REPRODUCTIVE PERFORMANCE AND POSTPARTUM SERUM BIOCHEMISTRY OF RABBIT DOES FED SUPPLEMENTAL MAGNESIUM

***Ikeh N. E., Anizoba N.W., Ezejiofor, M. C, Umeugokwe, P.I., Amaefule, B. C., Ali, L.C., and Machebe, N. S.**
Department of Animal Science, University of Nigeria, Nsukka
*Corresponding Author: nnanna.ikeh.pg80080@unn.edu.ng; +2347033085209

Abstract
A total of 16 matured female mixed-bred rabbits with an average weight of 2.1kg were used to evaluate the effect of supplemental magnesium (Mg) on the reproductive performance and postpartum serum biochemistry. The study lasted for twelve weeks and was designed as a completely randomized design (CRD) with four treatments having 4 female rabbits per treatment. The Mg was administered at 0, 250, 500 and 750 mg per litre of water to T1, T2, T3 and T4 respectively. Feed and water were supplied *ad libitum* throughout the study period. The results showed that gestation length, litter size at birth, litter size at weaning, mortality rate and kit weight at birth were not significant ($p>0.05$) among the treatment groups. However, Kit weight at weaning and kit weight gain of T1, T3 and T4, were similar, but significantly ($p<0.05$) lower than T2. Magnesium supplementation had no significant ($p>0.05$) effect on ALT, glucose, urea and total protein among treatment groups. The AST and ALP levels postpartum were however significantly different ($p<0.05$) among treatment groups. The AST level of T1 and T2 were similar, while T4 had the highest significant ($p<0.05$) value. The ALP levels of T1 and T2 were similar, whereas T3 and T4 were significantly ($p<0.05$) higher. Therefore, it was concluded that 250mg magnesium supplementation will improve the weight of the kits at weaning, and overall kits weight gain. Thus improving reproductive performance of breeding rabbit does.
Keywords: female rabbits, magnesium, reproductive performance, postpartum, serum biochemistry

Introduction

Efficient production is one of the main factors that assure high productivity in rabbit farms. To achieve this, breeders need to consider properly all the management practices that will promote the physiology, reproductive performance, behaviour and general well-being of the animal (Johnson, 2018). One such management practice involves reducing the effects of heat stress on the animals.

Rabbit Does are sensitive to heat stress, which is a major factor influencing their fertility, and also has negative effects on their reproductive and physiological traits as feed intake is reduced drastically; reduced feed intake ultimately leads to reduced nutrient levels and negative energy balance in rabbit does (which can lead to infertility and high culling rate because of the high energy demand for pregnancy and lactation). Hence, to compensate for the reduction in feed intake as a result of heat stress the farmer needs to increase the concentration of minerals and vitamins in their diet. Minerals such as magnesium, calcium and Iron have been used in the past to improve feed intake. The practice of supplementing feedstuffs with Mg is widely used, with the primary aim to avoid Mg deficiency and then improving animal performance (fertility and yield) and sometimes products' quality (Pinotti *et al.* 2021).

Adequate feed and nutritional plan will result in normal body physiology, metabolism and improved reproductive performance in terms of improved metabolic rate, increasing litter size, adequate maintenance of pregnancy and subsequent milk let down by the doe (Effiong and Wogar, 2007). Although not much has been documented on the impact of magnesium on the reproductive performance of rabbit does, possibly, dietary supplements such as magnesium with the intent to maintain healthy systems and deal with the effect of heat stress are necessary for better metabolic and reproductive performance of the rabbit. Hence, the need to evaluate the reproductive performance and postpartum serum biochemistry rabbit does fed supplemental magnesium.

Materials and methods

The research work was conducted in the Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Prolife magnesium (Mg) supplement was procured from Ogige Market, Nsukka. A total of 16 mature rabbit Does with an average weight of 2.1kg were randomly assigned to 4 treatment groups, (T_1, T_2, T_3, T_4,) with 4 replicates, having 1 doe per replicate. Immediately after acclimatization, four (4) bucks were used to randomly mate the Does, then the Mg was administered at 0, 250, 500 and 750 mg per litre of water to T1, T2, T3 and T4 respectively. Palpation of the lower abdominal region was done 14 days after mating to detect pregnancy, weight changes to diagnose pregnancy were also taken. Those that failed to conceive, were re-mated. Feed and water were supplied *ad libitum* throughout the twelve weeks study period. After parturition, about 2 ml of blood samples were obtained from the optical plexus of the does, postpartum, using a heparinized (plain) haematocrit capillary. Blood samples were collected from 3 Does per treatment, and samples were put in non-vacuum plain tubes without EDTA (Ethylenediaminetetraacetic acid) for serum analysis (aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), total protein, urea, and glucose). Reproductive Parameters such as Gestation length, Litter size at birth, Litter size at weaning, Mortality, Kit weight at birth, Kit weight at weaning, Kit weight gain were taken. The experimental design that was used for this study is the Completely Randomized Design (CRD) with model;

$$X_{ij} = \mu + T_i + \sum_{ij}$$

Where; X_{ij} = individual observation, μ = overall population mean, T_i = treatment effect, $\sum_{ij}$ = experimental error. The significant differences among the treatment means were separated using Duncan's New Multiple Range Test significance (Duncan, 1955).

Results and Discussion

The results of the effect of different levels of supplemental magnesium on the performance of rabbits, are presented in Table 1. This study shows that the different levels of supplemental magnesium fed to the rabbit Does had no significant (P>0.05) effect on the gestation length of the Does. This result is expected because gestation length is determined mostly by litter size and genetics and is fairly constant in animals (Omeje, 2014), thus, mineral supplementation has little effect on gestation length. The effect of supplemental magnesium on the litter size of the does at birth and weaning was non-significant (P>0.05). Zhang *et al.* (2014) also reported similar findings on the effect of magnesium on pigs and gilts. According to their report, the total number of piglets born and born alive, the number of piglets weaned, birth weight, and weaning weight were unaffected by magnesium supplementation for gilts. Mortality rate and kit weights at birth were found to be non-significant (P>0.05). Omeje (2014), reported similar findings in his study of the effect of using a mineral supplement on litter weight at kindling. The results showed that Kit weight at weaning and overall Kit weight gain differed significantly (P<0.05) between treatment means. The rabbit does in T2 produced the highest significant (P>0.05) value for both kit weight at weaning and kit weight gain compared to T1, T3, and T4 which were statistically similar. For both parameters, the control group had the lowest values. Similar to the findings of this research, Omeje (2014) reported significant (p < 0.05) differences in the mean body weight of litter at weaning in does fed mineral supplements in form of selenium.

Table 1: Performance of Rabbit Does Fed Supplemental Magnesium

Parameters	T1 (0mg)	T2 (250mg)	T3 (500mg)	T4 (750mg)	P-VALUE
Gestation Length (Days)	30.50 ± 0.65	31.50 ± 0.29	31.00 ± 0.58	31.67 ± 0.67	0.478^{NS}
Litter Size at Birth	4.69 ± 1.33	4.50 ± 1.50	4.50 ± 1.50	5.67 ± 0.88	0.885^{NS}
Litter Size at Weaning	3.33 ± 1.67	4.00 ± 1.00	4.50 ± 1.50	5.33 ± 1.20	0.762^{NS}
Mortality (%)	16.67 ± 0.00	8.33 ± 4.81	0.00 ± 0.00	8.33 ± 8.33	0.336^{NS}
Kit Weight at Birth(g)	42.81 ± 4.01	49.58 ± 5.13	39.47 ± 3.41	40.95 ± 2.17	0.247^{NS}
Kit Weight at Weaning(g)	323.60 ± 67.39^{b}	563.74 ± 63.32^{a}	333.71 ± 19.39^{b}	342.71 ± 15.36^{b}	0.000^{**}
Kit Weight gain(g)	282.63 ± 63.09^{b}	514.16 ± 58.45^{a}	294.24 ± 18.29^{b}	301.76 ± 14.50^{b}	0.000^{**}

$^{a,\ b}$: Means ± SEM in a row with different superscripts are statistically significant (**P <0.01). NS = Not significant

The results of the effect of different levels of supplemental magnesium on biochemical parameters (ALT, ALP, AST, etc.) in rabbit Does post-partum, are presented in Table 2. From the results, magnesium levels induced a significant (P<0.05) effect on postpartum AST and ALP levels and a non-significant effect (P>0.05) on ALT, urea, total protein, and glucose. The AST levels were highest in T4, followed by T3, T2 and T1. The ALP levels were highest in T4 and T3 while T1 had the least postpartum ALP levels. Magnesium (Mg) is involved in many biochemical processes, including the activation of phosphates and participation in carbohydrate metabolism. Its action is closely associated with calcium (Ca) and phosphorus (Kova´csne´ *et al.,* 2004). The concentration levels of both AST and ALP are between 21-25 iu/L and 21-26 iu/L, respectively. This ALP range is in tandem with the reports of Melillo (2007) in his book, Rabbit Clinical Pathology, where he reported the ALP range to be between 12 - 96 iu/L. The AST range does not, however, fall within his range of values; he reported the AST to be between 35 - 130 iu/L. Similarly, Hein and Hartmann (2003), reported laboratory ranges of AST and ALP for mixed rabbit breeds as, 0–28 iu/L, and 0–397 iu/L, respectively. These ranges agree with the results of this study for both parameters.

In rabbits, AST is distributed in many tissues, however, AST levels may rise in rabbits diagnosed with liver damage, when they are struggling during blood collection or hemolysis of the sample (Melillo, 2007), and with weight loss (Alina, 2022). ALP on the other hand is a widely distributed enzyme; liver and bone contain the highest concentrations. Rabbits produce two ALP isoenzymes in the liver. Serum ALP concentrations are actually a total of three isoenzymes because an intestinal isoenzyme is extremely abundant. This may further explain why many reference ranges are ambiguous and broad, and why elevated ALP levels are a frequent finding in clinically healthy animals (Melillo, 2007).

The effect of supplemental magnesium on concentration levels of ALT were non-significant (P>0.05). The ALT range obtained in this study falls within the range, 0-60 iu/L as reported by Hein and Hartmann (2003). Although it does not agree with the 45–80 iu/L range observed by Melillo (2007). The effect of supplemental magnesium on concentration levels of urea, total protein and glucose were non-significant (P>0.05). Urea and total protein range from 33-49 mg/dL and 5-9 g/dL, respectively. Values from T1, T3, and T4, fall within the 20- 45 mg/dl (for urea) and 5 - 7.5 g/dl (total protein) range reported by Melillo (2007). As in the case of glucose levels, T2 has a slight increase in urea and total protein levels, above the reference range (Melillo, 2007). Many factors (age of the animal, reproductive status, pregnancy) can affect TP (total protein) levels. Hemostasis at the sample site by fluid loss due to digital pressure on the vessel from which the blood is obtained can artificially elevate total serum protein levels. . The main cause of hyperproteinemia in rabbits is dehydration. Raised TP levels may also indicate a chronic infectious or metabolic process. Hypoproteinemia is usually due to chronic malnutrition or protein loss (Melillo, 2007). Urea levels in rabbits depend on many factors, such as circadian rhythm - peak in late afternoon and early evening (Alina, 2022), quantity and quality of proteins in the diet, nutritional status, liver function, intestinal absorption, urease activity of the caecal flora, and hydration status. Often, small changes in urea levels are difficult to interpret. Slight elevations in blood urea are a common finding (Melillo, 2007). The numerical but non-significant increase in urea and total protein concentration levels of the treatment group fed 250mg supplemental magnesium (T2) could be as a result of high magnesium intake and absorption. With the exception of the control group (T1), T2 had the highest water intake compared to T3 and T4. Magnesium is essential for carbohydrate and protein synthesis. Hence, an increased level of magnesium intake could improve protein synthesis and assimilation in the body, thus elevating the quantity of protein in the body.

Table 2: Postpartum Serum Biochemical Parameters of Rabbits Does Fed Supplemental Magnesium

Parameters	T1 (0mg)	T2 (250mg)	T3 (500mg)	T4 (750mg)	P-VALUE
AST (iu/L)	21.50 ± 0.29^{c}	22.50 ± 0.29^{bc}	23.50 ± 0.29^{b}	25.00 ± 0.58^{a}	0.00^{**}
ALT (iu/L)	12.00 ± 0.00	12.00 ± 0.58	12.50 ± 0.29	12.00 ± 0.00	0.62^{NS}
ALP (iu/L)	21.50 ± 0.87^{b}	25.50 ± 2.02^{ab}	27.50 ± 0.87^{a}	26.50 ± 1.44^{a}	0.04^{**}
Urea (mg/dL)	37.50 ± 0.87	49.00 ± 8.08	44.50 ± 6.06	33.50 ± 2.02	0.20^{NS}
Total Protein (g/dL)	5.40 ± 0.23	8.40 ± 0.52	7.55 ± 1.01	6.80 ± 1.09	0.10^{NS}
Glucose (mg/dL)	135.50 ± 2.59	170.00 ± 13.28	143.50 ± 8.37	153.50 ± 8.37	0.09^{NS}

$^{a,b,c:}$ Means in a row with different superscripts are statistically significant (P<0.05) NS = Not significant. AST - Aspartate Aminotransferase. ALT - Alanine Aminotransferase. ALP - Alkaline Phosphatase

Conclusion and Recommendations

In conclusion, supplementing magnesium significantly improved the kit weight and the kit weight gain at weaning. Aspartate transaminase (AST) and alkaline phosphatase (ALP) were also significantly influenced by Mg supplementation. The results therefore, concludes that: Kit weight at weaning, and kit weight gain, were better with 250mg magnesium supplementation. Also, increasing the levels of magnesium brought about a corresponding increase in levels of AST and ALP which may be detrimental. It can therefore be recommended that 250mg magnesium supplementation could be used by rabbit farmers who are ready to adopt improved nutritional strategies to improve reproductive traits.

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