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Influence of in ovo and pre-starter zinc and copper supplementation on growth performance and gastrointestinal tract development of broiler chickens

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Abstract: This experiment was on 350 uniform sized Cobb broiler hatching eggs (60 g) to assess the response of trace mineral supplementation (Zinc and copper) on growth performance and gastrointestinal tract development in broiler chicken. The fertile eggs were divided into groups with in ovo trace mineral solution containing zinc (80 µg) and copper (16 µg) and without in ovo administration. After hatching, the chicks were further divided into four groups: Group I served as control without in ovo and without post-hatch supplemented diet (WoINOVO-WoPHS), birds in Group II were without in ovo and with post-hatch supplemented diet (WoINOVO-WPHS) (100 % higher level of zinc 200 ppm, copper 30 ppm in diet), birds in Group III had in ovo (zinc, 80 µg; copper,16 µg) and without post-hatch supplemented diet (WINOVO-WoPHS) and birds in Group IV had in ovo and with post-hatch supplemented diet (WINOVO-WPHS). Data collected were subjected to completely randomized design. Hatchability, live weight gain, feed intake and feed conversion ratio at 0–3 wk were not affected ($p > 0.05$) by in ovo administration of the mineral. Post-hatch supplementation of zinc and copper without in ovo supplementation showed better feed conversion ratio at 3–5 wk of age. It could be recommended that for improved post-hatch performance, broiler chickens diets could be supplemented with inorganic zinc and copper.

Key words: poultry; broilers; animal nutrition; feed additives; in ovo; trace minerals; growth; gastrointestinal development; immune response

Vpliv dodatka cinka in bakra v jajce in v krmo po izvalitvi na rast in razvoj prebavil pri brojlerskih piščancih

Izvleček: Poskus je bil izveden na 350 valilnih jajcih pitovnih piščancev Cobb enotne velikosti, da bi ocenili odziv na dodatek mikromineralov (cink in baker) na rast in razvoj prebavil pri pitovnih piščancih. Oplojena jajca so bila razdeljena v dve skupini, ena je bila tretirana z raztopino cinka (80 µg) in bakra (16 µg), druga pa ne. Po izvalitvi so bili piščanci razdeljeni v štiri skupine: skupina I je služila kot kontrola brez poseganja v jajce in brez dodatka krmi po izvalitvi (WoINOVO-WoPHS), skupina II ni dobila cinka in bakra v jajce, ampak samo v krmo po izvalitvi (WoINOVO-WPHS), skupina III je dobila cink in baker v jajce, ne pa v krmo po izvalitvi (WINOVO-WoPHS), in skupina IV, ki je dobila dodatek cinka in bakra v jajce in v krmo po izvalitvi (WINOVO-WPHS). Zbrani podatki so bili uporabljeni za randomizirano zasnovo poskusa. Valilnost jajc, prirast, zauživanje in izkoriščanje krme v obdobju od izvalitve do treh tednov starosti niso kazali vpliva dodatka cinka in bakra ($p > 0,05$). Dodajanje cinka in bakra po izvalitvi brez dodajanja v jajca je bilo povezano z boljšim izkoriščanjem krme med 3. in 5. tednom starosti. Za boljše proizvodne rezultate priporočamo dodajanje anorganskega cinka in bakra v krmo za piščance.

Ključne besede: perutnina; pitovni piščanci; prehrana živali; krmni dodatki; in ovo; mikrominerali; rast; razvoj prebavil; imunski odziv

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1 INTRODUCTION

The perinatal period is a most crucial time in the development of a young chick as this is a transitional period in which the chicks undergoes metabolic and physiological shifts from the utilization of egg nutrients to exogenous feed (Ferket, 2012). However, with the current work flow of commercial hatcheries and considering time to transport and delivery of newly hatched chicks to broiler farms, the chicks are inevitably exposed to delayed feeding for 48–72 hrs (Noy et al., 2001; Panda et al., 2008; Uni and Smith, 2017). As a consequence of delayed feeding, chicks undergo starvation and allocate the limited reserves of nutrients to the upkeep of thermal regulation and metabolism which restricts growth and development (Ricklefs, 1987; Pinchasov and Noy, 1993). Delayed feeding causes poor viability and slow growth (Juul-Madsen et al., 2004), increases the weight loss (Bhanja et al., 2015), makes the hatchlings more susceptible to pathogens (Dibner, 1999), restricts the development of critical tissues (Halevy et al., 2000), influences the development of post-hatch gastrointestinal tract maturation (Geyra et al., 2001), increases mortality rate and consequently retards post-hatch growth of day old chicks (Careghi et al., 2005) as pronounced in present day stock of commercial broilers.

The development of the gut occurs throughout incubation (Romanoff, 1960), but the functional abilities of the gut only begins to develop about the time the amniotic fluid is orally consumed by the 18th day old embryo. The weight of the intestine, as a proportion of embryonic weight, increases from approximately 1 % at 17 days of incubation to 3.5 % at hatch. Rapid intestinal growth is due to great increase in cell numbers and size, due to accelerated enterocyte proliferation and differentiation and intestinal crypts formation (Uni et al., 2000, Geyra et al., 2001). Therefore, intestinal tissue growth, maturation and metabolism are of great importance in the last period on poultry embryonic development and the early post-hatch period. The sooner the intestine achieves functional capacity, quicker the chicks can utilize dietary nutrients, absorb minerals and vitamins and support the development of skeleton, immune system, breast muscle.

The current focus of broiler management needs to be shifted to the fortification of perinatal (last few days of pre-hatch to first few days post-hatch) nutrition so that the early given growth impetus results in achieving the targeted growth in less time. Accordingly, in ovo administration of nutrients in amnion prepares the opportunity for chicks to orally consume supplemented nutrients and develop their digestive and absorptive ability prior to hatch. Growth and development of the embryo and hatchling are dependent on the nutrients in the fertile egg (Richards, 1997). Residual yolk is the main source of nutrients during the transitional period between the hatch and grow-out phases (Gonzales et al., 2003; Henderson et al., 2008).

Table 1: Ingredient and nutrient composition (%) of experimental diets

	Post-hatch supplemented (0–3 days)	Starter (4–21 days)	Finisher (22–35 days)
Ingredients (%)			
Maize	57.53	58.06	62.31
Soybean meal	36.00	36.00	32.00
Sunflower oil	2.00	2.00	2.25
Limestone	1.00	1.00	1.00
Di-calcium phosphate	1.75	1.75	1.50
Salt (NaCl)	0.35	0.35	0.35
L-Lysine HCl	0.59	0.37	0.20
DL-Methionine	0.33	0.22	0.14
L-Threonine	0.20	0.00	0.00
Vitamin/mineral premix *	0.25	0.25	0.25
Analysed Nutrient composition (%)			
ME (MJ/kg) **	12.49	12.44	12.74
Crude protein	22.7	22.1	20.5
Lysine	1.68	1.34	1.11
Methionine	0.63	0.50	0.41
Threonine	0.97	0.77	0.73
Arginine	1.40	1.40	1.28
Calcium	1.04	1.04	0.98
Available phosphorus **	0.45	0.45	0.40
Zinc (ppm) ***	190.3	90.3	88.1
Copper (ppm) ***	29.3	14.3	13.8

* Trace mineral premix 0.1 %, Vit. Premix 0.1 %, B-Complex 0.02 %, Choline 0.05 %. Trace mineral premix supplied mg/kg diet: Mn = 75; Se = 0.2; Fe = 40; Zn = 70; Cu = 10. The vitamin premix supplied per kg diet: Vit. A = 8250 IU; Vit. D_3 = 1200 ICU; Vit. K = 1 mg; Vit. E = 40 IU; Vit. B_1 = 2 mg; Vit. B_2 = 4 mg; Vit. B_{12} = 10 mcg; niacin = 60 mg; pantothenic acid = 10 mg.
** calculated
*** Post hatch additional supplemental zinc @ 100ppm and copper @ 15 ppm

Micro-minerals that are important to bone formation and strength include Cu, Zn, and Mn, which are greatly reduced in concentration in the egg by the 17th day of incubation (doi) (Yair and Uni, 2011). These minerals also participate through their contribution to enzyme activity along metabolic pathways that are related to the formation of the skeletal system (Bao et al., 2007). Zinc participates in important regulatory pathways for bone and cartilage formation, such as collagen synthesis (Starcher et al., 1980), and hydroxyapatite crystallization (Sauer et al., 1997). Copper is part of the linkage between elastin and collagen, which gives the bone its tensile strength (Carlton and Henderson, 1964). Although Zn is important for collagen synthesis, Cu concentrations must be concomitantly sufficient so that fibrils are not weakened and become susceptible to breakage (Rath, 2000)

Currently, copper is added as copper sulphate to pre-mixes of blends for broiler chicken due to its antibacterial properties and to promote the effect of growth. Copper (Cu) is an essential micro element in poultry diets and is required to maintain the proper activities of metalloenzymes associated with iron metabolism. Tyrosinase, oxidase and feroxidase contain Cu, and their activities are dependent on this element, which is an integral part of the cytochrome oxidase system (Wang et al., 2013). Effects of dietary copper-loaded chitosan nanoparticle (CNP-Cu) supplementation on growth performance, haematological and immunological characteristics and the caecal microbiota in broilers were investigated. Results indicated that supplemental CNP-Cu could improve growth performance; affect the immune system (Wang et al., 2013). Varying hatchability with in ovo administration has been reported. There are reports of decreased (Ohta et al., 1999; McGruder et al., 2011) and increased (Bottje et al., 2010) and no effect (Zhai et al., 2011) of hatchability in literature. Therefore, the technique of in ovo administration, nutrient source or dose of nutrients should be perfected to the extent of reducing such loss. Hence, the present study was designed to evaluate if pre-conditioning remains as effective as in ovo supplementation of nutrients in terms of improved growth performance and feed conversion ratio.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE

The animal experimental procedure was approved by ethical committee of ICAR-National Institute of Animal Nutrition and Physiology, Bangalore, India.

2.2 INCUBATION AND IN OVO TREATMENT

Three hundred and fifty uniform sized Cobb broiler eggs of 60 g average weight (55–65 g) were procured from commercial hatchery. In the meantime, three hundred and forty-four eggs (98.29 %) were sorted and incubated with the dry bulb temperature ranging from 37.22–37.78 °C and wet bulb temperature of 29.44–30.56 °C from days 1 to 18. On day 14, all the unfertile eggs (40 eggs: 88.40 % fertility) were removed after candling. On embryonic day 18, fertile eggs were divided into two groups (152 eggs per group): one without supplementation and another supplemented with in ovo enriched solution containing zinc (80 µg) and copper (16 µg) into the amnion of the embryo under a laminar flow system and then transferred into the hatching trays. The relative humidity was increased by setting the wet bulb thermometer reading of more than 32.22 °C from day 18 till hatching. At hatching, 96.9 % hatchability was from group I (147 hatches) and 87.3 % hatchability was recorded from group II to give 132 hatches. One hundred and thirty-two (132) chicks were then selected per group and further divided into four (66 chicks each) groups; Group I served as control without in ovo and without post-hatch supplemented diet (WoINOVO-WoPHS), Group II composed of hatching eggs without in ovo and with post-hatch supplemented diet (100 % higher level of zinc 200 ppm, copper 30 ppm), Group III composed of hatching eggs with in ovo and without post-hatch supplemented diet. (WINOVO-WoPHS) and Group IV consisted of hatching eggs with in ovo and with post-hatch supplemented diet (WINOVO-WPHS). The required amount of trace minerals were weighed and dissolved in the deionized water in such a concentration that 0.5 ml contained the required amount of trace minerals to be injected in one egg. Before injection, the site was suitably sterilized with 70 % ethanol and the injections were done at the broad end of the egg using 25 mm needle and the pinhole site was sealed with sterile paraffin wax immediately, and eggs were transferred to the hatching trays in the incubator. The entire in ovo procedures were completed within 20 minutes after taking out of eggs from the incubator.

2.3 BIRDS AND HOUSING

The chicks (immediately from hatchery) from the different treatment groups were randomly distributed into battery cages (6 replicates with 11 chicks in each replicate), fitted with heating arrangements, feeders, waterers and dropping trays, with 24 hours light and proper air ventilation, and reared under standard manage-

Table 2: Hatchability and chick weight

Groups	Treatments	Egg wt (g)	Chick wt (%)	Hatchability (%)
I	WoINOVO	59.3	41.3	96.9
II	WINOVO	59.4	40.1	87.3
	SEM	0.224	0.575	
	Significance	0.759	0.601	

WoINOVO: Without INOVO; WINOVO : With INOVO

ment conditions. The temperature inside the cage was maintained at 33 °C on day 1 and gradually reduced to 24–25 °C by the end of the third week and maintained. The feed and fresh drinking water were provided ad libitum during the entire experimental period.

2.4 EXPERIMENTAL DIETS

Experimental diets were prepared with maize and soybean meal as major ingredients. The dietary treatments consisted of one normal prestarter diet for group I (WoINOVO-WoPHS) and group III (WINOVO-WoPHS) and one with post-hatch supplemented diet for group II (WoINOVO–WPHS) and group IV (WINOVO-WPHS). Ingredient and nutrient composition of experimental diets are given in Table 1.

2.5 MEASUREMENTS

Body weight changes were recorded every week to ascertain the weekly and overall body weight gain. The experimental diets were given ad libitum and the residue was weighed at weekly interval in order to arrive at feed intake. Based on the data pertaining to the feed intake and body weight gain, the weekly and period wise cumulative feed conversion ratio (FCR) was calculated.

2.6 GASTROINTESTINAL TRACT DEVELOPMENT

Six birds from each treatment were sacrificed by cervical dislocation at weekly interval (0–4 weeks of age) and twelve birds from each treatment at 5 wk of age (all week data not presented). Gut development was measured by recording the weights of gizzard, proventriculus, liver as well as weight and length of duodenum, jejunum, ileum and caecum. Immune organ weight (% of live weight) and meat yield (% of live weight) were recorded at the end of the trial.

Table 3: Growth performance of broiler chicken

	Live weight gain (g/b)			Feed intake (g/b)			Feed conversion ratio		
	0–3 wk	3–5 wk	0–5 wk	0–3 wk	3–5 wk	0–5 wk	0–3 wk	3–5 wk	0–5 wk
Effect of in ovo supplementation (In ovo)									
WoINOVO	829	1072	1901	1102	1765	2866	1.33	1.65	1.51
WINOVO	843	1089	1932	1106	1807	2913	1.31	1.66	1.51
Significance	0.50	0.66	0.52	0.88	0.35	0.44	0.35	0.66	0.97
Effect of post-hatch supplemented diet (PHS)									
WoPHS	841	1077	1918	1108	1805	2912	1.32	1.68	1.52
WPHS	831	1084	1916	1100	1767	2867	1.33	1.64	1.50
Significance	0.65	0.85	0.96	0.80	0.40	0.45	0.68	0.28	0.20
Interaction effect (In ovo × PHS)									
WoINOVO-WoPHS	833	1098	1932	1095	1845[a]	2940	1.31	1.68[a]	1.52
WoINOVO-WPHS	825	1046	1870	1109	1684[c]	2793	1.35	1.62[b]	1.49
WINOVO-WoPHS	849	1055	1904	1120	1765[b]	2885	1.32	1.67[a]	1.52
WINOVO-WPHS	838	1123	1961	1092	1849[a]	2941	1.30	1.66[a]	1.50
SEM	9.93	18.95	23.43	13.22	24.95	30.46	0.01	0.02	0.01
Significance	0.98	0.13	0.23	0.47	0.01	0.10	0.21	0.01	0.61

[a, b, c] = Means in the same column bearing different superscripts differ significantly ($p < 0.05$); WoINOVO-WoPHS = without in ovo and without post-hatch supplemented diet; WoINOVO-WPHS = without in ovo and with post-hatch supplemented diet; WINOVO-WoPHS = with in ovo and without post-hatch supplemented diet; WINOVO-WPHS = with in ovo and with post-hatch supplemented diet; g/b = grams / bird

Table 4: Digestive organ weight (% of live weight) and length (cm/100g live weight) at day 0

	Duodenum		Jejunum		Ileum		Caecum		Liver	Proven-triculus	Gizzard	Yolk
Treatment	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Weight	Weight	Weight	Weight
WoINOVO	19.50	1.69	42.37[b]	2.53[b]	34.43[b]	1.84[b]	8.68[b]	0.86[b]	3.03	1.09	9.24	10.05
WINOVO	19.95	1.98	46.21[a]	2.60[a]	35.47[a]	1.88[a]	10.70[a]	1.11[a]	3.24	1.26	9.59	7.13
SEM	0.59	0.08	2.05	0.16	1.49	0.09	0.62	0.07	0.08	0.12	0.35	0.95
Significance	0.18	0.17	0.04	0.04	0.04	0.01	0.01	0.01	0.65	0.37	0.13	0.09

[a, b] Means in the same column bearing different superscripts differ significantly ($p < 0.05$); WoINOVO = Without INOVO; WINOVO = With INOVO

2.7 STATISTICAL ANALYSIS

The data were subjected to one way analysis of variance (ANOVA) for completely randomized design and tested for significance between the dietary treatments means employing Tukey's HSD Post-hoc test (SAS, 2010).

3 RESULTS

3.1 HATCHABILITY AND CHICK WEIGHT

In Table 2, in ovo supplementation of trace mineral enriched solution did not show any significant difference ($p > 0.05$) in hatchability of in ovo injected group (87.3 %) compared to without in ovo supplementation group (96.9 %).

3.2 GROWTH PERFORMANCE

Live weight gain, feed intake and feed conversion ratio during 0–3 wk and overall phase was not affected ($p > 0.05$) either due to in ovo supplementation of enriched trace mineral solution, post-hatch supplemented diet or their interaction except in 0–3 wk as shown in Table 3. *In* case of feed intake, there was statistically significant differences only between groups WINOVO-WPHS, WINOVO-W0PHS and

Table 5: Digestive organ weight (% of live weight) and length (cm / 100g live weight) at 3rd wk

	Duodenum		Jejunum		Ileum		Caecum		Liver	Proven-triculus	Gizzard
	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Weight	Weight	Weight
Effect of in ovo supplementation (In ovo)											
WoINOVO	2.84	2.09	6.69	3.53	6.54	3.21	1.38	1.47	2.90	1.25	4.95
WINOVO	2.47	2.40	7.15	3.91	7.06	3.57	1.47	1.74	3.41	1.49	5.32
Significance	0.07	0.18	0.29	0.12	0.17	0.07	0.46	0.18	0.03	0.12	0.39
Effect of post-hatch supplemented diet											
WoPHS	2.54	2.26	6.85	3.98	6.43	3.66	1.33	1.53	3.27	1.43	5.13
WPHS	2.77	2.22	6.98	3.46	7.17	3.12	1.52	1.68	3.04	1.32	5.13
Significance	0.25	0.75	0.72	0.04	0.15	0.01	0.13	0.68	0.30	0.39	0.90
Interaction effect (In ovo*PHS)											
WoINOVO-WoPHS	2.69	2.14	6.21	3.57	5.91	3.17	1.37	1.29	2.94	1.29	4.99
WoINOVO-WPHS	2.99	2.03	7.17	3.50	7.17	3.25	1.40	1.64	2.85	1.21	4.90
WINOVO-WoPHS	2.39	2.38	7.50	4.39	6.95	4.14	1.28	1.77	3.60	1.56	5.27
WINOVO-WPHS	2.56	2.42	6.79	3.42	7.17	3.01	1.65	1.72	3.22	1.43	5.36
SEM	0.09	0.12	0.21	0.14	0.22	0.13	0.06	0.11	0.12	0.17	0.09
Significance	0.75	0.63	0.05	0.13	0.33	0.00	0.17	0.55	0.61	0.88	0.90

WoINOVO-WoPHS = Without in ovo and without post-hatch supplemented diet; WoINOVO-WPHS = without in ovo and with post-hatch supplemented diet; WINOVO-WoPHS = With in ovo and without post-hatch supplemented diet; WINOVO-WPHS = With in ovo and with post-hatch supplemented diet

W0INOVO-WPHS but not between groups WINOVO-WPHS and W0INOVO-W0PHS. Post-hatch supplemented group without in ovo supplementation showed better feed conversion ratio at 3–5 wk of age.

3.3 DIGESTIVE ORGAN DEVELOPMENT

In ovo supplementation significantly ($p < 0.05$) increased the weight (% of live weight) of jejunum, Ileum and caecum on the day of hatch in in ovo supplemented group compared to un-injected group (Table 4). At 3rd week of age, in ovo supplementation, post-hatch supplemented diet or their interaction groups did not differ significantly ($p > 0.05$) in all digestive organs weight (% of live weight) and length (cm/100g live weight) except weight of liver, jejunum and ileum (Table 5). Digestive organs length and weight did not show any significant difference at 5th week of age in in ovo supplemented, post-hatch supplemented or their interaction group except weight of duodenum (Table 6).

4 DISCUSSION

Bakyaraj et al. (2012) reported that hatchability of 81.3 % on in ovo feeding of enriched solution containing zinc 80 µg, copper 16 µg, selenium 0.3 µg and manganese 120 mg/egg compared to sham control group (97.3 %). Dzugan et al. (2014) evaluated effects of the injection of Zn and Cd, individually and in combination and reported that in ovo injection of individual minerals negatively affected hatchability, but had no effect when injected together. Oliveira et al. (2015) studied the in ovo injection of commercial diluent containing supplemental micro-minerals (Zn, Mn and Cu) on hatchability and concluded that in ovo injection of higher mineral concentrations into the amnion interfered with embryogenesis during late incubation, due to the creation of a mineral imbalance in the residual amnion. In ovo supplementation of trace mineral enriched solution did not show any significance ($p < 0.05$) on hatch weight. Oliveira et al. (2015) observed that injection of 0.5 mg of zinc along with manganese and copper did not influence the hatch weight of chicks compared to control. Joshua et al. (2016) also reported that in ovo nano zinc injection at a graded dose (8–20 mg) had no influence on hatch weight. Favero et al. (2013) resulted in no effect on hatchability, hatchling weight and Mn and Cu content in the egg. However, the Zn content in the egg was increased by the substitution.

Many of the earlier works (Tako et al., 2005; Goel et al., 2013; Yair et al., 2013; Oliveira et al., 2015) on in ovo injection of trace minerals individually or in combination have not reported increased growth performance of post-hatch

Table 6: Digestive organ weight (% of live weight) and length (cm/100 g live weight) at 5th wk

	Duodenum		Jejunum		Ileum		Caecum		Liver	Proventriculus	Gizzard
	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Weight	Weight	Weight
Effect of in ovo supplementation (In ovo)											
WoINOVO	1.52	0.99	3.38	1.92	3.22	1.79	0.94	0.82	1.72	0.41	1.93
WINOVO	1.59	1.04	3.31	2.07	3.35	1.72	0.92	0.78	1.74	0.40	2.01
Significance	0.17	0.31	0.57	0.20	0.51	0.55	0.66	0.73	0.58	0.43	0.48
Effect of post-hatch supplemented diet											
WoPHS	1.54	0.97	3.29	1.97	3.31	1.70	0.92	0.74	1.72	0.39	1.96
WPHS	1.57	1.06	3.39	2.02	3.25	1.80	0.94	0.85	1.74	0.42	1.97
Significance	0.67	0.03	0.54	0.59	0.81	0.23	0.54	0.01	0.78	0.12	0.65
Interaction effect (In ovo × PHS)											
WoINOVO-WoPHS	1.52	0.91	3.40	1.95	3.38	1.81	0.94	0.75	1.72	0.39	2.02
WoINOVO-WPHS	1.51	1.08	3.35	1.89	3.06	1.76	0.94	0.88	1.73	0.44	1.83
WINOVO-WoPHS	1.55	1.03	3.18	2.00	3.25	1.60	0.91	0.73	1.73	0.39	1.90
WINOVO-WPHS	1.63	1.05	3.43	2.15	3.45	1.84	0.93	0.83	1.76	0.41	2.11
SEM	0.03	0.02	0.11	0.05	0.09	0.05	0.02	0.02	0.03	0.01	0.05
Significance	0.93	0.04	0.46	0.34	0.40	0.33	0.86	0.36	0.91	0.59	0.22

WoINOVO-WoPHS = Without in ovo and without post-hatch supplemented diet; WoINOVO-WPHS = without in ovo and with post-hatch supplemented diet; WINOVO-WoPHS = With in ovo and without post-hatch supplemented diet; WINOVO-WPHS = With in ovo and with post-hatch supplemented diet

chicks. Joshua et al. (2016) observed a variable result on in ovo injection of graded level of nano zinc, group injected with 40 mg nano zinc showed significant increase in body weight compared to other groups at 5th week. Bakyaraj et al. (2012) reported that in ovo trace mineral supplemented group (Zinc 80 µg, selenium 0.3 µg iron 160 µg, iodine 0.7 µg per egg) showed significantly higher body weight (411.9) compared to sham control (367.8). In ovo inoculation of several nutrients (maltose, a multi-vitamin supplement, zinc-glycine, glutamine and a mixture containing all these elements and L-carnitine) to 18-day-old embryos did not influence feed intake and feed conversion ratio (dos Santos et al., 2010; Keralapurath et al., 2010; Dooley et al., 2011)

The discrepancies in various studies (Ohta and Kidd, 2001; Bhanja and Mandal, 2005; Shafey et al., 2012; Kop-Bozbay et al., 2013; Schulte-Drüggelte, 2015) could be explained by many intrinsic and extrinsic factors which affect performance of broiler birds on supplementation of in ovo nutrients. Intrinsic factor of in ovo supplementation includes the content of in ovo solution, pH of solution, osmolarity of solution, dose per egg, site of injection, day of injection, needle bore diameter, interaction effect in mixed two or more nutrients and extrinsic factor include source of hatching eggs, storage condition, weight and size of eggs, nutritive profile of hatching eggs, strain/ line/ breed of breeding birds, breeding age, feeding regimen followed by laying birds, time of hatch.

Lack of significant effects in growth performance on in ovo supplementation of trace minerals in this study may be due to ideal level of nutrient present in egg obtained from commercial hatchery. This explanation is supported by the findings of Kop-Bozbay and Ocak (2015) where they found no significant effect of in ovo supplementation of amino acids using eggs with ideal nutrients contents. Most of the researchers did not mention source of hatching eggs especially strain / breed of layer birds used. As in present trial, source of eggs is from commercial hatchery, so neutral effect on growth performance may be related to fast growing broiler strains. This explanation is in line with the findings (Sarica et al., 2009; Yamak et al., 2014; Baéza et al., 2015) that fast-growing birds were better able to perform with commercial basal diet due to the fact that nutrient requirements increase depending on growth rate and also they may be better able to digest the basal diet due to the development of the digestive tract and organs. Furthermore, Schulte-Drüggelte (2015) reported that well-nourished, healthy chicks do not respond to in ovo supplements and the degree of limiting protein synthesis of amino acids depend on the ratios and antagonistic relationship between each of these amino acids (Burnham et al., 1992; Dozier III et al., 2011) and the protein content and quality of poultry diets (Ospina-Rojas et al., 2014).

The significant difference in digestive organs weights at the 1st week of age supported by the findings of Uni et al. (2003) that *in ovo* feeding results in improved gastrointestinal tract development of hatchlings and functionally similar to that of conventional 2 day old chicks offered feed immediately after hatch. The authors also indicated that, during the last 3 days of incubation, the weight of the intestine as a proportion of embryo weight increased from approximately 1 % at 17 days of embryonic age to 3.5 % at hatch. In chicks, at 3–7 days of age, the digestive organs will grow at a faster rate as compared to other organs and the small intestine increases in weight more quickly than the body mass during the first week post-hatch (Sklan, 2001). Rapid intestinal growth is due to increase in cell number and size, accelerated enterocyte proliferation and differentiation and intestinal crypt formation (Uni et al., 2000; Geyra et al., 2001). Tako et al. (2005) observed that in ovo injection of Zn-methionine in amniotic fluid on 18th day of incubation increased the villus surface area and enhanced the expression of genes and biochemical activity of intestinal transporters and enzymes thus accelerated intestinal development.

Kop-Bozbay and Ocak (2015) observed in experiment of in ovo injection of branched chain amino acids on eggs having ideal levels of nutrient and found that in ovo injection had no effect on the hatchability, chick quality and the degree of growth promotion. Healthy chicks may not respond to in ovo supplements (Schulte-Drüggelte, 2015) and the degree of limiting protein synthesis of these amino acids depend on the ratios and antagonistic relationship between each of these amino acids in poultry diet (Burnham et al., 1992) and the protein content and quality of poultry diets (Corzo et al., 2010). The influence of in ovo supplement is greatly dependent on the maternal diet as any deficiency is overcome by extra supplementation.

4 CONCLUSIONS

In ovo supplementation of zinc and copper did not influence hatchability.

Birds on the supplementation of zinc and copper recorded better feed conversion ratio at 3–5 weeks of age.

In ovo supplementation of zinc and copper significantly increased the weight (% of live weight) of the jejunum, ileum and caecum on the day of hatch.

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