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Puberty attainment and growth performance in male rabbits fed zinc sulphate supplemented diets

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Abstract

Micronutrients are essential for reproductive performance in farm animals but often deficient in their diets especially in the tropics. This study was conducted to assess the effect of zinc supplementation on puberty attainment and growth indices in male rabbits. The experimental diets were supplemented with zinc as zinc sulphate at 0, 50, 100 and 150 mg/kg of diet and fed to twenty-four growing male rabbits aged 9-10 weeks old allotted to four groups in a Completely Randomized Design. Puberty attainment in the male rabbits was monitored at 72hours interval from 12weeks of age till sperm cells were first seen in their preputial fluid examined under a microscope. Body weight gain and feed intake were monitored weekly; and the feed conversion ratio was estimated. The study lasted for 10weeks. The data obtained were subjected to general linear model of analysis of variance at $p<0.05$. An insignificant impact of zinc supplementation on puberty attainment was recorded. Among all the growth parameters assessed, only feed intake was significantly ($p<0.05$) influenced by zinc supplementation. Average daily feed intake was higher (86.55g) in male rabbits fed Z150 compared to those on other groups. Weight gain was lower in rabbits fed zinc supplemented diets. It can be concluded that dietary zinc supplementation did not result in early attainment of puberty and no significant ($p>0.05$) effect on pubertal weight. Dietary, zinc supplementation did not improve growth performance, puberty attainment or pubertal weight in male rabbits. However, to meet nutritional requirement of growing rabbits, supplementation may be needed as many conventional feeds do not meet the recommended level.

Key words: Growing rabbits; Sperm cells; Weight gain; Zinc sulphate

Atteinte de la puberté et performances de croissance chez les lapins mâles alimentés avec des régimes complétés en sulfate de zinc

Résumé

Les micronutriments sont essentiels pour les performances de reproduction des animaux de ferme mais souvent déficients dans leur alimentation, en particulier sous les tropiques. Cette étude a été menée pour évaluer l'effet de la supplémentation en zinc sur l'atteinte de la puberté et les indices de croissance chez les lapins mâles. Les régimes expérimentaux ont été complétés avec du zinc sous forme de sulfate de zinc à 0, 50, 100 et 150 mg/kg de régime et donnés à vingt-quatre lapins mâles en croissance âgés de 9 à 10 semaines répartis en quatre groupes dans un plan entièrement randomisé. L'atteinte de la puberté chez les lapins mâles a été surveillée à intervalle de 72 heures à partir de l'âge de 12 semaines jusqu'à ce que les spermatozoïdes soient vus pour la première fois dans leur liquide préputial examiné au microscope. Le gain de poids corporel et la consommation d'aliments ont été surveillés chaque semaine; et le taux de conversion alimentaire a été estimé. L'étude a duré 10 semaines. Les données obtenues ont été soumises à un modèle linéaire général d'analyse de variance à $p<0,05$. Un impact insignifiant de la supplémentation en

zinc sur l'atteinte de la puberté a été enregistré. Parmi tous les paramètres de croissance évalués, seule la prise alimentaire était significativement (p<0,05) influencée par la supplémentation en zinc. La consommation alimentaire quotidienne moyenne était plus élevée (86,55 g) chez les lapins mâles nourris au Z150 par rapport à ceux des autres groupes. Le gain de poids était plus faible chez les lapins nourris avec des régimes enrichis en zinc. On peut conclure que la supplémentation alimentaire en zinc n'a pas entraîné l'atteinte précoce de la puberté et aucun effet significatif (p>0,05) sur le poids pubertaire. La supplémentation alimentaire en zinc n'a pas amélioré les performances de croissance, l'atteinte de la puberté ou le poids pubertaire chez les lapins mâles. Cependant, pour répondre aux besoins nutritionnels des lapins en croissance, une supplémentation peut être nécessaire car de nombreux aliments conventionnels ne répondent pas au niveau recommandé.

Mots-clés : Lapins en croissance ; les spermatozoïdes ; Gain de poids; Sulfate de zinc

Introduction

Poor nutrition may adversely affect the various stages of the reproductive event starting with delayed puberty (Smith and Akinbamijo, 2000; Kenny and Byrne, 2018). Supplying balanced diets that meets nutrient requirement is essential for profitable rabbit production. The nutrient requirement of an animal depends on its genetic makeup, its environment, and stage of life (Lebas et al., 1997). Along with carbohydrate and protein, micronutrients must be adequate in an animals' diet. Some impacts of micronutrients on reproduction in farm animals have been reported (Smith and Akinbamijo, 2000). Micronutrients are essential elements required by organisms in varying quantities throughout life to orchestrate a range of physiological functions to maintain health. Small quantities of micronutrients are however needed daily but its significant role in the overall performance of an animal cannot be underestimated. Micronutrients are provided to animals in food, by supplementation or in water. For intensive animal production, supplementation is necessary as it has been the only way to provide sufficient dietary quantities required for optimum health and productive performance (Chrastinova *et al.*, 2015). Micronutrients work as functional and structural cofactor in metal containing enzymes (Khan *et al.,* 2012). These elements either act directly on the gonads and other reproductive organs, or indirectly via the hypophyseal–pituitary–gonadal axis (Smith and Akinbamijo, 2000). Zinc as an essential trace element that plays important roles in different biological processes; maintenance of germ cells, progression of spermatogenesis and regulation of sperm motility (Yamaguchi *et al.,* 2009). It is the second most abundantly distributed trace element in the body after iron. Zinc as a hormone balancer helps hormones such as testosterone, prostate and sexual health (Fallah *et al*., 2018). Zinc is vital for growth and development, sexual maturation and reproduction, dark vision adaptation, olfactory and gustatory activity, insulin storage and release and for a variety of host immune defenses (Biswajit *et al*., 2013). Zinc is a modifier of wide spectrum of biological activities; its deficiency has been related to various dysfunctions and alterations of normal cell metabolism (Chrastinova *et al.*, 2015). Supplementation with 80 mg/kg Zn as Zinc lactate could improve growth performance (Yan *et al*., 2017); this may influence gonadal growth and development. Zinc gluconate supplementation increased seminal zinc concentration in mature male rabbits thus indicating its impact on prostrate health (Adeyemi *et al.,* 2020). Supplementing zinc in the diet of male rabbit may help to improve the use of available stock for breeding purpose in the

Nigeria. However, little has been reported on the impact of zinc supplementation on puberty attainment of farm animals in the developing countries where there are nutritional inadequacies that can influence performance. This study therefore assessed the effect of zinc supplementation as zinc sulfate on puberty attainment and growth performance in male rabbits.

Materials and Methods

The experimental diet was formulated and supplemented with zinc as zinc sulphate at 0, 50, 100 and 150 mg/kg respectively (Table 1). Samples from the diets were subjected to Atomic Absorption Spectrophotometry (AAnalyst 400 AAS) to determine the zinc concentration. The study was carried out at the Rabbitry unit of the Teaching and Research Farm and the Reproductive Physiology Laboratory of the Department of Animal Sciences, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria. The site is located between latitude 7° 32'N to 7°33'N and longitude 4°33'E to 4°40'E about 200 m above sea level. A total of twenty-four (24) growing male rabbits of heterogeneous stock aged 9 – 10 weeks were used for this experiment. The rabbits were acclimatized and given prophylactic treatments prior to the commencement of the experiment for a period of two weeks. After balancing for weight, the rabbits were allocated into four experimental groups with six rabbits per group in a Completely Randomized Design (CRD) and fed the experimental diets for 10 weeks.

Table 1: Composition of Experimental diets

INGREDIENTS	Z_0	Z_1	Z_2	Z_3
Maize (g)	17.00	17.00	17.00	17.00
Soya (g)	4.00	4.00	4.00	4.00
Wheat offal (g)	37.00	37.00	37.00	37.00
Groundnut cake (g)	4.00	4.00	4.00	4.00
PKC (g)	10.00	10.00	10.00	10.00
Rice bran (g)	26.00	26.00	26.00	26.00
Lysine (g)	0.25	0.25	0.25	0.25
Methionine (g)	0.25	0.25	0.25	0.25
Pre-mix (g)	0.50	0.50	0.50	0.50
Salt (g)	0.50	0.50	0.50	0.50
Limestone (g)	0.50	0.50	0.50	0.50
TOTAL	100	100	100	100
Zinc sulphate (mg/kg)	0.00	50.00	100.00	150.00
Nutrient Analysis:				
Crude protein (%)	16.19	16.42	16.65	16.88
Crude fibre (%)	9.75	9.90	10.06	10.12
Digestible energy (Kcal/Kg)	2479.56	2499.56	2501.70	2527.22
Zinc (mg/kg)	9.78	21.14	32.50	43.50

Z- Zinc sulphate supplement: Z_0- diet without Z; Z_1- diet with 50mg Z; Z_2- diet with 100mg Z; Z_3- diet with 150mg Z.

The rabbits were weighed weekly throughout the study using a digital scale; values were recorded in grams. The feed intake was recorded as amount consumed by each rabbit after deducting the left over feed from the quantity served per day. The feed conversion ratio (FCR) is calculated by dividing the feed intake (g) by weight gain of the rabbits. Puberty attainment in the rabbits was assessed from 12 weeks of age at 72hours interval by examining their preputial fluid smeared on a glass slide under a microscope for sperm cells. Pubertal age was established when at least 50%

of them showed the presence of sperm cells per group (Ewuola and Egbunike, 2010). Sample examination was done immediately after each collection at a magnification of x400. The weight of each animal when sperm cell was first seen in the semen was also recorded to determine the weight at puberty.

Data obtained from the study were subjected to analysis of variance (SAS, 2002) procedures and significantly different means were separated using the Duncan's multiple range test. Differences were considered to be significant at $p<0.05$.

Results and Discussion

The zinc content of the experimental diets in this study is shown in Table 2. Supplementation increased the zinc content of diets Z50, Z100 and Z150. Dietary zinc supplementation helps to provide the recommended level of zinc for rabbits (Sun *et al*., 2005; Adeyemi *et al*., 2020). Since 25 to 60 mg zinc/kg diet is recommended for rabbits depending on growth phase (Mateos *et al*., 2010), supplementing rabbits' diet with zinc sulphate from 100mg/kg upward will supply zinc at the minimum recommended level (32.50mg/kg) to male rabbits compared to 150mg/kg with zinc gluconate supplementation that also supply 32.7mg/kg in diet for male rabbits (Adeyemi *et al*., 2020).

Table 2: Zinc content of the Experimental diets

INGREDIENT	Z0	Z50	Z100	Z150
Level of Zinc sulphate supplementation (mg/kg diet)	0	50	100	150
Zinc concentration in diet (mg/kg)	9.78	21.14	32.50	43.50

Z- Zinc sulphate: Z0- diet without Z; Z50- diet with 50mg Z; Z100- diet with 100mg Z; Z150- diet with 150mg Z

The first evidence of efficient spermatogenesis in a male animal is seen at the onset of puberty as the presence of sperm cells in the preputial fluid indicates puberty attainment. Figure 1 shows the result of puberty attainment in male rabbits fed dietary levels of zinc sulfate. Dietary supplementation with zinc oxide at 150 mg/kg significantly ($p<0.05$) improved seminal vesicle volume in rabbit bucks (Emmanuel *et al*., 2019). Zinc supplementation had no significant impact on puberty attainment in this study which may be as a result of the form or level in which zinc was supplemented in this study. However, it was observed that rabbits fed 150mg of zinc as zinc sulphate attained puberty 8.5days earlier than those fed diet with no zinc supplementation. The onset of puberty in the rabbits ranged from 129 – 137.5 days. This implies that supplementing male rabbits' diets with zinc sulphate at the levels used in this study may not stimulate spermatogenic process to influence onset of puberty in male rabbits. Onset of puberty in male rabbits usually occurs between 12 to15 weeks of age (Lebas et al., 1997). The late onset of puberty recorded in this study may also be influenced by the genetics of the rabbit stock used. Zinc propionate at 35ppm had higher positive impact on seminal indices in crossbred bulls than zinc sulfate at 35ppm (Kumar *et al*., 2006). Rabbits fed 100mg of zinc sulphate diet had higher weight at the onset of puberty attainment. Zinc sulfate is an inorganic source of dietary zinc, this might have reduced the bioavailability of zinc to the rabbits lowered the absorption, distribution and uptake of zinc in the testicular tissues. An insignificant effect of zinc on puberty and weight at puberty (Figure 2) was recorded.

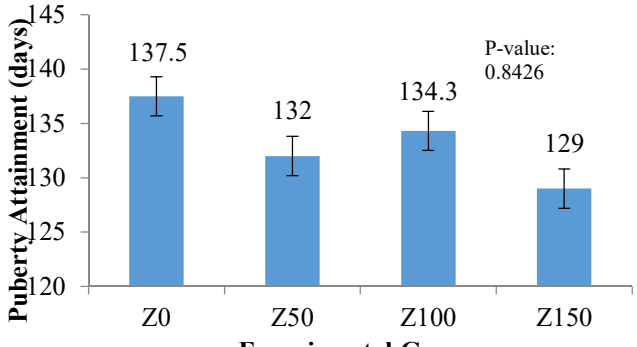

Figure 1: Puberty attainment in male rabbits fed zinc supplemented diet

The result on growth indices of male rabbit fed zinc supplemented diet shows (Table 3) that the final weight, total weight gain of the male rabbit and feed conversion ratio were not significantly ($p>0.05$) different among experimental groups. Rabbits fed Z100 diet had higher weight gain (894.0g) compared to that of those of other groups. Zinc supplementation had significant impact on feed intake of the male rabbits, those fed Z150 had significantly higher daily feed intake (86.54g) compared to those fed the Z0 diet (74.34g). The lowest feed conversion ratio was recorded in the Z0 group (6.58) with the highest in the Z150 group (7.69). However, rabbits fed Z150 attained puberty earlier (129 days) than those in other groups (132 - 137.5 days). Male rabbits fed Z100 had the highest weight at puberty (1879g) compared to others (1802 – 1867g). The weight gained by an animal is an important indicator of growth and development (Raji *et al.,* 2008); and nutrient bioavailability plays a significant role in the process. The results obtained from this study showed that zinc supplementation did not improve weight gain in the bucks till puberty. This may be due to the genetics (heterogeneous) of the rabbits used. Growing rabbits have been reported to respond positively to 100 mg/kg of supplemental zinc oxide, in terms of a significant improvement in live body weight gain (Nessrin *et al.,* 2012). Zinc supplementation (150mg $ZnSO_4$ /kg DM) decreased the average daily gain in young Holstein bulls (Fagari-Nobijari *et al*., 2012). Dietary supplementation with 80 mg/kg Zn as zinc lactate improved growth performance of growing rabbits compared to zinc sulphate (Yan *et al*., 2017). In this study, zinc supplementation significantly improved average daily feed intake in rabbits fed Z150 diet compared with those fed Z0. This is in contrast with the findings of Luis-chincoya *et al*. (2021) who reported that zinc source or concentration had no significant impact on daily weight gain and feed intake in growing rabbits. As observed in the study, Zinc sulphate supplementation may improve feed consumption since it helped to meet the zinc requirement of the rabbits as zinc levels in the Z100 and Z150 diet where within the range (25 to 60 mg zinc/kg) recommended for rabbits (Mateos *et al*., 2010). The non-significant effect of dietary zinc supplementation on feed conversion ratio (FCR) shows that supplementation did not boost nutrient utilization for muscle among the groups. FCR decreased in rabbits fed diet supplemented with zinc at 100 or 200 mg/kg (Nessrin *et al.,* 2012; Amen and Muhammad, 2016). However, Adeyemi *et al*., (2020) reported a significant reduction of FCR in mature male rabbits with 100mg/kg zinc gluconate supplementation. Rabbits fed diets supplemented with zinc lactate had greater villus height and lower crypt depth in the duodenum mucosa (providing wider surface for nutrient absorption) compared to those fed zinc sulphate supplemented diet; this may contribute to improved growth performance (Yan *et al*., 2017). In conclusion, the growth performance and onset of puberty of the male rabbits in all the experimental groups were independent of zinc supplementation as zinc sulphate. This may be as a result of the form of zinc supplement used or their genetics. However, to meet nutritional requirement of growing male rabbits, supplementation may be needed as many conventional feeds do not meet the recommended level.

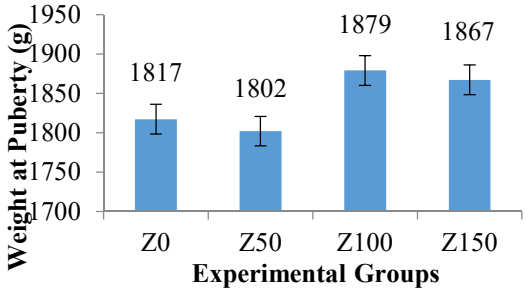

Figure 2: Weight at puberty in male rabbit fed zinc supplemented diet

Table 3: Growth Indices of male rabbits fed zinc supplemented diets

Parameters	Z0	Z50	Z100	Z150	SEM	P value
Initial body weight (g)	887.00	884.40	865.30	885.30	37.16	0.985
Final body weight (g)	1739.81	1734.00	1698.02	1723.60	37.43	0.932
Total body weight Gain (g)	852.81	849.62	832.71	837.71	48.04	0.999
Average daily body weight gain (g)	12.18	12.14	11.88	11.97	0.68	0.995
Average daily feed intake (g)	74.34^{b}	77.27^{ab}	81.08^{ab}	86.55^{a}	1.46	0.026
Feed Conversion Ratio	6.57	6.90	7.16	7.68	0.39	0.916

Z- Zinc sulphate: Z0- diet without Z; Z50- diet with 50mg Z; Z100- diet with 100mg Z; Z150- diet with 150mg Z

References

Adeyemi, A. A., Ibrahim, O. W., Ajayi, O. O. and Ayeni, S. T. 2020. Semen indices, growth response and sperm reserve of male rabbits fed zinc supplemented diets. *World Rabbit Science* 28: 199-206. doi:10.4995/wrs.2020.13507

Amen, M. H. M. and Muhammad, S. S. 2016. Effect of zinc supplementation on some physiological and growth traits in local male rabbit. *World Vet. J.*, 6: 151-155. https://doi.org/10.5455/wvj.20160881

Biswajit, R., Baghel, R. P. S., Mohanty, T. K. and Mondal, G. 2013. Zinc and male reproduction in domestic animals: A Review. *Indian J. Anim. Nutr*., 30: 339-350.

Chrastinova, L., Cobanova, K., Chrenkova, M., Polacikova, M., Formelova, Z., Laukova, A., Ondruska, L., Poganysimonova, M., Strompfova, V., Bucko, O., Mlynekova, Z., Mlynar, R. and Gresacova, L. 2015. High Dietary Levels of Zinc for Young Rabbits. *Slovak Journal of Animal Science*, 48: (2): 57-63.

Emmanuel, D. C., Amaka, A. E., Okezie, E. S., Sunday, U. P. and Ethelbert, O. C. 2019 Epididymal Sperm Characteristics, Testicular Morphometric Traits and Growth Parameters of Rabbit Bucks Fed Dietary Saccharomyces cerevisiae and/or Zinc Oxide. *Brazilian Journal of Poultry Science*. 21 (1) 001-010 http://dx.doi.org/10.1590/1806-9061-2018-0803

Ewuola, E. O. and Egbunike, G. N. 2010. Effects of dietary fumonisinB_1 on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits. Reproduction, *139: 439-445* doi: 10.1530/REP-09-0077.

Fagari-nobijari, H., Amanlou, H. and Dehghan-banadaky, M. 2012. Effects of zinc supplementation on growth performance, blood metabolites and lameness in young Holstein bulls. *Journal of Applied Animal Research*, 40(3): 222-228, doi:10.1080/09712119.2012.662776

Fallah, A., Mohammad-Hasani, A. and Colagar, A. H. 2018. Zinc is an Essential Element for Male Fertility: A Review of Zn Roles in Men's Health, Germination, Sperm Quality, and Fertilization. *Journal of Reproduction and Infertility* 19 (2): 69-81

Kenny D. A. and Byrne C. J. 2018 Review: The effect of nutrition on timing of pubertal onset and subsequent fertility in the bull. *Animal*. 12 (1) 36-44. doi:10.1017/S1751731118000514

Khan, R. U., Rahman, Z. U., Javed, I. and Muhammad, F. 2012. Effects of vitamins, probiotics and protein level on semen traits and some seminal plasma macro and micro minerals of male broiler breeders after zinc-induced molting. *Biol Trace Elem Res* 148, 44-52. doi: 10.1007/s12011-012-9341-9

Kumar, N., Verma R. P. Singh L. P., Varsheny V. P. and Dass R. S. 2006 Effect of different levels and sources of zinc supplementation on quantitative and qualitative semen attributes and serum testosterone level in crossbred cattle (*Bos indicus* × *Bos taurus*) bulls. Reprod. Nutr. Dev. 46 (2006) 663–675

Lebas, F., Coudert, P., de Rochambeau, H. and Thébault, R. G. (1997). The rabbit - Husbandry, health and production FAO Animal Production and Health Series No. 21 (new revised version) FAO - Food and Agriculture Organization of the United Nations Rome.

Luis-Chincoya H., Herrera-Haro J. G., Pro-Martínez A., Santacruz-Varela A. and Jerez-Salas M. P. 2021. Effect of source and concentration of zinc on growth performance, meat quality and mineral retention in New Zealand rabbits. *World Rabbit Sci*. 29: 151-159 doi:10.4995/wrs.2021.14095

Mateos, G. G., Rebollar, P. G., and de Blas, C. 2010. Minerals, vitamins and additives. In: de Blas C., Wiseman J. (Ed). The nutrition of the rabbit, CABI Publishing, Wallingford, UK, 3rd Edition, 126-158.

Murarka, S, Mishra, V, Joshi, P and Kumar S. 2015. Role of Zinc in Reproductive Biology - An Overview. *Austin J Reprod Med Infertil*. 2(2): 1009. ISSN: 2471-0393

Nessrin, S., Abdel-Khalek, A. M. and Gad S. M. 2012. Effect of supplemental zinc, magnesium or iron on performance and some physiological traits of growing rabbits. *Asian J. Poul. Sci., 6: 23-30. doi:10.3923/ajpsaj.2012.23.30*

Raji A. O., Igwebuike J. U. and Aliyu J., 2008. Testicular biometry and its relationship with body weight of indigenous goats in a semi-arid region of Nigeria. *Journal of Agricultural and Biological Science* 3(4), 6-9

SAS. 2002. SAS/STAT User's Guide.Version 9.2 for windows. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA.

Smith, O. B. and Akinbamijo, O. O 2000. Micronutrients and reproduction in farm animal development research center. *Animal Reproduction Science* 60-61 549-560. doi: 10.1016/s0378-4320(00)00114-7.

Sun, J. Y., Jing, M. Y., Weng, X. Y., Fu, L. J., Xu, Z.R., Zi, N.T. and Wang, J.F. 2005. Effects of dietary zinc levels on the activities of enzymes, weights of organs, and the concentrations of zinc and copper in growing rats. *Bio. Trace Elem. Res*., 107: 153-160. https://doi.org/10.1385/BTER:107:2:153

Wong, W. Y., Merkus, H. M., Thomas, C. M., Menkveld, R., Zielthuis, G.A. and Steegers-Theunissen, R.P. 2002. Effect of folic acid and zinc sulphate on male factor sub fertility, adouble blind, randomized placed controlled trial. *Fertil. Steril*. 77: 491–498.

Yamaguchi, S., Miura, C., Kikuchi, K, Celino, F. T., Agusa, T., Tanabe, S. and Miura, T. 2009. Zinc is an essential trace element for spermatogenesis. *Proceedings of the National Academy of Sciences USA*. 106(26):10859-10864. doi: 10.1073/pnas.0900602106

Yan, J. Y., Zhang, G. W., Zhang, C., Tang, l. and Kuang S. Y. 2017 Effect of dietary organic zinc sources on growth performance, incidence of diarrhoea, serum and tissue zinc concentrations, and intestinal morphology in growing rabbits *World Rabbit Science* 25: 43-49 doi:10.4995/wrs.2017.5770

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