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Evaluation of Ayurvedic Zinc (Yashada Bhasma) in Commercial Broilers

D.Padmaja[1], A.Ravi[*], B. Devasena[2], Ch.Sri Durga[3] and J .Suresh[4]

Department of Animal Nutrition, College of Veterinary Science, Tirupati

Sri Venkateswara Veterinary University, Tirupati

[1] Veterinary Assistant Surgeon AH Department, AP

[2] Professor & HOD, Department of Animal Nutrition, CVSc., Tirupati

[3] Professor & HOD , Department of Rasa Shastra and Bhaishajya Kalpana, S.V. Ayurvedic College, TTD, Tirupati, Andhra Pradesh, India

[4] Dean of Dairy Science (Retd), SVVU, Tirupati.

*Correspondence:raviakst@gmail.com

ABSTRACT

The objective of the study was to evaluate Ayurvedic Zinc [Yashada bhasma (YB)], a nano zinc oxide particle prepared by the Ayurvedic *bhasmikarana* process on growth, zinc retention and bioavailablity in commercial broiler chicken. The YB was prepared and characterized by traditional, physical, chemical methods and also by TEM, EDAX and DLS. In a growth trial, 360 commercial day old VENCOBB-400 broiler chicks were divided at random into four treatment groups (T1 to T4) such that each treatment contained six replicates with 15 birds per replicate. Basal diets for pre-starter (1-14 d), starter (15-21d) and finisher (22-42d) phases were formulated (ICAR, 2013) and they contained 33-34 mg/kg of Zn as against the recommended level of 80 mg/kg. The experimental diets were constituted by including $ZnSo_4$ (T1) or YB (T2,T3,T4) such that the diets contained 80,40,60 and 80 mg/kg, Zn in T1,T2,T3 and T4, respectively for feeding birds during prestarter, starter and finisher phases. The experiment was carried out for a period of 42 days to study the growth performance, the Zn, Ca, P, Mn and Fe concentration in serum, zinc retention by total excreta collection method and relative zinc bioavailability by slope ratio technique. The particle size of YB was 11.2 nm with a zeta potential of -36.3 mv and its elemental composition (g/kg) by EDAX analysis was Zn, 905, Oxygen, 28.3, Fe, 26.2 , Ca, 29.8 and P, 10.6. The TEM and SAED images revealed its crystalline nature and its zinc content was 975 g/kg DM when estimated by AAS. It contained 996 g total ash, 4.2 g AIA and 16.6 g water soluble ash per kg. The body weight gain, feed intake and feed conversion ratio were not significantly different among treatments. The relative Zn bioavailability was 130, 124 and 119.5 % in birds fed on T2, T3 and T4, respectively whereas the assumed bio-availability of Zn from ZnSo4 in T1was 100**%.** The results of the study suggested that *Yashada bhasma*, had the characteristics of nano zinc oxide and its inclusion at 2.5, 2.33 and 2.83 g/100 kg feed during prestarter, starter and finisher phase to increase the dietary zinc content to 60 mg/kg was beneficial to promote optimum growth and reduce environmental pollution of zinc in the excreta.

KEYWORDS: Commercial Broilers, Growth, Yashada Bhasma, Zinc Relative Bioavailablity

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INTRODUCTION

Several Zn sources (zinc acetate, zinc oxide and zinc sulphate), organic (including amino acid zinc chelates of glycine, lysine or methionine, and their sulphate and hydrate analogues) and nano Zn particles are used to enhance growth, improve feed efficiency, mitigate oxidative stress in poultry (Lina et al., 2009; Akash Mishra et al., 2014 and Naz et al., 2016). The bioavailability of inorganic zinc is low in broilers (Sandoval et al., 1997), requiring higher levels of supplementation (Bratz et al., 2013) and excretion resulting in the environmental pollution (Hanne Damgaard Poulsen , 1995; Broom et al., 2003). The nano mineral particles promote growth rate and feed efficiency (Oberdorster et al., 2005). Nano minerals can be synthesized by physical, chemical, and biological methods and for animal feeding a thorough study including toxicological effect is advocated before using these particles in the ration of livestock and poultry on a routine basis (Bahadar et al., 2016). *Bhasmas* are biologically produced nano particles of metal oxides through repeated incineration and during the *bhasmikarana* process zero valiant metal state is converted to higher oxidation state and the toxic nature of the resulting metal oxide is completely destroyed while medicinal properties are introduced and Bhasmas are potent in small quantity and can act quickly (Shebina Rasheed and Murugesh Shivashankar, 2015). *Yashada bhasma* is a zinc-based herbo metallic preparation that changes zinc metal into its oxide form and is being used for treating anaemia, anti-cancer activity, respiratory disorders, night sweating, menorrhagia, wound healing, parkinsonism (Chendran Seeta et al., 2016) diabetes mellitus (Umrani et al., 2013) and no major cytotoxicity was observed. Excessive Zn supplementation in chicken diets may cause environmental pollution due to Zn excretion in the feces, as fecal Zn content linearly increases with Zn dietary levels in broiler chickens and laying hens (Burrell et al., 2004; Kim and Patterson, 2004). Thus, there is a need to adopt new forms of zinc supplements that are highly bioavailable, in order that the wellbeing benefits of high zinc status may be achieved without excessive zinc excretion into the environment (Alkhtib et al., 2020**)**. In the last two decades, there has been a growing interest in the production of ZnO nano particles via biological methods. The fact that this novel technique avoids using hazardous chemicals or requiring lot of energy through physical processes makes it more practical, environmentally benign and economically viable (Khalid et al., 2017). The biogenic or green production of metal oxide nano particles has been explored by many research groups to replace the chemical and physical methods (Jha et al., 2023)

In light of the above developments, the present research work was planned to study the effect of ayurvedic zinc (Yashada bhasma) on the growth performance, its retention and bioavailability in broilers.

MATERIALS AND METHODS

The present study was carried out at the Department of Animal Nutrition and Department of Poultry Science, College of Veterinary Science, Tirupati. The *Yashada bhasma* was prepared from its ore in the Department of Rasa Sastra and Bhaishajya Kalpana, TTD S.V. Ayurvedic College, Tirupati, Andhra Pradesh

Preparation of Yashada bhasma

Raw zinc was subjected to *Samanya sodhana* (a general purification method for all metals), *Vishesha sodhana* (specific purification method for Zinc), *Jarana* (roasting) and *Marana* (incineration) to make it harmless, edible and a healthy zinc supplement (Goud and Sridurga, 2019). The raw zinc was melted in an iron pan and immediately poured into sufficient quantity (more than the volume of melted raw zinc) of sesame oil. The oil was taken in a stainless steel vessel covered with wooden plate with hole in its center through which liquids were poured. After cooling it was again heated and quenched. This procedure was repeated six more times. Each time the oil was changed. Likewise, the procedure was repeated with butter milk, cow urine, fermented rice gruel and decoction of horse gram (Kulkarni, 2010). The *samanya shodhita Yashada* was melted in an iron pan and quenched in sufficient quantity of lime water. The process was repeated six times. Each time the lime water was changed. The *visesha shodhita Yashada* was placed in an iron pan and heated to melt. After complete melting continuous stirring was done with fresh neem branches until all the *Yashada* turned into powder form. The powder was collected to the centre of the iron pan and subjected to intense heating for 3 hours . After self-cooling the powder was sieved through a cloth, the remains of metal particles on cloth were separated and again subjected to the same procedure. The obtained powder was washed with water for complete removal of alkali content. For washing, the powder was dissolved in water and left undisturbed for 3 hours and the clear supernatant liquid was drained. Water was tested each time for presence of *alkali* with a litmus paper. After complete removal of alkaline content it was dried (Pandit Kasinath Sastry, 1979) . The powder obtained after Jarana was taken in a mortar & pestle, added with sufficient quantity of aloe vera juice and triturated. It was made into small pellets of uniform size and dried well. The dried pellets were kept in an earthen saucer and another earthen saucer was used to cover them and the junction between two saucers was sealed with a double folded Multani mitti (Fuller's Earth) smeared cloth, dried and incinerated in a muffle (700^{0}C for 2 h) twice and one time through *gajaputa* i.e. the heating was done in a cubical pit of 2 ft using cow dung cakes (Trikamji Acharya, 1951).

Characterization of Yashada bhasma

The *yashada bhasma* was characterized by traditional methods of *Rekhapurnatva* and *Varitarq* , physical tests (color, odor, texture, taste, pH) and physical constants i.e. total ash, acid insoluble ash and water soluble ash (Arun Rasheed et al., 2011, Pareek and Bhatnagar, 2020). Chemical characterization (Williams and Carter, 1996) was done by using transmission electron microscopy (HRTEM, JEOL 3010, Jeol Ltd, Peabody, MA, USA), and energy dispersive analysis (Arun Rasheed et al., 2011) of X-rays (FEI Quanta 200). The particle size and zeta potential was tested by Horiba nanopartica SZ 100, using dynamic light scattering (DLS). The cost per gram of *Bashada bhasma* was calculated as Rs. 4.60.

Basal Diets

The basal diets (Table 1) for pre-starter (0-14 d), starter (15-21 d) and finisher (21-42 d) phases were formulated to meet ICAR (2013) specifications for broilers. The zinc (mg/kg) of basal diet was about 33-34 mg against the recommended 80 mg/kg for VENCOBB400 broiler birds (Venko Research and Breeding Farm Limited, 2017). The basal diet was supplemented with 12.5, 15.0 and 13.35 g / 100 kg of inorganic zinc source i.e. ZnSO4 to increase the Zn content to 80 mg/kg (T1) or with *YB* at 0.5,1.17, 0.78 g /100 kg to get a Zn content of 40 mg/kg (T2), 2.5, 3.3 and 2.83 g/100 kg to get a Zn content of 60 mg/kg (T3) and 4.5,5.58 and 4.88 g/100 kg to get 80 mg/kg (T4) during pre-starter, starter and finisher phases, respectively

Table 1. Ingredient composition (%) of experimental basal diets

Ingredient	Pre starter	Starter	Finisher
Maize	52.0	53.0	57.5
SBM	40.0	36.6	32.0
DORB	0	3	3
Palm oil	4.0	4.0	4.2
Calcite	1.2	0.74	0.9
DCP	2.0	2.0	1.9
Dl-methionine	0.36	0.25	0.15
L-Lysine	0.19	0.16	0.10
Salt	0.25	0.25	0.25
Total	100	100	100
Cost (Rs per kg)	25.8	24.25	23.30
Calculated values (DM basis)[1]			
CP %	22.2 (22.0)	21.6 (21.5)	19.5 (19.5)
ME (kcal/kg)	3040 (3000)	3040 (3050)	3106 (3100)
Zinc (mg/kg)	34.3 (60)	34.2 (60)	33.0 (60)
Calcium	1.04 (1.0)	0.92 (0.95)	0.89 (0.85)
Available Phosphorus	0.48 (0.45)	0.44 (0.4)	0.45 (0.38)
Lysine, (%) (Min)	1.22 (1.20)	1.12 (1.07)	0.97 (0.94)
Methionine, (%) Min	0.55 (0.52)	0.51 (0.48)	0.45 (0.41)

All diets contained :
Meriplex @ 0.25 g/ kg (each gram provided : Vit.A, 40,000 IU; Vit.D3, 10,000 IU; 3.2 mg Vit.B1, 20 mg Vit.B2, 6.4 mg Vit.B6, 82 mg Vit.B12, 48 mg Niacin, 32 mg Calcium pantothenate, 4 mg Vit.K, 32 mg Vit.E, 3.2 mg Folic acid. Cosmodot @ 0.5 g/ kg (3-5, Dinitro-O-Toluamide 25 % w/w)
[1] Values in parenthesis indicate ICAR, 2013 specifications for broiler diets except for Zn

Growth Performance

360 Commercial day old straight run Vencobb400 broiler chicks (Av. body weight of 50.5 ±5.5 g) were weighed individually, wing banded and distributed at random into four treatment groups (T1, T2, T3 and T4) with six replicates of 15 birds per replicate. The chicks in each replicate were housed in well ventilated, individual pens and reared in deep litter system with rice husk as litter material during growth study of 42 d. All pens were provided with uniform brooding facilities, linear feeders and trough waterers with sufficient feeder and water space per bird. Birds were fed starter (1-14 d), grower (15-21 d) and finisher (22-42 d) mash diets and had *ad libitum* access to feed and water. Weekly body weight of each bird and feed intake of each replicate were recorded and the FCR was calculated based on total feed consumed and total weight gain of each replicate. At the end of finisher phase, six birds per treatment were sacrificed (Sayda Ali et al., 2011) to collect breast and thigh muscles and also serum samples that were stored at -20°C until further analysis. Feathers and nails from each slaughtered bird were also collected for estimation of zinc concentration.

Zinc retention

Six birds per treatment i.e. one from each replicate was randomly selected and placed in separate cages on the last day of finisher phase. Excreta voided was collected from trays placed under each cage by the total fecal collection method for 3 days and stored at –20 °C (Han et al., 2009) and while in cages, the birds were fed the respective finisher diet *ad libitum* with free access to water.

Zn retention was calculated based on total feed DM intake and total excreta DM output of each bird (Zhang et al., 2018) **.**

Relative Zinc Bioavailability

Relative bioavailability was estimated by the slope ratio technique from regression of weight gain on Zn intake with Zinc Sulphate as the standard source at 100% availability (Finney, 1978 ; Littell et al., 1997). Because feed intake differences among treatments could affect zinc intake, regressions were calculated using dietary zinc intake (based on zinc assays of diets) as the independent variable rather than added zinc concentrations (Wedekind et al., 1992; Li et al., 2004). The total body weight gain and the zinc intake from pre starter, starter and finisher diets of each replicate were used for the slope ratio technique.

Methods of Analysis

Standard AOAC (2005) methods were used to analyze the experimental diet samples for DM (945.15), CP (990.03), ether extract (920.39), crude fiber (978.10), ash (942.05), Ca and P (Talapatra et al., 1940). The zinc content of YB, experimental diets, feathers, nails and excreta samples was analyzed using AAS (GBC AAS Avanta PM model). Serum zinc by colorimetric method using kit (Akita Abe and Yiamashita, 1989), Carbonic Anhydrase by a sandwich enzyme immunoassay (Develop Bovine Carbonic Anhydrase1 (CA1) ELISA kit) , Superoxide Dismutase (Marklund and Marklund, 1974) and serum Ca, P, Fe and Mn were estimated using AAS (GBC AAS Avanta PM model).

Statistical Analysis

The data were subjected to one-way analysis of variance (Snedecor and Cochran, 1994) and the differences between means were tested using LSD test and stated as significant when P was less than 0.05. All the statistical procedures were carried out using SPSS, version 23.0.

RESULTS AND DISCUSSION

The physical and chemical characteristics of Yashada bhasma

The *YB*, prepared from raw zinc was reddish orange in color with no taste and odor, fine in texture and floated on stagnant water. Its elemental composition (%) as per the energy dispersive X-ray analysis (EDAX) was Zn, 90.5, oxygen, 2.83, Fe, 2.62, Ca, 2.98 and P, 1.06 (Fig 1). Its internal structure was crystalline in nature as per transmission electron microscope (TEM) imaging (Fig 2). The mean hydrodynamic diameter (size) of the *YB* particles was 11.2 nm (Fig 3) with a zeta potential of -36.3 mV by the dynamic light scattering technique (Fig 4). YB contained 975 g of zinc , 996 g total ash, 4.2 g AIA and 16.6 g water soluble ash per kg DM. In simple terms, nano mineral particles have particle size in the range of 1-100 nm, have large surface area, small sized with uniform shape, potent in small dose and can be synthesized by physical, chemical and biological methods for animal feeding (Swain et al., 2015). *Yashada Bhasma (YB) is* a biologically produced nano Zn particle through repeated purification, frying and calcination processes which change zinc metal into its oxide form (Chendran Seetha et al., 2016, Goud and Sridurga, 2019). The procedures adopted to prepare *YB* as per the ancient ayurveda literature includes samanya *sodhana, jarana* and *marana* (Pareek and Bhatnagar, 2020) . *Bhasmas* are characterized by both classical and modern methods of analysis (Pal et al., 2014). In the present study, the *Yashada bhasma* , after complete preparation had reddish orange color, floated on water and entered furrows of finger when rubbed between thumb and index finger and these characteristics are in agreement with earlier reports (Arun Rasheed et al ., 2011; Pareek and Bhatnagar, 2018). The EDAX analysis of *YB* (Fig. 1) revealed its chemical composition as Zinc, 90.5, oxygen, 2.83, iron, 2.62, calcium, 2.98 and phosphorus, 1.06 g/kg and these values were comparable with earlier reports (Babita and Nilima, 2018) while Santhosh et al. (2013) reported that the XRD spectra of *YB* revealed major concentration with zinc, 95.08 ppm, other elements like Tin (Sn), 0.27 ppm , Lead (Pb), 0.14 ppm and iron, 1.69 ppm. Sharma et al. (2015) reported the chemical composition of *YB* through EDAX analysis as zinc, 61 to 68% and oxygen, 20 to 30% and *49* to 80 nm particle size with XRD test. Babita and Neelima (2018) reported that the transmission electron microscopy (TEM) of *YB* showed crystalline nature of *bhasma* particles. Similar results were obtained in the present study also indicating the polycrystalline nature of the YB.

Particle size and Zeta potential

The particle size and zeta potential of *YB* in the present study were 11.2nm and -36.3 mv. A zeta potential value other than -30 mv to +30 mv is generally considered to have sufficient repulsive force to attain better physical colloidal stability .

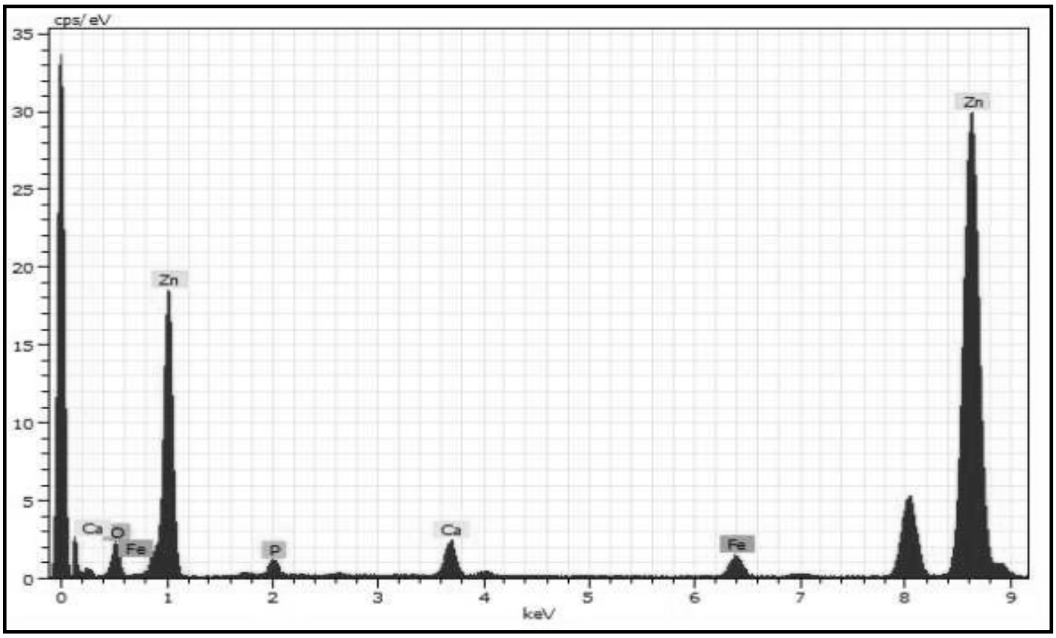

Fig 1 EDAX analysis Y*ashada Bhasma*

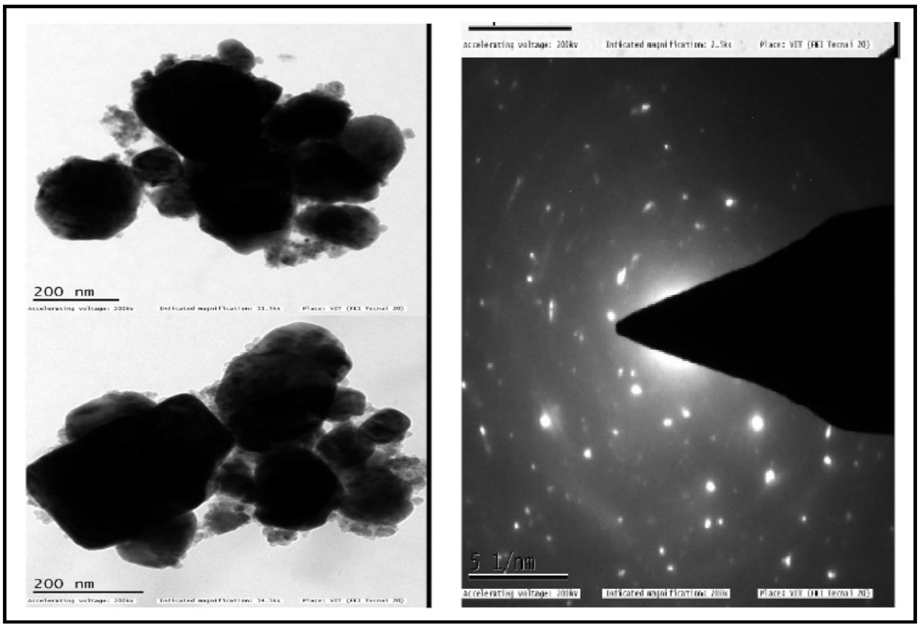

Fig 2.TEM and Selected Area Electron Diffraction (SAED) images of Y*ashada Bhasma*

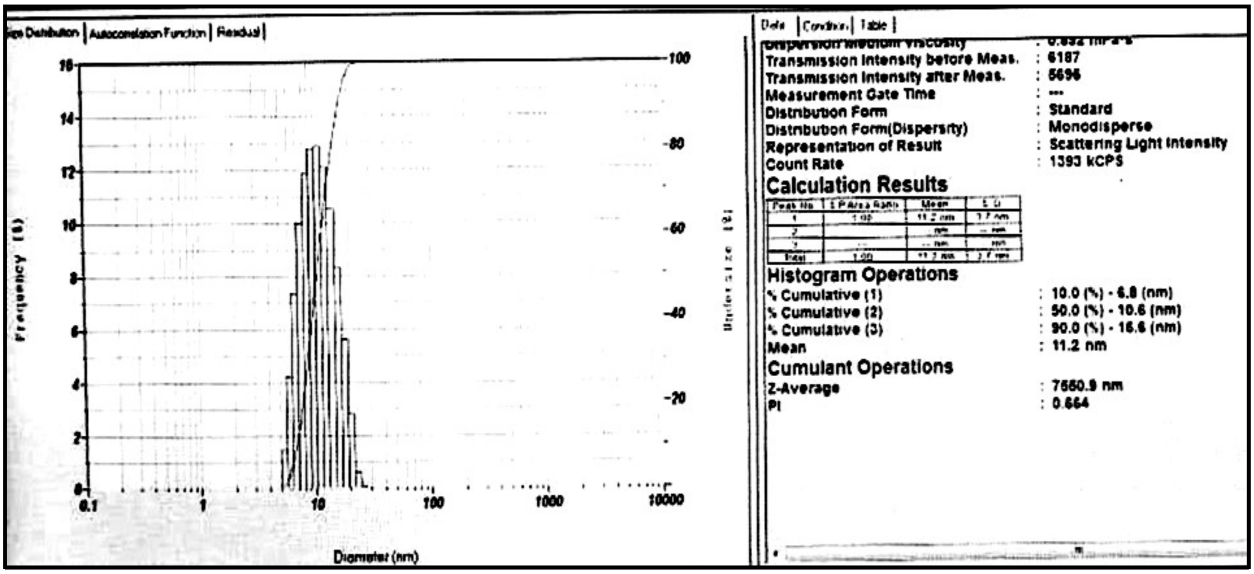

Fig 3 Particle size distribution pattern of Yashada bhasma

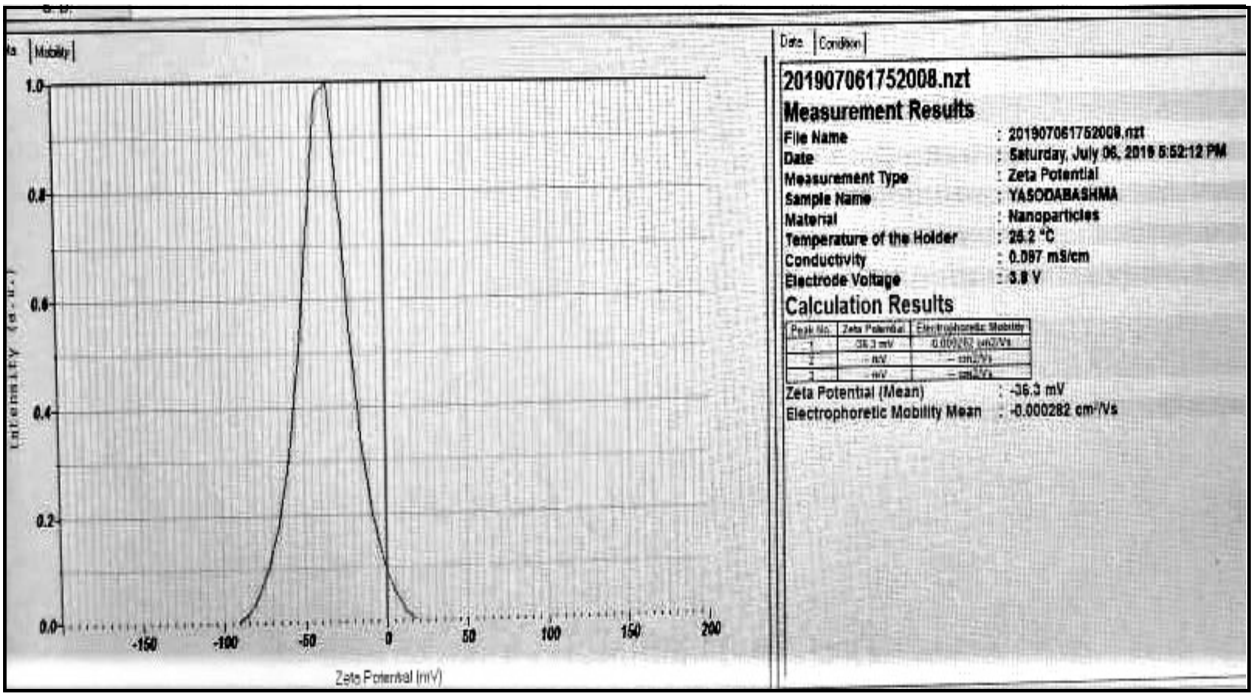

Fig 4 Particle size and micrograph representing zeta potential of *yashada bhasma* particles

Zinc content of experimental diets

The zinc requirement of chicks (mg/kg feed) was defined to be 30 (Roberson and Schaible, 1958), 40 (NRC, 1994), 60 (ICAR, 2013) and 80 (Venko Research and Breeding, 2017). The calculated zinc content of basal diets was 33.0 to 34.0 mg/kg (Table 1) and several previous studies (Bartlett and Smith , 2003, Ao et al., 2006, Huang et al., 2007, Star et al., 2012, and Pacheco et al., 2017) reported that the Zn content of corn-soybean meal basal diets for broilers ranges from 25 to 35 mg/kg, indicating an average Zn content of 30 mg/kg. The Zn content of the basal diet cannot be overlooked when formulating broiler chicken feeds. Hence, in the present study, the basal diets (Table 1) were supplemented with ZnSO4 or *yashada bhasma* at different levels such that the experimental diets T1 to T4 contained 80, 40, 60 and 80 mg/kg Zinc, respectively. However, the analysis of the diets for zinc content by AAS (Table 2) revealed slightly higher Zn content. The analyzed chemical composition (% DM) of pre starter, starter and finisher basal diets is presented in Table 2.

Table 2. Analyzed chemical composition (% DM) of diets

Nutrient	Pre Starter	Starter	Finisher
DM	90.9	88.7	88.7
CP	22.0	21.4	19.4
EE	2.76	2.65	2.85
CF	5.66	6.05	6.12
TA	7.86	8.76	8.91
AIA	1.91	1.97	1.92
NFE	61.2	61.4	61.7
Zinc (ppm)	T1 – 80.6	89.6	80.4
	T2 – 39.5	47.9	44.5
	T3 – 62.3	70.3	66.7
	T4 – 82.4	88.7	80.8
Calcium	1.39	0.85	0.90
Phosphorus	0.72	0.58	0.48
GE (kcal/kg)	4178	4244	4030

Growth performance

Birds were weighed at weekly intervals, weekly feed intake of each replicate was recorded and the FCR was calculated based on total feed consumed and total weight gain of each replicate. The average body weight gain, feed intake and feed conversion ratio during different growth phases and the overall study period were not impacted (P>0.05) by the zinc source or level of zinc (Table 3). Mohanna and Nys (1999), Ao et al. (2006), Batal et al. (2001) and Huang et al. (2007) reported positive response to zinc supplementation to increase dietary Zn content upto 40-50 mg/kg feed beyond which no significant effect was observed.

Table 3. Growth performance of birds fed experimental diets

Phase		T1	T2	T3	T4	SEM	P value
Pre starter	Weight gain (g)	8.03	7.74	7.75	7.59	0.17	0.84
	Feed intake (g)	8.94	8.83	8.12	8.10	0.19	0.25
	FCR (g/g)	1.12	1.14	1.06	1.07	0.01	0.84
	Feed cost/kg gain (Rs)	28.7	29.4	27.3	27.5	0.61	0.12
Starter	Weight gain (g)	8.11	8.03	7.86	8.29	0.18	0.88
	Feed intake (g)	10.32	9.57	9.70	10.20	0.20	0.41
	FCR (g/g)	1.27	1.19	1.23	1.24	0.02	0.52
	Feed cost/kg gain (Rs)	30.9	29.5	31.7	33.0	0.68	0.10
Finisher	Weight gain (g)	15.24	15.10	15.27	14.55	0.44	0.94
	Feed intake (g)	43.00	42.30	42.48	40.25	1.22	0.31
	FCR (g/g)	2.83	2.81	2.79	2.77	0.03	0.08
	Feed cost/kg gain (Rs)	65.8	66.4	67.4	65.5	0.91	0.14
Overall	Weight gain (g)	31.23	30.33	30.79	30.10	0.76	0.96
	Feed intake (g)	62.26	60.69	60.17	58.55	1.48	0.44
	FCR (g/g)	2.00	2.01	1.96	1.92	0.02	0.10
	Feed cost/kg gain (Rs)	47.3	47.5	47.1	47.3	0.42	0.06

Serum minerals, anti-oxidant enzymes, Zinc content of breast and thigh muscle, feathers and nails

The serum zinc (mg/L) was significantly (P<0.05) higher in birds fed T2 to T4 than in T1 and the values were 1.48, 1.58, 1.78 and 1.85 , respectively for T1 to T4 fed birds (Table 4) due to better zinc retention (Table 5) and bio-availability (Table 6) in birds fed YB containing diets. It was also reflected by the higher Zn content of breast and thigh muscles, nails and feathers in birds fed on T3 and T4 (Table 5). Lower (P<0.05) serum calcium and iron (P<0.01) was observed in T4 than in other treatments while the phosphorus (mg/L) and Mn (µg/L) were not significantly different among treatments. Pigs, poultry, sheep and cattle exhibit considerable tolerance to high intake of Zn, the extent of the tolerance depending partly on the species but mainly on the nature of the diet, especially its relative content of Ca, Cu, Fe, and Cd with which Zn interacts in the processes of absorption and utilization (Underwood and Suttle, 1999), which was reflected by the lower Ca, P, Mn and Fe concentration in T4 fed birds in which maximum serum Zn concentration was observed.

Table 4. Serum mineral and enzyme concentration

Treatments	Zinc (mg/L)	Ca (mg/L)	P (mg/L)	Mn (µg/L)	Fe (mg/L)	SOD (Units/ml)	Serum CA1 (ng/ml)
T1	1.48^{b}	106.91^{a}	74.9	45.5	12.8^{a}	24.5	126.83
T2	1.58^{b}	102.49^{a}	72.42	44.8	11.1^{ab}	23.0	125.19
T3	1.78^{a}	111.89^{a}	74.3	44.6	11.8^{ab}	24.6	127.88
T4	1.85^{a}	86.6^{b}	67.1	38.6	7.80^{b}	20.8	128.73
SEM	0.001	12.4	6.11	5.26	1.51	3.25	1.64
P value	0.01	0.04	0.86	0.65	0.01	0.852	0.547

^{ab} Values in a column not sharing common superscripts differ significantly

Zinc balance

The zinc intake (mg/d/bird) was significantly lower (P<0.01) in birds fed on T2, containing 40 mg/ kg diet Zn than in other treatments (Table 5) and the values were 9.86,7.24,9.67 and 10.24 in T1 to T4 fed birds, respectively. The zinc outgo in excreta (mg/d/bird) was lower (P<0.05) in T2 and T3 fed birds than in T1 whereas the zinc retention (mg/d/bird) and as percent of intake was significantly lower (P<0.01) in T1 than in other treatments. Mohanna and Nys (1999) reported that under normal commercial dietary conditions, 94% of the zinc ingested is excreted by broiler chicks. The low percentage of body retention (6%) may result firstly, from the high amount of zinc ingested, and secondly, from the low utilization of this element. Therefore, the excretion of zinc can be reduced by lowering the dietary zinc concentration or by using the sources of zinc with higher availability to achieve the wellbeing benefits of high zinc status without excessive zinc excretion into the environment (Alkhtib et al., 2020)

Table 5 Zinc balance and tissue concentration in broilers fed experimental diets

Treatments	Zn intake (mg/d/bird)	Zn in excreta (mg/d/bird)	Zn retention (mg/d/bird)	Zn retention (%intake)	Breast	Thigh	Feathers	Nails
T1	9.86 ^{a}	6.03^{a}	3.83 ^{a}	38.9^{b}	27.07^{c}	68.1^{c}	35.1^{c}	145.89^{d}
T2	7.24 ^{b}	3.89^{b}	3.35 ^{b}	46.5^{a}	26.1^{c}	65.7^{c}	42.2^{c}	151.26^{c}
T3	9.67 ^{ab}	4.88^{b}	4.79 ^{a}	49.8^{a}	30.2^{a}	74.1^{a}	53.1^{a}	196.3^{a}
T4	10.2 ^{a}	5.02^{ab}	5.22 ^{a}	50.7^{a}	28.2^{b}	71.2^{b}	47.3^{b}	179.0^{b}
SEM	0.42	0.61	0.75	6.16	0.17	0.18	0.14	0.23
P value	0.014	0.048	0.004	0.011	0.04	0.05	0.03	0.05

^{abcd} Values in a column not sharing common superscripts differ significantly

Relative Zinc Bioavailability

The P values indicated that the regression model was significant. The relative bioavailability (RBV) of Zn was higher in birds fed on T2 than in T3 and T4 treatments and the values were 130, 124 and 119.5%, respectively when compared with birds fed on T1 in which the bioavaialbility of Zn from ZnSO4 was assumed to be 100%. The relative bioavailability of zinc from *yashada bhasma* was better than that of 44.1% for zinc sulphate (Wedekind and Baker, 1990), 93.8 % for zinc sulphate and 74.0% for Zn oxide (Sandoval et al., 1997), 101, 107, and 49% for basic Zn sulfate, basic Zn chloride, and Zn oxide, respectively (Cao et al., 2000) and comparable to the values of 116-119 % for Zn propionate (Brooks et al., 2013**),** 111% for zinc-lysine complex (Aoyagi and Baker,1993) but lower than 183% based on the weight gain and 157% based on the total tibia zinc content of broiler chicks for zinc proteinate (AO et al., 2006).

Table 6 Estimated relative zinc bioavailability in broilers[a]

Treatment	Total zinc intake (mg/42 d)	Total weight gain(g/42 day)	Estimate of slope ± SE [b]	RBV(%)	R^2	P value
T1 (ZnSO4)	5102[a]	31238	5.24±1.08	100.0	0.85	0.008
T2	2791[d]	30336	6.81±2.04	130.0	0.73	0.02
T3	4183[c]	30823	6.53±1.75	124.0	0.78	0.02
T4	4826[b]	30103	6.26±1.41	119.5	0.83	0.01
SEM	50.4	398.2				

[a] Data are mean of six replicates of 15 chicks per replicate fed the experimental diets for 42d
[b] Standard curve for weight gain (Y, in g/42 d) regressed on total zinc intake (X, in mg/42 d)
[abcd] Values in a column with different superscripts differ significantly (P<0.01)

CONCLUSION

Yashada bhasma, prepared by the *Ayurvedic bhasmeekarana* process had the characteristics of nano zinc oxide (11.2 nm with a zeta potential of -36.3 mV) and *its inclusion* at 2.5, 2.33 and 2.83 g/100 kg feed during prestarter, starter and finisher phase (T3) to increase the dietary zinc content to 60 mg/kg was beneficial without detrimental effect on growth with higher bioavailabity of Zinc.

REFERENCES

Akash Mishra., Swain, R.K., Mishra,S.K., Panda, N. and Sethy, K. 2014. Growth performance and serum biochemical parameters as affected by nano zinc supplementation in layer chicks. Indian Jornal of Animal Nutrition. 31 (4): 384-388.

Akita Abe and Yiamashita. 1989. Colorimetric method for the estimation of zinc. Clinical Chemistry. 35(4):552–554.

Alkhtib, A., Scholey, D., Carter, N., Cave, G.W.V., Hanafy, B.I., Kempster, S.R.J., Mekapothula, S., Roxborough, E.T. and Burton, E.J., 2020. Bioavailability of methionine coated Zinc nanoparticles as a dietary supplement leads to improved performance and bone strength in broiler chicken production. Animals (Basel). 24;10(9):1482.

Ao, T., Pierce, J. L., Power, R., Dawson, K. A., Pescatore, A. J., Cantor, A. H. and Ford, M. J. 2006. Evaluation of Bioplex Zn as an organic zinc source for chicks. International Journal of Poultry Science*,* 5(9):808-811.

AOAC. 2005. Official Methods of Analysis of the Association of Official Analytical Chemists.18th Edn,Washington, D.C.

Aoyagi, S and Baker, D.H. 1993. Nutritional evaluation of copper-lysine and zinc-lysine complexes for chicks. Poultry Science. 72 (1): 165– 171.

Arun Rasheed., Anvesh Marri and Madhu Naik, M. 2011. Standardization of Bhasma-Importance and Prospects. Journal of Pharmacy Research. 4 (*6*):1931-1933.

Babita Kale and Nilima Rajurkar. 2018. Yashad bhasma: Synthesis and characterization. Pharma Innovation. 7(1):119-122.

Bahadar, H., Maqbool, F., Niaz, K. and Abdollahi, M. 2016. Toxicity of Nanoparticles and an Overview of Current Experimental Models. Iranian Biomedical Journal. 20 (1) : 1–11.

Bartlett, J. R. and Smith, M. O. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poultry Science. 82(10): 1580–1588.

Batal, A.B., Parr,T.M. and Baker, D.H. 2001. Zinc bioavailability in tetra basic zinc chloride and the dietary zinc requirement of young chicks fed a soy concentrate diet. Poultry Science. 80: 87 – 90.

Bratz, K., Golz,G., Riedel, C., Janczyk, P., Nockler, K. and Alter, T. 2013.Inhibitory effect of high-dosage zinc oxide dietary supplementation on *Campylobacter coli* excretion in weaned piglets. J ournal of Appllied Microbiology.115 (5) :1194-1202.

Broom, L., Mille, H., Kerr, K. and Toplis, P.2003. Removal of both zinc oxide and avilamycin from the post-weaning piglet diet: Consequences for performance through to slaughter. Animal Science. 77(1): 79-84.

Brooks, M.A., Grimes, J.L., Lloyd, K.E., Verissimo, S. and Speers, J.W. 2013. Bioavailability in chicks of zinc from zinc propionate. Journal of Applied. Poultry Research. 22:153–159.

Burrell, A.L., Dozier, W.A., Davis, A.J., Compton, M.M., Freeman, M.E., Vendrell, P.F. and Ward, T.L. 2004. Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. British Poultry Science. 45:225-263.

Cao, J., Henry, P. R., Guo, R., Holwerda, R. A., Toth, J. P., Littell, R. C., Miles, R. D. and Ammerman, C. B., 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. Journal of Animal Science. *78*(8), 2039–2054

Chendran Seetha., Biswajyothi Patgiri., Galib, R. and Prasanth, D. 2016. A Review through therapeutic attributes of *YashadaBhasma*. International Journal of Pharmaceutical and Biological Archives.7(5):6-11.

Finney,D.J.1978.Statistical Method in Biological Assay (3rdEd.). Charles Griffin and Co., London

Goud, S. and Sridurga Ch. 2019. Pharmaceutical Standardization of Y*ashadabhasma* International Journal of Ayurveda and Pharma Research. 7(10) : 13-20.

Han,J.C., Yang,X.D., Zhang,T., Li,H., Li,W.L. and Zhang,Z.Y.2009. Effects of 1*á*-hydroxy cholecalciferol on growth performance, parameters of tibia and plasma, meat quality and type II bisodium phosphate co transporter gene expression of one to twenty-one-day-old broilers. Poultry Science. 88:323–329.

Hanne Damgaard Poulsen.1995. Zinc oxide for weanling piglets. Acta Agriculturae Scandinavica, Section A - Animal Science. 45(3):159-167.

Huang, Y. L., Lu, L., Luo, X. G. and Liu, B. 2007. An optimal dietary zinc level of broiler chicks fed a corn-soybean meal diet. Poultry Science. 86(12): 2582–2589.

ICAR. 2013.Nutrient Requirements of Poultry ICAR Publications, New Delhi. http://krishi.icar.gov.in/jspui/handle/123456789/9857.

Jha, S., Rani, R. and Singh. S. 2023. Biogenic Zinc Oxide Nanoparticles and Their Biomedical Applications: A Review. Journal of Inorganic and Organometallic Polymers and Materials. 1-16.

Khalid, A., Khan, R., Ul-Islam, M., Khan, T. and Wahid, F. 2017. Bacterial cellulose-zinc oxide nano composites as a novel dressing system for burn wounds. Carbohydrate polymers. 164: 214-221.

Kim,W.K. and Patterson,P.H. 2004. Effects of dietary zinc supplementation on broiler performance and nitrogen loss from manure. Poultry Science.83:34-38.

Kulkarni, D.A. 2010. Vigyanbodhini Commentary on Rasaratnasamuchchya of Vagbghata. 2nd Edn.1,Ver. 8-10. New Delhi: Meharchanda Lachhamanadas Publication.

Li, S., Luo, X., Liu, B., Crenshaw, T.D., Kuang, X., Shao, G. and Yu,S. 2004.Use of chemical characteristics to predict relative bioavailability of supplemental organic manganese sources for broilers. Journal of Animal Science. 82:2352-2363.

Lina,T., Jianyang,J., Fenghua,Z., Huiying, R. and Wenli, L.2009. Effect of nano zinc oxide on the production and dressing performance of broiler. Chinese Agricultural Science Bulletin. 2: 003

Littell, R.C., Henry, P.R., Lewis, A.J. and Ammerman, C.B., 1977. Estimate of relative bioavailability of nutrients using SAS procedures. Journal of Animal Science. 75:2672-2683

Marklund, S. and Marklund, G.1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry. 47(3) : 469-74.

Mohanna, C. and Nys Y. 1999. Effect of dietary zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. British Poultry Science. 40: 108–114.

Naz.S., Idris, M. and Khalique, M.A. 2016.The activity and use of zinc in poultry diets. World's Poultry Science Journal. 72(1): 159-167.

NRC. 1994. Nutrient Requirements of Poultry: Ninth Revised Edition, 1994. Washington, DC: The National Academies Press.

Oberdörster,G., Oberdörster, E. and Oberdörster, J. 2005. Nanotoxicology : an emerging discipline evolving from studies of ultra fine particles. Environ Health Perspect. 113(7): 823-839.

Pacheco, B.H.C., Nakagi, V.S., Kobashigawa, E.H., Caniatto, A.R.M., Faria, D.E. and Faria Dilho, D.E. 2017. Dietary levels of zinc and manganese on the performance of broilers between 1 to 42 days of age. Brazilian Journal of Poultry Science,19:171–178.

Pal, D., Sahu, C. K. and Haldar. A. 2014. Bhasma : The ancient Indian nanomedicine. J Adv Pharm Technol Res, *5*(1), 4–12.

Pandit Kashinath Shastri.,1979. Rasa Taringini panchamtarang shloka no 27-30. Motilal Banarsidass Publishing House, New Delhi, India.

Pareek, A. and Bhatnagar N. 2018. Revisiting ancient therapeutic potential of ayurvedic bhasma. International Journal of pharmaceutical Sciences Research 9(8):3150-65.

Pareek, A. and Bhatnagar, N. 2020. Physico-chemical characterization of traditionally prepared Yashadabhasma. Journal of Ayurveda and Integrative Medicine 11 (3):228-235.

Roberson, R.H. and Schaible, P.J. 1958. The zinc requirement of the chick. Science (New York, N.Y.) 127(3303):875.

Sandoval, M., Henry, P. R., Ammerman, C. B., Miles, R. D. and Littell, R. C., 1997. Relative bioavailability of supplemental inorganic zinc sources for chicks. Journal of Animal Science.75, 75: 3195-3205

Santhosh, B., Raghuveer., Jadar Prashanth and Rao Nageswar. 2013. Analytical study of *Yashadabhasma* with ayurvedic and modern parameters. International Ayurvedic Medical Journal. 1(2): 114-116

Sayda Ali , A.M., Hyder O. Abdalla. and Ibrahim Mahgoub.M.2011. Effect of slaughtering method on the keeping quality of broiler chickens' meat. Egyptian Poultry Science Journal 31(4):727-736

Sharma, K.C, Usha Sharma., Vipin Kumar. and Yadavendra Yadav. 2015. Some observations of the Zinc-Metals Based Preparations and its Properties in Ayurvrda with regard to stability of medicines in the nano state. International Journal of Research in Ayurveda and Pharmacy. 6(1):30-34

Shebina, P Rasheed. and Murugesh Shiva Shankar. 2015. Evaluation of Herbo Mineral Formulations (Bhasma): An Overview. International Journal of Research in Ayurveda and Pharmacy. 6 (3) : 382-386.

Snedecor, G. W. and Cochran, W. S. 1994. Statistical Methods. 8th Edn. Oxford and IB Publishing Co., Kolkata.

Star, L., van der Klis, J. D., Rapp, C. and Ward, T. L. 2012. Bioavailability of organic and inorganic zinc sources in male broilers. Poultry Science. 91(12): 3115–3120.

Swain, P.S., Rajendran, D., Rao,S.B.N. and Dominic,G. 2015. Preparation and effects of nano mineral particle feeding in livestock: A review. Veterinary World. 8(7): 888-891.

Talapatra, S. K., Roy, S. C. and Sen, K. C. 1940. Estimation of phosphorous, chlorine, calcium, magnesium and potassium in foodstuffs. Indian Jurnal of Veterinary Science and Animal Husbandry. 10: 243-258.

Trikamji Acharya.,1951. *Rasamritam*. Motilal Banarasi Das , Nirnaya Sagar Press, Kolbhat Street, Bombay..

Umrani, R.D., Agrawal, D.S. and Paknikar, K.M. 2013. Anti-diabetic activity and safety assessment of Ayurvedic Medicine, Jasadabhasma (zinc ash) in rats. Indian Journal of Experimental Biology. 51(10):811-22. PMID: 24266105.

Underwood, E. and Suttle,N. 1999. The Mineral Nutrition of Livestock. Zinc

Absorbability. 3rd Edn, CABI Publishing , New York, NY. pp 490-491

Venko Research and Breeding Farm . 2017. Vencobb400 Management Guide. vencobb 400_22 Feb. (tirumalagroup.com)

Wedekind, K. J . and Baker,D.H. 1990. Zinc bioavailability in feed-grade sources of zinc Journal of Animal Science. 68:684-689.

Wedekind, K.J., Hortin, A.E. and Baker, D.H.1992. Methodology for assessing zinc bioavailability: Efficacy estimates for zinc–methionine, zinc sulfate and zinc oxide .Journal of AnimalScience.70:178-87

Williams, D.B. and Carter, C.B. 1996. The Transmission Electron Microscope, Transmission Electron Microscopy: A Textbook for Materials Science (Springer US, Boston, MA, 1996) p: 3-17

Zhang ,T.Y., Liu, J.L., Zhang, J.L., Zhang, N., Yang, X., Qu, H.X., Xi, L. and Han, J.C. 2018. Effects of Dietary Zinc Levels on the Growth Performance, Organ Zinc Content and Zinc Retention in Broiler Chickens. Brazilian Journal of Poultry Science 20(1): 127-132