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RESEARCH ARTICLE

Effect of inorganic, organic and nano zinc supplemented diets on bioavailability and immunity status of broilers

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Abstract

Two hundred and eighty unsexed broiler chicks were distributed randomly into seven dietary treatments (D). The diets were D_1: basal diet + mineral mixture without Zn; D_2: D_1 + 15ppm inorganic Zn; D_3: D_1 + 15ppm organic Zn; D_4: D_1 + 7.5ppm organic Zn; D_5: D_1 + 0.3ppm nano Zn; D_6: D_1 + 0.06ppm nano Zn; D_7: D_1 + 0.03ppm nano Zn. Zn concentration in tibia bone, liver and blood serum were the highest in the birds of D_6 which differed significantly ($P<0.05$) with the remaining dietary groups. In case of muscles, the Zn level was highest in the birds of D_3 which varied significantly ($P<0.05$) with that of D_1 and D_7. The antibody titre to SRBC shows significantly ($P<0.05$) higher titer levels in D_3 and D_6 when compared to that of D_1 and D_2. The CBH response was found to be highest in D_3 which differed significantly ($P<0.05$) with that of D_1 and D_2. The relative weight of spleen in D_3 was significantly ($P<0.05$) higher than all the other groups. The percent weight of bursa was highest in the birds of D_6 which varied significantly ($P<0.05$) with D_1, D_4 and D_5. Significantly higher ($P<0.05$) relative weight of thymus was observed in the broilers of D_3 when compared to that of D_1, D_4 and D_5. Hence, it was found that 15 ppm organic Zn and 0.06 ppm nano Zn when added to the basal diet improved the health status of the broiler birds.

Introduction:

Trace minerals are essential feed additives in the diets of broiler birds to ensure better health and productivity. Zinc (Zn) is the most commonly added trace mineral in poultry feeds. It provides immunity (Kidd et al., 1996) and adequate Zn consumption is crucial to the development, maintenance and efficient functioning of the immunological system and the cells associated to it. A number of researchers documented that dietary zinc in its organic form was found to be more bioavailable than its inorganic form, hence provides better immunity (Lim and Paik, 2003; Ao et al., 2006; Yan and Waldroup, 2006; Tronina et al., 2007; Shyam Sunder et al., 2008; Swiatkiewicz and Koreleski, 2008; Gheisari et al., 2010; Britanico et al., 2012). Recently trace minerals in the form of nanoparticles can be effectively used to fulfill the requirement of minerals in the poultry diets. Due to their extreme small size and unique physical properties, the nanoparticles are likely to be different when compared to their conventional forms. As a feed additive, these are expected to have the advantage of better bioavailability, small dose rate and stable interaction with other components. The nanoparticles can effectively fulfill the requirement of minerals in the animals, promote growth rate and feed efficiency (Oberdörster, G. et al., 2005). With low use dosage, they can replace antibiotics as growth promoters, eliminate the residue of the antibiotics in the animal products, reduce the environmental contamination and produce pollution-free animal products (Hett, 2004 and Schmidt, C.W., 2009). With a view to enhance the immunity status it has been attempted for a comparative study of inorganic, organic and nano zinc supplemented diets in broiler chickens.

Materials and methods

Diet and management of experimental birds

A total number of two hundred eighty unsexed day old broiler chicks were weighed, wing banded and distributed randomly into seven dietary treatments with two replicates having 20 chicks in each replicate. Seven dietary treatments (D) were: D_1: Basal diet + mineral mixture without any zinc, D_2: D_1 + 50% of Zn present in basal diet through inorganic zinc ($ZnSO_4$) [15ppm], D_3: D_1 + 50% of Zn present in basal diet through organic zinc (Zn-Met) [15ppm], D_4: D_1 + 25% of Zn present in basal diet through organic zinc (Zn-Met) [7.5ppm], D_5: D_1 + nano ZnO @ 1/100th of Zn content in basal diet [0.3ppm], D_6: D_1 + nano ZnO @ 1/500th of Zn content in basal diet [0.06ppm], D_7: D_1 + nano ZnO @ 1/1000th of Zn content in basal diet [0.03ppm]. Basal diet for broiler starter and finisher was prepared as per BIS (1992) specification. The broilers were reared in deep litter system and fed with ad libitum feed and water for 42 days. Experimental feed samples were analyzed for proximate composition according to the AOAC (Association of Official Analytical Chemists). The ingredient and proximate composition of the experimental basal diet for the birds are presented in Table 1. Zinc concentration of the basal diet was estimated to be 30 ppm by atomic absorption spectrophotometer.

Bioavailability study

On 42nd day, four birds were randomly selected from each replicate and slaughtered by modified Kosher's method. Liver, spleen, thymus, bursa, muscle and both the tibiae bones were collected and weighed on a top pan electric balance. In case of liver and other organs 2 g of sample was taken in the digestion tube. In case of blood serum, 1 ml of serum was measured by micropipette and 9 ml of distilled water was added to dilute it up to 10 ml of volume. Fifteen ml of nitric acid (HNO_3), 10 ml of per-chloric acid ($HClO_4$)and 5 ml of hydrochloric acid (HCl) (HNO_3:$HClO_4$:HCl = 3:2:1) were added to a beaker. The tri-acid is added to the KEL plus digestion system containing the samples and digested till it became colourless. The digested samples were diluted and aspirated into an atomic absorption spectrophotometer (Perkin Elmer) to estimate the concentration of zinc (Shahjalal, M. et al., 2008). The right and left tibiae from each slaughtered bird were pooled group-wise and pressure cooked for 1 hour. The bones were allowed to cool and cleaned manually clearing off all the attached muscles, tissues, including cartilage caps and washed with distilled water. Then the bones were dried in a hot air oven at 105^{0}C for 24 hours. The tibiae were weighed and ashed at 600^{0}C for 24 hours in a muffle furnace. The total ash was weighed and expressed on percent dry weight basis (AOAC, 2000). Approximately 0.2 g of ash sample from each replicate was solubilised in 5 ml of 50% HCl and the mineral extract was filtered into a volumetric flask. The extract was then diluted using deionised water to the required volume and after suitable dilution it was used for mineral analysis by the help of an atomic absorption spectrophotometer (Perkin Elmer).

Cellular immunity

The cellular immune response is usually assessed by cutaneous basophilic hypersensitivity (CBH) test in vivo by using PHA-P (Phytohaemagglutinin phosphate). On 40th day of age, 2 birds from each replicate of each treatment were taken for CBH response. 10 mg of PHAP-P was dissolved in 10 ml of normal saline solution. 100 µg of PHAP-P in 0.1 ml of normal saline was injected intradermally in the right wattle. The normal thickness of the right wattle and 24 hrs of post injection were measured by using a digital slide caliper (Mitituyo, Japan) and CBH response was calculated using the formula:

$$\text{CBH response} = \frac{\text{Post injection thickness of right wattle}}{\text{Pre injection thickness of right wattle}} \times 100$$

Humoral immunity

The humoral immune response is measured by the antibody production in response to sheep red blood corpuscles (SRBC). About 0.1 ml of SRBC (0.5%) was injected into the wing vein of each bird. After five days of post inoculation, blood sample was collected from the SRBC injected birds and antibody titer was determined by HA titer methods (Siegel and Gross, 1980 and Shyam Sunder et al., 2008). The titer was expressed as log_2.

Weight of lymphoid organs

At the end of sixth week, four birds from each treatment were slaughtered by modified Kosher's method. The weights of bursa, thymus and spleen were recorded and expressed in per cent of live weight.

Statistical analysis

Data obtained from the experiment were subjected to statistical analysis wherever required. Analysis of Variance was obtained according to the method of Snedecor and Cochran (1998).

Results

Zinc concentration in tibia, liver, serum and muscle

The zinc concentration in tibia, liver, serum and muscles of six week old broiler birds are depicted in Table 2. In tibia bone, the zinc concentration was found to be highest in treatment D_6 (135.38 ± 3.18 ppm) which varied significantly (P<0.05) with all the other treatments, whereas the lowest concentration was observed in the treatment D_1 (80.15 ± 1.55 ppm) which was found to be differed significantly (P<0.05) from the other treatments. In case of liver, the zinc concentration was observed to be the highest in D_6 (93.63 ± 2.06 ppm) which was insignificant (P>0.05) to all the other treatment groups except D_5 and the lowest zinc concentration was found to be present in the treatment D_1 (55.49 ± 3.33 ppm) which varied significantly (P<0.05) with that of all other treatment groups. The zinc concentration in serum was the highest in the birds of treatment D_6 (23.60 ± 0.37 ppm) which differed significantly (P<0.05) with the remaining treatment groups and the lowest zinc concentration was found in that of D_1. In case of muscles, the concentration of zinc was observed to be the highest in the birds of treatment D_3 (36.07 ± 0.79 ppm) which varied significantly (P<0.05) with the treatments D_1 and D_7 whereas the lowest zinc content was seen in treatment D_1 (30.73 ± 0.71 ppm) which was significantly (P<0.05) different from all the other groups except D_7.

Effect of zinc on tibia bone

Tibia bone weight (g), tibia ash (%), tibia calcium (%) and tibia phosphorus (%) of six week old broiler birds varied insignificantly (P>0.05) and are presented in Table 3. The tibia weight of 6 week old broilers ranged between 5.03 ± 0.08 and 5.36 ± 0.06 g. The average tibia ash (%) ranged from 47.75 ± 1.92 to 52.97 ± 1.52 %. Tibia calcium (%) and tibia phosphorus (%) ranged from 31.61 ± 0.85 to 35.31 ± 0.79 % and 13.69 ± 0.38 to 15.15 ± 0.42 %, respectively.

Immunity Status

The antibody titers (log_2) against SRBC inoculation and the CBH responses of 6 week old broiler birds under different dietary treatments are depicted in Table 4. Significantly (P<0.05) higher titer levels were observed in the treatments D_3 (8.83 ± 0.40) and D_6 (8.83 ± 0.48) when compared to that of treatments D_1 and D_2. The CBH response was found to be the highest in the treatment D_3 (171.25 ± 16.95) which differed significantly (P<0.05) with that of D_1 and D_2. The lowest CBH response was observed in the treatment D_1 (120.79 ± 5.37) which showed significant difference (P<0.05) with all the other treatments except D_2.

The average weights of lymphoid organs viz., spleen, bursa and thymus expressed as percentage of live weight of 6 week old broiler birds are presented in Table 5. The relative weight of spleen of broilers in D_3 was significantly (P<0.05) higher than all the other treatments. The percent weight of bursa was found to be the highest in the birds of treatment D_6 which varied significantly (P<0.05) with D_1, D_4 and D_5. Significantly higher (P<0.05) relative weight of thymus was observed in the broilers of D_3 when compared to that of D_1, D_4 and D_5.

Table 1: Ingredients and proximate composition of broiler basal ration on dry matter basis

Ingredients	Starter (%)	Finisher (%)	Proximate composition	Starter mash	Finisher mash
Maize	57	64	Crude protein	23.02	20.04
Soyabean meal	38	30	Ether extract	4.94	5.06
De oiled rice bran	2	3	Crude fibre	3.95	3.70
Mineral mixture#	2.7	2.7	Total ash	7.78	7.94
Common salt	0.3	0.3	Acid insoluble ash	3.17	3.2
Total	100	100	Nitrogen-free extract	60.29	63.27
Feed additives	0.09	0.04	Calcium	1.87	1.84
			Total phosphorus	0.49	0.52
			ME (kcal/kg)*	2800	2900

Mineral mixture without zinc * Calculated value

Table 2: Average zinc concentration (ppm) in different organs, serum and tibia bone of broiler birds under different dietary treatments

Concentration of zinc (ppm)	Treatments							P value
	D_1	D_2	D_3	D_4	D_5	D_6	D_7	
Tibia bone	80.15^{f} ±1.55	109.33^{d} ±1.08	121.14^{b} ±1.16	108.05^{de} ±1.29	114.90^{c} ±2.70	135.38^{a} ±3.18	106.35^{de} ±2.41	1.47E-12
Liver	55.49^{e} ±3.33	70.25^{bc} ±1.94	75.41^{b} ±4.24	67.73^{cd} ±0.79	89.73^{a} ±1.76	93.63^{a} ±2.06	69.03^{bcd} ±1.04	3.58E-09
Serum	8.46^{d} ±0.41	13.44^{c} ±0.33	19.00^{b} ±0.29	13.20^{c} ±0.51	17.80^{b} ±0.37	23.60^{a} ±0.37	12.97^{c} ±0.59	9.45E-16
Muscle	30.73^{b} ±0.71	35.25^{a} ±0.79	36.07^{a} ±0.79	35.80^{a} ±0.73	35.15^{a} ±0.62	35.93^{a} ±0.57	32.27^{b} ±0.57	5.16E-05

Values bearing different superscripts in a row differ significantly ($P<0.05$)

Table 3: Effect of zinc on tibia bone of broiler birds under different dietary treatments

Parameters	Treatments							P value
	D_1	D_2	D_3	D_4	D_5	D_6	D_7	
Tibia bone weight (g)	5.08 ± 0.09	5.36 ± 0.06	5.03 ± 0.08	5.22 ± 0.09	5.10 ± 0.09	5.06 ± 0.08	5.11 ± 0.12	0.219
Tibia ash (%)	52.91 ± 2.46	47.75 ± 1.92	52.69 ± 1.67	51.14 ± 1.25	52.97 ± 1.52	50.76 ± 1.93	52.07 ± 1.86	0.439

Tibia calcium (%)	31.61 ± 0.85	33.66 ± 1.15	35.31 ± 0.79	32.62 ± 0.56	31.85 ± 0.61	33.12 ± 1.42	32.39 ± 0.43	0.379
Tibia phosphorus (%)	13.69 ± 0.38	14.28 ± 0.75	15.14 ± 0.41	15.07 ± 0.66	15.15 ± 0.42	14.25 ± 0.39	13.71 ± 0.35	0.172

Values bearing different superscripts in a row differ significantly (P<0.05)

Table 4: Immunity status of the broiler birds under different dietary treatments

Parameters	Treatments							P value
	D_1	D_2	D_3	D_4	D_5	D_6	D_7	
Antibody titers (log_2)	5.5^b ± 0.62	6.67^b ± 0.49	8.83^a ± 0.40	7.83^a ± 0.60	8.67^a ± 0.42	8.83^a ± 0.48	8.33^a ± 0.61	0.00056
CBH response	120.79^b ± 5.37	129.76^b ± 3.99	171.25^a ± 16.95	162.91^a ± 5.32	158.63^a ± 3.35	161.58^a ± 3.94	160.16^a ± 4.22	0.0007

Values bearing different superscripts in a row differ significantly (P<0.05)

Table 5: Lymphoid organs (% of live weight) of broiler birds under different dietary treatments

Parameters	Treatments							P value
	D_1	D_2	D_3	D_4	D_5	D_6	D_7	
Spleen (%)	0.098^f ±0.005	0.148^d ±0.005	0.200^a ±0.011	0.120^e ±0.007	0.160^c ±0.006	0.178^b ±0.006	0.145^d ±0.006	1.4836E-08
Bursa (%)	0.130^d ±0.004	0.188^{abc} ±0.013	0.198^{ab} ±0.009	0.150^{cd} ±0.011	0.180^{bc} ±0.007	0.213^a ±0.014	0.195^{abc} ±0.010	0.0001
Thymus (%)	0.358^d ±0.035	0.515^{ab} ±0.025	0.575^a ±0.012	0.413^c ±0.012	0.468^{bc} ±0.018	0.538^{ab} ±0.015	0.505^{ab} ±0.043	8.42E-05

Values bearing different superscripts in a row differ significantly (P<0.05)

Discussion

Underwood and Shuttle (1999) stated that estimating the deposition of minerals in selected tissues like tibia, serum, muscle and liver is the most common method of analysis in mineral relative bioavailability experiments. In this experiment zinc content in tibia bone, liver, serum and muscles of broiler birds under different dietary treatments of zinc differed significantly (P<0.05) between the groups. The zinc levels in tibia, liver, muscles and serum of all the Zn supplemented groups were significantly (P<0.05) higher than control (D_1). With inclusion of organic and inorganic Zn in the diet, it was observed that no significant difference was there in tibia, serum and muscle zinc levels between D_2 and D_4. But the Zn levels of tibia and serum were found to be significantly higher in D_3 than D_2 and D_4. This increased level of zinc in tibia and serum could be due to higher level of organic zinc in the diet of D_3 than D_4 and between D_3 than D_2 might be due to higher bioavailability of organic zinc. Based on tibia zinc content, Wedekind et al. (1992) reported that zinc from Zn-Met was more bioavailable than zinc from $ZnSO_4$ or ZnO. This finding is more or less similar with the findings of a number of researchers (Ao et al., 2006; Tronina et al., 2007; Cao et al., 2000; Shyam Sunder et al., 2008 and Idowu et al., 2011). In contrast to this finding, Zhao et al. (2010) reported that by replacing 50% of the inorganic Zn, Cu and Mn in the control ration with chelated minerals in dietary treatments, no significant difference was observed between the control and treatment groups for tibia zinc content which might be due to lower duration of feeding. Pimentel et al. (1991) reported that feeding broiler chicken with feed containing either Zn-Met or ZnO had no significant effect on the presence of microelements in the bone.

Zinc content of basal diet was estimated to be 30 ppm. By supplementing nano zinc @ $1/500^{th}$ of the zinc content of basal diet i.e. 0.06 ppm over and above the basal concentration had significantly ($P<0.05$) increased the Zn concentration of tibia, liver and serum compared to inorganic zinc supplemented (D_2), organic zinc supplemented groups (D_3 and D_4) and control birds (D_1). As regard to nano zinc feeding, higher zinc levels in tibia, liver, serum and muscle were observed in D_6 than D_5 and D_7. The explanation for higher zinc levels in D_6 than D_7 might be due to higher level of dietary zinc. But for higher zinc levels in tibia and serum of D_6 than that of D_5 could not be explained even on higher level of zinc supplementation in D_5. In this context, the findings of Ao et al. (2006) and Rossi et al. (2007) explained that dietary supplementation of Bioplex Zn® and $ZnSO_4$ linearly ($P<0.01$) increased total tibia Zn content.

Moreover, levels of trace mineral in tibia, muscles, serum and liver were solely not dependent upon the dietary levels rather on many other factors. In this regard, Bao et al. (2007) reported that at 29 day of age, the body weight of birds fed with control diet was only 70% that of birds on supplemental treatments, but there was no significant difference ($P>0.05$) in plasma trace mineral concentrations. They concluded that chickens give priorities to their mineral requirements for vital functions in compromise of body growth as indicated by the normal concentrations of the minerals in the plasma of the control birds. Hence, assessment of trace mineral status was found to be difficult yet an important challenge. According to a report of Hett (2004), it can only be assumed that these nanoparticles for their extreme small size and unique physical properties, their behaviour in the environment, uptake, distribution and bioavailable effects within the bodies of living organisms are likely to be different when compared to their inorganic and organic counterparts.

In this study, there was no significant difference in tibia ash % among the dietary treatments. Swiatkiewicz and Koreleski (2008) in their experiment reported that organic zinc supplementation had no significant effect on tibia length, relative weight of tibia, ash content of tibia and ash content of toe of the laying birds.

Higher antibody titers against SRBC were observed in the organic and nano zinc supplemented groups when compared to the control D_1. Beach et al. (1980) reported that diets supplemented with zinc tend to improve the ability of birds to produce antibodies. No significant difference was observed between D_1 and D_2 even on inorganic zinc supplementation in D_2. Pimentel et al. (1991) observed that birds fed with diet having Zn from an inorganic source, have not shown any improvement in their immunity status. The highest antibody titers were observed in the treatments D_3 and D_6 where birds were supplemented with dietary zinc in the form of Zn-Met and nano Zn, respectively. The antibody titer of D_4 was found to be non-significantly lower than D_3 even both the groups were supplemented with organic zinc, but the level of organic zinc was higher in D_3 than D_4. Smith (2003) showed that birds receiving a high zinc diet had significantly higher titers of total antibodies than those receiving adequate or low zinc diets. However, higher levels of Zn beyond the physiological limits of body might have no beneficial effect. Thus, in our study it can be assumed that dietary nano particles of zinc might have elicited a better immune response even at lower physiological limits.

The lowest CBH response was observed in the treatment D_1. Sunder et al. (2008) reported that the birds supplemented with diets without any zinc elicited the lowest CBH response. The highest CBH response was observed in D_3 which was statistically non-significant ($P>0.05$) with D_4, suggesting the effect of organic zinc particles in triggering immune response to be at par with Zn-Met. Hudson et al. (2004) reported that immune response to PHA-P injection was enhanced when dietary zinc was solely from Zn-AA. The non-significant difference ($P>0.05$) between organic and nano zinc fed groups might be due to better bioavailability which was earlier discussed in antibody titer response to SRBC. It can be predicted that the cell-mediated immunity was possibly related to the production of interleukin-2, which was supported by higher Zn level in the diet (Kidd et al., 1996). Overall, Zn-Met seems to provide more bioavailable zinc than inorganic zinc sources. However, it may be assumed that the cell-mediated response in nano zinc fed birds of this study was more intense even at lower levels of zinc in the form of nano particles which might be due to higher production of interleukin-2. In this regard O'Dell (1992) stated that the immune system is dependent on the functions of cellular metabolism where role of zinc in metalloenzymes is indispensable both structurally and catalytically.

The lowest weights of lymphoid organs were observed in D_1 where no dietary supplementation of zinc was made in the ration. Low levels of supplemental Zn showed a relative reduction in the size of lymphoid organs with the possible decrease in T-cell function (Kidd et al., 1996). Earlier researchers in this context reported that, a diet deficient in zinc leads to atrophy of the thymus and a reduction in spleen weight (Mengheri et al., 1988 and Arshami, J., 2010). The relative weights of spleen and thymus were higher in D_3 than D_2 where birds in D_3 were supplemented with Zn-Met but the levels of zinc in both the groups were same. Similar results were also reported when birds were fed diets with zinc from amino acid complexes or chelates that had increased relative thymus weights when compared to their inorganic counterparts (Virden et al., 2002; Moghaddam and Jahanian, 2009 and Feng et al., 2010). In contrast to our results, Moghaddam and Jahanian (2009) and Feng et al. (2010) reported non-significant ($P>0.05$) effect on the weight of spleen and bursa of Fabricius on organic mineral supplementation. Dietary supplementation with a more bioavailable zinc source like Zn-Met caused a significant ($P<0.05$) increase in relative weight of thymus. In contrast to this, Smith (2003) reported that none of these studied organs (thymus, bursa, and spleen) were significantly ($P<0.05$) affected by the levels of zinc in the diet. Even if insignificant, birds on a high zinc diet showed a slight increase in relative weight of thymus when compared with lower levels of zinc supplementation. Though, birds of both the treatments D_3 and D_4 were supplemented with organic zinc, a significant ($P<0.05$) increase in weight of lymphoid organs was observed in D_3 when compared to D_4. This implied that the level of zinc in D_4 might not be sufficient enough to boost the immune response following which lower weight of lymphoid organs was observed in D_4. Similar results were also reported by Shyam Sunder et al. (2008), where the weights of bursa and spleen were higher at 40 ppm compared with lower levels. The supplementation of nano zinc increased the weight of the bursa of Fabricius in treatments D_5, D_6 and D_7 even at a very lower level. This might be due to increased bioavailability of zinc nanoparticles when compared to its inorganic and organic counterparts. The highest weight of bursa was observed in D_6 compared to D_5 and D_7 where zinc nanoparticles were supplemented in the diet @ $1/500^{th}$ of the zinc content of basal diet. The reason behind a higher weight of bursa might be due to the antimicrobial properties of nano zinc that might have killed the pathogenic microbes and improved gut health thereby contributing a healthy bursa. But it contradicts the reports of Ahmadi and Kurdestany (2010) who reported a significant ($P<0.05$) decrease in the relative weights of bursa of birds when supplemented with silver nanoparticles. The lower weight of lymphoid organs observed in treatment D_5 might be due to lower level of zinc availability from dietary sources. The explanation for these lower relative weights of the lymphoid organs in D_5 is that a larger portion of dietary zinc might have diverted to develop body weight and the immune organs comparatively needed only a small fraction of it (Smith, 2003). The non-significant difference ($P>0.05$) in the weights of thymus and bursa in T_6 and T_7 might be due to higher bioavailability of dietary zinc.

Conclusion

From this experiment it can be concluded that organic zinc and nano zinc supplemented @ 15 ppm and 0.06 ppm, respectively to the basal diet of broiler birds were observed to be more bioavailable when compared to inorganic zinc and consequently improved the immunity status of the broiler birds.

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