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The effect of micronutrient supplementation on bioavailability, antioxidants activity, and weight gain in response to Infectious Bursal Disease vaccination in commercial broilers

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ABSTRACT

The effect of supplementing organic selenium and zinc on bioavailability, oxidative stress, weight gain in commercial broilers was studied. A total of 180-day-old chicks were divided into six groups: NSUV (Not supplemented, unvaccinated), NSV (Not supplemented, vaccinated), VS (vaccinated, supplemented selenium), VZ (vaccinated supplemented zinc), VSZ (vaccinated supplemented selenium and zinc), UVSZ (unvaccinated supplemented selenium and zinc). 1 mg/kg selenium and 60 mg/kg zinc were added to the feed of supplemented groups. The concentration of selenium (0.05 \pm 0.00 mg/L) in VS and zinc (0.66 \pm 0.13 mg/L) in VZ were lower on day 27 post-vaccination compared to day 10 (VS= 0.07 ± 0.01 mg/L; VZ= 1.46 ± 0.30 mg/L). Glutathione peroxidase and catalase concentrations were highest in the supplemented groups compared to unsupplemented groups on day 27 post vaccination, expressing a similar trend with the micronutrients. There was no difference $(P \ge 0.05)$ in the glutathione concentration between all groups except on day 27 post vaccination where SZV group was significantly higher (P=0.02) compared to the NSV group. Catalase concentration was significantly decreased in the NSV group compared to SZV (P=0.04) on day 27 post vaccination. The NSV group (1.64 ± 0.13 kg) weighed significantly lower (P=0.02) than the VSZ (2.00 ± 0.12 kg) in the fifth week, while on the sixth week, the SZV group gained the highest weight (2.04 \pm 0.18 kg). The supplementation of organic selenium and zinc in broilers increased the serum micronutrients bioavailability, decreased oxidative stress, increased weight gain, thus, enhancing immunity in the broilers.

1. Introduction

Poultry industry is one of the agriculture subsector's fastest expanding sectors in Nigeria. It contributes 9–10% of the agricultural Gross Domestic Product (GDP) and has a net worth of more than \$250 million US (Folajinmi et al., 2020). Nigeria is home to an estimated 140 million chickens, of which 25% are kept for commercial purposes, 15% are kept for semi-commercial purposes, and 60% are kept in rural areas or backyards (Malik et al., 2021).

Infectious diseases like Infectious bursal disease (IBD), which is second to Newcastle disease in prevalence, pose major threats to Nigeria's poultry stock (Shekaro & Josiah, 2015). IBD is one of the most prevalent immune-suppressing avian diseases, that causes substantial morbidity and mortality in commercial broilers and even up to 100% mortality in vulnerable chickens (Wagari, 2021; Thomrongsuwannakij et al., 2021). IBD is caused by the Infectious bursal disease virus, a single-shelled, non-enveloped, with a double-stranded RNA genome (El-shall et al., 2018). The virus targets the bursa of Fabricius destroying the B-lymphocyte thus resulting in immunosuppression in young chickens (Dey et al., 2019). In addition, macrophages and monocytes may be susceptible to IBDV infection, according to a report (Oláh et al., 2020). It has been hypothesized that macrophages act as IBDV transporters from the gut infection site to the bursa of Fabricius and other peripheral organs (Orakpoghenor et al., 2020). The infection is characterized by immune deficits and increased mortality in chicks between the ages of three and six weeks.

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Infectious Bursal Disease has become prevalent in various chicken farms in Nigeria (Arowolo et al., 2021; Sabitu et al., 2022), and vaccination with a single indigenous prototype and various imported IBD vaccine types is the main method of IBD prevention in Nigeria (Balami et al., 2018). Nevertheless, there are still major outbreaks with substantial mortality rates in both vaccinated and unprotected flocks, making it almost hard to manage IBD on most farms (Sadiq & Mohammed, 2017).

Nutrients in the form of carbohydrates and proteins are necessary for the animal to produce antibodies and cells, while micronutrients like minerals (zinc, copper, iron, and selenium) and vitamins (A and E) are necessary for portions of the animal's body to communicate with one another and fight diseases (Gupta & Mishra, 2021). Specifically, micronutrients are important nutrients that birds must have little amounts to carry out a variety of crucial physiological processes. Balanced feeding is required for ensuring that the birds are productive, healthy, resistant to harmful environmental influences, and produce raw meat and feather-down products of higher quality. The genetic potential of chickens cannot be achieved without well-planned bird feeding (Rao et al., 2014). Experience both domestically and internationally demonstrates that great care is taken in the selection of vitamins and minerals when formulating feed regimens for broiler chickens. To date, these minerals have not been taken into consideration while creating healthy regimens, but it has been demonstrated that they have a substantial impact on the body. Zinc and selenium are two of these mineral elements.

Selenium is an element of glutathione peroxidase, which eliminates peroxides from cells before they damage unsaturated lipids that are protected by vitamin E (Surai et al., 2016). The ability of selenium to boost immunity may result from increased peripheral blood lymphocyte response to phytohaemoglobin, higher activity of natural killer cells, and accelerated development of cell-mediated immunity (Rao et al., 2014). While the effect of zinc on immunity is mediated through an increase in thymocyte and peripheral T-cell counts, activity of natural killer cells and heterophils, macrophage function, and antibody formation (Jarosz et al., 2017).

Infectious Bursal Disease vaccine breaks and failures are more frequently reported by professionals and farmers. The outbreak has persisted despite the development of numerous vaccination strategies and delivery methods, appearing in flocks that have received vaccinations as well as those that have not been vaccinated (Shekaro & Josiah, 2015). Hence, a further study on the IBD vaccine response in commercial broilers. Therefore, this study aimed at investigating the effect of supplementing organic selenium and zinc on weight gain, antioxidants, selenium and zinc bioavailability, and IBD vaccine response in commercial broilers.

2. Materials and methods

2.1. Study location

This experiment was carried out at the Animal Research House, Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

2.2. Ethical approval

This research was approved and conducted according to the guidelines and recommendations of the University of Ibadan, Animal Use and Welfare Committee with approval number UI-ACUREC/20/042.

2.3. Experimental chicks and housing

A total of 180-Day-Old Chicks (DOC) were purchased from a reputable commercial hatchery in Ibadan for this study. The chickens were housed and managed intensively; commercial feed and water were provided ad libitum.

2.4. Grouping and micronutrient supplementation

The chickens were divided into six groups of 30 chickens each. Chickens in all groups were vaccinated against Newcastle Disease on day 7 using LaSota vaccine administered through drinking water. Infectious Bursal Disease Vaccine (Abic®) was administered orally on days 10 and 20 to chickens in groups NSV, VS, VZ and VSZ only. Organic selenium and zinc at 1 and 60 ppm respectively were added to the feed of chickens in all groups except NSUV and NSV daily throughout the study period. All chickens were weighed weekly throughout the experiment and were terminated on day 42 (six weeks). (Table 1)

2.5. Determination of selenium and zinc bioavailability

Five chickens were selected randomly from each group on each sampling day and blood samples were taken from the brachia vein. The blood samples were dispensed into plain tubes to allow harvesting of serum used for selenium and zinc analysis. Atomic Absorption Spectrophotometry (AAS) was used for the analysis of selenium and zinc using serum preserved at -20 °C after collection following methods described by Fawzy et al. (2016). For the determination of zinc, 0.5 ml of the serum samples were diluted 5-fold with deionized water and introduced into nebulizer burner system by the injection method. 3% (v/v)glycerin was added to the standard solutions and calibrators for Zn to determine their levels at wavelength at 213.9 nm. While a nitrate mixture was added as a modifier to both standard solution and serum samples (which was diluted 2-fold with deionized water) for determination of selenium at wavelength 196.0 nm. A blank was used for setting of zero absorbance of spectrophotometer before reading the measurements of each element as described Al-Assaf (2010).

2.6. Determination of antioxidant concentration

The serum concentration of catalase and glutathione peroxidase concentration was assayed using colorimetric assays kits (Cayman Chemicals, Ann Arbor, MI). There were thirty chickens per group and five chickens were randomly selected on each sampling day per group. Fifty microliter of each serum sample was used. Fe-EDTA mixture and hydrogen peroxide was added after 20% of acetic acid. 1 mmol/L uric acid was used for calibration. The mixture was incubated for 10 min at 100 °C in a boiling bath then cooled with ice bath. Thereafter, the absorbance was measured at 532 nm against denoised water. All analysis and specific calculated glutathione and catalase activities was carried out according to the protocol outlined by the manufacturer and methods described by Koraceciv et al. (2001).

2.7. Determination of body weight

Chickens were weighed individually once weekly using a measuring scale and units were presented in grams following methods described by Heckert et al. (2002).

Table 1

Grouping and supplementation of chickens with micronutrients.

Groups	Treatment
One	Not supplemented and Unvaccinated (NSUV)
Two	Not supplemented and vaccinated (NSV)
Three	Vaccinated and supplemented with Organic selenium only (VS)
Four	Vaccinated and supplemented with organic zinc only (VZ)
Five	Vaccinated and supplemented with organic selenium and zinc (VSZ)
Six	Not vaccinated and supplemented with selenium and zinc (NVSZ)

2.8. Statistical analysis

The data generated were expressed as mean \pm SD and performed using the SPSS statistical software for Windows (version 16; SPSS Inc., Chicago, IL, USA). The data were analyzed using One-way ANOVA and post-hoc test (Duncan Multiple Range Test). Values of ($P \le 0.05$) were considered significant.

3. Result

The mean± SD of selenium and zinc concentration on days 10, 27 and 42 post-supplementation is presented in Table 2. The groups supplemented with selenium and zinc had the highest concentrations of these micronutrients respectively on the three sampling days. On days 10, 27 and 42 post-supplementations, chickens in the group supplemented with selenium and zinc had significantly higher concentration ($P \le 0.05$) compared to chickens in the other groups. Also, selenium and zinc concentrations were observed to be highest on day 10 post supplementation, it decreased on day 27 post vaccination and supplementation and then increased on day 42 post supplementation. Although, the increase on the last sampling day (day 42) was not so high to what was observed on day 10 post supplementation. In addition, chickens in the group vaccinated and not supplemented, had the lowest concentration of selenium and zinc on the three sampling days.

The trend in the concentration of glutathione peroxidase was observed to be higher on day 10 post supplementation, which then reduced on day 27 post vaccination and supplementation. There was an increase in the concentration of this antioxidant on day 42 post supplementation but not as high as was observed on day 10 post supplementation. Chickens in the groups supplemented with selenium and zinc had a higher glutathione concentration compared to the groups not supplemented on all sampling days. Also, the group vaccinated and not supplemented (NSV) had the lowest glutathione peroxidase concentration on all the sampling days while chickens in the group vaccinated and supplemented with selenium and zinc (VSZ), had the highest concentration on each of the sampling days. There was no significant difference (P > 0.05) in the glutathione concentration between all the groups on all the sampling days except on day 27 post vaccination when the SZV group was significantly higher (P=0.02) compared to the NSV group. (Fig. 1)

The trend in the concentration of catalase is shown in Fig. 2. Catalase concentration was higher on day 10 post-supplementation, reduces on day 27 post-vaccination and supplementation with an eventual increase on day 42 after both IBD vaccination has been completed. The chickens supplemented had a higher catalase concentration on all sampling days. The chickens in the group vaccinated and supplemented with selenium and zinc (VSZ), had the highest catalase concentration when compared to all the other groups on all sampling days. In addition, there was a significant decrease in the catalase concentration of the NSV group compared to SZV (P=0.04), VS (P=0.00) and VZ (P=0.01)) on day 27 post vaccination. On day 10 post supplementation, catalase concentration was significantly lower (P=0.03) in NSV group compared to SZV group, UVSZ was significantly higher (P=0.01) than NSUV group on day

27 post vaccination. While at the end of the experiment on day 42 post vaccination and supplementation, the NSV had significantly lower (0.00) catalase concentration compared to the SZV group.

The mean \pm SD of the weekly body weight gain is illustrated in Table 3. There was a gradual increase in the weight gain of the chickens from week one up to week six. Chickens in the supplemented groups had a higher weight gain compared to the groups not supplemented. Chickens in the supplemented groups gained more weight compared to the unsupplemented groups weekly. There was a significant increase weight gain ($P \le 0.05$) in the vaccinated selenium with zinc group (VSZ) compared to the other five groups on weeks four and five. Chickens in the vaccinated and unsupplemented group (NSV) had a significant ($P \leq$ 0.05) lower weight gain compared to vaccinated selenium with zinc group (VSZ). Amongst the vaccinated groups, the chickens that were not supplemented (NSV) with selenium or zinc had the lowest weight gained on all sampling days. The body weight gained on the last day (day 42) amongst all the groups is presented in Fig. 3. The chickens supplemented and vaccinated had a higher weight gain while chickens vaccinated and supplemented with selenium and zinc gained the highest weight. Amongst the vaccinated groups chickens vaccinated and not supplemented gained the least weight at the point of terminating the experiment.

4. Discussion

Micronutrients make up only a small portion of the total diet, but they are crucial nutrients involved in a variety of physiological and metabolic processes that are necessary for all phases of poultry production (Nunez et al., 2022). Endogenous antioxidant levels in tissues are efficiently maintained by natural and synthetic antioxidants in the feed, as well as appropriate amounts of micronutrients such as manganese, copper, zinc, and selenium. The antioxidants in food can be efficiently absorbed and metabolised when the diet is composed optimally with sufficient micronutrients (Suraj, 2016).

In this study, chickens supplemented with selenium and zinc had higher bioavailability of these micronutrients compared to the unsupplemented groups. The increase in the serum concentration of selenium and zinc in the chickens supplemented with micronutrients is attributed to the addition of selenium and zinc to their feed. Also, the decreased concentration of selenium and zinc in the vaccinated and unsupplemented chickens on days 10, 27, and 42 could be due to a lack of additional selenium and zinc in their diet. This finding differs from the report of Yoon et al. (2007) and this could be associated with the lower dose of selenium (0.3 mg/kg) used in their study.

Various antioxidant activities in chickens are regulated by glutathione peroxidase, superoxide dismutase, and catalase. They are known to prevent protein and lipid oxidation, protect cells against oxidative damage and remove reactive oxygen species. The levels of these antioxidants are determined by the nutritional status of the chickens, their level of exposure to stressors and varying disease conditions (Wang et al., 2023). It was observed that chickens supplemented with dietary selenium and zinc had higher glutathione peroxidase and catalase concentrations on days 10, 27, and 42. The concentration of glutathione

Table 2

Mean± standard deviation (SD) of serum selenium and zinc concentration in broilers on day	y 10, 27	7 and 42 p	ost supplementations.

DAYS	MN (mg/L)	GROUPS NSUV	NSV	VS	VZ	VSZ	UVSZ
Day 10	Selenium	$0.03{\pm}0.09^{ac}$	$0.03{\pm}0.01^{ab}$	$0.07{\pm}0.01^{a}$	$0.04{\pm}0.02^{ae}$	$0.04{\pm}0.00^{ad}$	$0.05{\pm}0.01^{af}$
	Zinc	$0.94{\pm}0.09^{ m ac}$	$0.85{\pm}0.18^{\mathrm{af}}$	$1.03{\pm}0.00^{\rm ad}$	$1.46{\pm}0.30^{ m a}$	$0.05{\pm}0.08^{\mathrm{ab}}$	$1.10{\pm}0.10^{\rm ae}$
Day 27	Selenium	$0.03{\pm}0.00^{\mathrm{af}}$	$0.01{\pm}0.00^{\rm ac}$	$0.05{\pm}0.00^{\rm a}$	$0.03{\pm}0.00^{\mathrm{ad}}$	$0.04{\pm}0.00^{ m ae}$	$0.04{\pm}0.01^{ m ab}$
	Zinc	$0.35{\pm}0.13^{ m ad}$	$0.28{\pm}0.12^{\rm ab}$	$0.29{\pm}0.15^{\rm ac}$	$0.66{\pm}0.13^{a}$	$0.58{\pm}0.27^{ m aa}$	$0.53{\pm}0.28^{\mathrm{aa}}$
Day 42	Selenium	$0.02{\pm}0.01^{ m ab}$	$0.02{\pm}0.00^{\mathrm{ac}}$	$0.06{\pm}0.01^{a}$	$0.03{\pm}0.02^{ m ad}$	$0.04{\pm}0.00^{aa}$	$0.05{\pm}0.00^{aa}$
	Zinc	$1.00{\pm}0.78^{ae}$	$0.28{\pm}0.06^{\rm af}$	$0.40{\pm}1.00^{ac}$	$1.38{\pm}0.03^{a}$	$0.61{\pm}0.09^{ab}$	$1.33{\pm}0.03^{ad}$

Means in the same row with different superscripts are significantly different (P<0.05). MN (micronutrient), NSUV (Not Supplemented, Unvaccinated), NSV (Not Supplemented Vaccinated), VS (Vaccinated + selenium), VZ(Vaccinated+zinc), VSZ (Vaccinated + selenium + zinc), UVSZ (Unvaccinated + selenium + zinc).

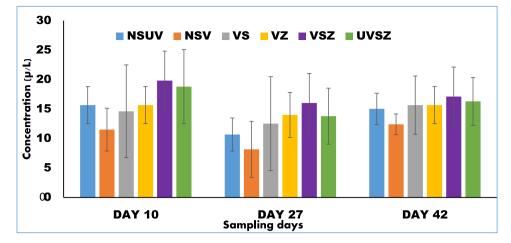


Fig. 1. The concentration of glutathione peroxidase between the supplemented and non-supplemented broilers on days 10, 27, and 42 post-supplementations.

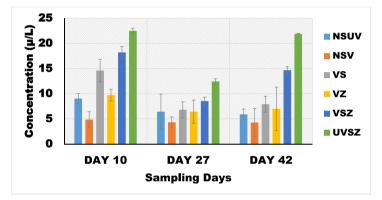


Fig. 2. The concentration of catalase between the supplemented and non-supplemented broilers on days 10, 27, and 42 post-supplementations.

Table 3
Mean \pm standard deviation of weekly broiler weight (kg) in non-supplemented
and supplemented broilers.

Groups	Week one	Week two	Week three	Week four	Week five	Week six
NSUV	0.16	0.46	0.74	1.25	1.82	1.94
	± 0.05	± 0.05	± 0.13	$\pm 0.15^{aa}$	$\pm 0.17^{\mathrm{aa}}$	± 0.18
NSV	0.16	0.44	0.70	1.22	1.64	1.86
	± 0.00	± 0.05	± 0.05	$\pm 0.13^{a}$	$\pm 0.13^{a}$	± 0.08
VS	0.18	0.46	0.80	1.26	1.88	2.02
	± 0.04	± 0.06	± 0.14	$\pm 0.18^{aa}$	$\pm 0.16^{\mathrm{ab}}$	± 0.14
VZ	0.18	0.48	0.78	1.36	1.84	2.00
	± 0.04	± 0.04	± 0.15	$\pm 0.08^{aa}$	$\pm 0.21^{\mathrm{aa}}$	± 0.14
VSZ	0.20	0.50	0.82	1.38	2.00	2.04
	± 0.07	± 0.07	± 0.15	$\pm 0.08^{\mathrm{ab}}$	$\pm 0.12^{\mathrm{ac}}$	± 0.18
UVSZ	0.20	0.47	0.78	1.36	1.84	1.97
	± 0.03	± 0.05	± 0.07	$\pm 0.06^{aa}$	$\pm 0.17^{\mathrm{aa}}$	± 0.25

Means in the same column with different superscripts are significantly different (P<0.05). MN (micronutrient), NSUV (Not Supplemented, Unvaccinated), NSV (Not Supplemented Vaccinated), VS (Vaccinated + selenium), VZ (Vaccinated+zinc), VSZ (Vaccinated + selenium + zinc), UVSZ (Unvaccinated+ selenium + zinc).

peroxidase was observed to be highest in chickens supplemented with combined selenium and zinc while chickens supplemented with zinc alone had a higher concentration of catalase. The increase concentration of these antioxidants in the supplemented chickens is associated with the role of these micronutrients in relation to the enzymes. Selenium is known to be an integral part and a co-factor for glutathione peroxidase. This study is consistent with the report of (Li et al., 2018) who observed

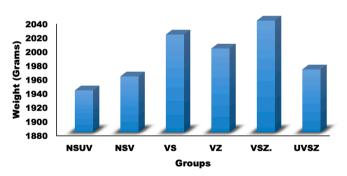


Fig. 3. Mean weight gain of selenium and zinc-supplemented and nonsupplemented broilers at six weeks of age.

increase antioxidant activity of glutathione peroxidease in local Chinese Subei chickens when administered organic selenium compared to inorganic selenium while (Ismail et al., 2013) observed an increase in catalase in chickens supplemented with organic zinc only.

Furthermore, the trend of antioxidant activities was observed to be highest on day 10 prior to vaccination, reduced on day 27 post vaccination, and gradually increased on day 42 post vaccination. Various stressors in chickens including temperature changes, environmental factors, transportation, debeaking, heat stress, diseases, and vaccination are reported to influence the oxidative status of chickens as they result in increased free radical release with a resultant increase in antioxidants usage in order to mitigate oxidative stress (Li et al., 2017). Therefore, the decrease in glutathione peroxidase and catalase concentrations on day 27 could be associated with the role of antioxidants in clearing the free radicals released and used up following the stress associated with vaccination as these chickens are maintained off water a few hours prior to vaccination and introduction of a live attenuated virus with the affinity of causing possible infection but not the disease (Muller et al., 2012).

In addition, enhanced biosecurity measures and vaccination are key steps in protecting chickens against Infectious Bursal Disease. However, routine vaccination has been associated with some level of immunosuppression commonly resulting from oxidative stress, thus necessitating the need for increased antioxidant usage to ensure balance between the oxidant/antioxidant system (Liu et al., 2020; Bi et al., 2018). Findings by Dukare et al. (2021) are consistent with this study as administration of zinc lower than 60 ppm did not result in increased activity of catalase while chickens supplemented with zinc at a dose of 80 ppm expressed a significant increase in the antioxidant activity.

Chickens supplemented with selenium and zinc had a higher weight gain weekly compared to the unsupplemented group. This could be associated with the role of selenium in maintaining and enhancing the integrity of intestinal villi length thus increasing nutrient absorption Krisnan et al. (2021). Selenium is reported to enhance the activity of the thyroid gland by efficiently protecting the thymocytes in the thyroid gland from oxidative stress and enhancing the activity of iodothyronine deiodinase thus enhancing metabolism (Huang et al., 2016; Ibrahim et al., 2019). Also, it is important in the creation of epithelial cells, one of the intestinal defenses against hazardous external organisms, as well as the regulation of insulin levels, which is crucial in the glucose route, and depends on zinc (Shakeri et al., 2022). This finding is similar to the reports by Khan et al. (2023); Li et al. (2018) who observed an increase in weight gain and feed conversion ratio in chickens supplemented with organic selenium.

5. Conclusion

The findings from this study indicate the efficacy of organic selenium and zinc in enhancing bioavailability, reducing oxidative stress, and increasing weight gain especially when organic selenium and zinc were combined. Plant species, soil status, region of growth affect the quantity of selenium and zinc concentration available in plants used in making in poultry feed. Therefore, to maintain enhanced immunity and reduce the effect of oxidative stress on immune cells associated with vaccination and other stressors accompanying intensive poultry management systems, selenium and zinc supplementation in poultry feed is still highly recommended even with routine vaccination practice in intensive poultry production.

Ethnical statement

This research was approved and conducted according to the guidelines and recommendations of the University of Ibadan, Animal Use and Welfare Committee with approval number UI-ACUREC/20/042.

CRediT authorship contribution statement

Latifat Ajoke Adekunle: Conceptualization, Investigation, Software, Resources, Writing – original draft, Writing – review & editing, Data curation, Formal analysis. Olawale Olawumi Ola: Investigation, Writing – review & editing, Writing – original draft. Ridwan Olamilekan Adesola: Investigation, Writing – original draft, Writing – review & editing, Data curation, Formal analysis. Usman Abdulrauf Adekunle: Investigation, Writing – original draft, Writing – review & editing. Olusegun Victor Taiwo: Writing – review & editing, Supervision, Writing – original draft. Afusat Jagun Jubril: Supervision, Writing – original draft, Writing – review & editing. Oniginal draft, Writing – review & editing. Software, Resources, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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