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To cite this article: Ilkay Yalçinkaya, Miyase Çınar, Ebru Yildirim, Serkan Erat, Mehmet Başalan & Tülin Güngör (2012) The effect of prebiotic and organic zinc alone and in combination in broiler diets on the performance and some blood parameters, Italian Journal of Animal Science, 11:3, e55, DOI: [10.4081/ijas.2012.e55](https://doi.org/10.4081/ijas.2012.e55)

To link to this article: <https://doi.org/10.4081/ijas.2012.e55>



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Published online: 18 Feb 2016.



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## PAPER

## The effect of prebiotic and organic zinc alone and in combination in broiler diets on the performance and some blood parameters

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### Abstract

This study was conducted to investigate the effects of prebiotic and organic zinc alone and in combination in broiler diets on the live weight gain (LWG), feed consumption (FC), feed consumption ratio (FCR), carcass yield, some relative organ weights and blood parameters. A total of 160 Ross 308 one-day old male chicks were assigned to 4 treatment groups with 4 replicates of 10 birds each. Treatment for each group consisted of: first group (control group) received basal diet without supplementation; second group received 1 g/kg Mannan-oligosaccharide (MOS); third group received 80 mg/kg organic zinc (OZn); and fourth group received 80 mg/kg organic zinc + 1 g/kg Mannan-oligosaccharide (MOS+OZn). The study lasted 42 days. The supplementation of MOS and OZn had no effect on the LWG, FC, FCR, carcass yield, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and triglyceride levels during the experiment ( $P>0.05$ ). Relative organ weights (liver, spleen, pancreas) were significantly higher in OZn group than those in the other groups ( $P<0.001$ ). There was a significant difference in serum total cholesterol and glucose levels between treatment groups and control group ( $P<0.001$ ). Serum Zn and Fe levels were significantly lower in control group than those in OZn and MOS+OZn groups ( $P<0.001$ ). The highest and the lowest Cu levels were in the MOS+OZn and control groups, respectively ( $P<0.001$ ). These data suggest that OZn with MOS combination may have a beneficial effect on serum mineral level in broilers.

### Introduction

Prebiotics can be defined as non-digestible food that have a beneficial effect on the host by selectively stimulating the growth and/or activity of one, or a limited number, of bacteria in the colon (Gibson and Roberfroid, 1995). Mannan-oligosaccharide (MOS), prebiotic, is derived from the cell wall of the yeast *Saccharomyces cerevisiae*, improves intestinal environment and promotes growth and feed conversion in broilers (Yang *et al.*, 2007; Benites *et al.*, 2008; Markovic *et al.*, 2009).

Organic trace mineral sources, such as proteinate and amino acid chelate, have been used in broiler feeds for recent years, showing promise in improving live performance, bird health, processing yield and meat quality characteristics. Zinc sources are the most studied mineral among these compounds. Some researchers have reported improved growth rate and/or feed conversion with organic zinc sources in broiler (Cao *et al.*, 2000; Hess *et al.*, 2001; Leeson, 2005; Ao *et al.*, 2006; Novak and Troche, 2007; Bao *et al.*, 2007; Rossi *et al.*, 2007).

Poultry industry uses higher dietary trace mineral sources. On the other hand supplemental inorganic trace minerals result in a high level of mineral excretion. Therefore, it may be preferable to use organic complexed trace minerals as they can provide alternative pathways for absorption, thus leading to reduction in excretion (Scott *et al.*, 1982; Leeson, 2003). At the same time, poultry industry shows a tendency to supplement prebiotics to broiler diet. Several studies indicated that prebiotics had a beneficial effect on mineral absorption by lowering intestinal pH (Scholz-Ahrens *et al.*, 2007; Demigne *et al.*, 2008; Ortiz *et al.*, 2009).

Literature regarding the effect of MOS with OZn in broilers is scarce. Therefore, the objective of present study was to examine the performance, carcass yield, some relative organ weight and blood parameters in broiler chicken fed on an experimental diet supplementation prebiotic (Bio-Mos<sup>®</sup>, Alltech Inc., Nicholasville, KY, USA) and organic Zn (Bioplex<sup>®</sup> Zn, Alltech Inc.).

### Materials and methods

#### Animal and diets

A total of 160 Ross 308 one-day-old male chicks were used. Chicks were weighed and assigned to 4 treatment groups with 4 repli-

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Key words: Broiler, Organic zinc, Prebiotic, Performance.

Acknowledgments: this study was funded by the Kırıkkale University Research Fund, project n. 2008/38.

Received for publication: 29 March 2012.

Revision received: 5 June 2012.

Accepted for publication: 13 June 2012.

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Italian Journal of Animal Science 2012; 11:e55  
doi:10.4081/ijas.2012.e55

cates of 10 birds per replicated. The temperature was  $34\pm 1^\circ\text{C}$  up to 7 d of age and gradually reduced to  $26\pm 1^\circ\text{C}$  by 21 d of age. All birds had *ad libitum* access to water and diets from 0 to 42 d of age. Liveweight and feed consumption (FC) of animals were weekly recorded. Feed consumption ratio (FCR) was calculated by dividing feed consumption by live weight gain (LWG). The experiment was conducted under protocols by The University of Kırıkkale, Ethical Commission Accession (n. 08/31/41).

The basal diet was presented in Table 1. First group (control group) received a basal diet with no supplementation during the experiment. Second group received 1 g/kg mannan-oligosaccharide (MOS), 3<sup>rd</sup> group received 80 mg/kg organic zinc (OZn) and 4<sup>th</sup> group received 80 mg/kg organic zinc + 1 g/kg mannan-oligosaccharide (MOS+OZn) in basal diet. Zinc, Fe and Cu levels were detected in starter and grower broiler diets by ICP (Inductively coupled plasma atomic emission spectrometer; AES Varion Vista Model, Sydney, Australia).

#### Carcass yield and relative organ weight

At the end of the study (day 42), all birds were weighed individually and 12 birds from each treatment group were randomly slaughtered for carcass data. Carcasses were cleaned by removing feathers (wet), feet, and visceral

organs. Carcass yield was calculated as carcass weight/liveweight x 100 at slaughter and expressed as percentage .

The liver, spleen and pancreas were removed, weighed and expressed as relative organ weights (gram of per 100 of live-weights).

### Blood sample collection

At the end of the 42 d, 3 birds from each replicate of each treatment group (12 birds/group) were randomly selected, and blood samples were individually collected into serum test tubes from brachial vein for biochemical analysis during slaughter. Sera were separated by centrifugation for 10 min at 1600xg after 1 h incubation at room temperature and stored at -20°C until the analysis. Aspartate aminotransferase (AST) (EC 2.6.1.1), alanine aminotransferase (ALT) (EC 2.6.1.2), alkaline phosphatase (ALP) (EC 3.1.3.1) activities, triglyceride, glucose (Biolabo, Maizy, France), total protein (TP), total cholesterol (DDS, Dusseldorf, Germany) concentration were determined by a Shimadzu UV 1700 spectrophotometer (Kyoto, Japan) with diagnostic kits. Concentrations of Zn, Fe and Cu in serum were determined by ICP (Inductively coupled plasma atomic emission spectrometer; AES Varion Vista Model, Sydney, Australia).

### Statistical analyses

The data were analyzed using SPSS 10 for Windows (SPSS Inc., Chicago, IL, USA). Statistically significant differences between group means were determined by analysis of variance (ANOVA). Duncan's multiple range test was performed when the differences were significant. Mean values were considered significantly different at P<0.05. The data were expressed as mean values ±SE.

## Results and discussion

The results of broiler performance are given in Table 2. Live weight gain, FC, FCR were not influenced by addition dietary MOS and OZn (P>0.05). Similar results were obtained in broiler fed with 1 g/kg MOS (Bozkurt *et al.*, 2008). Also Yang *et al.* (2007) reported that 0.5-2 g/kg MOS supplementation had no effect on FC and FCR but tended to improve LWG as compared to control group. Waldroup *et al.* (2003) demonstrated that supplementation of MOS (1 g/kg) in broiler diet did not improve BW and FCR. Khalaji *et al.* (2011) showed that MOS (0.5, 1 and 1.5 g/kg) had no significant effect

on growth performance and FCR. Mohanna and Nys (1999) reported that LWG, FC and FCR in broilers were not influenced by 40 mg/kg Zn sulfate or Zn-Met. Rossi *et al.* (2007) reported that LWG, FC and FCR were not statistically influenced by addition of organic Zn supplementations (0, 15, 30, 60 ppm) in diet. These results are also consistent with our study. On the contrary, some investigators, who added organic zinc to broiler diets, observed an improvement in growth performance (Ao *et al.*, 2006; Bao *et al.*, 2007;). This difference can be attributed to the varying protocols and dose regimens of the experiments. In Bao *et al.* (2007) study, not only 80 mg/kg Zn but also other minerals like Fe, Cu and Mn were used,

and the study lasted 29 d, in our study we used only Zn and the study lasted 42 d. In Ao *et al.* (2006) study, it was concluded that feed intake and weight gain were linearly increased by dietary supplementing Bioplex Zn until 10 mg/kg after which no further increase occurred.

Carcass yield and relative organ weights were shown in Table 3. In this study carcass yield was not influenced by addition of MOS and OZn (P>0.05). Similarly some researchers (Eren *et al.*, 1999; Waldroup *et al.*, 2003; Yalcinkaya *et al.*, 2008) reported no significant improvement in carcass yield of broilers when fed with 1 g/kg Bio-Mos. In addition, Rossi *et al.* (2007) reported that carcass yield was not

**Table 1. Composition of the basal diets.**

	Starter, 0-21 days	Finisher, 22-42 days
Ingredients, %		
Corn	48.50	56.00
Soybean meal	42.40	34.40
Vegetable oil	5.50	6.00
Limestone	1.30	1.30
Dicalcium phosphate	1.50	1.50
Salt	0.25	0.25
Vitamin+mineral premix <sup>o</sup>	0.25	0.25
DL-Methionine	0.30	0.30
Nutrient composition		
Crude protein, %	23.00	20.10
Metabolizable energy, kcal/kg	3090	3209
Zinc, mg/kg	23.70	29.05
Iron, mg/kg	83.46	86.15
Copper, mg/kg	9.15	8.20

<sup>o</sup>Vitamin-mineral premix: vit. A, 12,000 U; vit. D<sub>3</sub>, 1500 U; vit. E, 30 mg; vit. K<sub>3</sub>, 5 mg; vit. B<sub>1</sub>, 3 mg; vit. B<sub>2</sub>, 6 mg; vit. B<sub>6</sub>, 5 mg; vit. B<sub>12</sub>, 0.03 mg; nicotinic acid amine, 40 mg; D-Ca-pantothenate, 10 mg; folic acid, 0.075 mg; choline, 375 mg; manganese, 80 mg; iron, 80 mg; copper, 8 mg; iodine, 0.5 mg; cobalt, 0.2 mg; selenium, 0.15 mg.

**Table 2. Effects of mannanoligosaccharide and organic zinc supplementation on feed consumption, liveweight gain and feed consumption ratio of broilers.**

Parameter	Period, days	Control	MOS	OZn	MOS+OZn	P
FC, g/bird/d	1-21	1221.18±24.72	1249.07±26.55	1291.42±19.63	1254.91±15.45	ns
	21-42	3098.32±49.19	3175.33±64.77	3143.62±77.69	3182.19±68.72	ns
	1-42	4319.49±51.71	4424.40±82.54	4435.04±76.21	4437.11±81.80	ns
LWG, g/bird/d	1-21	902.68±22.04	942.59±25.85	944.18±20.40	937.40±14.70	ns
	21-42	1640.12±22.17	1694.43±39.43	1681.96±19.43	1711.78±46.35	ns
	1-42	2540.70±27.64	2636.04±21.93	2622.61±15.88	2646.38±49.66	ns
FCR	1-21	1.35±0.02	1.33±0.03	1.37±0.02	1.34±0.01	ns
	21-42	1.89±0.04	1.87±0.01	1.87±0.04	1.86±0.01	ns
	1-42	1.70±0.02	1.68±0.03	1.69±0.02	1.68±0.01	ns

MOS, mannanoligosaccharide; OZn, organic zinc; MOS+OZn, mannanoligosaccharide + organic zinc; FC, feed consumption; LWG, liveweight gain; FCR, feed consumption ratio; ns, not significant.

influenced by supplementation of increasing levels of dietary organic zinc. But relative organ weights (liver, spleen, pancreas) were significantly higher in OZn group than those in other treatment groups ( $P < 0.001$ ). Also Jahanian *et al.* (2008) fed chicks on increasing levels of OZn, (40-80-120 mg/kg) and demonstrated that dietary zinc source affect liver weight. The heaviest livers were found in chicks fed on 80 mg/kg ZnMet supplemented diets. In our study the combined supplementation of MOS and OZn reduced the increase found in the liver of OZn group.

Blood parameters were presented in Table 4. In the present study, serum glucose concentration decreased in OZn and MOS+OZn groups ( $P < 0.001$ ) compared with control group. Our results were similar to those of Uyanik *et al.* (2001) who found that Zn supplementation decreased serum glucose concentration in broilers. This decrease might have been resulted from a possible relationship between Zn and insulin. Salgueiro *et al.* (2001) suggested a close relation among zinc, glucose metabolism and insulin physiology. Zinc induced pancreatic islet cells to produce and secrete insulin by

playing crucial role in the synthesis, storage, and secretion of insulin. The zinc deficient rats had an impaired glucose metabolism. The deficiency of zinc increased the level of glucose in rats (Søndergaard *et al.* 2006).

In this study, the concentration of serum cholesterol was increased in MOS+OZn group as compared to MOS and control group ( $P = 0.004$ ). This result was consistent with the results of studies in goat (Keskin *et al.*, 1999), rat (Allen and Klevay, 1980; Samman and Roberts, 1988) pig (Eiseman *et al.*, 1979) and human (Hooper *et al.*, 1980; Samman and Roberts, 1988) which showed an increased serum cholesterol concentration due to Zn supplementation ( $< 300$  mg/kg diet) to the diet. The relationship between dietary zinc and plasma cholesterol is not well understood (Uyanik *et al.*, 2001). Zinc supplementation did not influence serum cholesterol in chicks (Lu and Combs, 1988). Uyanik *et al.* (2001) indicated that inorganic zinc decreased serum cholesterol concentration. In contrast, Kaya *et al.* (2001) reported that adding 50 and 200 mg/kg Zn to the diet increased plasma total cholesterol level in laying hens.

Total protein level in serum was higher in OZn group than those in control and MOS groups ( $P = 0.043$ ). This increase was consistent with the studies of Feng *et al.* (2010) who used 90 mg/kg Zn-Gly in broiler and Bülbül and Küçükersan (2004) who used 140 mg/kg Zn-proteinat in laying hens and suggesting an increase in total plasma protein. In our study, serum Zn and Fe levels were lower in control group than those in OZn and MOS+OZn groups ( $P < 0.001$ ). The Cu level was the highest and the lowest in MOS+OZn and control groups respectively and significantly differed from the other groups ( $P < 0.001$ ). Ghosh *et al.* (2008) found that, Cu, Zn and Mn in the plasma were not affected by different dietary supplementation of MOS in Japanese quail. Also, mannanoligosaccharide in the diet improved plasma Fe level (3.68 ppm) as compared to control (3.07 ppm). Leeson and Summers (2001) suggested that binding of the organic ligands to the chelated trace minerals in the upper gastrointestinal system minimize the mineral losses to antagonists and allowing the complex to be delivered to the absorptive epithelium of the small intestine for mineral uptake. An improvement of mineral absorption by prebiotics in rats, human and layers have been reported by some researchers (Delzenne *et al.*, 1995; Chen and Chen, 2004; Scholz-Ahrens *et al.*, 2007; Ortiz *et al.*, 2009). These data suggested that using organic zinc might reduce Cu-Zn antagonism and MOS supplementation improved Fe and Cu absorption.

**Table 3. Effects of mannanoligosaccharide and organic zinc supplementation on carcass yield<sup>c</sup> and some relative organ weights<sup>d</sup> for broilers.**

	Control	MOS	OZn	MOS+OZn	P
Carcass yield, %	79.21±0.50	80.35±0.95	78.45±1.21	80.32±0.43	ns
Liver, %	1.81±0.09 <sup>b</sup>	1.83±0.08 <sup>b</sup>	2.28±0.04 <sup>a</sup>	1.86±0.06 <sup>b</sup>	0.000**
Spleen, %	0.12±0.01 <sup>c</sup>	0.25±0.05 <sup>b</sup>	0.43±0.01 <sup>a</sup>	0.27±0.05 <sup>b</sup>	0.000**
Pancreas, %	0.24±0.01 <sup>c</sup>	0.36±0.05 <sup>b</sup>	0.53±0.01 <sup>a</sup>	0.42±0.05 <sup>b</sup>	0.000**

<sup>c</sup>Carcass yield was calculated as carcass weight / liveweight x 100 at slaughter and expressed as percentage. <sup>d</sup>Organ weights, specific organ weight/100 g liveweight. MOS, mannanoligosaccharide; OZn, organic zinc; MOS+OZn, mannanoligosaccharide + organic zinc. <sup>abc</sup>Means in the same row having different superscripts are significantly different; ns, not significant. \*\* $P < 0.001$ .

**Table 4. Effects of mannanoligosaccharide and organic zinc supplementation to diet on some blood parameters of broilers.**

Parameters	Control	MOS	OZn	MOS+OZn	P
AST, U/L	213.03±10.62	236.69±7.08	238.18±12.55	231.38±8.55	ns
ALT, U/L	8.36±1.17	7.33±1.39	5.97±0.95	5.93±0.70	ns
ALP, U/L	2632.48±140.14	2577.38±183.49	2637.10±120.46	2555.68±73.92	ns
Glucose, mg/dL	261.74±8.59 <sup>a</sup>	233.72±5.16 <sup>b</sup>	205.89±4.05 <sup>c</sup>	208.55±8.24 <sup>c</sup>	0.000**
Triglyceride, mg/dL	48.79±1.70	45.43±2.44	45.34±4.11	43.87±2.08	ns
Total cholesterol, mg/dL	126.53±2.98 <sup>b</sup>	122.14±5.42 <sup>b</sup>	128.84±3.29 <sup>ab</sup>	139.99±3.15 <sup>a</sup>	0.004*
Total protein, g/dL	3.49±0.10 <sup>b</sup>	3.42±0.12 <sup>b</sup>	3.96±0.17 <sup>a</sup>	3.73±0.17 <sup>ab</sup>	0.043
Zinc, ppm	1.83±0.11 <sup>b</sup>	2.12±0.11 <sup>ab</sup>	2.24±0.12 <sup>a</sup>	2.38±0.13 <sup>a</sup>	0.034*
Iron, ppm	3.38±0.50 <sup>b</sup>	4.82±0.79 <sup>ab</sup>	5.84±0.49 <sup>a</sup>	5.97±0.59 <sup>a</sup>	0.017*
Copper, ppm	0.28±0.03 <sup>c</sup>	0.68±0.09 <sup>b</sup>	0.71±0.09 <sup>b</sup>	0.96±0.09 <sup>a</sup>	0.000**

MOS, mannanoligosaccharide; OZn, organic zinc; MOS+OZn, mannanoligosaccharide + organic zinc; AST, aspartate aminotransferase, ALT, alanine aminotransferase, ALP, alkaline phosphatase. <sup>abc</sup>Means in the same row having different superscripts are significantly different; ns, not significant. \* $P < 0.05$ ; \*\* $P < 0.001$ .

## Conclusions

Mannanoligosaccharide and organic zinc had no significant effect on growth performance but changed some biochemical parameters in broilers, and Cu level in MOS+OZn group was higher than those in the other groups whereas Fe level in MOS+OZn group was only higher than that of control group. These data suggest that OZn with MOS combination facilitate the Fe and Zn absorption and aid in the retention of Cu. MOS and OZn combination exhibited synergistic effect on blood mineral level, which would provide more alternative choice to broiler producers for preparation of environment-friendly feed.

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