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Dietary selenium and zinc supplementation alters growth and immunity of broiler chicken

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ABSTRACT

This study evaluated the influence of varying dietary levels of selenium (Se) and Zinc (Zn) on the performance index, carcass characteristics, and immune response in broiler chicken. The experimental trial was carried for 42 days with a 3 × 3 factorial design involving three levels of each Se (0.15, 0.30, and 0.45 mg/kg diet) and Zn (40, 80, and 120 mg/kg diet) resulting in nine treatments. Six replicate groups with eight birds in each were assigned to each treatment (48 birds/treatment). During starter phase (0-3 weeks), optimum growth performance and growth efficiency of chicken was observed at 0.30 mg Se/kg diet. However, during finisher phase (4-6 week) and overall growth phase (0-6 week), 0.15 mg Se/kg diet was found to be optimum. Similarly, weight gain during starter phase was higher in birds fed at least 80 mg Zn/kg diet. The carcass characteristics did not reveal significant effect of Se and Zn supplementation in broiler chicken. And, on similar lines, cell mediated immunity remained unaffected. However, better humoral immunity was observed in birds supplemented with NRC recommended Se level in diet, but the NRC recommendation of 40 mg Zn/kg diet was inadequate for better humoral immune response compared to 80 or 120 mg level. Further, higher spleen and thymus weight was observed at 0.3 mg Se/kg diet and higher spleen weight at 80 mg Zn/kg diet. In conclusion, Se supplementation of 0.30 mg /kg diet and Zn supplementation of 80 mg/kg diet resulted in optimum growth performance, efficiency, and immunity of broiler chicken.

Keywords: Broiler chicken, Growth performance, Immune response, Selenium, Zinc

Interactions among nutrients such as vitamins and minerals are of great importance in poultry nutrition and can negatively affect the performance of birds if not considered (Mir *et al*. 2021). Providing protection to the cells against the oxidative damage by free radicals and lipo-peroxides, selenium (Se) is an integral part of Se-dependent antioxidant enzymes, and thus involved in the antioxidant defense system of the body (Finkel and Holbrook 1991). Traditionally, in poultry diets, Se supplementation, in inorganic forms, are recommended at 0.15 mg/kg diet level (NRC 1994, BIS 2007). However, basal diet containing 0.24-0.25 mg Se/kg diet was found optimum under normal conditions or under heat stress for broiler chicken (Ghazi *et al*. 2012). Furthermore, Se supplementation increased relative weights of immune organs of chicken under heat stress condition (Mahmoud *et al*. 2016) and improved cellular and humoral immunity was reported in layer chicken fed ration containing 0.3 mg Se/kg diet (Mohapatra *et al*. 2014) with higher IgG and IgM levels in broiler chicken (Cai *et al*. 2012) compared to control diets.

Similarly, in mammals, the second most abundant trace mineral is zinc (Zn) which has structural and/or functional importance in more than 300 enzymes involved in antioxidant defense mechanisms (Mir *et al*. 2013). In poultry nutrition, the role of dietary Zn has been established in the growth, bone development, feather development, enzyme structure and function, immunomodulation, and appetite regulation. Though NRC has recommended 40 mg Zn/kg diet as optimum for growth performance of broiler chickens, there is no concurrence among the poultry nutrition researchers whose recommendations vary from 40 to 120 mg/kg diet (Burrell *et al*. 2004, Zhang *et al*. 2006). BIS has recommended 80 mg Zn/kg diet for optimum growth and immunity of broiler chicken (BIS 2007, Ghazi *et al*. 2012). Furthermore, Zhang *et al*. (2006) reported that in broiler chicken Zn supplementation should be 80-120 mg/kg diet to get better growth performance, immune competence, and other physiological indexes. Therefore, the deficiency of one or both micronutrients may reasonably affect the health and production of the broiler chicken. Since, the optimization of dietary requirement of nutrients in broiler chicken is a continuous process because of the continuous genetic improvement of birds and at the same time BIS (2007) recommendation is now 16 years old. Thus, the

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present study was undertaken to optimize requirement of dietary levels of Se and Zn in broiler chicken diet.

MATERIALS AND METHODS

The ethical approval for this study was provided by Animal Ethics Committee of IVRI, Izatnagar, India (Approval number 491/01/ab/CPCSEA). This study was carried out on straight run CARIBRO Vishal broiler chicken (n=432), which were distributed at random into 54 groups based on their initial body weight having 8 birds in each group. The basal diets of similar energy and protein levels were formulated as starter and finisher with ingredients and nutrient composition given in Table 1. A 3×3 factorial experimental design was followed by employing three levels of each Se (0.15, 0.30, and 0.45 mg/kg) and Zn (40, 80, and 120 mg/kg) which resulted in nine experimental diets. Each treatment was assigned six groups of birds at random (48 birds/treatment). Selenium (Se) and zinc (Zn) levels in the diets were analyzed and the supplementation was done accordingly to achieve the desired levels of Se and Zn in the respective diets. The source of Se and Zn supplementation was in the form of Sodium selenite and Zinc sulphate, respectively. The respective diets were offered to birds *ad lib.* in mash form with uniform managemental conditions.

The feed intake (FI) of birds was recorded group-wise and daily feed intake per bird was calculated. The body weight of birds was recorded on weekly basis followed by body weight gain (BWG) and feed conversion ratio (FCR) calculation for each treatment group wise separately. The effects of Se and Zn supplementation on the growth efficiency of broiler chicken were assessed by calculating production efficiency factor (PEF), protein efficiency ratio (PER), and energy efficiency ratio (EER) as follows (Kumar *et al*. 2021):

$$PEF = [\text{Final body weight (kg)} \times \text{Livability (\%)} \times 100]/\text{Age in days} \times FCR$$

$$PER = \text{Weight gain}/\text{Protein intake}$$

$$EER = [\text{Weight gain (g)}/\text{Total energy intake (ME kcal)}]/ \times 100$$

For the study of carcass characteristics, 12 birds (2 birds per group) per treatment were selected at random, feed was withdrawn 12 h before sacrificing, but drinking water was provided *ad lib.* at the end of 42 d experimental trial. However, male and female were selected in equal number to avoid the influence of sex on the results of slaughter traits of birds.

The immunity of birds in response to Se and Zn supplementation was assessed in terms of cell mediated immunity (CMI) against the mitogen {Phyto-haemagglutinin-P (PHA-P)}, humoral immunity (HI) against sheep red blood corpuscles (SRBC), and the development of lymphoid organs. The relative weight of lymphoid organs (thymus, spleen, and bursa) was measured at the time of bird sacrifice. The foot web index (mm)

Table 1. Ingredients and nutrient composition of basal diets for broiler chicken

Ingredient (g/kg)	0 - 21 days	22 – 42 days
Maize	535.7	616.5
Soybean meal	420	340
Oil	10	13
Limestone	9.2	11
Di-calcium phosphate	17.3	12.85
Salt	3.0	3.0
DL-Methionine	1.65	0.50
TM- premix[1]	1.0	1.0
Vitamin premix[2]	1.5	1.5
B complex[3]	0.15	0.15
Choline chloride	0.5	0.5
Nutrient composition (analyzed values)		
Crude protein	232.2	204
Calcium	10.2	9.5
Total phosphorus	7.2	6.6
Zinc (mg/kg)	23.87	22.42
Selenium (mg/kg)	0.15	0.13
Metabolizable energy (Kcal/kg)	2905	3006
Available phosphorus (calculated)	4.5	3.6
Lysine (calculated)	12.5	10.6
Methionine (calculated)	5.3	3.8
Threonine (calculated)	9.9	8.7

[1] Trace mineral mixture (100 g): $FeSO_4.7H_2O$-8 g, $ZnSO_4.7H_2O$-10 g, $MnSO_4$. H_2O- 10 g, $CUSO_4.5H_2O$-1 g, KI- 30 g. [2] Vitamin premix (1 g): Vitamin A-82.5 IU, Vitamin E 50% -160 mg, Vitamin D3-12000 unit, Vitamin K-10 mg. [3] Vitamin B complex (1 g): Vitamin B_1-8 mg, Vitamin B_6-16 mg, Vitamin B_{12}-80 mcg, Niacin-120 mg, Calcium panthotheonate-80 mg , Vitamin B_2-50 mg, L-lysine-10 mg and DL- Methionine- 10 mg.

measurement was done in 12 birds (2 birds per group) from each dietary treatment at 22^{nd} day to assess CMI. 0.2 ml of PHAP-P mitogen (1 mg/ml PBS) was injected in left foot web of birds and 0.2 ml PBS was injected in right foot web to serve as positive control. The thickness of foot webs for each bird was measured prior to injection and 24 h post injection in both legs and difference between the two legs was calculated as a response to PHA-P injection Bera *et al.* (2019).

The HI was measured in terms of HA titre against SRBC in 12 birds (not used for CMI assay) from each treatment (2 birds per group). About 1 ml of 1% suspension of SRBC was injected via wing vein of birds. Blood samples were collected at 6^{th} day post injection for harvesting of serum followed by storage at -20°C until further use. The HA antibody titre estimation was done in fresh U-bottom micro titre plates by making two-fold serial dilution of sera and reciprocal of the highest dilution showing clear agglutination was taken as end point of titre and the values were expressed as $\log_2$.

A completely randomized design was followed for the data analysis via two-way ANOVA by adopting General Linear Model procedure of IBM SPSS software-20. For the data pertaining to feed intake, FCR, and efficiency parameters each group was designated as an experimental unit and for the data pertaining to weight gain, carcass characteristics, and immune response experimental unit was sampled bird.

RESULTS AND DISCUSSION

Growth performance: The BWG during 0-3 weeks of age was higher (P<0.01) in birds fed 0.15 mg Se/kg diet along with 120 mg Zn/kg diet and 0.30 mg Se/kg diet along with either of 40 mg, 80 mg, or 120 mg Zn/kg diet. Whereas, lower BWG was observed in birds fed 0.15 mg Se/kg diet along with 40 mg Zn/kg diet (Table 2). The other dietary combinations of Se and Zn yielded intermediate values. However, during 4-6 weeks and 0-6 weeks of age, higher BWG was observed in birds fed 0.15 mg Se/kg diet along with 80 mg or 120 mg Zn/kg diet while lower BWG was observed upon supplementation of 0.45 mg Se/kg diet along with 80 mg Zn/kg diet. The other combinations of Se and Zn resulted in intermediate values of BWG. The 0-3 week FI of birds was (P<0.05) higher at 0.15 mg Se/kg with 120 mg Zn/kg diet, 0.30 mg Se/kg with 80 mg Zn/kg, and 0.45 mg Se/kg with 0.40 mg Zn/kg diet compared to 0.45 mg Se/kg with 80 mg Zn/kg diet and other combinations resulted in intermediate values. During 4-6 weeks of age, higher (P<0.05) FI was observed in birds fed 0.45 mg Se/kg with 80 mg Zn/kg diet compared to birds fed 0.30 mg Se/kg with 40 mg Zn/kg diet whereas, other combinations resulted in intermediate FI of birds. During 0-6 weeks age, higher (P<0.05) FI was observed at 0.30 mg Se/kg with 80 mg Zn/kg diet followed by 0.45 mg Se/kg with 80 mg Zn/kg diet compared to other combinations which did not differ significantly from each other. The FCR during 4-6 weeks and 0-6 weeks of age

Table 2. Effect of different levels of selenium (Se) and zinc (Zn) on growth performance of broiler chicken

Se (mg/kg)	Zn (mg/kg)	Body weight gain (g)			Feed intake (g)			Feed conversion ratio		
		0-3 week	4-6 week	0-6 week	0-3 week	4-6 week	0-6 week	0-3 week	4-6 week	0-6 week
0.15	40	676^{a}	1190^{c}	1866^{d}	728^{b}	2396^{bc}	3174^{a}	1.08	2.01^{ab}	1.70^{b}
	80	702^{c}	1255^{d}	1957^{e}	718^{ab}	2424^{c}	3192^{a}	1.02	1.93^{a}	1.63^{a}
	120	731^{d}	1238^{d}	1968^{e}	769^{c}	2434^{c}	3253^{b}	1.05	1.97^{a}	1.65^{a}
0.30	40	728^{d}	1110^{b}	1838^{cd}	711^{ab}	2319^{a}	3180^{a}	0.98	2.09^{bc}	1.73^{bc}
	80	733^{d}	1114^{b}	1847^{cd}	781^{c}	2419^{c}	3250^{b}	1.07	2.17^{cd}	1.76^{c}
	120	720^{d}	1086^{ab}	1805^{bc}	714^{ab}	2434^{c}	3198^{a}	0.99	2.24^{d}	1.77^{cd}
0.45	40	702^{c}	1085^{ab}	1787^{b}	767^{c}	2365^{b}	3182^{a}	1.09	2.18^{cd}	1.78^{cd}
	80	696^{bc}	1040^{a}	1736^{a}	696^{a}	2483^{d}	3229^{ab}	1.00	2.39^{e}	1.86^{e}
	120	684^{ab}	1080^{ab}	1764^{ab}	730^{b}	2418^{c}	3198^{a}	1.07	2.24^{d}	1.81^{d}
Pooled SEM		2.9	9.3	10.4	12.9	16.5	20.9	0.016	0.028	0.016
Main effect										
Selenium										
0.15		703^{n}	1228^{o}	1930^{o}	738	2418	3206	1.05^{n}	1.97^{m}	1.66^{m}
0.30		727^{o}	1103^{n}	1830^{n}	735	2391	3176	1.01^{m}	2.17^{n}	1.74^{n}
0.45		694^{m}	1068^{m}	1762^{m}	731	2422	3203	1.05^{n}	2.27^{o}	1.82^{o}
Zinc										
40		702^{x}	1128	1830	735	2360^{x}	3145^{x}	1.05	2.09	1.72
80		710^{y}	1136	1847	732	2442^{y}	3224^{y}	1.03	2.15	1.75
120		712^{y}	1135	1846	738	2429^{y}	3216^{y}	1.04	2.14	1.74
Probability										
Selenium		P<0.01	P<0.01	P<0.01	P<0.05	P<0.05	P<0.05	P<0.05	P<0.01	P<0.01
Zinc		P<0.05	NS	NS	NS	P<0.05	P<0.05	NS	NS	P<0.05
Interaction		P<0.01	P<0.01	P<0.01	P<0.05	P<0.05	P<0.05	NS	P<0.01	P<0.01

Value bearing different superscripts within a column differ significantly, NS-Non-significant.

was better (P<0.01) in birds fed 0.15 mg Se/kg with 80 mg and 120 mg Zn/kg diet compared to 0.45 mg Se/kg with 80 mg Zn/kg diet and the other combinations resulted in intermediate FCR values in birds. The main effect of Se at 0-3 weeks of age resulted in better BWG (P<0.01) and FCR (P<0.05) at 0.30 mg Se/kg diet followed by 0.45 mg Se/kg diet compared to 0.15 mg Se/kg diet. However, at 4-6 weeks and 0-6 weeks of age, better (P<0.01) BWG and FCR of birds was observed at 0.15 mg Se/kg diet followed by 0.30 mg Se/kg diet compared to 0.45 mg Se/kg diet. No significant effect of Se supplementation was observed on the FI of birds. The main effect of Zn supplementation revealed lower (P<0.05) 0-3 week BWG and 4-6 week and 0-6-week FI at 40 mg/kg diet compared to other two higher levels which did not differ significantly from each other.

The growth efficiency parameters have shown significant interaction effects and main effects of Se supplementation only (Table 3). The PEF at 3rd week of age was higher (P<0.01) in birds fed 0.30 mg Se/kg with 40 mg Zn/kg diet followed by 0.15 mg and 0.30 Se/kg with 120 mg Zn/kg diet compared to birds fed 0.45 mg Se/kg with 40 or 120 mg Zn/kg diet. At 6th week of age, higher (P<0.01) PEF was observed in birds fed 0.15 mg Se/kg with 80 or 120 mg Zn/kg diet compared to birds fed 0.45 mg Se/kg with 80 or 120 mg Zn/kg diet. Higher (P<0.01) PER and EER during 0-3 weeks of age were observed in birds at 0.30 mg Se/kg with 40 or 120 mg Zn/kg diet compared to birds fed 0.45 mg Se/kg with 40 or 120 mg Zn/kg diet and 0.15 mg Se/kg with 40 mg Zn/kg diet. During 4-6 weeks of age, higher (P<0.01) PER and EER were observed in birds fed 0.15 mg Se/kg with 80 or 120 mg Zn/kg diet compared to birds fed 0.45 mg Se/kg with 80 mg Zn/kg diet. Similarly, during 0-6 weeks of age, birds fed 0.15 mg Se/kg with 80 or 120 mg Zn/kg diet resulted in higher (P<0.01) PER and EER compared to birds fed 0.30 mg Se/kg with 120 mg Zn/kg diet and 0.45 mg Se/kg with 40, 80, or 120 mg Se/kg diet. Other combinations yielded intermediate PEF, PER, and EER values of birds. The main effects of Se supplementation resulted in higher (P<0.01) 3rd week PEF in birds at 0.30 mg Se/kg diet followed by 0.15 mg Se/kg level compared to 0.45 mg Se/kg diet. But higher PEF at 6th week (P<0.01), 4-6 week and 0-6 week PER (P<0.05) were observed at 0.15 mg Se/kg diet followed by 0.30 mg Se/kg diet compared to 0.45 mg Se/kg diet. During 0-3 weeks of age, higher PER (P<0.01) and EER (P<0.05) were observed in birds fed 0.30 mg Se/kg diet compared to other two levels, whereas, higher EER during 4-6 weeks (P<0.05) and 0-6 weeks (P<0.01) of age were observed at 0.15 mg Se/kg diet compared to other levels.

Therefore, during 0-3 weeks of age, optimum growth performance and growth efficiency of chicken were observed at 0.30 mg Se/kg diet. However, during 4-6 weeks of age and overall growth phase (0-6 week), 0.15 mg Se/kg diet turned out to be optimum level which shows the age dependency of Se requirement of broiler chicken. Similarly, the BWG was higher in birds fed at least 80 mg Zn/kg diet. According to this study, for optimum growth performance and efficiency of broiler chicken, 0.30 mg Se/kg diet must be supplied in the ration along with 80 mg Zn/kg diet. On similar lines, better growth of broiler chicken was reported at a level of 0.25 mg Se/kg

Table 3. Effect of different levels of selenium (Se) and zinc (Zn) on growth efficiency parameters of broiler chicken

Se (mg/kg)	Zn (mg/kg)	Production efficiency factor		Protein efficiency ratio			Energy efficiency ratio		
		3rd week	6th week	0-3 week	4-6 week	0-6 week	0-3 week	4-6 week	0-6 week
0.15	40	298.9^{b}	261.2^{d}	4.00^{ab}	2.43^{d}	2.84^{bc}	32.0^{a}	16.5^{d}	20.0^{bc}
	80	318.7^{cd}	278.5^{e}	4.21^{c}	2.54^{e}	2.96^{d}	33.7^{b}	17.2^{e}	20.9^{c}
	120	330.9^{d}	283.5^{e}	4.09^{b}	2.49^{de}	2.92^{d}	32.7^{ab}	16.9^{de}	20.6^{c}
0.30	40	355.0^{e}	252.9^{d}	4.41^{e}	2.35^{cd}	2.88^{cd}	35.2^{d}	15.9^{cd}	20.3^{bc}
	80	311.2^{bc}	237.4^{c}	4.04^{ab}	2.26^{bc}	2.74^{ab}	32.3^{a}	15.3^{bc}	19.4^{b}
	120	328.5^{d}	230.4^{bc}	4.34^{de}	2.19^{b}	2.73^{a}	34.7^{cd}	14.8^{ab}	19.2^{ab}
0.45	40	283.0^{a}	221.0^{ab}	3.94^{a}	2.25^{bc}	2.71^{a}	31.5^{a}	15.3^{bc}	19.1^{ab}
	80	323.1^{cd}	216.7^{a}	4.31^{d}	2.05^{a}	2.60^{a}	34.4^{cd}	13.9^{a}	18.3^{a}
	120	282.3^{a}	214.3^{a}	4.04^{ab}	2.19^{b}	2.66^{a}	32.3^{a}	14.9^{ab}	18.8^{ab}
Pooled SEM		4.91	4.36	0.061	0.039	0.051	0.70	0.41	0.051
Main effect									
Selenium									
0.15		316.1^{n}	274.4^{o}	4.10^{m}	2.49^{o}	2.90^{o}	32.8^{m}	16.9^{n}	20.5^{n}
0.30		330.9^{o}	242.7^{n}	4.26^{n}	2.26^{n}	2.78^{n}	34.0^{n}	15.4^{m}	19.6^{mn}
0.45		295.4^{m}	217.4^{m}	4.09^{m}	2.16^{m}	2.65^{m}	32.7^{m}	14.7^{m}	18.7^{m}
Probability									
Selenium		P<0.01	P<0.01	P<0.01	P<0.05	P<0.05	P<0.05	P<0.05	P<0.01
Zinc		NS	NS	NS	NS	NS	NS	NS	NS
Interaction		P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05	P<0.01

Value bearing different superscripts within a column differ significantly, NS-Non-significant.

diet (Sagar *et al*. 2020). However, the results of this study do not concur with the recommendation of 0.15 mg Se/kg diet by NRC (1994) and BIS (2007) in all phases of chicken growth. There are the reports of non-significant effects on the BWG and FCR of chicken (Payne *et al*. 2005, Li *et al*. 2018) which can be associated with the different genetic makeup of birds, Se status of hatching eggs, and the managemental conditions (Attia *et al*. 2010). Similar to the results of present study, 80 mg Zn/kg diet resulted in improved growth performance and growth efficiency of broiler chicken (Burrell *et al*. 2004, Mohammadi *et al*. 2015). Improved feed efficiency has been reported in broiler chicken fed 60-90 mg Zn/kg diet (Ahmadi *et al*. 2013, Zhao *et al*. 2014). Even an earlier study reported 80-120 mg Zn/kg diet to be optimum for better growth performance and other physiological indices (Zhang *et al*. 2006). The results of this study contradicted the recommendation of 40 mg Zn/kg diet by NRC (1994) but supported the recommendation of 80 mg Zn/kg diet by BIS (2007). Contrary to the results of the present study, 40 mg Zn/kg diet was found optimum for broiler chicken (Mir *et al*. 2013).

Carcass characteristics: None of the carcass characteristics were influenced by Se or Zn supplementation or by their interaction (Supplementary Table 1). This study did not reveal any significant effect of Se and Zn supplementation on carcass characteristics of broiler chicken. On the similar lines, supplementation of Zn (Collins and Moran 1999, Kumar 2007, Kumar *et al*. 2009) and Se (Khajali *et al*. 2010, Rajashree *et al*. 2014) beyond NRC recommendations do not affect the carcass characteristics of chicken. The absolute weight of different internal organs was also not influenced by Se (Sevcikova *et al*. 2006, Haug *et al*. 2007) or Zn (Mir *et al*. 2013) supplementation in broiler chicken diet.

Immunity of birds: No effect was observed on the CMI and bursa weight of birds (Table 4). However, higher (P<0.05) antibody titre was observed in birds fed 0.15 mg Se/kg with 80 or 120 mg Zn/kg diet compared to birds fed 0.15 mg Se/kg with 40 mg Zn/kg diet and 0.30 mg Se/kg with 120 mg Se/kg diet, whereas, other combinations resulted in intermediate values. The main effect of the Se revealed higher (P<0.05) antibody titre at 0.15 mg level compared to other two levels. But, the main effect of Zn revealed lower (P<0.05) antibody titre at 40 mg Zn/kg diet as compared to other two levels which did not differ significantly from each other. The relative weights of thymus and spleen were lower at 0.15 mg Se/kg diet compared to other two higher levels and Zn supplementation revealed lower spleen weight at 40 mg Zn/kg diet as compared to other two higher levels which were statistically similar to each other.

Table 4. Effect of different levels of selenium (Se) and zinc (Zn) on cellular and humoral immune response and immune organs of broiler chicken

Se (mg/kg)	Zn (mg/kg)	Immune status		Immune organs (% of live weight)		
		Cell mediated immunity[1]	Humoral immunity[2]	Thymus	Spleen	Bursa
0.15	40	0.65	1.99[a]	0.17	0.08	0.08
	80	0.91	2.91[c]	0.14	0.17	0.08
	120	0.95	3.02[c]	0.15	0.14	0.08
0.30	40	0.64	2.06[ab]	0.15	0.16	0.08
	80	0.69	2.28[b]	0.18	0.15	0.08
	120	0.77	1.99[a]	0.19	0.21	0.08
0.45	40	0.81	2.27[b]	0.20	0.18	0.08
	80	0.60	2.09[ab]	0.20	0.18	0.09
	120	0.45	2.29[b]	0.18	0.19	0.09
Pooled SEM		0.044	0.048	0.007	0.007	0.002
Main Effect						
Selenium (mg/kg)						
0.15		0.83	2.64[n]	0.15[m]	0.13[m]	0.08
0.30		0.70	2.11[m]	0.18[n]	0.17[n]	0.08
0.45		0.62	2.22[m]	0.19[n]	0.18[n]	0.09
Zinc (mg/kg)						
40		0.70	2.11[x]	0.17	0.14[x]	0.08
80		0.73	2.43[y]	0.17	0.17[y]	0.08
120		0.72	2.43[y]	0.17	0.18[y]	0.08
Probability						
Selenium		NS	P<0.05	P<0.01	P<0.01	NS
Zinc		NS	P<0.05	NS	P<0.05	NS
Interaction		NS	P<0.05	NS	NS	NS

Value bearing different superscripts within a column differ significantly, NS-Non-significant.[1] Measured as foot-web index (mm). [2] Measured as antibody titre expressed in Log_2 values.

Adaptive immunity, composed of humoral and cell mediate immunity, is determined by the activity of B- and T-lymphocytes by producing antibodies against the pathogens or by directly attacking them, respectively. In this study, no significant dietary effects were observed on the CMI of broiler chicken. However, higher humoral immunity was observed in birds supplemented with NRC recommended Se level in diet, but the NRC recommendation of 40 mg Zn/kg diet was inadequate for better humoral immune response compared to 80 or 120 mg Zn/kg diet. Further, higher spleen and thymus weight was observed at 0.3 mg Se/kg diet and higher spleen weight at 80 mg Zn/kg diet. However, earlier studies have reported that Se supplementation at levels higher than the recommended dose significantly increased the antibody titre against SRBC antigen (Nageswara *et al*. 2003, Yamuna *et al*. 2011). Further, the supplementation of chicken diets with 0.3 ppm Se improved the cellular as well as humoral immunity (Mohapatra *et al*. 2014). Dietary Zn is essential for normal immune functioning of the body (Dardenne *et al*. 1993) as evident from the present study. Better immune response was observed in chicken supplemented with 80-120 mg Zn/kg diet (Zhang *et al*. 2006) or 60-80 mg Zn/kg diet (Kumar 2007, Kumar *et al*. 2009) with respect to control diet. The increase of dietary Zn supplementation from 40 to 80 mg Zn/kg diet significantly increased the HA titre against SRBC in Aseel chicken (Deo *et al*. 2009).

Similarly, the Se supplementation in broiler chicken diets increased the relative weight of immune organs of birds (Hegazy *et al*. 2000, Mahmoud *et al*. 2016) which was observed in case of spleen and thymus weights only in this study. In line with the results of this study, Zn supplementation of 60 or 120 mg Zn/kg diet was reported to have no significant effects on the relative weights of thymus and bursa at 42 days of age in broiler chicken (Kulkarni *et al*. 2017). Contrary to the results of this study, the weight of immune organs, cellular and humoral immunity increased by supplementation of 40 ppm Zn in broiler chicken (Bartlett *et al*. 2003, Sunder *et al*. 2008).

In conclusion, Se supplementation of 0.30 mg /kg diet and Zn supplementation of 80 mg/kg diet resulted in optimum growth performance and growth efficiency of broiler chicken. No significant effect was observed on the carcass characteristics. Better humoral immunity was observed at 0.15 mg Se and 80 mg Zn/kg diet. However, 0.3 mg Se/kg diet resulted in higher spleen and thymus weights and 80 mg Zn/kg diet resulted in higher spleen weights.

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