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Replacement of Inorganic Zinc with Various Organic Zinc Sources on Haematological Constituents, Antioxidant Status, Immune Response and Reproductive Efficiency in Rats

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A B S T R A C T

The present study was undertaken on 60 adult female Sprague Dawley rats (275±2.04 g) for an experimental duration of 84 days to evaluate various organic sources of zinc on immunity, reproduction, oxidative defense mechanism in rats. The rats were randomly allotted to 5 dietary treatments (6 replicates in each) prepared by varying the source of Zn supplementation (12 ppm) either from inorganic (Zn carbonate) or sources (Zn proteinate, Zn propionate, Zn amino acid complex and Zn methionine). Supplementation of organic zinc had no significant effect on performance, haematological and serum biochemical parameters, TBARS and protein carbonyls concentration in liver. The glutathione reductase activity ($P<0.05$) in haemolysate and reduced glutathione concentration in liver ($P<0.01$) was higher in organic Zn fed rats, while highest ($P<0.01$) RBC catalase activity was noticed with Zn methionine supplementation. The humoral immune response against sheep RBC was increased ($P<0.05$) with organic Zn supplementation. The cell mediated immune response was higher ($P<0.05$) in Zn propionate group. The serum progesterone concentration was higher ($P<0.05$) in rats fed organic Zn sources. Organic Zn supplementation increased ($P<0.05$) the number of graafian follicles and corpus luteum. The study indicated that supplementing 12 ppm of Zn from various organic sources had higher antioxidant enzyme activities, immune response and serum progesterone concentration with higher number of mature follicles in ovaries compared to inorganic.

Introduction

Zinc (Zn) by being a component of more than 300 metalloenzymes influences the growth, immunity, antioxidant mechanism and reproductive performance of animals (Hafeez *et al.*, 2013). In addition, previous experiments conducted at author's laboratory indicated improved immunity, reproduction and oxidative defense mechanism in rats fed

diets containing Zn (Zinc carbonate) up to 36 ppm (Nagalakshmi *et al.,* 2009, 2012, 2013 a&b). However, over supplementation of Zn may interfere with the absorption and availability of other minerals which are also essential for normal physiological activities of animals (Nitrayova *et al.,* 2012; Sridhar *et al.,* 2015a). Hence, to overcome this concept of organic minerals was established, in which Zn supplementation recommended or lower than the recommendations may sufficient for better immunity, antioxidant status and reproductive performance (Sridhar *et al.,* 2016; Nagalakshmi *et al.,* 2015a,b; 2016a,b). Even though this concept well established, there are conflicts among the researches on relative bioavailability/effectiveness among organic Zn sources, because the availability of mineral is mainly depend on the physic-chemical bonding between ligand and mineral. Therefore, here authors made an attempt to evaluate the various organic sources (Zn proteinate, Zn propionate, Zn amino acid complex and Zn methionine) of zinc on immunity, reproduction, oxidative defense mechanism in rats

Materials and Methods

A study was conducted on 60 adult female Sprague Dawley rats (SD) strain with an average body weight of 275±2.04 g. The rats were housed in polypropylene cages in the Animal House of College of Veterinary Science, Hyderabad under hygienic conditions with controlled temperature (22-23^{o}C) and photoperiod (12h/d). The rearing and care of rats and procedures adopted were according to the guidelines of Institutional Animal Ethics Committee and permission has been taken from the same for experimentation. The approval number from ethics committee for this experiment was I/2/11 on dated 14.03.2011. The rats were provided with respective diet *adlibitum* and with free access to wholesome clean deionized water. Water was provided in polypropylene bottles having provision for nipples. The animals were randomly divided into 30 replicates with 2 rats per replicate and these 30 replicates in turn were randomly allotted to 5 dietary treatments varying in source of Zn so as to supply 12 ppm Zn as per NRC (1995) recommendation. The rats were fed with the respective diets for an experimental duration of 12 weeks. A basal diet (BD) based on AIN- 76A rodent diet for adult rats was formulated using purified ingredients (Table 1) to this 12 ppm Zn was added from inorganic source (Zn carbonate) and this served as control diet. The other 4 experimental diets were similar to control diet except the $ZnCO_3$ was replaced with various organic Zn sources viz., zinc proteinate (Zn prot), zinc propionate (Zn prop), zinc amino acid complex (Zn AA) and zinc methionine (Zn met) supplementing 12 ppm Zn. During experimentation, weekly body weights and daily feed intake were recorded. At 50th d, blood was collected by retro-orbital puncture to analyze the haematological and biochemical constituents. At 55th d of experiment, all rats were antigenically challenged with sheep RBC to assess the humoral immune response. The cell mediated immune response was assayed by footpad reaction method on 80th day of the experiment. On 83rdd of the experiment, blood was collected to assess the antioxidant enzymes. After 12 weeks of feeding, rats were sacrificed and livers and ovaries were collected for antioxidant enzymes in liver and ovarian histology, respectively.

Haematological constituents

For haematology, blood was collected on 50th d from all rats after overnight fasting in heparinized vacutainers. Haemoglobin (Hb) content, red blood cell (RBC) and white blood cell (WBC) counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin

(MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocyte and granulocyte percentage were determined by automatic blood analyser (Huma count, Med source Ozone biomedical Pvt. Ltd, India).

Serum biochemical constituents

After collecting blood in heparinized vacutainers, blood was also collected in serum vacutainers with no anticoagulant and then centrifuged at 3500 rpm for 15 minutes. The serum was collected and stored at 20°C for analysis. The glucose was determined by O-toludine method (Cooper and Mc Daniel, 1970) cholesterol by the method described by Wybenga and Pileggi's (1970). Serum total protein and albumin levels were estimated according to the methods described by Reinhold (1953) and Gustafsson (1956) respectively. The globulin was determined as the difference between total protein and albumin concentration in the serum. Serum alkaline phosphatase (ALP) activity was determined by the method of Kind and King (1954). Serum progesterone concentration was estimated by using commercial ELISA kit (Omega diagnostics, pathozyme, progesterone, Scotland, UK).

Immune response

For assaying the immune response, the rats were challenged twice with sheep RBC (0.5 x 10^9 cells/100 g, I/P), as an antigen and second challenge was given 7 days after first challenge. The blood was withdrawn from retroorbital plexus from all antigenically challenged rats after one week of primary and secondary challenge and HA titers were estimated as per method given by (1942). 25 µl of serum was serially diluted with 25 µl of phosphate-buffered saline (PBS). Sheep RBC (0.025 x 10^9 cells) were added to each of these dilutions and incubated at 37°C for one hour. The rank of minimum dilution that exhibited hemagglutination was considered as the antibody titer. The CMI was assayed by footpad reaction method in all rats. The increase in the paw volume induced by an injection of sheep RBC (0.025 x 10^9 cells), in the subplantar region of right hind paw, was assessed after 48 h. The mean percent increase in paw volume was considered as DTH reaction and represented as an index of CMI. The volume of the left hind paw, injected similarly with PBS served as control

Antioxidant enzyme activity in haemolysate

The blood was collected from all rats on 83rdd in clean heparinized vacutainers, was centrifuged at 2000 rpm for 15 minutes at 4^{o}C to separate buffy coat and erythrocyte pellet. The erythrocytes were washed thrice with phosphate buffer saline (PH 7.4). The packed RBC obtained was mixed with an equal volume of phosphate buffer saline and then diluted as per requirement with distilled water. The antioxidant enzymes viz., RBC catalase, glutathione peroxidase (GPx) and glutathione reductase (GSH-Rx) in haemolysate were estimated as per the procedures of Bergmeyer (1983), Paglia and Valantine (1967) and Horn and Burns (1978), respectively and the enzyme activity was expressed as units per g Hb, µM/mg protein and µM/mg protein, respectively. The Hb and protein concentration in haemolysate was estimated colorimetrically as per the procedure described by Cannan (1958) and Lowry *et al.,* (1951).

Oxidative stress markers in liver

After 12 weeks of experiment, all rats were sacrificed. Immediately after slaughter, liver was removed and perfused with normal saline (0.9%) to reduce red blood cell contamination. The samples were then fixed in liquid nitrogen and stored at -20^{o}C for

analysis. The estimation of oxidative stress markers i.e., thiobarbituric acid reacting substances (TBARS) and protein carbonyls were estimated as per the procedures of Balasubramanian *et al.,* (1988) and Levine *et al.,* (1990) respectively and was expressed as nmole per mg protein and nmole per mg protein, respectively. The reduced glutathione (GSH) was estimated by method described by Moron *et al.,* (1979).

Ovarian histology

At end of experiment all rats were sacrificed and ovaries were collected and fixed in buffered formalin. The fixed tissues were embedded in paraffin (58.6^{o}C) to make paraffin blocks and were cut by a rotator microtome (5µ). The sections were stained by eosine and haematoxylin and examined under a compound microscope. During microscopic examination, diameters and morphologies of the follicles were used to classify the follicles. The quantification study of folliculogenesis was performed according to Patil *et al.,* (1988).

Statistical analysis

The results obtained were statistically analyzed by using one way ANOVA according to the method of Snedecor and Cochran (1980). The means were compared by Duncan's multiple range test (1955).

Results and Discussion

Effect of zinc on body weight and feed intake

No significant effect on weekly body weight changes was observed in rats fed Zn from various organic sources compared to Zn carbonate (inorganic source) throughout the feeding trial o (Table 2). Similarly, throughout the experiment the average daily feed intake (FI) in rats was statistically comparable among the dietary treatments, except on4thand 12th weeks in which ZnAA and Zn prop supplemented rats showed lower ($P<0.05$) FI compared to control group (Table 3). Our results are in agreement with the findings of several researchers (Sridhar *et al.,* 2014; Nagalakshmi *et al.,* 2015a; 2016a,b) who observed no significant effect of source level of Zn supplementation on growth and feed intake of animals.

Haematological and serum biochemical constituents

Serum biochemical constituents in the present study were not affected by source of zinc in the diet (Table 4). The serum concentration of glucose, cholesterol, total protein, albumin, globulin and alkaline phosphatase activity was comparable between organic and inorganic sources. In corroborating with our findings, earlier researchers indicated no change in serum biochemical parameters in guinea pigs (Shinde *et al.,* 2006) and rats (Nagalaskhmi *et al.,* 2015a) fed diets were supplemented with organic Zn.

Similarly, haematological constituents analyzedin preset study was also not affected by source of zinc in diet (Table 5). All the parameters were comparable among various groups and were within the normal physiological limits (Hrapkiewicz and Medina, 2007). Scanty data available to compare these parameters, however in our previous studies, observed no significant difference in broiler chicken hematological parameters though reduced Zn supplementation by 25%of requirement using Zn glycinate (Sridhar *et al.,* 2015b). Similarly Nagalakshmi *et al.,* (2015a) observed no significant difference in RBC, Hb concentration, haematocrit, MCV, MCH, lymphocyte, monocyte and granulocyte values among rats supplemented with 12 ppm Zn either from organic or inorganic source.

Oxidative stress markers and antioxidant enzyme activity

The RBC catalase activity was higher (P<0.01) with Zn methionine compared to $ZnCO_3$ and other organic sources supplementation. The glutathione reductase activity was higher (P<0.05) with supplementation of Zn from organic source (except Zn propionate) compared to inorganic source (Table 6). Similarly, the reduced glutathione concentration in liver was higher (P<0.05) in rats fed organic sources of Zn compared to inorganic Zn. However, the TBARS and protein carbonyls concentration in liver were comparable between inorganic and various organic sources (Table 6). Overall trend of antioxidant parameters in current experiment, indicating that the supplementation of Zn from organic source improved the antioxidant status of rats, though they were reared in environmentally controlled conditions (environmental stress free condition). Moreover better antioxidant status was observed with Zn-methionine supplementation compared to other Zn sources. Similarly, Nagalakshmi *et al.,* (2015a &2016a) observed better antioxidant activity in rats supplemented with Zn methionine compared to Zn carbonate. Furthermore, Sridhar *et al.,* (2016) observed improved antioxidant status of broiler chicken though reducing the Zn supplementation using organic Zn (Zn glycinate).

Immune response

The relationship between dietary Zn and the immune response has received much attention. The results of this study indicated this relationship is a crucial even among Zn sources. Zinc is an essential cofactor for thymulin, a thymic hormone that promote improved immune response through maturation of T-Cells and activation B-Cells by T-helper cells (Fraker *et al.,* 1986). As noted in table 7 the primary immune response was not affected by source of Zn in the diet. While the secondary immune response was higher (P<0.05) when fed organic sources of zinc compared to inorganic source. However, no difference in antibody titers was observed among various organic sources of Zn tested. Similarly Zn sources had no much effect on primary immune response against sheep RBC, while secondary response found to be stronger in broiler birds fed on ZnMet containing diets (Moghaddam and Jahanian, 2009). Further, several earlier reports indicated improved humoral immune response with ZnMet (Moghaddam and Jahanian, 2009), ZnAA (Hudson *et al.,* 2004), Zn propionate (Nagalakshmi *et al.,* 2016b) and Zn proteinate (Mandal *et al.,* 2011) supplementation. Although there is conflicting data regarding relative bioavailability among organic versus inorganic Zn sources, the present study indicated organic sources supplemented had considerable relative efficacy in promoting immune response. The CMI response expressed as skin delayed type hypersensitivity (DTH) reaction was higher (P<0.05) in rats fed Zn propionate as Zn source (Table 7). While, no effect of feeding other Zn sources was observed on CMI and it was comparable to inorganic source. Similarly, Nagalakshmi *et al.,* (2016b) reported improved DTH response against PHA-P in buffalo heifers fed diets containing Zn propionate even at lower concentration (75% of inorganic).

Ovarian folliculogenesis and progesterone concentration

Zinc has important role in female reproduction by influencing estrous cycle, gonadotropic hormones and ovarian folliculogenesis (Nagalakshmi *et al.,* 2013b). Zinc has beneficial role in improving follicular number and regulate the survival

and maturation of follicles at any stage of their development (Nagalakshmi *et al.*, 2013b). The serum progesterone concentration in the present study was higher (P<0.05) in rats fed organic source of zinc compared to inorganic zinc and it was comparable among rats fed on various organic sources of zinc (Fig. 1). The data on ovarian folliculogenesis in the present study indicated that organic Zn had no effect on primary, secondary and tertiary follicle number while, graafian follicles and corpus luteum numbers were higher (P<0.05) in rats fed organic sources of zinc compared to inorganic source (Table 8).

Table.1 Ingredient composition of purified diet (AIN-76A)

Ingredient	Proportion, g/kg diet
Sucrose	500.0
Casein	200.0
Corn starch	150.0
Oil	50.0
Cellulose	50.0
Mineral mixture*	35.0
Vitamin mixture*	10.0
DL-methionine	3.0
Choline chloride	2.0

*Mineral and vitamin mixture was prepared as per specifications for AIN-76A

Table.2 Body weight changes (g) in rats fed various organic sources of zinc

Week	Zn source					SEM	P value
	Zn met	Zn aa	Zn prot	Zn prop	$ZnCO_3$		
Start	275.0	275.1	275.0	275.0	275.0	2.039	0.999
1	276.7	275.7	277.4	275.6	275.5	2.132	0.998
2	277.8	276.5	278.4	275.9	275.7	2.019	0.993
3	277.6	277.8	281.5	276.3	275.9	2.030	0.921
4	280.6	279.2	281.9	277.2	276.0	1.956	0.883
5	282.2	282.3	282.9	277.7	276.1	1.840	0.700
6	282.3	282.4	283.7	278.6	277.6	1.867	0.822
7	283.9	284.6	286.5	280.1	279.0	1.986	0.741
8	270.4	271.8	269.4	268.5	269.7	2.260	0.994
9	269.9	265.4	264.2	262.9	265.8	2.406	0.992
10	274.6	269.9	273.4	263.2	266.7	2.244	0.532
11	279.8	274.1	284.6	271.5	276.0	2.228	0.384
12	288.3	280.8	287.8	281.3	281.4	2.317	0.725

SEM: Standard errors of mean.

Zn met: Zn methionine; Zn aa: Zn amino acid complex; Zn prot: Zn proteinate; Zn prop: Zn propionate

Table.3 Daily feed intake (g) in rats fed various organic sources of zinc

Week	Zn source					SEM	P value
	Zn met	Zn aa	Zn prot	Zn prop	$ZnCO_3$		
1	15.12	13.70	14.93	15.11	16.92	0.411	0.179
2	16.58	15.15	17.83	16.84	18.05	0.497	0.381
3	14.81	13.60	14.39	13.64	15.91	0.400	0.351
4	17.21^{ab}	14.57^{b}	15.38^{ab}	14.65^{b}	17.96^{a}	0.456	0.043
5	15.30	15.67	14.58	17.23	16.62	0.318	0.054
6	16.06	16.32	17.36	16.02	17.06	0.325	0.616
7	14.81	14.67	14.02	13.49	15.65	0.399	0.519
8	12.60	11.87	12.08	11.44	13.89	0.286	0.054
9	13.40	11.69	12.33	11.44	11.62	0.307	0.244
10	14.08	15.33	15.49	14.94	15.02	0.382	0.826
11	17.73	15.49	16.90	16.45	18.17	0.361	0.136
12	18.89^{ab}	17.14^{bc}	19.92^{a}	16.13^{c}	18.81^{ab}	0.436	0.032

^{ab}Means with different superscripts in a row differ significantly: P<0.05; SEM: Standard errors of mean.
Zn met: Zn methionine; Zn aa: Zn amino acid complex; Zn prot: Zn proteinate; Zn prop: Zn propionate.

Table.4 Biochemical constituents in rats fed various organic sources of zinc

Attribute	Zn source					SEM	P value
	Zn met	Zn aa	Zn prot	Zn prop	$ZnCO_3$		
Glucose, mg/dl	152.9	149.0	149.8	140.2	164.9	7.257	0.893
Cholesterol, mg/dl	117.6	130.0	144.6	119.0	128.9	5.309	0.530
Alkaline phosphatase, IU/L	475.0	465.8	468.1	488.7	462.9	25.19	0.998
Total protein, g/dl	9.47	10.68	9.34	11.38	9.20	0.535	0.656
Albumin, g/dl	3.12	3.12	3.38	3.02	3.23	0.081	0.706
Globulin, g/dl	6.35	7.56	5.96	8.36	5.97	0.571	0.616
Albumin globulin ratio	0.518	0.497	0.664	0.494	0.664	0.0542	0.739

SEM: Standard errors of mean.
Zn met: Zn methionine; Zn aa: Zn amino acid complex; Zn prot: Zn proteinate; Zn prop: Zn propionate.

Table.5 Haematological constituents in rats fed various organic sources of zinc

Attribute	Zn source					SEM	P value
	Zn Met	Zn AA	Zn prot	Zn prop	$ZnCO_3$		
Haemoglobulin (%)	17.42	16.76	17.66	17.08	17.96	0.193	0.320
RBC ($x10^6$/cumm)	10.66	10.65	11.18	10.68	11.40	0.156	0.420
WBC ($x10^3$/cumm)	5.20	6.79	6.63	5.82	6.13	0.282	0.403
HCT (%)	63.44	63.08	66.56	64.64	67.10	0.981	0.634
MCV (%)	59.40	59.6	59.0	60.40	58.40	0.349	0.538
MCH (%)	16.30	15.78	15.72	16.16	15.72	0.099	0.183
MCHC (%)	27.44	26.66	26.50	26.42	26.92	0.178	0.388
Lymphocytes (%)	52.60	50.36	54.92	56.20	50.64	2.962	0.969
Monocytes (%)	7.32	5.86	7.48	6.94	7.26	0.810	0.977
Granulocytes (%)	40.14	43.80	37.60	36.84	42.12	3.061	0.955

SEM: Standard errors of mean.
Zn met: Zn methionine; Zn aa: Zn amino acid complex; Zn prot: Zn proteinate; Zn prop: Zn propionate.

Table.6 Oxidative enzyme activities in haemolysate and liver of rats fed various organicsources of zinc

Attribute	Zn source					SEM	P value
	Zn Met	Zn AA	Zn prot	Zn prop	$ZnCO_3$		
Haemolysate							
RBC Catalase (Umole/min/Hb)	17.30[a]	6.27[b]	6.22[b]	6.77[b]	5.48[b]	1.171	0.001
Glutathione reductase (uM/mg protein)	9.59[ab]	12.34[a]	12.33[a]	4.83[b]	5.19[b]	1.094	0.044
Livers							
TBARS (nM MDA/mg protein)	0.026	0.027	0.024	0.024	0.029	0.0025	0.962
Protein carbonyls (nM/mg protein)	1.18	1.13	1.03	1.13	1.07	0.211	0.475
Reduced glutathione (uM/mg protein)	40.36[b]	43.76[b]	43.22[b]	62.83[a]	26.71[c]	2.601	0.001

[abc]Means with different superscripts in a row differ significantly: P<0.01; P<0.05; SEM: Standard errors of mean.
Zn met: Zn methionine; Zn aa: Zn amino acid complex; Zn prot: Zn proteinate; Zn prop: Zn propionate.

Table.7 Humoral and cell mediated immune response in rats fed various organic sources of zinc

Attribute	Zn source					SEM	P value
	Zn Met	Zn AA	Zn prot	Zn prop	$ZnCO_3$		
HA titres (log_2)							
Primary response	5.00	5.17	4.83	5.00	5.00	0.179	0.989
Secondary response	7.00[a]	6.83[a]	6.00[ab]	6.67[a]	5.50[b]	0.170	0.015
Cell mediated immune response (% increase in paw volume)							
48h	8.69[b]	8.06[b]	8.20[b]	10.17[a]	8.65[b]	0.239	0.037

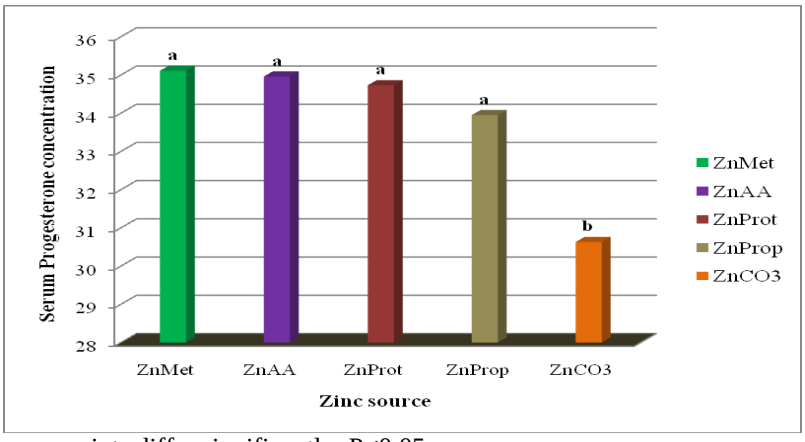
[abc]Means with different superscripts in a row differ significantly: P<0.01; P<0.05;SEM: Standard errors of mean.
Zn met: Zn methionine; Zn aa: Zn amino acid complex; Zn prot: Zn proteinate; Zn prop: Zn propionate.

Table.8 Number of different follicles (%) in ovary of rats fed diets supplemented with various organic sources of zinc

Attribute	Zn source					SEM	P value
	Zn Met	ZnAA	Zn prot	Zn prop	$ZnCO_3$		
Primary follicles	66.81	66.82	68.35	63.12	76.95	2.038	0.281
Secondary follicles	4.38	7.99	4.92	6.08	9.54	1.018	0.478
Tertiary follicles	3.75	3.55	2.62	1.47	1.61	0.516	0.513
Graafian follicle	8.85[a]	8.36[a]	7.96[a]	7.46[a]	2.47[b]	0.762	0.047
Corpus luteum	16.22[ab]	13.27[b]	16.14[ab]	21.13[a]	9.43[b]	1.679	0.021

[ab]Means with different superscripts in a row differ significantly: P<0.05; SEM: Standard errors of mean.
Zn met: Zn methionine; Zn aa: Zn amino acid complex; Zn prot: Zn proteinate; Zn prop: Zn propionate.

Fig.1 Progesterone concentration (ng/ml) in serum of rats fed diets supplemented with various organic sources of zinc



[ab]Bars with different superscripts differ significantly: P<0.05
Zn Met: Zn methionine; Zn AA: Zn amino acid complex; Zn Prot: Zn Proteinate; Zn prop: Zn propionate.

Similarly, supplementation of Zn as ZnO in Baladi ewes at 100 and 150 ppm increased the large follicles and ovulation rate subsequently improved the reproductive performance by decreasing the numbers of days to oestrus, increasing the incidence of oestrus, pregnancy, lambing rates compared to 50 ppm supplemented ewes (Monem and El-Shahat, 2011). Further, earlier reports on chelated Zn supplementation in heifers exhibited higher number of mature follicles and corpus lutea (Manspeaker *et al.*, 1987). Thus it indicated that higher concentration or more bioavailable organic Zn sources had beneficial effect on maturation of preantral (primary, secondary and tertiary) follicles into subsequent stages such as antral (graafian) follicles followed by ovulation to form corpus lutea. Furthermore, Nagalakshmi *et al.*, (2015b) reported that Zn concentration in diets could be reduced by 75% when supplemented as Zn nicotinate (organic Zn) without affecting the oestrus cycle and follicular population. In addition they observed increased follicular population in rats with 100% substitution of inorganic Zn with organic Zn.

In conclusion, the present study indicated that rats fed diets containing various organic sources of Zn had higher antioxidant enzyme activities, immune response and serum progesterone concentration with higher number of mature follicles in ovaries compared to inorganic. Though no differences among various organic Zn sources was observed on these attributes, the cell mediated immunity and number of follicles converting to corpus luteum in ovaries was comparatively higher with Zn propionate.

Competing interests

The authors declare that they have no competing interests.

References

Balasubramanian, K.A., Manohar, M. and Mathan, V.I. 1988. An unidentified inhibitor of lipid peroxidation in intestinal mucosa. *Biochimicaet. Biophysica Acta,* 962: 51-58.

Bergmeyer, H.U. 1983. Methods of enzymatic analysis. In: Catalase (Weinheim, VerlagChemie), 2: 165-166.

Cannan, R.K. 1958. Laboratory Methods, Proposal for a certified standard for use in hemoglobinometry second and final report. *J. Lab. Clin. Med.*, 52(3): 471-476.

Cooper, G. R. and Mc Daniel, V. 1970. Assay methods. In: Mc Donald R.P. (Ed.) Standard methods for clinical chemistry. John Wiley and Sons, New York. 159-170.

Duncan, D.B. 1955. Multiple 'F' test. *Biometrics*, 1: 142.

Fraker, P.J., Gershwin, M.E., Good, R.A. and Prasad, A.S. 1986. Interrelationships between zinc and immune function. *FASEB. J.*, 45: 1474-1479.

Gustafson, E.J. 1976. Improved specificity of serum albumin determination and estimation of acute phase of reactants by use of the bromocresol green. *Clin. Chem.*, 22: 616-622.

Hafeez, B., Khanif, Y. M. and Saleem, M. 2013. Role of zinc in plant nutrition-a review. *American J. Experimental Agri.*, 3(2): 374.

Hirst, G.K. 1942. The quantitative determination of influenza virus and antibodies by means of red cell agglutination. *J. Exper. Med.*, 75: 49–64.

Horn, H.D. and Burns, F.H. 1978. Methods of Enzymatic Analysis. In: Bergmeyer H V, editor. Academic Press, New York, p. 875.

Hrapkiewicz, K. and Medina, L. 2007. Clinical laboratory animal medicine - an introduction.3rd Ed. Blackwell publishers, USA.

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 wk of age. *J. Appl. Poult. Res.*, 13: 349–359.

Kind, P.R. and King, E.J. 1954. Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrine. *J. Clin. Path.*, 7: 322-326.

Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S. and Stadtman, E.R. 1990. Determination of carbonyl content in oxdatively modified proteins. *Methods in Enzymol.*, 186: 464-478.

Lowry, O.H., Rosenberg, N.J., Farr, A.L. and Randal, R.J. 1951. Protein measurement with the Folin-Phenol reagent. *J. Biol. Chem.*, 193: 265-275.

Mandal, G.P., Roy Amitava., Samanta, I. and Biswas, P. 2011. Influence of dietary zinc and its sources on growth, body zinc deposition and immunity in broiler chicks. *Indian J. Anim. Nutr.*, 28(4): 432- 436.

Manspeaker, J.E., Robl, M., Edwards, G.H. and Douglass, W.G. 1987. Chelated minerals: their role in bovine fertility. *Vet. Med.*, 82: 951–956.

Moghaddam, H.N. and Jahanian, R. 2009. Immunological responses of broiler chicks can be modulated by dietary supplementation of zinc-methionine in place of inorganic zinc sources. *Asian-Aust. J. Anim. Sci.*, 22(3): 396-403.

Monem, A.U.M. and El-Shahat, K.H. 2011.Effect of different dietary levels of inorganic zinc oxide on ovarian activities, reproductive performance of Egyptian Baladi ewes and growth of their lambs. *Bulg. J. Vet. Med.*, 14(2): 116−123.

Moron, M.J., Diperre, J.W., Mannerv, K.B. 1979.Levels of glutathione, Glutathione reductase and Glutathione-s-transferase activities in rat lungs and liver. *Biochimicaet Biophysica Acta.*, 582: 67-71.

Nagalakshmi, D., Dhanalakshmi, K. and Himabindu, D. 2009.Effect of dose and source of supplemental zinc on immune response and oxidative enzymes in lambs. *Vet. Res. Commun.*, 33(7): 631-644.

Nagalakshmi, D., ParashuRamulu, S. and Usha Rani, M. 2012.Effect of graded levels of zinc supplementation on growth performance and oxidative defense mechanism in rats. *IOSR J. Pharm.*, 2(6): 36-41.

Nagalakshmi, D., Parashuramulu, S., Usha Rani, M., Kalakumar, B., Shivakumar, P. and Reddy A Gopala. 2013a. Effect of zinc supplementation on immune response, haematological and serum biochemical constituents in rats. Inventi Rapid: *Biomed. Analysis*, 1: 1-4.

Nagalakshmi, D., SadasivaRao, K., Aruna, G. and Parashuramulu, S. 2013b. Influence of dietary zinc supplementation on ovarian folliculogenesis in rats. *Int. J. Recent Scientific Res.*, 4(4): 1-3.

Nagalakshmi, D., Sridhar, K. andParashuramulu,

S. 2015a. Replacement of inorganic zinc with lower levels of organic zinc (zinc nicotinate) on performance, hematological and serum biochemical constituents, antioxidants status, and immune responses in rats. *Vet. World, 8*(9): 1156-1162.

Nagalakshmi, D., Rao, K.S., Aruna, G. and Sridhar, K. 2015b. Effect of Replacing Inorganic Zinc with Lower Levels of Organic Zinc on Zinc Retention and Follicular Population in Rats. *J. Anim. Res.*, 5(4): 743-748.

Nagalakshmi, D., Sridhar, K., Swain, P. S. and Reddy, A. G. 2016a. Effect of substituting increasing levels of organic Zn for inorganic Zn on performance, hematological and serum biochemical constituents, antioxidant status and immune response in rat. *Iranian J. Vet. Res.*, 17(2): 111-117.

Nagalakshmi, D., Rao, K. S., Kumari, G. A., Sridhar, K., &Satyanarayana, M. 2016 b. Comparative evaluation of organic zinc supplementation as proteinate with inorganic zinc in buffalo heifers on health and immunity. *The Indian J. Anim. Sci.*, 86(3): 322-328.

NRC. 1995. Nutrient requirements of laboratory animals. 4th revised edition, National Academy of Science, Washington, DC.

Nitrayova, S., Windisch, E., Von Heimendahl, A., Müller.andBartelt, J. 2012.Bioavailability of zinc from different sources in pigs. *J. Anim. Sci.*, 90: 185-187.

Paglia, D.E. and Valentine, W.N. 1967.Studies on the quantitative and qualitative of erythrocyte glutathion peroxidase. *J. Lab. Clin. Med.*, 70: 158-168.

Patil, S.R., Ravindra Patil, S.R., Londonkar, R. and Patil, S.B. 1988. Nicotine induced ovarian and uterine changes in albino mice. *Indian J. Physiol. Pharamacol.*, 42: 503-508.

Reinhold, J.G. 1953. Standard methods of clinical chemistry C, (Ed.) Rynner, M, New York, Academic Press.p. 88.

Shinde, P., Dass, R.S., Garg, A.K., Chaturvedi, V.K. and Kumar, R. 2006.Effect of zinc supplementation from different sources on growth, nutrient digestibility, blood metabolic profile and immune response of male guinea pigs. *Biol. Trace Elem. Res.*, 112(3): 247-262.

Snedecor, G. W. and Conchran, W. G. 1980.Statistical method 7th Ed. Iowa State Univ Press. Ames. Iowa, USA.

Sridhar, K., Nagalakshmi, D., Rao, D. S. andRao, S. V. 2014. Effect of dietary addition of organic zinc on performance and carcass traits in commercial broiler chicken. *Indian J. Poult. Sci.*, 49(2): 155-158.

Sridhar, K., Nagalakshmi, D., SrinivasaRao, D. and Rama Rao, S.V. 2015a. Effect of supplementation of graded levels of organic zinc on nutrient utilization and retention of minerals in broiler chicken. *Indian J. Anim. Nutr.*, *3*2(1): 80-85.

Sridhar, K., Nagalakshmi, D and Rama Rao, S.V. 2015b. Effect of graded concentration of organic zinc (Zn-glycinate) on skin quality, haematological and serum biochemical constituents in broiler chicks. *Indian J. Anim. Sci.*, 85(6): 643-648.

Sridhar, K., Nagalakshmi, D., SrinivasaRao, D. and Rama Rao, S.V. 2016.Effect of graded concentration of organic zinc (zinc glycinate) on antioxidants status and immune response in commercial broilers. *Indian J. Anim. Res.*, 50(4): 471-475

Wybenga, D.R. and Pileggi, V.J. 1970.Estimation of cholesterol. *Clin. Chem.*, 16: 980-981.

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