

## Dietary Zinc Oxide and 6-Phytase Effects on Fertility Rate in Old Broiler Breeder Hens

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### ABSTRACT

The effects of Zinc Oxide (ZnO) and *E. Coli*-derived 6-phytase supplemented-diet on the reproductive performance in aged broiler breeder hens and on immune responses of their progeny chicks were studied. In a 2×4 factorial arrangement, 2 inclusion levels (0 or 300 U kg<sup>-1</sup> diet) of *E. coli*-derived phytase and 4 inclusion levels (30, 60, 90, and 120 mg kg<sup>-1</sup> diet) of ZnO were provided from 62 throughout 72 weeks of age. A total of 128 hens were randomly assigned into 8 dietary treatments with 4 replicates of 4 hens each. During 66 to 71 weeks of age, egg production and egg weights were daily recorded. Results showed that ZnO and ZnO×phytase interaction affected the egg weight and fertility rate ( $P < 0.01$ ). Adding ZnO and phytase to diet increased the relative weight of bursa of Fabricius and liver in the hatchlings. Also, the interactive effect of ZnO and phytase on the relative weight of heart was significantly affected by the hatchlings ( $P < 0.01$ ). In conclusion, dietary supplementation of ZnO and *E. coli*-derived 6-phytase profoundly improved the fertility rate in aged broilers breeder hens, although the effects on immunity of their progeny were negligible.

**Keywords:** Aging, Dietary supplementation, Hatchability, Late production, Reproduction.

### INTRODUCTION

The age-related decline in fertility rate of breeder stocks has been a major concern to breeder producers (Kirk *et al.*, 1980). The higher body weight of the broiler breeder hens at the late production period is associated with a decreased fertility (Hocking, 1990). The reproductive performance and immune responses in most of animal species were significantly influenced by nutrition (Armstrong and Benoit, 1996; Kidd, 2004). Hence, using the adequate level of nutrients in the diet would improve the reproductive function and immune responses in broiler breeders.

Zinc (Zn) is present in all living organisms and acts either structurally and catalytically in metalloenzymes (Zaghari *et al.*, 2015).

Zinc is distributed in the tissues and shares in various reproductive functions (Hudson *et al.*, 2004). Zinc deficiency may depress the DNA synthesis as it is required for the binding of the protein to DNA and the role of the DNA binding proteins with "Zn fingers" regulates genetic expression (McDowell, 2003). Inadequate intracellular concentration of zinc also compromises the lymphocyte function as it is responsible for T-cell and B-cell proliferation (Vruwink *et al.*, 1993). Dietary supplementation of zinc in turkey hens improved the immune status in their progeny chicks (Kidd *et al.*, 2000). The Zn deficiency in the broiler breeder diet resulted in a decreased hatchability, weak hatchlings, and an increased embryonic mortality rate (Wilson, 1997).

Recent studies show that ingestion of phytate influences the excretion and

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digestibility of minerals (Beutler, 2009). Phytate-phosphorus (P) cannot be fully utilized by poultry essentially due to a lack of effective endogenous phytase (Maenz and Classen, 1998). Phytate is a poly-anionic molecule which has several binding sites for chelating cationic nutrients in the digestive tract, making them unavailable to the bird (Beutler, 2009). Phytase is an enzyme capable of degrading phytate in the digestive tract to yield lower inositol and orthophosphoric acid (Liu et al., 1998). *Escherichia Coli*-derived 6-phytase remains active in a very broad range of pH i.e. 2.5 to 6.0 (Beutler, 2009). This property may give an advantage to *E. Coli*-derived 6-phytase to have a long-lasting effective function within the gastro-intestinal tract.

Hudson et al. (2004) reported that cutaneous hypersensitivity response to Phytohemagglutinin-P (PHA-P) and antibody titers to Newcastle Disease Virus (NDV) were increased in birds fed on diets supplemented with 160 ppm zinc. The higher immune response and antibody titer in broiler breeder hens affect the immunity of their progeny chicks (Leeson and Summers, 1997). Dietary supplementation of ZnO and phytase improved the humoral and cell mediated immune responses in breeder hens (our unpublished data). On the other hand, effects of a simultaneous use of dietary ZnO and *E. Coli*-derived 6-phytase on the reproductive performance of aged broiler breeder hens have not been well addressed. In the present study, we hypothesized that using *E. Coli*-derived 6-phytase may be associated with an increase in the bioavailability of zinc. Thus, the aim of this study was to evaluate the effects of dietary supplementation of ZnO and *E. Coli*-derived 6-phytase on the reproductive performance of aged broiler breeder hens during the later part of the production period, in which the hatchability rate as well as the differential leukocyte numbers and weight of immune organs of their progeny chicks were also evaluated.

## MATERIALS AND METHODS

### Birds and Experimental Treatments

A total of 128 Cobb 500 broiler breeder hens (weighing  $5,200 \pm 200$  g) were selected at 59 week of age from a commercial flock and were kept under a 16L:8D photoschedule at  $22 \pm 2^\circ\text{C}$  environmental temperature. The birds were used in a  $2 \times 4$  factorial arrangement where 2 inclusion levels of phytase (0 or  $300 \text{ U kg}^{-1}$ , AB Vista Feed Ingredients, Wiltshire, UK) and 4 inclusion levels of Zinc Oxide (ZnO: 30, 60, 90, and  $120 \text{ mg kg}^{-1}$  diet) were provided. The birds were randomly assigned to the dietary treatments with 4 replicates of 4 hens each. All hens received a diet containing 11.513 MegaJoules (MJ) Metabolizable Energy (ME)  $\text{kg}^{-1}$  and 14.5% crude protein (CP, Table 1). Prior to start of the dietary treatments, all hens were fed a depletion diet with 11.513 MJ of AMEn  $\text{kg}^{-1}$ , 14.5% CP, and  $9.5 \text{ mg ZnO kg}^{-1}$  for 2 weeks.

### Reproductive Performance

From 66 to 71 weeks of age, the number and weight of laid eggs were recorded daily. Three sexually-mature Ross 308 breeder roosters (56 week old) were used to artificially inseminate the hens. The males were habituated to abdominal massage for semen collection. Two hens per experimental unit were randomly selected at 67 week of age for artificial insemination. The hens were inseminated with  $200 \mu\text{L}$  of pooled (diluted with a modified Beltsville extender; unpublished data) on a weekly basis until 71 week olds. The eggs ( $n = 40$  to  $50$  per treatment, Total eggs = 360) were collected during the last 10 days of the experiment and stored at  $12^\circ\text{C}$  (75% RH) with their blunt ends up prior to incubation. The eggs set in the same incubator (specific dry-bulb temperature of  $37.7^\circ\text{C}$  and wet-bulb temperature of  $29^\circ\text{C}$  for 18 days). The

**Table 1.** Ingredients and chemical composition of diets fed to aged Cobb 500 breeder hens (as fed basis).

Ingredient (%)	Depletion diet	Experimental diet
Corn	0	64.61
Corn starch	46.1	0.35
Soybean meal (42.6% CP)	11.16	16.22
Corn gluten meal (62% CP)	14.7	0.27
Alfalfa meal (24% CF)	0	8.78
Cellulose (89% CF)	13	0
Corn oil	2.26	1
Sodium bicarbonate (NaHCO <sub>3</sub> )	0.28	0.22
Dicalcium phosphate	1.48	1.48
Calcium carbonate	7.78	6.52
Potassium sulfate <sup>a</sup> (K <sub>2</sub> SO <sub>4</sub> )	0.94	0
Phosphoric acid <sup>b</sup> (H <sub>3</sub> PO <sub>4</sub> )	1.09	0
Common salt	0.20	0.17
Mineral premixes <sup>c</sup>	0.13	0.17
Vitamin premix <sup>d</sup>	0.02	0.02
DL-Methionine, (99%)	0.30	0.11
L-Threonine	0.30	0
L-Lysine HCl (78%)	0.26	0.08
Calculated nutrient content		
AME (MJ kg <sup>-1</sup> )	11.513	11.513
Crude protein (%)	14.5	14.41
Calcium (%)	3	3
Available phosphorus (%)	0.35	0.38
Sodium (%)	0.16	0.15
Lys (%)	0.64	0.65
Met (%)	0.58	0.32
Met+Cys (%)	0.81	0.35
Thr (%)	0.78	0.46
Arg (%)	0.62	0.74
Zinc (mg kg <sup>-1</sup> )	9.5	30

<sup>a</sup> Available Potassium 44.6%. <sup>b</sup> Available phosphorus 27.5%. <sup>c</sup> Provides (per kg of diet): Copper (CuSO<sub>4</sub>·5H<sub>2</sub>O), 10 mg; Iodine (KI), 2 mg; Iron (FeSO<sub>4</sub>·7H<sub>2</sub>O), 50 mg; Manganese (MnSO<sub>4</sub>·H<sub>2</sub>O), 120 mg; Selenium (Na<sub>2</sub>SeO<sub>3</sub>), 0.3 mg. <sup>d</sup> Provides (per kg of diet): Vitamin A (Retinyl acetate), 12,000 IU; Cholecalciferol, 3,000 IU; Vitamin E (DL- $\alpha$ -tocopheryl acetate), 2.5 mg; Vitamin K, 0.5 mg; Thiamin, 2.0 mg; Riboflavin, 10 mg; D-Pantothenic acid, 25 mg; Niacin, 40 mg; Pyridoxine, 6 mg; Biotin, 0.66 mg; Folic acid, 4 mg; Vitamin B12, 0.035 mg.

eggs from each hen were placed under a pedigree basket and transferred to the hatcher for the remaining 3 days. Fertility rate [Fertilized eggs/Total eggs set]×100 and hatchability of fertile eggs [(Hatched eggs/Fertilized eggs)×100] were calculated at emergence.

### Ovary and Abdominal Fat Pad

At 72 week of age, 8 birds per treatment were weighed and humanely killed by decapitation. The abdominal fat pad, ovary, and oviduct were dissected and weighted. The weight and diameter of non-hierarchical ovarian follicles were also determined as



follows: large yellow follicles number and weight (> 11 mm), small yellow follicles number (5 to 10 mm), and large white follicles number (2 to 4 mm) (Romero *et al.*, 2009). The largest ovarian follicles ( $F_1$ ) and the stroma (ovary without the large yellow follicles) were also weighted.

### Progeny Chicks

At hatching, 4 hatchlings per treatment were randomly selected and bled from the jugular vein in EDTA-coated tubes for differential leukocyte enumeration. Using the Wright's differential staining (Akhlaghi *et al.*, 2013), leukocytes were counted. Further, 8 chicks per treatment were weighed and slaughtered to weigh the liver, heart, spleen, and bursa of Fabricius as a ratio of the body weight.

### Statistical Analysis

All data were analyzed by the GLM procedure of SAS software (2002) and LSMEANS were compared using the Tukey's test.

## RESULTS

The effect of dietary ZnO and phytase on egg production, egg weight, fertility rate and hatchability of fertile egg are shown in Table 2. Dietary supplementation of ZnO had a significant effect on egg weight ( $P < 0.01$ ). Zinc oxide and phytase supplementation increased fertility rate ( $P < 0.01$ ,  $P < 0.05$ , respectively), but their effect on hatchability of fertile egg was not significant. The interactive effect of ZnO and phytase on fertility and egg weight were significant. Dietary supplementation of ZnO and phytase on ovarian morphology, oviduct weight, and abdominal fat weight were not significant (Table 3).

The effect of adding ZnO and phytase to diet on relative weight of bursa, liver,

spleen, heart, and body weight of chickens are shown in Table 4. Adding ZnO and phytase to diet increased relative weight of bursa of Fabricius ( $P < 0.01$ ,  $P < 0.05$ , respectively). Phytase supplementation increased the relative liver weight ( $P < 0.05$ ). Zinc oxide supplementation had a significant effect on relative liver weight ( $P < 0.05$ ). The interactive effect of ZnO and phytase was only significant ( $P < 0.01$ ) on relative heart weight of chicks.

Effect of ZnO and phytase supplementation on the granulocytes and mononuclear cells percentage in hatchlings are shown in Table 4. Adding ZnO and Phytase to diet had no significant effect on the granulocytes and mononuclear cells percentage in hatchlings.

## DISCUSSION

Zinc oxide supplementation had a significant effect on egg weight. Reduction in egg weight was statistically significant once total dietary ZnO was  $60 \text{ mg kg}^{-1}$ . Supplementary ZnO and phytase was associated with an increased fertility rate, but their effect on hatchability of fertile eggs was not significant. It seems to be unnecessary to supplement a corn-soybean diet with zinc to obtain normal hatchability (Wilson, 1997). Interactions between ZnO and phytase were significant on fertility and egg weight. This indicates that effects of ZnO and phytase were apparent together on fertility rate and egg weight. From this result, it seems that phytase could improve Zn availability and absorption. Therefore, Zn could increase fertility rate. Of requirements for prolonged sperm survivability in hen's oviduct is a local suppression of the immune system to sperm stored in the sperm storage tubules. Das *et al.* (2006) reported that the leukocyte number was increased in the UteroVaginal Junction (UVJ) following the artificial insemination in infertile laying hens. Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) is one of the possible factors to suppress

**Table 2.** Reproductive performance in aged broiler breeder hens fed diets supplemented with different levels of zinc oxide and E. Coli-derived 6-phytase.<sup>a</sup>

Treatment	Factor		Egg production (%)	Egg weight (g)	Fertility (%)	Hatchability of fertile egg (%)
	Phytase (U kg <sup>-1</sup> )	Zinc (mg kg <sup>-1</sup> )				
1	0	30	54.61	68.74 <sup>ab</sup>	28.32 <sup>d</sup>	86.66
2	300	30	57.93	70.88 <sup>a</sup>	54.04 <sup>c</sup>	87.91
3	0	60	59.28	69.60 <sup>ab</sup>	60.71 <sup>bc</sup>	92.50
4	300	60	64.28	64.77 <sup>c</sup>	64.13 <sup>abc</sup>	94.00
5	0	90	53.96	68.15 <sup>b</sup>	80.36 <sup>a</sup>	94.16
6	300	90	55.20	69.48 <sup>ab</sup>	76.25 <sup>ab</sup>	93.00
7	0	120	48.21	69.84 <sup>ab</sup>	79.72 <sup>a</sup>	96.66
8	300	120	57.73	69.49 <sup>ab</sup>	78.33 <sup>ab</sup>	92.58
SEM			4.07	0.57	3.9	3.27
Main effect						
Zinc						
		30	56.27	69.81 <sup>a</sup>	41.19 <sup>c</sup>	87.29
		60	61.78	67.18 <sup>b</sup>	62.42 <sup>b</sup>	93.25
		90	54.58	68.82 <sup>a</sup>	87.30 <sup>a</sup>	93.58
		120	52.97	69.67 <sup>a</sup>	79.02 <sup>a</sup>	94.62
SEM			2.90	0.37	2.76	2.31
Phytase						
	0		54.02	69.08	62.28 <sup>b</sup>	92.50
	300		58.79	68.65	68.19 <sup>a</sup>	91.87
SEM			2.05	0.27	1.95	1.63
P-value						
Zinc			NS	**	**	NS
Phytase			NS	NS	*	NS
Zinc × Phytase			NS	**	**	NS

<sup>a</sup> Within each column for each effect, LS means with no common superscript are significantly different \* (P<0.05) and \*\* (P<0.01), NS: Not Significant.



**Table 3.** The number and weight of ovarian follicles along with the weight of other parts of genital tract and fat pad in broiler breeder hens fed diets supplemented with different levels of zinc oxide and phytase.

Treatment	Factor		Number of ovarian follicles			Weight of ovarian follicles			Stroma (g)	Ovary (g)	Oviduct (g)	Fat pad (% BW)
	Phytase U kg <sup>-1</sup> )	Zinc (mg kg <sup>-1</sup> )	LYF (>11 mm)	SYF (5–10 mm)	LWF (2–4 mm)	LYF (g)	F1 (g)					
1	0	30	3.00	11.42	15.60	28.81	15.03	8.08	34.65	56.21	1.76	
2	300	30	4.25	13.87	15.37	43.24	18.58	7.78	51.79	61.55	2.31	
3	0	60	4.00	11.00	14.83	36.76	15.72	7.53	41.79	51.11	1.52	
4	300	60	3.28	13.42	15.42	29.71	15.20	7.37	41.81	52.50	1.77	
5	0	90	3.42	10.62	14.00	35.21	16.94	5.97	44.97	51.30	2.09	
6	300	90	2.66	11.75	14.00	27.36	14.18	7.03	39.55	56.62	1.88	
7	0	120	3.83	14.28	13.66	41.04	20.48	7.84	46.09	63.83	1.85	
8	300	120	3.20	13.28	15.25	32.16	16.16	6.37	36.79	56.45	2.69	
SEM			0.48	2.40	2.01	7.99	3.43	0.50	6.60	6.82	0.35	
Main effect												
Zinc												
		30	3.62	12.65	15.48	36.02	16.80	7.93	43.22	58.88	2.04	
		60	3.64	12.21	15.13	33.24	15.46	7.45	41.80	51.80	1.65	
		90	3.04	11.18	14.00	31.28	15.56	6.50	42.26	53.96	1.99	
		120	3.51	13.78	14.45	36.60	18.32	7.10	42.94	60.14	2.27	
	SEM		0.35	1.83	1.90	4.89	2.03	0.39	4.55	4.61	0.24	
Phytase												
	0		3.56	11.83	14.52	35.46	17.04	7.35	42.62	55.61	1.81	
	300		3.35	13.08	15.01	33.12	16.032	7.13	42.49	56.78	2.16	
	SEM		0.26	1.32	1.41	3.68	1.45	0.28	3.87	3.41	0.17	
P-value												
Zinc			NS <sup>a</sup>	NS	NS	NS	NS	NS	NS	NS	NS	NS
Phytase			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Phytase×Zinc			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

<sup>a</sup> NS: Not Significant.

**Table 4.** Organs weight and percentages of granulocytes and mononuclear cells of progeny from broiler breeder hens fed diets supplemented with different levels of zinc oxide and phytase.

Treatment	Factor		Bursa of Fabricius (% BW)	Liver (% BW)	Spleen (% BW)	Heart (% BW)	chicken weight (g)	chicken carcass weight (g)	Granulocytes (%)	WBC Mononuclear cells (%)
	Phytase (U kg <sup>-1</sup> )	Zinc (mg kg <sup>-1</sup> )								
1	0	30	0.079	2.73	0.082	0.376 <sup>ab</sup>	49.66	18.48	72.79	27.20
2	300	30	0.109	2.91	0.054	0.393 <sup>a</sup>	49.06	19.73	73.28	26.71
3	0	60	0.108	2.70	0.045	0.39 <sup>a</sup>	49.99	19.74	74.26	25.73
4	300	60	0.108	2.77	0.048	0.341 <sup>ab</sup>	47.14	18.36	67.81	32.18
5	0	90	0.119	2.68	0.043	0.381 <sup>ab</sup>	46.22	18.15	69.75	30.24
6	300	90	0.121	2.89	0.042	0.366 <sup>ab</sup>	47.26	18.23	68.16	31.83
7	0	120	0.106	2.47	0.051	0.318 <sup>b</sup>	49.61	19.51	72.81	27.18
8	300	120	0.115	2.66	0.047	0.377 <sup>ab</sup>	47.90	18.26	70.28	29.71
SEM			0.01	0.08	0.002	0.02	1.09	0.53	1.98	1.98
Main effect										
Zinc		30	0.09 <sup>b</sup>	2.82 <sup>a</sup>	0.069	0.38	48.86	19.11	73.03	26.96
		60	0.11 <sup>a</sup>	2.74 <sup>ab</sup>	0.047	0.37	48.56	19.05	71.03	28.96
		90	0.12 <sup>a</sup>	2.79 <sup>a</sup>	0.043	0.37	46.78	18.19	68.96	31.03
		120	0.11 <sup>a</sup>	2.57 <sup>b</sup>	0.049	0.35	48.75	18.88	71.54	28.45
SEM			0.005	0.06	0.0012	0.01	0.77	0.38	1.47	1.47
Phytase		0	0.10 <sup>b</sup>	2.65 <sup>b</sup>	0.056	0.37	48.62	18.97	72.40	27.59
		300	0.11 <sup>a</sup>	2.81 <sup>a</sup>	0.048	0.37	47.84	18.64	69.88	30.11
SEM			0.004	0.04	0.009	0.01	0.55	0.27	0.99	0.99
P-value	Zinc		**	*	NS	NS	NS	NS	NS	NS
	Phytase		*	*	NS	NS	NS	NS	NS	NS
	Phytase x Zinc		NS	NS	NS	**	NS	NS	NS	NS

<sup>a</sup>LS means within each column without common superscript are significantly different \* (P<0.05) and \*\* (P< 0.01), NS: Not Significant.



immune response (Das *et al.*, 2006). On the other hand, Sundaresan *et al.* (2008) reported that the expression of TGF- $\beta$ 2 mRNA in the oviduct was up-regulated during the zinc-induced molting. These results indicated that increased expressions of TGF- $\beta$  and their receptors (T $\beta$ Rs) in UVJ may protect sperm in sperm storage tubules (SST), probably by suppressing the immune response against sperm. In the current study, ZnO supplementation might enhance the expressions of TGF- $\beta$ s and T $\beta$ Rs in the UVJ of hens, although the level of TGF- $\beta$  and its receptors were not assayed. Thus, zinc may protect sperm in SST after each artificial insemination, probably by suppressing anti-sperm immunoreactions. Dietary phytase reduced inositol phosphate esters and released cations such as calcium, zinc, amino acids, and proteins, which could contribute to increased fertility (Liu *et al.*, 2008; Beutler, 2009).

Dietary supplementation of ZnO and phytase on ovarian morphology, oviduct weight, and abdominal fat weight were not significant. Overweight in broiler breeder hens is associated with ovarian dysfunction (Chen *et al.*, 2006). A previous report has shown that the heavy strains of breeders (5.6 kg) had the highest incidence of follicular atresia at sexual maturity (Hocking and Robertson, 2005). Hocking (2004) reported that yellow follicles number and the weight of abdominal fat in broiler breeders were influenced by the body weight and feed intake. In the current study, ZnO and phytase alone had no effect on the above-mentioned parameters, because of the same energy level and other nutrients in the experimental diet.

Zinc oxide and phytase supplementation increased the relative weight of the chick's bursa of Fabricius. Phytase-supplemented diet increased the relative liver and heart weight, but dietary supplementation of ZnO reduced the relative heart weight in the chicks. Also, the interactive effect of ZnO and phytase on relative heart weight was significant. Kidd *et al.* (2000) showed that progeny of turkeys that received Zn from

amino acid complexes had higher bursa weights, but no differences in the weights of spleen, liver, and heart were observed. An increased bursa weight may be due to increased Zn content in the egg yolk (McDowell, 2003). Zyla *et al.* (2000) showed that the addition of phytase to diets lacking phosphorus enhanced the bursa weight in 21-day-old Hubbard broilers. In the present study, phytase supplementation might have increased the absorption of certain nutrients by hydrolysis of phytate and increased them in the eggs.

Dietary ZnO had no effect on the granulocytes and mononuclear cells percentage in the chicks. The current results are consistent with Virden *et al.* (2004). Zinc is required for the normal development of lymphocytes, and its deficiency leads to thymocyte depletion in the thymus and a reduction in peripheral T-cell numbers and T-cell helper functions (Kidd *et al.*, 1996). Also it has been reported that Zn-deficient animals have decreased total numbers of lymphocytes and higher total numbers of neutrophils, the avian equivalent of heterophils (Vruwink *et al.*, 1993). Kidd *et al.* (2000) showed that the progeny of turkeys receiving Zn from amino acid complexes had a higher number of macrophage recruited in the peritoneal cavity. Also, Liu *et al.* (2008) reported that phytase-supplemented in broiler diet increased the percentages of lymphocyte T-helper (CD4<sup>+</sup>) and lymphocyte T-cytotoxic (CD8<sup>+</sup>) cells, but it did not affect the ratio of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells. Although macrophages and percentages of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells were not enumerated in the current study, mononuclear cells, which include the subpopulation monocytes and lymphocytes, were counted and showed no differences among the treatment groups.

## CONCLUSIONS

Obtained data indicated that adding ZnO and *E. Coli*-derived 6-phytase to diet could improve the fertility of aged broilers breeder



hens at the late stage of egg production, although dietary supplementation of ZnO and phytase had no effect on ovarian morphology, egg production, and immunity in their progeny hatchlings. Further studies are recommended to investigate the mechanism of phytase and ZnO impact on fertility and prolonged sperm survivability in the hen's oviduct.

## REFERENCES

1. Akhlaghi, A., Zamiri, M. J., Jafari Ahangari, Y., Atashi, H., Ansari Pirsaraei, Z., Deldar, H., Eghbalian, A. N., Akhlaghi, A. A., Navidshad, B., Yussefi Kelarikolaei, K. and Hashemi, S. R. 2013. Oral Exposure of Broiler Breeder Hens to Extra Thyroxine Modulates Early Adaptive Immune Responses in Progeny Chicks. *Poult. Sci.*, **92**: 1040-1049.
2. Armstrong, J. D. and Benoit, A. M. 1996. Paracrine, Autocrine, and Endocrine Factors that Mediate the Influence of Nutrition on Reproduction in Cattle and Swine: An *In vivo*, IGF-I Perspective. *J. Anim. Sci.*, **74**: 18-35.
3. Beutler, A. L. 2009. The Efficacy of Quantum™ Phytase in Laying Hens Fed Corn-Soybean Meal Based Diets. Master of Science (MSc.) Theses, University of Saskatchewan.
4. Chen, S., McMurtry, J. and Walzem, R. 2006. Overfeeding-Induced Ovarian Dysfunction in Broiler Breeder Hens is Associated with Lipotoxicity. *Poult. Sci.*, **85**: 70-81.
5. Das, S. C., Isobe, N., Nishibori, M. and Yoshimura, Y. 2006. Expression of Transforming Growth Factor- $\beta$  Isoforms and Their Receptors in Utero-vaginal Junction of Hen Oviduct in Presence or Absence of Resident Sperm with Reference to Sperm Storage. *Reproduction*, **132**: 781-790.
6. Hocking, P. M. 1990. The Relationships between Dietary Crude Protein, Body Weight, and Fertility in Naturally Mated Broiler Breeder Males. *Br. Poult. Sci.*, **31**: 743-757.
7. Hocking, P. M. 2004. Roles of Body Weight and Feed Intake in Ovarian Follicular Dynamics in Broiler Breeders at the Onset of Lay and after a Forced Molt. *Poult. Sci.*, **83**: 2044-2050.
8. Hocking, P. M. and Robertson, G. W. 2005. Limited Effect of Intense Genetic Selection for Broiler Traits on Ovarian Function and Follicular Sensitivity in Broiler Breeders at the Onset of Lay. *Br. Poult. Sci.*, **46**: 354-360.
9. Hudson, B. P., Dozier, W. A., Wilson, J. L., Sander, J. E. and Ward, T. L. 2004. Reproductive Performance and Immune Status of Caged Broiler Breeder Hens Provided Diets Supplemented with Either Inorganic or Organic Sources of Zinc from Hatching to 65 week of Age. *J. Appl. Poult. Res.*, **13**: 349-359.
10. Kidd, M. 2004. Nutritional Modulation of Immune Function in Broilers. *Poult. Sci.*, **83**: 650-657.
11. Kidd, M. T., Ferket, P. R. and Qureshi, M. A. 1996. Zinc Metabolism with Special Reference to Its Role in Immunity. *World Poult. Sci. J.*, **52**: 309-324.
12. Kidd, M. T., Qureshi, M. A., Ferket, P. R. and Thomas, L. N. 2000. Turkey Hen Zinc Source Affects Progeny Immunity and Disease Resistance. *J. Appl. Poult. Res.*, **9**: 414-423.
13. Kirk, S., Emmans, G. C., McDonald, R. and Arnot, D. 1980. Factors Affecting the Hatchability of Eggs from Broiler Breeders. *Br. Poult. Sci.*, **21**: 37-53.
14. Leeson, S. and Summers, J. D. 1997. *Commercial Poultry Nutrition*. 2<sup>nd</sup> Edition, University Books.
15. Liu, B. L., Rafiq, A., Tzeng, Y. M. and Rob, A. 1998. The Induction and Characterization of Phytase and Beyond. *Enzyme Microb. Tech.*, **22**: 415-424.
16. Liu, N., Ru, Y. J., Cowieson, A. J., Li, F. D. and Cheng, X. C. 2008. Effects of Phytate and Phytase on the Performance and Immune Function of Broilers Fed Nutritionally Marginal Diets. *Poult. Sci.*, **87**: 1105-1111.
17. Maenz, D. and Classen, H. 1998. Phytase Activity in the Small Intestinal Brush Border Membrane of the Chicken. *Poult. Sci.*, **77**: 557-563.
18. McDowell, L. R. 2003. *Minerals in Animal and Human Nutrition*. 12. Zinc. Second Edition, Elsevier, Amsterdam, PP. 357-395.
19. Romero, L. F., Renema, R. A., Naeima, A., Zuidhof, M. J. and Robinson, F. 2009. Effect of Reducing Body Weight Variability on the



- Sexual Maturation and Reproductive Performance of Broiler Breeder Females. *Poult. Sci.*, **88**: 445-452.
20. Sundaresan, N. R., Anish, D., Sastry, K. V. H., Saxena, V. K., Nagarajan, K., Subramani, J., Leo, M. D. M., Shit, N., Mohan, J., Saxena, M. and Ahmed, K. A. 2008. High Doses of Dietary Zinc Induce Cytokines, Chemokines, and Apoptosis in Reproductive Tissues during Regression. *Cell Tissue Res.*, **332**: 543-554.
21. Virden, W. S., Yeatman, J. B., Barber, S. J., Willeford, K. O., Ward, T. L., Fakler, T. M., Wideman, R. F. and Kidd, M. T. 2004. Immune System and Cardiac Functions of Progeny Chicks from Dams Fed Diets Differing in Zinc and Manganese Level and Source. *Poult. Sci.*, **83**: 344-351.
22. Vruwink, K. G., Keen, C. L., Gershwin, M. E., Mamihi, J. P. and Hurley, L. S. 1993. The Effect of Experimental Zinc Deficiency on Development of the Immune System. In: "Nutrient Modulation of The Immune Response", (Ed.): Cunningham-Rundles, S.. Marcel Dekker, Inc., New York, NY, 2634 PP.
23. Wilson, H. 1997. Effects of Maternal Nutrition on Hatchability. *Poult. Sci.*, **76**: 134-143.
24. Zaghari, M., Avazkhanlou, M. and Ganjkhani, M. 2015. Reevaluation of Male Broiler Zinc Requirement by Dose-response Trial Using Practical Diet with Added Exogenous Phytase. *J. Agr. Sci. Tech.*, **17**: 333-343.
25. Zyla, K., Wikiera, A., Koreleski, J., Swiatkiewicz, S., Piironen, J. and Ledoux, D. 2000. Comparison of the Efficacies of a Novel *Aspergillus Niger* Mycelium with Separate and Combined Effectiveness of Phytase, Acid Phosphatase, and Pectinase in Dephosphorylation.

## اثر روی و ۶- فیتاز جیره‌ای روی باروری مرغ‌های مادر گوشتی مسن

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### چکیده

اثرات مکمل جیره‌ای اکسید روی و آنزیم ۶- فیتاز گرفته شده از باکتری *E. Coli* بر عملکرد تولید مثلی مرغ‌های مادر گوشتی مسن و پاسخ‌های ایمنی جوجه‌های آنها، مورد مطالعه قرار گرفت. آزمایش در قالب طرح فاکتوریل ۲×۴، با دو سطح (۰ یا ۳۰۰ واحد/کیلوگرم جیره) فیتاز گرفته شده از باکتری *E. Coli* و چهار سطح (۳۰، ۶۰، ۹۰ و ۱۲۰ میلی گرم/کیلوگرم جیره) اکسید روی از سن ۶۲ تا ۷۲ هفتگی، طراحی شد. مجموع ۱۲۸ مرغ به صورت تصادفی در هشت تیمار غذایی با چهار تکرار و چهار مرغ در هر تکرار اختصاص داده شدند. طی سن ۶۶ تا ۷۱ هفتگی، تولید تخم مرغ روزانه و وزن تخم مرغ‌ها اندازه‌گیری شد. نتایج نشان دادند که اکسید روی و اثر متقابل روی×فیتاز تولید تخم مرغ و نرخ باروری را تحت تاثیر قرار دادند ( $P < 0.01$ ). افزودن اکسید روی و فیتاز به جیره وزن نسبی بورس فابریسیوس و کبد جوجه‌های هیچ شده را افزایش داد. همچنین، اثر متقابل روی×فیتاز بر وزن نسبی قلب به طور معنی‌داری در جوجه‌های هیچ شده تحت تاثیر قرار گرفت ( $P < 0.01$ ). در نتیجه، مکمل جیره‌ای اکسید روی و ۶- فیتاز گرفته شده از باکتری *E. Coli* به شدت نرخ باروری مرغ‌های مادر گوشتی مسن را بهبود دادند، اگرچه اثرات ناچیزی بر ایمنی جوجه‌های آنها داشتند.