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Influence of Combined Yeast-derived Zinc, Selenium and Chromium on Performance, Carcass Traits, Immune Response and Histomorphological Changes in Broiler Chickens

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ABSTRACT

This study was carried out to assess the effects of dietary supplementation of yeast-based Zn, Se, and Cr on growth performance, carcass characteristics, immune response, and histomorphological changes in broiler chickens. A total of 1092 one-day-old unsexed broilers of Ross 308 strain were randomly allocated into two equal treatment groups (6 equal replicates/ group). Birds were fed the dietary treatments from day 1 to day 35 of age. The first group was fed a basal diet without yeast-based Zn, Se and Cr supplementation (control). The second group was fed a basal diet supplemented with 1.5 mg, 0.15 mg and 2 µg / kg diet of Zn, Se, and Cr, respectively. The yeast-based Zn, Se and Cr increased ($P<0.05$) the growth rate, carcass traits and improved feed conversion ratio compared to control. The antibody titer against avian influenza and the relative weight of bursa, thymus and spleen were increased ($P < 0.05$) in broilers supplemented with yeast-based Zn, Se, and Cr compared with the control. The intestinal villi height and crypt depth and bursa diameters were increased in broilers fed the yeast-based Zn, Se and Cr supplemented diet. In addition, there was an increase in the lymphocytic infiltration in the bursa and a decrease in germinal centers in the submucosal lymph nodules of the ileum of birds supplemented with yeast-based Zn, Se and Cr compared to the control. In conclusion, dietary supplementation of yeast-based Se, Cr, and Zn in combination could improve growth performance, carcass traits, immune responses and histo-morphological parameters in broiler chickens.

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Introduction

Zinc (Zn), selenium (Se) and chromium (Cr) are essential micronutrients for optimum performance and health of poultry (Park *et al*., 2004; Huang *et al*., 2019; Hidayat *et al*., 2020). These trace minerals have been traditionally used in the form of inorganic salts in practical poultry diets. However, inorganic trace minerals are of little efficiency due to both the low bioavailability and interactions with other nutrients with subsequent high excretion rate into the environment. Other forms of trace minerals, including organic, nanoparticles, or yeast-derived forms are successfully replacing the inorganic ones with promising results in growth, nutrient utilization and immunity (Świątkiewicz *et al*., 2014; Hassan *et al*., 2019; Patra and Lalhriatpuii, 2020). By growing in trace element-enriched media, yeast can accumulate large amounts of these trace minerals and incorporate them into organic compounds such as selenomethionine, Zn methionine and Cr lysine (Stehlik-Tomas *et al*., 2004; Sillerová *et al*., 2012).

Saccharomyces cerevisiae, can convert inorganic micro minerals to organic ones through absorption from cultivation media enriched with inorganic sources of trace minerals and the yeast-derived organic trace minerals are currently safely used as feed additives (Sillerová *et al*., 2012; Wu *et al*., 2019). In addition to the yeast-derived specific trace elements, the functional components of the yeast cells (i.e., Amino acids, enzymes, nucleic acids) and yeast cell walls (i.e., glucans, mannans, nucleotides) may contribute in improving animal health and performance (Alexandre and Guilloux-Benatier, 2006; Shurson, 2018).

Incorporating Zn, Se, and Cr together with yeast extract (mannans and B glucans) could have a synergistic effect since each component of them works via a different mechanism. As

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far as the authors' concerns, there have not been any reports on products composed of Se, Zn, Cr and yeast. Therefore, this study was carried out to assess the effects of dietary supplementation of Zn, Se, and Cr enriched-yeast as one product on zoo-technical performance, carcass characteristics, immune responses, and histomorphological parameters in broiler chicken.

Materials and methods

Birds and experimental diets

This feeding trial was carried out at the Poultry and Animal Research Center, Faculty of Veterinary Medicine, Cairo University, Egypt. The protocol was approved by the Animal Care Committee of the Faculty of Veterinary Medicine (Vet CU23012020110).

A total of 1092 one-day-old chicks (unsexed) of Ross 308 strain were randomly allocated to two equal treatment groups, each group contained 6 equal replicates (91 chicks / each). Replicates were randomly allocated in a floor pen equipped with wheat straw litter, with a bird density of 10 birds/ m^2. The first treatment served as control where chicks were fed the basal diet (Table 1) without yeast-based Zn, Se and Cr supplementation. In the second treatment, chickens were fed the control diet supplemented with a yeast-based Zn (1.5 mg/kg diet), Se (0.15 mg/kg diet) and Cr (2 µg /kg diet) product as one commercial mix added at the rate of 50 g/ton (Fertapromine®, obtained from the American Microbiotech Company; Microbiotech USA, Inc.). Birds were fed the dietary treatments from day 1 to day 35 of age. Each 1 g of the product contained 950 mg yeast extract (Saccharomyces cerevisiae), 30 mg Zn methionine, 9 mg mannan oligosaccharide, 8 mg β-glucan, 3 mg selenomethionine, 40 µg Cr lysine and dextran (up to 1 g) (Microbiotech USA, Inc.). Birds were subjected to 1 h darkness and 23 h light during the first week of the experiment followed by 18 h light and 6 h darkness for the rest of the experimental period (35 days). Feed and fresh water were provided ad libitum throughout the whole experiment, which lasted for 35 days. Birds were vaccinated against H5N1 Avian influenza (AI H5N1: at 7 days old) and Newcastle disease (NDV: at 7, 13 and 20 days old) viruses. The basal diet was formulated according to Ross 308 broiler nutrient specification Manual 2014 (AVIAGEN, 2014) with recommended levels of inorganic trace minerals provided by a premix.

Sampling and measurements

Growth performance

Feed intake and the change in body weight were recorded weekly to calculate the body weight gain and feed conversion ratio (FCR).

Carcass characteristics

At the end of the experiment, three birds from each replicate (18 birds per treatment) were randomly selected for slaughter to determine carcass weight, dressing percentage, and the breast and thigh yield. While the lymphoid organs (spleen, thymus, and bursa of Fabricius) were collected, weighed and expressed as a percentage of live body weight.

Immune response

On days 7, 14, 21, 28 and 35, blood samples were collected from the wing vein of two birds per replicate, 12 birds per treatment, (using EDTA as an anticoagulant). Samples were centrifuged at 3500 × g for 15 min to determine antibody titers against NDV and AI H5N1. Antibody titers against NDV and AI H5N1 were determined by the haemagglutination inhibition test and were expressed as the logarithm base 2. The reciprocal of the highest dilution where there was complete

Table 1. Ingredients composition and chemical analysis of the control diet

Item	Diet		
	Starter	Grower	Finisher
Ingredients			
Yellow corn	56.59	59.62	63.71
Corn gluten meal 60%	4	3	2.5
Soybean meal 47%	35	32.6	28.2
Soybean oil	0	0.8	1.8
Monocalcium phosphate	1.55	1.3	1.2
Limestone, Ground	1.8	1.7	1.6
Common salt	0.4	0.4	0.4
DL-Methionine	0.14	0.12	0.12
L-Lysine	0.17	0.11	0.12
L-Threonine	0.05	0.05	0.05
Broiler premix[1]	0.3	0.3	0.3
Total	100	100	100
Calculated chemical analysis (% of dry matter)			
Dry matter%	88.9	88.9	90
Metabolizable energy (MJ/kg)	12.7	13	13.4
Crude protein	23.1	21.5	19.6
Crude fat	2.54	2.6	2.68
Crude fiber	2.33	2.29	2.22
Calcium	1.02	0.93	0.87
Available phosphorus	0.5	0.44	0.41

[1]per Kg premix: 1200000 IU Vit. A, 350000 IU Vit. D3, 4000 mg Vit. E, 250mg Vit. B1, 800 mg Vit. B2, 600 mg Vit. B6, 3.2 mg Vit. B12, 450 mg Vit. K3, 4.5g nicotinic acid, 1.5g Ca pantothenate, 120 mg folic acid, 5 mg biotin, 55 mg choline chloride, 3g Fe, 2 g Cu, 10 g Mn, 8 g Zn oxide, 0.15 mg sodium selenite, 120 mg I, 40 mg Co for the control diet. In the experimental diet, a commercial product (Fertapromine®; Microbiotech USA, Inc.) containing yeast-based trace minerals Zn, Se and Cr was added (50 g/ton) to provide a final concentration of 1.5 mg, 0.15 mg and 2 µg/kg diet of Zn, Se and Cr respectively

agglutination was taken as titer (Wegmann *et al*., 1966).

Histo-morphometric examination of the small intestine and bursa

Three birds from each replicate (n=18) were slaughtered for the histomorphological studies. The villus height (from the villus tip to the bottom) and the depth of the intestinal gland (crypt) were measured. Five stained sections per bird of each segment (duodenum, jejunum, and ileum) were examined using Leica Quin 500 analyzer computer system (Leica Microsystems, Switzerland) in the Faculty of Veterinary Medicine, Cairo University. Automatic calibration of the image analyzer was performed to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. The images of each section were captured for each sample with a final magnification of 40x. The bursa of Fabricius, the follicle, cortical and medullary diameters were also measured.

Histological examination of the trachea, lung tissue, breast muscle, kidney, liver, and ileum

Three birds from each replicate (n=18) were slaughtered. Then, the gastrointestinal tract, trachea, lung, liver, kidney, breast muscle and bursa were dissected out. Sections from the middle of the duodenum, jejunum (at the midpoint between the bile duct entry and the Meckel's diverticulum), and ileum (about 0.5 cm in length) were excised and opened longitudinally at the anti-mesenteric attachment and gently flushed with 0.1 M phosphate-buffered saline (pH 7.4). All specimens were fixed immediately in 10% neutral buffered formalin solution. Then, the specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Sections of 6-7 μm thick were obtained and mounted on clean glass slides and stained with Delafield's iron Haematoxylin and Eosin (H&E) to verify general histological structure details. Methods were adopted according to Bancroft and Gamble (2008).

Statistical analysis

Data obtained from the experiment were subjected to statistical analysis using SPSS Software. The two treatments were compared using the independent T-test with pre-set significance at $P \leq 0.05$.

Results

Growth performance

Changes in growth and feed utilization of broilers in response to feeding a diet supplemented with yeast-based Zn, Se, and Cr are illustrated in Table 2. The yeast-based Zn, Se and Cr significantly increased the growth rate, but decreased feed intake, feed conversion ratio and mortality rate compared to control (P < 0.05, Table 2). Feeding the yeast-based Zn, Se and Cr increased final body weight and body weight gain by 8% and decreased the feed intake and feed conversion ratio by 5 and 12 %, respectively compared to the control. Also, the mortality rate was decreased by 50% in broilers fed the yeast-based Zn, Se and Cr compared to that in those fed the control diet.

Carcass characteristics

The carcass characteristics of broilers fed either control or yeast-based Zn, Se, and Cr supplemented diet are shown in Table 3. The carcass weight, dressing percentage, and both breast and thigh yield and percentage were higher (P < 0.05) in broilers receiving yeast-based Zn, Se and Cr compared to the control (Table 3). The carcass, breast and thigh weights were increased by 17, 38 and 21%, respectively in broilers fed the yeast-based organic Zn, Se, and Cr supplemented diet compared to those fed the control diet.

Table 2. Growth performance, feed utilization and mortality of broiler chickens fed diets supplemented with yeast-based Zn, Se and Cr (Fertapromine®)

Item	Experimental diets[1]	
	Control	Y-TM
Initial body Weight (g)	42.2 ± 0.27^a	41.9 ± 0.26^a
Final body weight (g)	1915 ± 1.56^b	2081 ± 3.19^a
Weight gain (g)	1874 ± 0.45^b	2028 ± 0.27^a
Feed intake (g)	3305 ± 0.60^a	3151 ± 0.65^b
Feed conversion ratio	1.73 ± 0.003^a	1.52 ± 0.004^b
Mortality%	5.6	2.6

[1]Control basal diet without supplementation, Y-TM supplemented with the yeast-based trace minerals Zn, Se and Cr at a rate of 1.5 mg, 0.15 mg and 2 µg/kg diet, respectively.
[a,b] Different subscripts within a row indicate significant effect (P< 0.05)
Values are expressed as Mean±SE (Standard error)

Table 3. Carcass traits of broiler chickens fed diets supplemented with yeast-based Zn, Se and Cr (Fertapromine®)

Item	Experimental diets[1]	
	Control	Y-TM
Live body weight (g)	1926 ± 7.98^b	2050 ± 13.2^a
Carcass weight (g)	1522 ± 4.66^b	1773 ± 7.14^a
Dressing %	79 ± 0.15^b	85.9 ± 0.49^a
Breast weight (g)	452.5 ± 0.96^b	623 ± 0.96^a
Breast yield%	29.6 ± 0.07^b	34.3 ± 0.21^a
Thigh weight (g)	612.9 ± 1.64^b	738.5 ± 0.69^a
Thigh yield%	40.3 ± 0.28^b	41.7 ± 0.25^a

[1]Control basal diet without supplementation, Y-TM supplemented with the yeast-based trace minerals Zn, Se and Cr at a rate of 1.5 mg, 0.15 mg and 2 µg/kg diet, respectively.
[a,b] Different subscripts within a row indicate significant effect (P< 0.05)
Values are expressed as Mean±SE (Standard error).

Immune response

The changes of the antibody titers against NDV and AI H5N1 and in the relative weight of the lymphoid organs in response to supplementation with yeast-based Zn, Se, and Cr are illustrated in Table 4. Supplementation with yeast-based Zn, Se and Cr significantly decreased (P < 0.05) the antibody titers against the NDV (at the first- and second-weeks following vaccination) but increased (P < 0.05) the antibody titer against AI H5N1 (at from the third to the fifth week following vaccination) (P < 0.05). The relative weight of bursa, thymus and spleen were significantly increased (P < 0.05) in broilers supplemented with yeast-based Zn, Se, and Cr compared to those fed the control diet (Table 4).

Histomorphology of intestine and bursa

The histomorphometry of the intestine and bursa are illustrated in Table 5, there was a significant increase in the villi length of duodenum, jejunum, and ileum and in the crypt depth of duodenum and ileum in broilers fed the yeast-based Zn, Se and Cr supplemented diet compared to those fed the control diet (Table 5). There was a significant increase in the follicular, medullary and cortical diameter of the bursa in broilers fed the yeast-based Zn, Se and Cr supplemented diet compared to those fed the control diet (Table 5).

Table 4. Antibody titers against Newcastle disease and Avian influenza H5 vaccines and the relative weight of lymphoid organs in broilers fed diets supplemented with yeast-based Zn, Se and Cr (Fertapromine®)

Item	Experimental diets[1]	
	Control	Y-TM
Newcastle Disease		
First week	4.6 ± 0.05^{a}	4.6 ± 0.06^{a}
Second week	3.0 ± 0.06^{a}	2.8 ± 0.1^{b}
Third week	3.0 ± 0.06^{a}	2.7 ± 0.06^{b}
Fourth week	4.9 ± 0.06^{a}	4.7 ± 0.06^{a}
Fifth week	4.0 ± 0.06^{a}	3.8 ± 0.06^{a}
Avian influenza H5		
First week	1.4 ± 0.06^{a}	1.4 ± 0.06^{a}
Second week	1.5 ± 0.1^{a}	1.5 ± 0.1^{a}
Third week	0.3 ± 0.03^{b}	1.2 ± 0.06^{a}
Fourth week	0.9 ± 0.05^{b}	1.4 ± 0.05^{a}
Fifth week	1.9 ± 0.06^{b}	2.5 ± 0.06^{a}
Lymphoid organs relative weight (at the fifth week)		
Bursa	0.06 ± 0.005^{b}	0.11 ± 0.003^{a}
Thymus	0.08 ± 0.004^{b}	0.13 ± 0.006^{a}
Spleen	0.28 ± 0.004$_{b}$	0.44 ± 0.004^{a}

[1]Control basal diet without supplementation, Y-TM supplemented with the yeast-based trace minerals Zn, Se and Cr at a rate of 1.5 mg, 0.15 mg and 2 µg/kg diet, respectively
[a,b] Different subscripts within a row indicate significant effect ($P < 0.05$)
Values are expressed as Mean±SE (Standard error)

Table 5. Histomorphology of the duodenum, jejunum, ileum and bursa of broiler chickens fed diets supplemented with yeast-based Zn, Se and Cr (Fertapromine®)

Item	Experimental diets[1]	
	Control	Y-TM
Duodenum (µm)		
Villus length	1006 ± 197^{b}	1740 ± 183^{a}
Crypt depth	117.4 ± 18.1^{b}	165.0 ± 17.6^{a}
Villus crypt ratio	8.87 ± 2.72^{b}	10.7 ± 2.03^{a}
Jejunum (µm)		
Villus length	989 ± 63.2^{b}	1350 ± 94.3^{a}
Crypt depth	93.4 ± 7.73^{a}	90.8 ± 5.13^{a}
Villus crypt ratio	10.7 ± 1.13^{b}	14.9 ± 1.14^{a}
Ileum (µm)		
Villus length	747.2 ± 29.7^{b}	839.5 ± 74.0^{a}
Crypt depth	95.5 ± 9.82^{b}	117.0 ± 10.2^{a}
Villus crypt ratio	7.89 ± 0.78^{a}	7.23 ± 0.99^{a}
bursa (µm)		
Follicle diameter	213.6 ± 33.57^{b}	509.0 ± 81.1^{a}
Medullary diameter	222.7 ± 42.45^{b}	419.4 ± 60.1^{a}
Cortex diameter	44.1 ± 13.2^{b}	112.4 ± 13.9^{a}

[1]Control basal diet without supplementation, Y-TM supplemented with the yeast-based trace minerals Zn, Se and Cr at a rate of 1.5 mg, 0.15 mg and 2 µg/kg diet, respectively
[a,b] Different subscripts within a row indicate significant effect ($P < 0.05$)
Values are expressed as Mean±SE (Standard error)

Histo-morphological examination of the trachea, lung tissue, breast muscle, kidney, liver, and ileum

The histo-morphological changes of the trachea, lung tissue, breast muscle, kidney, liver, and ileum are illustrated in Figures A, B and C. Trachea showed a normal structure with pseudostratified columnar ciliated epithelium rest on a relatively thin lamina propria with infiltrating lymphocytes in the control birds (Arrow; Fig.A1). In birds fed the yeast-based Zn, Se and Cr supplemented diet, the lamina propria showed an increase in the mononuclear cells (thicker lamina propria than in the control group) (Arrow, Fig. A2). Also, some regions of lamina propria showed the presence of lymph nodules (Ln; Fig A2) and there was an increase in the goblet cells (star, Fig A3) and intraepithelial mucous glands (arrow; Fig A3) in birds supplemented with the yeast-based Zn, Se and Cr. For lung tissue, the control birds showed a normal cytoarchitecture (Arrow; Fig.A4) whereas the birds supplemented with the yeast-based Zn, Se and Cr showed an increase in the lymphocytic infiltration in the inter-parabronchus septum surrounding each lobule, together with a slight increase in the lymphocyte infiltration in the lamina propria of the primary, secondary and tertiary bronchi (arrow, star; Fig.A5). For breast muscle fibers, no changes in the histological structure of the breast muscle fibers in both groups (Fig. B1 & B2). For kidney, the control birds showed normal renal corpuscle (RC) and renal tubules (RT) and slight lymphocytic cells (arrow) (Fig.B3) but there was an increase in the lymphocytic infiltration (arrow; Fig. B4) of the interstitial tissue surrounding the renal tubules (RT) of birds supplemented with the yeast-based Zn, Se and Cr. For the liver, the control birds showed normal hepatocytes (HC) and central vein (CV) (Fig. B5) but a lymphocytic infiltration surrounding the central vein of the liver was noticed in birds supplemented with the yeast-based Zn, Se and Cr (Fig. B6). Moreover, a low number of germinal centers appeared in the submucosal lymph nodule of the ileum in birds supplemented with the yeast-based Zn, Se and Cr (arrow, Ln; Fig.C1 & C2).

Discussion

Results of this study revealed that supplementation of organic Se, Cr, and Zn as yeast-based increased final body weight and body weight gain but decreased the feed intake and improve the FCR. These findings are consistent with previous studies, which indicated an improvement in growth performance and feed utilization in broilers fed diets supplemented with yeast-derived Zn, Se and Cr compared to those fed inorganic counterparts (Suksombat and Kanchanatawee, 2005; Nollet *et al*., 2008; Rao *et al*., 2016 a; Amer *et al*., 2019). The positive effect of yeast-based Se, Cr, and Zn supplementation on growth performance parameters could be partially explained to the improved utilization of trace minerals in the organic form (i.e., higher bioavailability) and consequently their roles in enhancing certain enzyme systems involved in nutrient metabolic pathways, cell proliferation, and muscle metabolism. It has been reported that Zn and Se are involved in endocrine regulation of cell proliferation induced by growth hormone and insulin-like growth factor pathways (MacDonald, 2000; Ren *et al*., 2016). Also, Cr has been shown

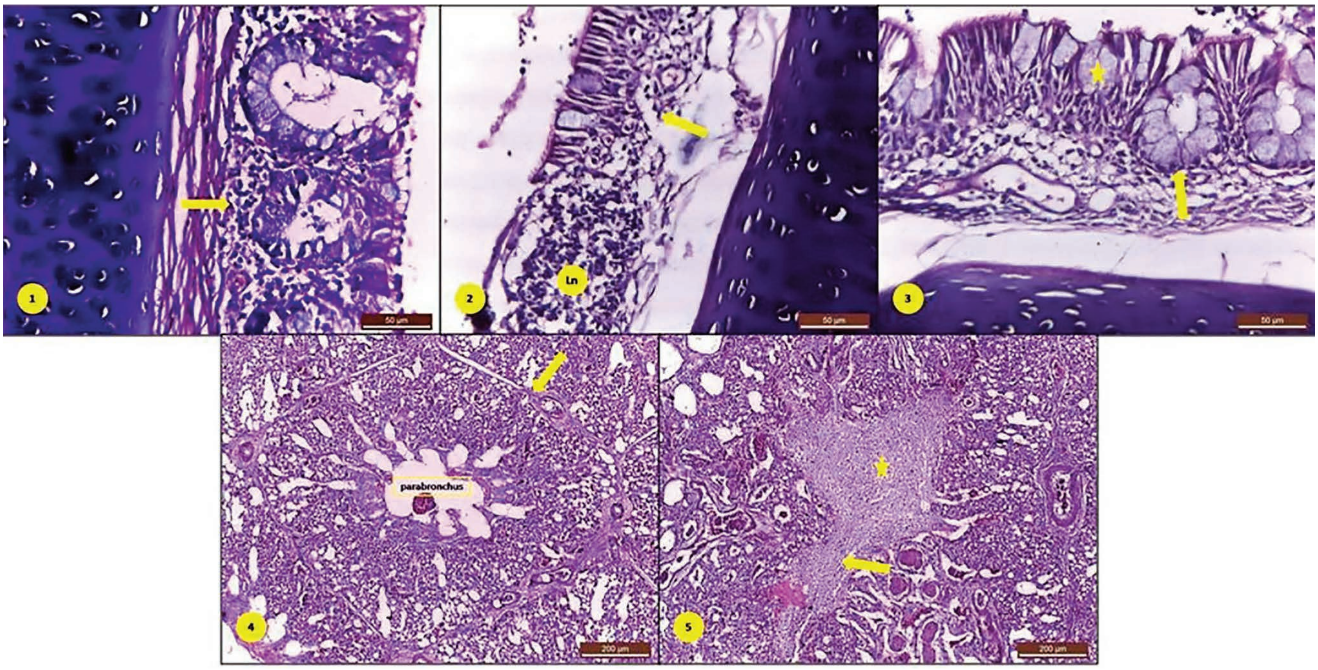


Fig. A. 1) Normal histological structure of trachea with few lymphocytic infiltrations in control birds; 2) lymphocytic infiltration (arrow) and lymph nodule (Ln) in lamnia propria in birds fed the yeast-based organic Zn, Se, and Cr supplemented diet; 3) Goblet cells (star) and intraepithelial mucous glands (arrow) in birds fed the yeast-based organic Zn, Se, and Cr supplemented diet; 4) Normal lung tissue in control birds; 5) Lymphocytic infiltration (star) in inter-parabronchus septum surrounding each lobules (arrow) in birds fed the yeast-based organic Zn, Se, and Cr supplemented diet. H&E stain, Figs. 1, 2 & 3, at 40x, Figs. 4 & 5, at 100x.

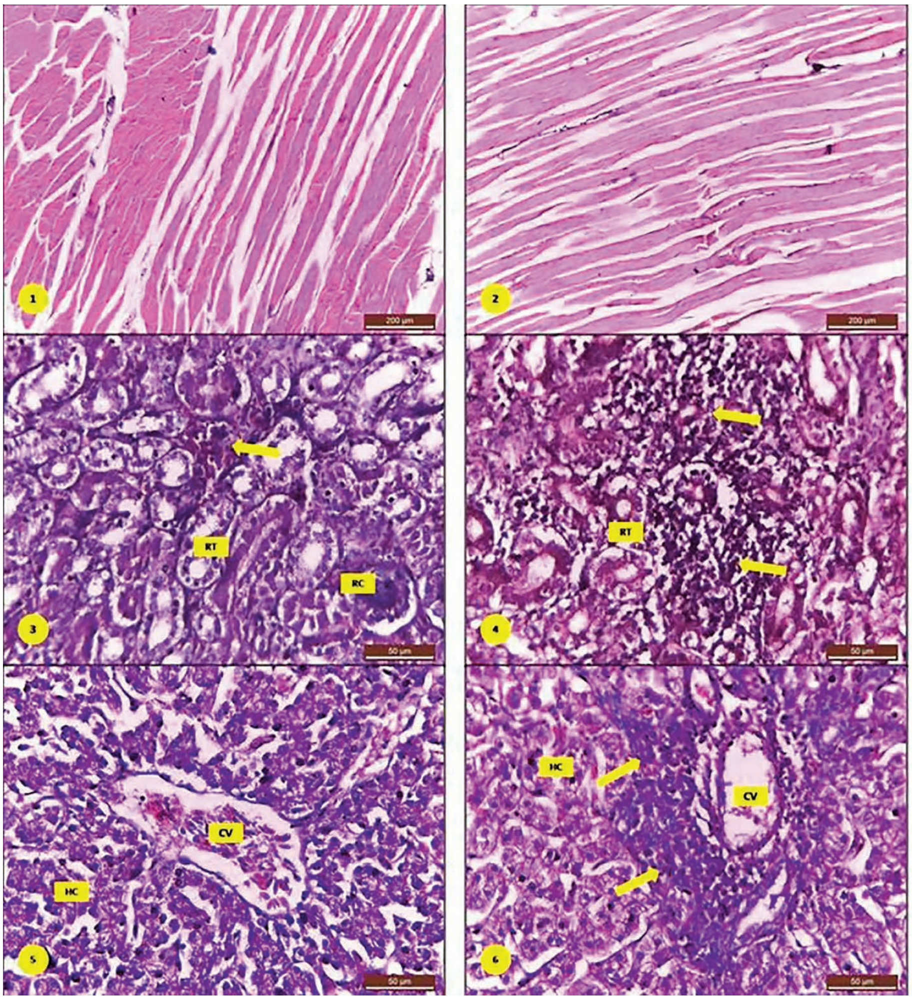
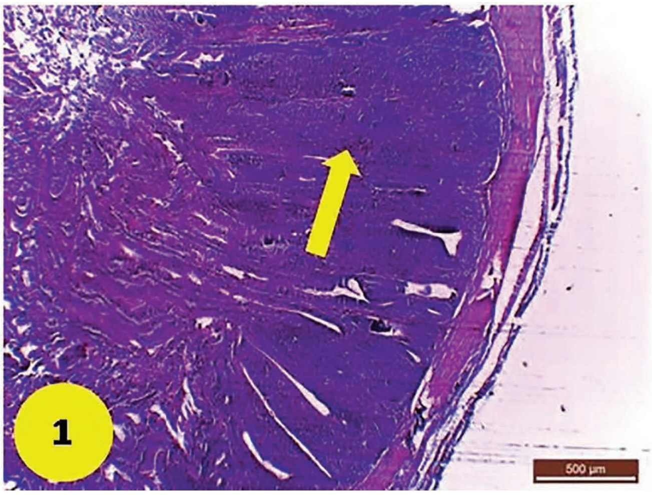


Fig. B. 1 & 2), Normal histological structure of breast muscle fibers in both groups; 3) Kidney tissue with normal (arrow) renal corpuscle (RC) and renal tubules (RT) in control birds; 4) Kidney tissue with lymphocytic infiltration (arrow) surrounding renal tubules (RT) in birds fed the yeast-based organic Zn, Se, and Cr supplemented diet; 5) Normal hepatocytes (HC) in control birds. 6) Lymphocytic infiltration (arrow) surrounding central vein (CV) in birds fed the yeast-based organic Zn, Se, and Cr supplemented diet. H&E stain, Figs. 1 & 2 at 100x, Figs. 3,4,5 & 6, at 40x.



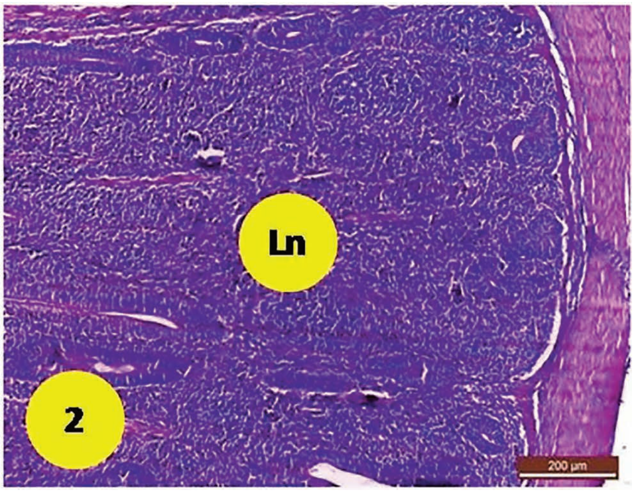


Fig. C. 1 & 2) Ileum in birds (n.=18) fed the yeast-based organic Zn, Se, and Cr supplemented diet with normal structure notice submucosal lymph nodules (arrow) no germinal center in lymph nodules (Ln). H&E stain, Fig. 1, at 40x, Fig. 2 at 100x.

to enhance glucose metabolism through the activation of insulin release and action (Lien *et al*., 1999). Supplemental Cr and Se have been shown to increase protein synthesis in muscles and accordingly enhance growth efficiency (Ahmed *et al*., 2005; Pan *et al*., 2013). Contrary to the results of the present study, Suksombat and Kanchanatawee (2005) did not observe significant differences in the growth performance of broilers fed Cr yeast or Cr picolinate vs Cr chloride. Besides, dietary supplementation of organic zinc at 25 mg/kg to a basal diet containing 32.55 mg/kg zinc did not affect the growth performance of broiler chickens (Salim *et al*., 2012). Further, other reports indicated that Se yeast did not offer extra benefits than sodium selenite for the performance of broilers from 1-42 d old (Silva *et al*., 2019). Also, organic Se addition up to 400 µg/kg diet vs 0 µg/kg diet did not affect the growth performance of broiler chickens (Rao *et al*., 2013). Despite there is no concrete conclusion regarding the Cr supplementation to poultry diet and the findings are largely inconsistent (White eand Vincent, 2019), the obtained results suggest the nutritional relevance of Cr supplementation in broilers' diet. As further as we know, this is the first trial to use the three trace minerals in one product and the results obtained could be explained in part due to the probable synergism among the Zn, Se and Cr, in addition to the other components (β-glucan and mannan oligosaccharide) of yeast extract.

The supplementation of yeast-based Zn, Se, and Cr increased the carcass weight, dressing percentage, and both breast and thigh yield and percentage, which is considered a reflection of the overall improvement in growth performance. Current observations of carcass trait parameters are consistent with previous reports, which stated that supplementation of Cr yeast vs inorganic Cr increased the dressing yield and carcass quality of broilers (Kroliczewska, 2005; Suksombat and Kanchanatawee, 2005). Furthermore, Selenium yeast has been reported to promote muscle growth in broilers (Jin *et al*., 2019).

In this study, supplementation of yeast-based Cr, Se, and Zn in broiler diet increased antibody titers against the AI H5N1 and caused an increase in the relative weight of lymphoid organs (bursa, spleen, and thymus), but decreased the antibody titer against the NDV. Similar increases in antibody titers against Avian influenza were reported in broilers fed diets supplemented with organic Cr picolinate and Cr nanoparticles (Hajializadeh *et al*., 2017). The relative weight of the lymphoid organs was increased in the broilers fed organic vs inorganic zinc (Sahoo *et al*., 2014). The findings regarding the influence of trace minerals on the immune response are largely inconsistent, probably because of the supplementing level and form of the mineral in addition to the variations in experimental diets and the challenging/stressor agents. In contrast to our results, the antibody titers against the NDV and the relative weight of lymphoid organs were not affected in broilers supplemented with organic Cr (Rao *et al*., 2012), Se (Rao *et al*., 2013) or Zn (Rao *et al*., 2016b) offered either individually or in combination. Also, the relative weights of thymus and bursa were decreased, but the spleen relative weight was increased by Se supplementation in broilers challenged with salmonella and Aflatoxin B1 (Hegazy and Adachi, 2000).

Small intestinal histomorphology, including villus height and crypt depth, is one of the important indications of gut health in broiler chickens. Increased villus height is directly correlated with increased epithelial turnover, and longer villi are associated with activated cell mitosis. Histomorphometric studies for the small intestine, especially the villus length and crypt depth considered as an important tool for the determination of the available area for digestion and absorption (Swatson *et al*., 2002; Franco *et al*., 2006). The histological results revealed that the integrity of the intestinal mucosa was maintained in chickens fed the yeast-based Zn, Se and Cr-supplemented diet. In the present study, there was an increase in the intestinal villus length together with deeper crypts in both duodenum and ileum in birds supplemented with yeast-based Zn, Se, and Cr when compared with the control. Longer villi enhance gut health and improve the efficiency of digestion and absorption (Baurhoo *et al*., 2007). The increase in the crypt depth is an indicator of faster turnover and so renewal of the villi, which is considered as a response mechanism to overcome normal sloughing or atrophy of the villi due to inflammation (Gao *et al*., 2008). Intestinal morphology was improved (increased villus height) in broilers fed Zn amino acid complex vs Zn sulfate at a rate of 60 ppm (De Grande *et al*., 2020) or Zn proteinate vs Zn sulfate at 90 mg/kg (Bortoluzzi *et al*., 2019). Similarly, villus height was increased in duodenum and jejunum but crypt depth was decreased in jejunum and ileum of broilers fed Zn glycine at a rate of 90 mg/kg (Ma *et al*., 2011).

From the light microscopic results, there was an improvement in lymphocytic infiltration in the different organs (trachea, lung tissue, kidney, liver, and bursa) of birds supplemented with the yeast-based trace minerals. This could be attributed to the ameliorative effect of Cr on the proliferation of peripheral lymphocytes and the immune functions

(Uyanik *et al*., 2002; Bahrami *et al*., 2012). The lower number of the germinal centers in the submucosal lymph nodules of the ileum in birds supplemented with yeast-based Zn, Se and Cr might indicate a state of healthy nutrition provided by the yeast-derived trace minerals, which might enhance the immune performance of birds; similar morphological changes were observed by (Dibner *et al*., 1998).

Conclusion

Dietary supplementation of yeast-based Se, Cr, and Zn in combination significantly improve growth performance, carcass traits, immune responses and histo-morphological parameters in broiler chickens. Further research is needed to clarify the mechanism underlying these effects and whether these micronutrients work in synergism.

Conflict of interest

The authors declare that they have no conflict.

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