0.05) influenced. The level of significance was observed throughout at 50 mg level of inclusion of dietary cerium oxide while the values observed at 100 and 150 mg levels of inclusion were similar compared with the control diet.

The length of left epididymis ranged from 11.79, 11.05 and 10.82cm for diets 2, 3 and 4 respectively as against 10.82 for control diet and 11.66, 10.92 and 10.64cm for diets 2, 3 and 4 for the right epididymis as against 10.65cm for the control diet. The length of the paired epididymis ranged between 23.44-21.97cm across the dietary treatments compared with 21.47cm for control diet. The weight of the epididymis were significantly increased at 50mg (diet 2) level of inclusion of dietary cerium oxide with the values 1.44, 1.32 and 2.76g for left, right and paired epididymis respectively as against 1.19, 1.08 and 2.27g for control diet.

***Table 4.** Epididymal Morphometrics of Bucks fed Dietary Cerium Oxide*

	Diet 1	Diet 2	Diet 3	Diet 4	±SEM
Parameters	**Control**	**50 mg**	**100 mg**	**150 mg**	
Length (cm)					
Left epididymis	10.82^{b}	11.79^{a}	11.05^{ab}	10.82^{b}	0.55
Right epididymis	10.65^{b}	11.66^{a}	10.92^{ab}	10.64^{b}	0.54
Paired epididymis	21.47^{b}	23.44^{a}	21.97^{ab}	21.46^{b}	1.08
Weight (g)					
Left epididymis	1.19^{b}	1.44^{a}	1.20^{b}	1.25^{b}	0.11
Right epididymis	1.08^{b}	1.32^{a}	1.15^{b}	1.19^{ab}	0.10
Paired epididymis	2.27^{b}	2.76^{a}	2.35^{b}	2.44^{b}	0.20

ab: Means on same row with different superscripts differ significantly (P<0.05)
SEM- Standard Error of Mean

4. Discussion

4.1. Organ and Relative Organ Weight

The results of this study on organ weights were contrary to the reports of [2] that the absorbed amounts of REE were higher in liver, kidney and fat compared to muscle or heart in the range of 5 - 100 µg/kg after oral administration of rare earths to broilers. The report of this study is also contrary to the reports of [12,13,14] that reported accumulation of lanthanides in liver, kidney, bone and teeth of rats after oral administration and that of [12] that rare earth contents of up to 0.607 mg were observed in liver, kidney, lungs, spleen and bone in rats fed rare earths at 1800 mg/kg over eight months.

Although, the organ weights were not significantly influenced in this study, the relative liver and kidney weights were influenced significantly indicating possible impacts of dietary cerium oxide on liver and kidney, as both weights increased but insignificantly at 100 mg of cerium oxide inclusion. These results indicate minimal organ absorption of rare earths after oral administration.This might be explicable by the higher affinity of light rare earths for liver tissue [15].

4.2. Testis Morphometrics

[16] reported that the testis size is related to age of the animal. This report made the significant influence of dietary cerium oxide on the testes weight, its length and width unexpected because the animals used in this research work are of the same age. The results of this study showed that dietary cerium oxide influenced testis morphometrics significantly, although the testicular volume increased insignificantly at 50 and 100mg levels of cerium oxide inclusion. [16] also reported that testicular weight and sperm production are related. [17] described the mammalian testes as infallible predictors of spermatozoa production. The results of this study on testis morphometrics therefore, indicate that REE is capable of improving sperm production in rabbits, as [18] and [19] reported that increase in relative testis size are assumed to reflect increased investment in sperm production. [18] also stated that testicle size is a good indication of sperm producing ability. The significant improvement in testes morphometrics observed in this study could possibly be due to the influence of REE on the hormones of spermatogenesis such as testosterone; as [2] suggested that REE may be capable of influencing sex hormones in animals. Moreso, [18] also stated that testosterone controls testicular volume and also support spermatogenesis.

4.3. Epididymal Morphometrics

[17] (2003) asserted that the knowledge of the basic morphometric characteristics of the reproductive organs is mandatory for assessment and prediction not only of sperm production but also of the storage potential and fertilizing ability of the breeder male. The significant improvement of epididymal morphometrics of the buck rabbits fed dietary REE in this study indicates the possibility of REE influencing sperm maturation and storage, as epididymis is the site of gametes maturation and storage before ejaculation.

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