Reproductive Performance of Growing Female Rabbits (Oryctolagus Cuniculus) Fed Diets Supplemented with Cerium Oxide

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***Abstract*—In a four-month feeding experiment, 32 growing female rabbits (*Oryctolagus cuniculus*) of an average weight of 1366.56±37.54 g were used to evaluate the inclusion of different dietary concentrations of cerium oxide (CeO) on fertility and post-partum performance of animals. The animals were randomly allotted to four dietary groupssupplemented with 0, 100, 200 and 300 ppm CeO, each consisting of eight animals and were fed for eight weeks before mating.At the end of the 1st trimester, 16 out of the 32 mated does were sacrificed and their uteri cut open and the remaining 16 pregnant does were raised to full gestation to evaluate the fertility and post-partum parameters, respectively. The results revealed that the dietary REE, especially at 200 ppm/kg feed significantly (P<0.05) increased the litter size, litter weight, total litter weight, embryo survival rate, average litter size and weight post−partum except for the conception rate, gestation length, foetal crown-rump length and embryo weight that were not significantly (P>0.05) influenced. Dietary REE is therefore capable of enhancing fertility in rabbits particularly at 200 ppm/kg feed and at the same time reduce mortality in kittens by 20.88 - 68.01 % and 34.14 - 45.10 % at 100-200 ppm/kg feed.**

***Index Terms*—cerium oxide, fertility, rabbit, Rare earth element, reproduction**

I. Introduction

The use of antibiotics as feed additives in farm animals was banned by the European Union in early 2006 [1]. Hence, there was a need to develop an alternative feed additive which promotes growth and also enhances feed efficiency. Probiotics, prebiotics, organic acids and enzymes are already known as replacements for antibiotics as feed additives but rare earth elements (REEs) might be the new generation of growth promoters [1].

Rare earth elements are the 15 lanthanide elements with atomic numbers 57 (lanthanum) through 71 (lutetium), which are in group III A of the periodic table. Despite their name, the REEs are not rare. In China, REEs have been in use forover 50 years as performance enhancers in crop production and remarkable results have been reported from Chinese agricultural operations [2]. In animal production, excellent results have also been obtained by incorporating REE in animal diets in Chinese literatures [1]. It was reported that proper concentrations of REE in diet can improve animal growth performance without any form of interference with the quality of the products [3].

Moreover, it was reported that REE may also increase milk production in dairy cows and egg production in laying hens [1] and at the same time improved fertility in hens [4]. Feeding experiments performed under Western conditions showed that dietary supplementation of REE had positive effects on both animal growth and feed conversion in pigs and poultry [5]-[8]. Based on results obtained from Western feeding experiments, the effects of dietary REE vary with the animal species. Concentration and type of rare earths as well as the compositions of individual rare earth elements have also been shown to be important factors influencing performance enhancing effects of REE on animals [1], [8], [9]. Also, it is generally agreed that the efficiency of growth- promoting agents highly correlates with keeping, housing, hygiene and feeding conditions [10] and the genetic variations in animals which might lead to differences in enzyme activities, and keeping conditions which may affect the composition of microbial populations within the gastrointestinal tract [1].

Performance enhancements of rare earth containing feed additives could be achieved in a great variety of farm animals as well as in aquaculture [11], [12]. However, not only the effectiveness but also the safety of REE application has been assessed in China, prior to their commercial utilization. Additionally, adverse effects for consumers may neither be expected from possible accumulation of rare earth elements in animal tissues as their concentration is generally reported to be low in the products [1]. This present study was therefore designed to evaluate the effects of different concentrations of cerium oxide on the reproductive performance of growing female rabbits fed diets supplemented with cerium oxide.

II. Materials and Methods

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A. Animals and Treatment

Thirty-two female rabbits of about 12 weeks of age of an average weight of 1366.56±37.54g were used. The animals were housed individually in a wire-mesh cage in an in-door pen and were fed *ad libitum* at 0800 and 1600h for two weeks of physiological adjustment period and Kepromec Oral (Ivermectin®) was administered through drinking water against potential ecto and endo-parasites for two days at recommended dosage by the manufacturer. Four experimental diets were formulated: control (diet 1) with non-inclusion of REE, diets 2, 3 and 4 had 100, 200 and 300ppm inclusion of REE (Cerium oxide) respectively, as shown in Table I. The animals were randomly assigned into one of the four diets (eight per treatment) after the 2weeks of physiological adjustment period. The diets were isocaloric, isonitrogenous and satisfied the nutrient requirements of the animals as recommended by National Research Council [13]. The animals were provided with fresh, clean water and appropriate feed throughout the feeding period.

TABLE I. GROSS COMPOSITION (%) OF GROWING RABBIT TEST DIETS

Ingredients	Diet 1 Control	Diet 2 100ppm CeO	Diet 3 150ppm CeO	Diet 4 200ppm 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
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
CeO (ppm)	-	100	200	300
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.

TABLE II. FERTILITY OF GROWING FEMALE RABBITS FED VARIED CONCENTRATIONS OF DIETARY CERIUM OXIDE

Parameters	Diet 1 Control	Diet 2 100ppm	Diet 3 200ppm	Diet 4 300ppm	±SEM
Conception Rate (%)	93.75	100.00	100.00	96.88	6.99
Embryo Survival Rate (%)	67.22^{b}	63.20^{ab}	72.79^{a}	58.78^{b}	11.92
Embryo Weight (g)	34.63	34.44	34.25	34.50	1.17
Foetal Crown Rump Length (cm)	8.4	8.0	8.1	8.4	0.4
Litter Size	4.38^{b}	5.25^{a}	5.25^{a}	4.75^{ab}	0.61
Gestation Length (days)	30.75	30.25	30.00	30.00	0.54
Litter Weight (g)	42.00^{b}	41.00^{b}	46.75^{a}	43.50^{ab}	2.64
Total Litter Weight (g)	183.75^{b}	215.25^{ab}	245.44^{a}	206.63^{ab}	21.58

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

B. Parameter Analysis

To determine the fertility rate of growing female rabbits fed varied levels of dietary REE, after 8 weeks of feeding the respective experimental diets, 4 bucks were randomly mated to 8 does per treatment (i.e. 1 buck to 2 does). At the end of the 1st trimester, 16 out of the 32 mated does (4 per treatment) were sacrificed and their uteri cut open longitudinally to check for conception, count the embryos therein and measure the crown-rump lengths as well as the weights of the embryos. The embryo survival rate was determined as described by Adu [14]. The remaining 16 pregnant does were raised to full gestation to determine thegestation length, litter size and litter weight. The breeder females were then maintained on their respective diets through gestation to 21-day post-partum and the kittens were weighed on weekly basis.

C. Statistical Analysis

The design used for this experiment was Completely Randomized Design (CRD). Data collected were subjected to statistical analysis using one way analysis of variance (ANOVA) procedure of SAS [15]. The significant treatments were compared using Duncan's multiple range test of the same software.

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III. Results

A. Reproductive Performance

Table II shows the effects of different concentrations of dietary cerium oxide on the fertility of does. The results showed that dietary cerium oxide at various concentrations significantly (P<0.05) improved the reproductive parameters in terms of the litter size which improved by 8.45 - 19.86 %, litter weight and total litter weight by 3.57 - 11.31 % and 12.45 - 33.57 %, respectively and embryo survival rate increased by 8.29 % at 200ppm level of inclusion. Though the conception rate was not significantly (P>0.05) influenced, it improved by 3.13-6.25 % across the treatments. The improvements observed for all the reproductive parameters determined were most pronounced at 200 ppm level of inclusion of cerium oxide.

B. Post−Partum Performance

The post-partum performance of the kittens is as shown in Table III. The results showed that the average litter weight and size at birth, 7 and 21, daily weight gain and mortality at 7 and 21 day post-partum were significantly (P<0.05) influenced. The average weights at birth, and at 7 and 21 day post-partum improved by 2.38 - 11.31 %, 6.77 - 8.70 % and 0.44 - 7.78 %, respectively across the treatments.The result showed 2.02 - 8.89 % improvement in daily weight gain at 200 and 300 ppm/kg feed concentration of dietary cerium oxide with no improvement at 100 ppm level of inclusion.

TABLE III. Post-Partum Performance of Kittens and Milk Production of Does Fed Varied Levels of Dietary Cerium Oxide

Parameters	Diet 1 Control	Diet 2 100ppm	Diet 3 200ppm	Diet 4 300ppm	±SEM
Weight at birth(g)	42.00^{b}	41.00^{b}	46.75^{a}	43.50^{ab}	2.64
Weight at 7th day(g)	90.74^{b}	97.88^{ab}	98.63^{a}	96.88^{ab}	6.69
Weight at 21st day(g)	197.75^{b}	196.88^{b}	205.63^{a}	213.13^{a}	7.46
Daily Weight Gain(g)	7.42^{c}	7.42^{c}	7.57^{b}	8.08^{a}	0.52
Litter size at birth	4.38^{b}	5.25^{a}	5.25^{a}	4.75^{ab}	0.61
Litter size at 7th day	4.25^{b}	5.20^{a}	5.15^{a}	4.58^{b}	0.58
Litter size at 21st day	4.00^{b}	5.00^{a}	4.95^{a}	4.33^{b}	0.70
Mortality at 7th day (%)	2.97^{b}	0.95^{d}	1.90^{c}	3.59^{a}	0.28
Mortality at 21st day (%)	8.67^{a}	4.76^{b}	5.71^{c}	8.84^{a}	0.07
Milk production (%)	126.52^{b}	116.61^{c}	125.75^{b}	136.73^{a}	0.57

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

The litter size improved by 8.45 - 19.86 %, 7.76 - 22.35 % and 8.25 - 25 % at birth, 7and 21 day post-partum for 100, 200 and 300 ppm, respectively. The mortality on the 7 and 21 days post-partum reduced by 20.88 - 68.01 % and 34.14 - 45.10 %, respectively and increased at 300 ppm level of cerium oxide inclusion. The milk production of the does increased (P<0.05) significantly at 300 ppm concentration of dietary cerium oxide and decreased significantly (P<0.05) at 100 ppm inclusion level but similar at 200 ppm compared with the control diet.

IV. Discussion

The few researches carried out to show the effects of dietary rare earth elements on fertility parameters have been on carps [16], [17], prawns [16], [18] and poultry [19]. In this study, the increment observed in litter size as a result of dietary cerium oxide was more at lower concentrations than at high concentrations. This is in consonance with the report of Redling [1] that lower concentrations of REE has been reported by most authors for optimum performance and the ranges of 100 and 200 mg/kg were described in most experiments.The enhanced reproductive performance of rabbits fed varied levels of dietary cerium oxide in this study, which showed improvement in litter size and embryo survival rate, agreed with the report of Wu *et al*. [4] that dietary REE enhanced egg production and fertilization rate of hatching eggs in poultry after oral rare earth administration and the report of Chen and Xiong [19] that dietary REE improved the laying rate of hens by 8.18 and 6.41 %, respectively. The significant improvement observed for embryo survival rate in this study is in line with the reports of Xin *et al*. [18] and Shao *et al.* [16] that REE could promote egg embryo development in oriental prawns after being absorbed from seawater. The significantly improved litter size observed in this study correlates with the findings of Gong [20] that reported an increase of 4.7 % in laying rate of breeding hens after being administered REE supplemented diet. The results of this study on embryo survival rate and average litter weight are also in line with the findings of Guo *et al.* [21] that reported improved survival rate and body weight by 4.5 – 10 % and 4 – 8 % respectively in chickens fed rare earth supplemented diet. The significant increase in litter weight corresponds with the report of Zhang *et al*. [22] that improvements in fertilization, hatching rate as well as the percentage of healthy birds and at the same time a significant increase in individual egg weight were observed in laying hens fed dietary REE.

Possible interaction of dietary REE with reproductive hormones is suspected as the reason for the improved fertility performance observed in this study. This is consistent with several Chinese reports [23], [24] proposing changes in hormonal activities as mechanism underlying performance enhancement of rare earth elements. Thus, REEs may affect the intermediate metabolism in many different ways including hormone levels, enzyme activities, protein or lipid metabolism [1].

The results obtained on daily weight and average weight gains in does are in line with the reports of Guo *et*

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al. [21] that dietary REE increased the daily body weight gain by 8 – 10 % and 4 – 8 % in pigs and chicken respectively, and Adu *et al.* [14] that reported 4.4 – 13.5 % improvement in the daily weight gain of rabbits fed rare earth supplemented diet. Xu *et al.* [25] also reported similar result in growing pigs. The improvements observed in the daily and average weight gain from birth to 21 day post-partum could be attributed to the fact that the REE is capable of improving nutrients utilization in the diets and also increase the secretion of digestive fluids thereby leading to increased digestibility and subsequent influence on live weight gain [26].The result of this study on mortality agreed with the findings of Shao *et al.* [16] that reported inhibitory effects of REE supplemented diets at high concentration in carps and shrimp, respectively as against positive effects obtained at low concentrations. However, the significant reduction in percentage mortality observed in this study, is in line with the findings of Gong [20] that in breeding hens, the incidence of damaged eggs decreased by 1.5 % after rare earth supplementation. This could be due to the influence of dietary REE on the activities of several hormones and enzymes. The result of this study on milk production concurred with the report of Redling [1] that dietary rare earth element increased milk yield in dairy cow. This could be as a result of possible influence of cerium oxide on some hormones such as prolactin that enhances milk secretion and oxytocin that releases the milk.

It can be concluded that dietary REE is capable of enhancing fertility in rabbits by significantly increasing the litter size, litter weight, total litter weight and embryo survival rate and at the same time reduced mortality at concentrations of 100 – 200 ppm/kg feed. It also increased milk yield in rabbits at 300 ppm/kg feed but could increase mortality at same concentration in kittens.This study has therefore, shown clearly that dietary cerium oxide is capable of promoting reproductive performance in rabbits with no detrimental effects at 200 ppm/kg feed.

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