IN OVO VITAMIN B6 SUPPLEMENTATION AT DIFFERENT EMBRYONIC DAYS INFLUENCES DUODENAL MORPHOLOGY IN GUINEA FOWL

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Abstract
This study investigated the effects of *in ovo* vitamin B6 administration at different embryonic days on duodenal morphology of guinea fowl keets (*Numida meleagris*). A total of 160 fertile eggs from a commercial breeder in Abeokuta, Ogun State, Nigeria, were incubated under standard conditions. Fertility was 73.68% (n = 112). Eggs were assigned to four groups: Control (no injection), and *in ovo* injection of 0.05 ml vitamin B6 (100 µg in deionized water) into the amnion on embryonic days (EDs) 21, 23, and 25. The keets hatched were 26 in the control, 16 keets from eggs injected on embryonic day 21, 14 keets from eggs injected on embryonic day 23, and 26 keets from eggs injected on embryonic day 25. The keets were reared for 10 weeks, and at day 70, two keets per group were slaughtered for duodenal histomorphology. Data obtained were subjected to Analysis of Variance in a Completely Randomized Design. Vitamin B6 significantly ($p<0.05$) increased smooth muscle thickness of the guinea fowls' duodenum, with those from eggs injected on ED23 having the highest value (0.26 µm), while the villus and crypt were unaffected. The study concluded that *in ovo* injection of 0.05 ml of vitamin B6 on ED23 could improve intestinal muscle development and potentially enhance post-hatch nutrient utilization in guinea fowl.

Keywords: *in ovo*, guinea fowl, post-hatch, gut histomorphology

Description of problem

Guinea fowl (*Numida meleagris*) are valued in sub-Saharan Africa and tropical regions for their adaptability, disease resistance, and high-quality meat (Moreki and Radikara, 2013). However, slow growth, late maturity, and poor feed conversion limit their productivity, particularly during the early post-hatch period when rapid intestinal development is critical for nutrient utilization (Nahashon *et al*., 2005; Moreki, 2009). Intestinal morphology villus height, crypt depth, and mucosal surface area directly influence nutrient absorption, growth, and health (Uni *et al*., 2003; Smirnov *et al*., 2006). *In ovo* technique, the administration of nutrients into the egg during embryonic development, has improved gut maturation, digestive enzyme activity, and early growth in chickens and quail (Uni and Ferket, 2003; Foye *et al*., 2006), yet remains underexplored in guinea fowl. Vitamin B6 is a coenzyme in over 140 enzymatic reactions essential for amino acid, carbohydrate, and lipid metabolism (Combs and McClung, 2016). It supports protein metabolism, hemoglobin synthesis, immune function, and antioxidant defense (Moneva *et al*. 2008; Hellman and Mooney, 2010). Post-hatch supplementation of vitamin B6 improves growth performance, feed efficiency, and gut morphology (Ouyang *et al*., 2018; Youssef *et al*. 2021), but its *in ovo* effects in guinea fowl are unknown. Evidence from other poultry species shows that *in ovo* vitamins can enhance villus architecture, absorptive capacity, and mucosal barrier function (Zamani *et al*., 2018; Reicher *et al*., 2022). Hence, this study evaluated the effects of *in ovo* vitamin B6 at different embryonic stages on gut histomorphology in guinea fowl, aiming to identify the optimal timing to improve intestinal development and production efficiency.

Materials and Methods

Experimental Site

The hatchery study was carried out at the Hatchery Unit, College of Animal Science and Livestock Production. The field experiment was carried out at the Poultry Unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta.

Source of fertile eggs and *in ovo* injection procedure

A total of 160 fertile guinea fowl eggs were sourced from a commercial breeder in Abeokuta, Ogun State, Nigeria, weighed, sorted, and incubated in a Chick Master® incubator under controlled conditions (37.5–37.8 °C; 45–55% humidity). On day 21 of incubation, candling identified 112 fertile eggs (73.68%), which were randomly assigned to four treatment groups: Control (no injection), and *in ovo* injection of 0.05 ml vitamin B6 (100 µg in deionized water) into the amnion on embryonic days (EDs) 21, 23, and 25. The keets hatched were 26 in the control, 16 keets from eggs injected on embryonic day 21, 14 keets from eggs injected on embryonic day 23, and 24 keets from eggs injected on embryonic day 25. A 24-gauge hypodermic needle was used for the injection, after which the injection sites were sealed with sterile paraffin wax and the injected eggs were returned to the hatching compartment.

Management of post-hatched keets

The newly hatched keets were transferred to the farm, and raised on deep litter. Keets in each treatment were divided into three replicates i.e. Control (9,9, 8), day 21 (5,6,5), day 23 (5,5,4) and day 25 (8,8,8). Birds were supplied commercial feed and water *ad libitum*; and all necessary vaccinations were strictly adhered to. The birds were intensively raised for 10 weeks.

Evaluation of gut histomorphology

On day 70 post-hatch, two guinea fowl per treatment were randomly selected, and duodenal morphology was assessed following the procedure described by Gasomji *et al*. (2015).

Statistical analysis

Data collected were subjected to One Way Analysis of Variance in a Completely Randomized design as described in Minitab (2013). Significantly different means (P<0.05) were separated using Tukey's Honest Significant Difference Test of the same statistical tool.

Results and Discussion

Results showed that *in ovo* vitamin B6 administration significantly (p<0.05) influenced smooth muscle thickness, with the highest value recorded in keets from eggs injected on day 23 (0.26 µm) compared to other treatments. This suggests that mid-to-late embryonic injection enhances muscular layer development, potentially improving peristalsis and nutrient absorption post-hatch. The improved muscle thickness may be linked to vitamin B6 role in protein metabolism and tissue synthesis. However, other morphological parameters including villus height, villus width, crypt height, and crypt width were not significantly affected, aligning with the findings by Yin *et al*. (2020), who reported no notable changes in duodenal morphology of weaned piglets supplemented with vitamin B6. Contrastingly, Ouyang *et al*. (2018) observed improved villus height and villus-to-crypt ratios in broilers supplemented with dietary vitamin B6, suggesting that species differences, nutrient interaction, or timing of supplementation may influence morphological outcomes. The absence of significant changes in villi and crypt dimensions in this study may indicate that vitamin B6's primary influence during embryonic development is on the muscularis layer rather than mucosal architecture. This underscores the importance of nutrient type, dose, and embryonic stage of administration in shaping gut development.

Table 1: Effect of *in ovo* injection of vitamin B6 at different embryonic days on gut morphology of guinea fowl

Parameters	Days of *in ovo* injection					
	Control	**21**	**23**	**25**	**SEM**	**P-Value**
Smooth muscle thickness (µm)	0.09[b]	0.09[b]	0.26[a]	0.12[b]	0.03	< 0.001
Crypt width (µm)	0.18	0.12	0.17	0.13	0.02	0.108
Crypt height (µm)	0.14	0.14	0.24	0.21	0.03	0.084

Villi width (µm)	0.10	0.11	0.10	0.12	0.01	0.595
Villi height (µm)	1.30	0.71	1.16	0.82	0.14	0.054

[ab]Means with different superscripts differ at $p<0.05$

Conclusion and Application

The study demonstrated that *in ovo* vitamin B6 injection at embryonic day 23 enhanced smooth muscle development of guinea fowl's gut, which could improve post-hatch gut motility and nutrient utilization.

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