SOLITARY AND COMBINED EFFECT OF TWO LEVELS OF DIETARY ZINC-METHIONINE AND PREBIOTICS SUPPLEMENTATION ON BROILER GROWTH PERFORMANCE, INTESTINAL MORPHOLOGY, CARCASS TRAITS, AND IMMUNE RESPONSE

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ABSTRACT

Dietary zinc-methionine, when combined with prebiotics, may enhance its bioavailability in organs, thereby improving overall performance, immunity, gut development, and carcass traits in broilers. With this objective in mind, the study was conducted to assess the effects of solitary and combined supplementation of prebiotics at two distinct levels of zinc-methionine. Two hundred and forty-day-old chicks (Ross-308) were obtained from a local hatchery and randomly assigned to four dietary treatments in a 2x2 factorial arrangement, with four replicates of 15 birds each. The dietary treatments included: Zn40, containing supplemental zinc-methionine at 40 mg/kg; ZnP40, a combination of supplemental zinc-methionine at 40 mg/kg and MOS at 1 g/kg; Zn80, comprising supplemental zinc-methionine at 80 mg/kg; and ZnP80P, supplemental zinc-methionine at 80 mg/kg in combination with MOS at 1 g/kg. The chicks were reared in two distinct phases: starter

(0 to 21 days) and grower (21 to 35 days). Ad-libitum feed and water were provided throughout the experimental period. Parameters such as feed intake and weight gain were recorded daily, while FCR was calculated weekly. Gut morphology, immune status, and carcass characteristics were assessed at day 35. The ZnP40 treatment significantly affected ($P < 0.05$) FCR, feed intake, carcass weight, and breast weight yield. Villus height and VH:CD ratio were also significantly influenced ($P < 0.05$) by the ZnP40 treatment. Additionally, the antibody titer against NDV at days 21 and 35 was significantly affected by the ZnP80 treatment. The findings suggest that supplemental zinc-methionine at a level of 40 mg/kg of diet, along with the prebiotic combination (ZnP40), had a significant positive impact not only on broiler performance but also on carcass traits and immune status. Therefore, the use of zinc-methionine at 40 mg/kg in combination with MOS at 1 g/kg is recommended based on the conclusions drawn from the study.

INTRODUCTION

Poultry farming plays a significant role in Pakistan's meat production, contributing 33.9% (1518 thousand tons) of the total meat output of 4478 thousand tons. However, the average consumption of animal protein in Pakistan falls below the World Health Organization's recommended daily intake of 37 grams per capita, with only 17 grams consumed, of which broiler meat accounts for a mere 5 grams. Given the affordability and nutritional value of broiler meat, improving cost-effective production methods could help bridge this consumption gap.

Historically, antibiotics growth promoters (AGPs) were used to enhance meat production, but their use is now discouraged due to health concerns. Consequently, finding effective alternatives to AGPs is a priority in poultry nutrition worldwide. Proper mineral supplementation is essential for optimal performance, immune function, and overall health in chickens. Therefore, a systematic approach to supplementing key minerals and prebiotics could potentially replace AGPs without compromising broiler health or production performance.

Zinc, a critical micronutrient, plays a vital role in broiler production. It serves as a cofactor for over 300 enzymes, contributing to protein structure maintenance and gene expression regulation. Zinc also supports the integrity of epithelial cells, which serve as barriers against infectious agents in the gastrointestinal tract and skin. Additionally, zinc is essential for maintaining immune function, as it supports the activity of various immune cells such as macrophages, T lymphocytes, and neutrophils.

Traditionally, inorganic zinc salts have been used in feed formulations due to their lower cost. However, zinc-methionine, an organic form of zinc, offers higher absorption rates and cellular availability. On the contrary, the over-supplementation of inorganic zinc-methionine can disrupt mineral balance and interfere with nutrient absorption and metabolism. Furthermore, dietary fiber and phytates present in feed can inhibit inorganic zinc absorption which can be avoided by zinc-methionine supplementation.

Prebiotics, such as mannon-oligosaccharides (MOS), help to stimulate the growth of beneficial gut microflora and improve mineral absorption by reducing intestinal pH. Supplementation of MOS, extracted from yeast cell wall, has been shown to reduce pathogenic microorganisms in the gut and enhance broiler immune response and growth performance.

Despite individual studies demonstrating the efficacy of zinc-methionine and prebiotics in broiler diets, little research has explored their combined effects. Therefore, our study aims to investigate the solitary and combined effects of prebiotics at two distinctive levels of zinc-methionine supplementation on broiler production performance, carcass traits, immunity, and gut development.

MATERIALS AND METHODS

The study was executed at Experimental Broiler House, an environmentally controlled broiler shed, A block, UVAS Ravi Campus Pattoki. The rearing span of birds was 35 days, with prior approval obtained from the Ethical Review Committee of UVAS, Lahore, for all experimental procedures.

Management practices: Experimental procedures commenced with the preparation of the experimental house 7-8 days before the arrival of the birds. The house underwent thorough disinfection using bleaching powder followed by washing with water. Subsequently, the walls were whitewashed, and a disinfectant spray was applied. Fumigation was conducted after disinfection. Approximately 8-10 cm of rice husk litter was spread on the floor 24-48 hours before the birds' arrival. The brooding temperature was set at 33°C±2, and water lines were inspected for leaks. Each replicate was equipped with two feed bins, and temperature and humidity were adjusted according to the Ross 308 recommended guidelines prior to bird placement.

Experimental Design: A total of 240-day-old mixed-sex chicks were procured from the hatchery and allocated to four dietary treatments in a completely randomized design. Each treatment consisted of four replicates of 15 birds each. The dietary treatments included: Zn40 (zinc-methionine supplementation at 40 mg/kg), Zn40P (zinc-methionine at 40 mg/kg + MOS at 1 g/kg), Zn80 (zinc-methionine at 80 mg/kg), and Zn80P (zinc-methionine at 80 mg/kg + MOS at 1 g/kg). The starter phase lasted from 1 to 21 days, the finisher phase continued from 21 to 35 days. Birds had ad-libitum access to feed and water throughout the trial, with a 23-hour light period maintained at 30-40 Lux light intensity. The temperature of the controlled environment house was decreased to 0.3°C daily to that of the start.

Experimental Parameters: Growth performance indicators including feed intake, weight gain, and feed conversion ratio (FCR) were recorded weekly. Mortality rates were documented daily. At the trial's conclusion, three birds per replicate were randomly selected and slaughtered for carcass evaluation. Three birds weighing to the mean live weight of each respective replicate were slaughtered to evaluate the carcass parameters including hot carcass yield, weight without giblets, giblet weight (heart, liver, and gizzard), breast yield, and leg quarter weight following the guidelines of (Ojewola et al. 2001) at 35d. Additionally, gut development was assessed by histological examination of small intestine samples, focusing on villus height (VH), crypt depth (CD), and villus height to crypt depth ratio (VD: CD). A 2 cm portion of the small intestine (from duodenum and jejunum) was taken and flushed with normal saline via a sterile syringe and preserved in a container with 10 ml of 10% formalin for 48 hours for histological examination. Histological slide preparation went through the processes of drying, clearing, and infiltrating with wax. Respective values of VH, CD, and VH: CD were measured in micrometers and calibrated using the software PixelPro®. Immune response was evaluated by measuring antibody titers against Newcastle disease virus (NDV) at days 21 and 35. From each replicate 3 ml blood from three representative birds was collected in an anticoagulant gel-coated vacutainer. For serum separation, samples were centrifuged at 2000 rpm for ten minutes, shifted to labeled Eppendorf, and stored at -20°C until further analysis (Muthusamy et al. 2011) Serum samples were analyzed for NDV titer using the hemagglutination test. Moreover, the weight of immune organs (spleen and bursa of fabricius) was recorded from the same birds.

Feed Formulation: The basal diet for the starter and grower phases was formulated as per the recommendation of Ross 308 and is elaborated in Table-1.

Data Analysis: Data were analyzed through the One-way ANOVA technique using PROC GLM in SAS Software (version 9.1). Significant treatment means were compared through Duncan's Multiple Range test assuming probability at $p \leq 0.05$. The following mathematical model was used:

$$Yijk = \mu + \alpha i + \beta j + (\alpha\beta)ij + \epsilon ijk$$

RESULTS AND DISCUSSION

Growth Performance:

From the data presented in table-2, it is evident that both the main effects of zinc-methionine levels (ZL) and prebiotic presence (PP), as well as their interaction (ZL × PP), significantly influenced the performance parameters of broilers.

For feed intake, a significant difference was observed among treatments ($P < 0.05$). Birds supplemented with ZnP80 exhibited the highest feed intake, followed by Zn80, ZnP40, and Zn40 treatments, respectively. This suggests a potential interactive effect between zinc-methionine levels and prebiotic presence on feed intake.

Similarly, for weight gain, significant differences were detected among treatments ($P < 0.05$). Broilers supplemented with ZnP40 showed the highest weight gain, followed by ZnP80, Zn80, and Zn40 treatments, respectively. In terms of FCR, significant differences were observed among treatments ($P < 0.05$). Broilers supplemented with ZnP40 exhibited the lowest FCR, indicating better feed efficiency compared to the other treatments.

These findings align with previous studies. For instance, Amira et al. (2009) reported increased feed consumption with diets supplemented with bio-zinc (Zn Methionine), while Liu et al. (2013) observed higher daily feed intake with diets supplemented with zinc proteinate, consistent with our results. Additionally, Anderson et al. (2000) demonstrated that prebiotics enhance feed consumption when used in combination with zinc-methionine.

Regarding body weight gain, our findings are in line with Saleh et al. (2018), who observed higher body weight gain with supplementation of zinc methionine. Similarly, Jahanian et al. (2015) concluded that replacing inorganic zinc with zinc methionine in broiler feed significantly impacted weight gain, supporting our observations. Moreover, Mookiah et al. (2014) noted increased body weight gain and feed intake after supplementation of prebiotics, consistent with our study.

The improvement in FCR with ZnP40 supplementation contrasts with findings by Hess et al. (2001), who observed higher FCR with the addition of organic Zn-amino acid complexes in feed. However, our results are consistent with literature suggesting that the utilization of zinc-methionine in broiler diets leads to improved FCR. Furthermore, Shendare et al. (2008) concluded that the addition of prebiotics (MOS) significantly improved FCR, supporting the findings of our experiment.

Carcass Traits:

Results on the carcass traits are illustrated in Table 4.2. The results show significant main effects of zinc-methionine levels (ZL) and prebiotic presence (PP), as well as their interaction (ZL × PP), on live weight, carcass weight, breast weight, and thigh weight of broilers.

For live weight, broilers supplemented with ZnP40 had the highest ($P < 0.05$) live weight, followed by ZnP80, Zn80, and Zn40 treatments, respectively. Similarly, for carcass weight, breast weight, and thigh weight ZnP40 exhibited the highest values ($P < 0.05$), followed by ZnP80, Zn80, and Zn40 treatments, respectively. This highlights a potential synergistic effect between zinc-methionine levels and prebiotic presence carcass yield. There was an overall non-significant effect of treatments on giblet weights including the liver, gizzard and heart.

Carcass parameters exhibited significance with the lower level of zinc-methionine in conjunction with prebiotics (ZnP40), a result consistent with the findings of Jahanian et al. (2008), who demonstrated that supplementation of zinc from organic sources improved live weight and breast yield. Similarly, Jahanian and Rasouli (2015) reported an increase in carcass yield and liver weight in broilers fed zinc methionine as a replacement for zinc from inorganic sources. Additionally, Ashraf et al. (2019) highlighted the significant impact of prebiotics on dressing percentage and thigh muscle yield, aligning with our observations of progressive impacts on carcass traits due to prebiotics. However, in contrast to our study, Yalçın et al. (2013) found that the combination of prebiotics and zinc-methionine did not improve carcass yield and visceral organ percentage in broilers.

Gut Development: Table 4.5 presents the results on Gut development. The interaction between zinc-methionine levels and prebiotic presence significantly influenced villus height (VH) and the villus height to crypt depth ratio (VH:CD). The VH was significantly higher ($P < 0.05$) in the ZnP40 treatment (1353.06 µm) compared to Zn40 (1204.54 µm) and Zn80 (1237.40 µm), but was not significantly different from ZnP80 (1287.18 µm). This indicates that the combination of a lower level of zinc-methionine with prebiotic significantly enhances villus height. No significant differences were observed in CD across all treatments.The VH:CD ratio was significantly higher ($P < 0.05$) in ZnP40 (8.89) compared to Zn40 (7.63) and Zn80 (7.79), but not significantly different from ZnP80 (8.28), indicating an improved gut morphology with the combined supplementation of zinc-methionine and prebiotic.

The interactive effects of dietary zinc-methionine and prebiotics on gut development parameters reveal noteworthy improvements, particularly in villus height and VH:CD ratio, when both supplements are used in combination at lower levels. This synergistic effect suggests enhanced nutrient absorption and overall gut health in broilers. This is consistent with Bao et al. (2009), who found that zinc-methionine supplementation enhanced villus height and intestinal architecture in broilers. El-Katcha et al. (2017) also reported that zinc-methionine or nano zinc improved intestinal villi length, width, and crypt depth.

The significant increase in villus height with ZnP40 is consistent with findings by Jahanian et al. (2008), who reported improved intestinal morphology with zinc from organic sources. The enhancement of VH:CD ratio in the presence of prebiotics corroborates the work of Ashraf et al. (2019), who demonstrated the positive impact of prebiotics on gut health and morphology. Conversely, Yalçın et al. (2013) found no improvement in gut development with combined prebiotics and zinc-methionine supplementation, which may be attributed to differences in experimental conditions or broiler strains. Oliveira et al. (2008) observed that mannan oligosaccharides in broiler diets improved gut health, aligning with our findings. However, the current study did not observe significant improvements in CD with zinc-methionine or prebiotic supplementation, similar to the findings of Yang et al. (2009), who reported minimal improvement in crypt depth in broilers fed with MOS.

Immune Response and Livability: In Table 4.4 it can be observed that the supplementation of zinc-methionine and prebiotic showed significant interactive effects on immune organ weights and antibody titers. The spleen weight was significantly higher ($P < 0.05$) in the ZnP80 group (3.05 g) compared to other treatments. Similarly, bursa weight was highest in ZnP80 (2.27 g). Antibody titers at 20 and 35 days were also significantly elevated in the ZnP80 group, with titers of 6.55 and 7.35, respectively. Livability remained unaffected across all treatments.

The increased spleen and bursa weights in the ZnP80 group align with previous findings by Bao et al. (2009), who reported improved immune organ development with zinc-methionine supplementation. El-Katcha et al. (2017) also noted that zinc-methionine enhances immune response, supporting the observed increase in antibody titers. Oliveira et al. (2008) highlighted the positive impact of mannan oligosaccharides on gut health and immune function, which corresponds with our findings of enhanced immune organ weights and antibody titers.

In a study by Zakeri et al. (2011), elevated cellular and humoral immune potential was observed with the addition of MOS in the broiler diet. Lopetuso et al. (2019) reported that prebiotics improve the bird's immune system by promoting the growth of beneficial microflora, which produce short-chain fatty acids and stimulate immune organs, leading to an enhanced immune response. These findings support the current study, where supplementation of MOS significantly improved the immune response against the NDV vaccine. Thus it can be concluded that the combination of zinc-methionine and prebiotics potentially creates a synergistic effect that boosts the immune system more effectively than either component alone.

CONCLUSION

The study found that combining lower doses of supplemental zinc-methionine (40mg/kg, ZnP40) with prebiotics significantly improved broiler performance, carcass traits, and immunity. This suggests a more effective approach compared to using zinc-methionine alone or at higher doses. Such precision in dietary supplementation could optimize broiler production outcomes.

IMPLICATIONS

The implications of the study are twofold. Firstly, it underscores the importance of considering synergistic effects between dietary supplements, such as zinc-methionine and prebiotics, in broiler nutrition. Secondly, the study suggests a more efficient and cost-effective approach to broiler nutrition management. Rather than relying solely on higher doses of individual supplements, such as zinc-methionine, the combination with prebiotics at lower doses yields comparable or even superior results.

Overall, the findings emphasize the importance of precision in dietary formulation and the potential benefits of synergistic supplement combinations in enhancing broiler production efficiency while minimizing resource inputs. Further research in this area could lead to refined dietary strategies for maximizing broiler health and productivity.

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TABLES

Table 1. Feed Formulation of basal diet for Starter and grower phases as per the recommendation of Ross 308

Ingredient %	Starter	Grower
Maize	39.18	42.18
Wheat grains	10.00	8.00
Rice Polish	7.70	9.70
Soy Bean Meal	23.40	19.40
Canola Meal	11.00	12.00
Guar Meal	3.00	3.00
Oil	2.50	2.50
Limestone	0.60	0. 60
DL-Meth	0.10	0.10
Lysine Sulphate	0.40	0.40
Premix [1]	0.20	0.20
Choline	0.10	0.10
Soda Bicarb	0.30	0.30
Salt	0.10	0.10
DCP	1.42	1.41
Total	**100.00**	**100.00**
Composition		
Energy (ME, Kcal/kg)	3000	3050
CP %	23.00	21.50
EE %	4.15	4.85
CF %	3.65	4.50
Ash%	3.00	4.00
Calcium %	0.96	0.87
Phosphorus %	0.48	0.43
Zinc mg/kg 2	26.24	28.67
Lysine %	1.44	1.29
Methionine %	1.64	1.46

1. Zn free premix was supply per kg of diet: manganese 80mg, copper 8mg, iron 45 mg, iodine 1 mg, selenium 0.2 mg, cobalt 0.1 mg, choline chloride 1000 mg, vit. A 12,000 IU, vit. D3 4000IU, vit. E 60 mg, vit K3 3 mg, vit. B1 2 mg, vit. B2 6.5 1 mg; vit. B 6 5 mg; vit. B12 0.02 mg, pantothenic 12 mg, nicotinic 45 mg, folic acid 2 mg, biotin 0.08 mg.
2. Availa Zinc® was supplemented at a dose rate of 335gm/ton of feed to make the supplemental zinc-methionine at 40mg/kg while Availa Zinc® was supplemented at a dose rate of 670gm/ton of feed to make the supplemental zinc-methionine at 80mg/kg in the experimental.
3. ActiveMos® as prebiotic was supplemented at a dose of 1g/kg in experimental diet of ZnP40 and in ZnP80.

Table 2: Effects of supplementation of zinc-methionine and prebiotic on performance of broilers

	Treatments[1]							
	Zn40		Zn80			[2]P-value		
Parameters	Zn40	ZnP40	Zn80	ZnP80	***SEM***	ZL	PP	ZL × PP
Feed Intake *(g/bird)*	3207.91[c]	3186.77[c]	3291.72[b]	3392.94[a]	***23.00***	*0.000*	*0.083*	*0.032*
Weight Gain *(g/bird)*	1995.07[c]	2182.83[a]	2046.82[c]	2110.38[b]	***19.91***	*0.583*	*0.000*	*0.005*
FCR *(intake/gain)*	1.61[a]	1.46[b]	1.60[a]	1.61[a]	***0.02***	*0.001*	*0.001*	*0.000*

[a-c] different superscript within a row indicate significantly difference ($P \leq 0.05$)

[1]Treatments, Zn40 = Diet containing 40 mg/kg supplemental zinc-methionine, ZnP40 = Diet containing 40 mg/kg supplemental zinc-methionine + 1 mg/kg Mannon Oligo Saccharides (MOS), Zn80 = Diet containing 80 mg/kg supplemental zinc-methionine, ZnP80 = Diet containing 40 mg/kg supplemental zinc-methionine + 1 mg/kg Mannon Oligo Saccharides (MOS)

[2]ZL= Zinc-methionine Level (40mg *vs.* 80mg); PP= Prebiotic Presence [not added (-) *vs.* added (+)], ZL× PP = Zinc-methionine Level × Prebiotic Presence

Table 3: Solitary and combined effect of dietary zinc-methionine and prebiotic on carcass traits in broilers

Parameters	Treatments[1]				SEM	[2]P-value		
	Zn40		Zn80					
	Zn40	ZnP40	Zn80	ZnP80	*SEM*	ZL	PP	ZL × PP
Live wt. (g)	2035.08[c]	2222.84[a]	2086.83[c]	2150.36[b]	***19.91***	*0.582*	*0.000*	*0.005*
Carcass wt. (g)	1359.75[b]	1596.64[a]	1386.88[b]	1530.39[a]	***27.48***	*0.419*	*0.000*	*0.000*
Carcass (%)	66.83[b]	71.82[a]	66.46[b]	71.17[a]	***0.75***	*0.589*	*0.000*	*0.002*
Breast (g)	334.78[b]	417.61[a]	337.43[b]	403.34[a]	***10.47***	*0.523*	*0.000*	*0.001*
Breast (%)	24.61[b]	26.16[a]	24.33[b]	26.36[a]	***0.30***	*0.923*	*0.001*	*0.008*
Thigh (g)	204.25[b]	252.71[a]	209.42[b]	241.38[a]	***5.65***	*0.488*	*0.000*	*0.000*
Thigh (%)	15.03	15.84	15.11	15.79	***0.19***	*0.967*	*0.058*	*0.853*
Liver (g)	45.11	48.39	48.25	49.13	***0.99***	*0.360*	*0.328*	*0.567*
Liver (%)	3.32	3.04	3.48	3.21	***0.08***	*0.303*	*0.099*	*0.962*
Gizzard (g)	38.65	39.25	39.15	39.00	***0.91***	*0.623*	*0.549*	*0.827*
Gizzard (%)	2.58	2.52	2.51	2.54	***0.04***	*0.827*	*0.868*	*0.636*
Heart (g)	11.38	11.84	11.19	11.55	***0.21***	*0.476*	*0.239*	*0.584*
Heart (%)	0.76	0.75	0.81	0.76	***0.01***	*0.277*	*0.173*	*0.482*

[a-c] different superscript within a row indicate significantly difference ($P \leq 0.05$).
[1]Treatments, Zn40 = Diet containing 40 mg/kg supplemental zinc-methionine, ZnP40 = Diet containing 40 mg/kg supplemental zinc-methionine + 1 mg/kg Mannon Oligo Saccharides (MOS), Zn80 = Diet containing 80 mg/kg supplemental zinc-methionine, ZnP80 = Diet containing 40 mg/kg supplemental zinc-methionine + 1 mg/kg Mannon Oligo Saccharides (MOS).
[2]ZL= Zinc-methionine Level (40mg *vs.* 80mg); PP= Prebiotic Presence [not added (-) *vs.* added (+)], ZL× PP = Zinc-methionine Level × Prebiotic Presence

Table 4. Solitary and combined effect of dietary zinc-methionine and prebiotic on gut development in broilers

	Treatments[1]					[2]P-value		
	Zn40		Zn80					
Parameters	Zn40	ZnP40	Zn80	ZnP80	***SEM***	ZL	PP	ZL × PP
VH	1204.54[b]	1353.06[a]	1237.40[b]	1287.18[ab]	***20.47***	*0.620*	*0.009*	*0.035*
CD	158.81	154.01	161.38	157.09	***2.39***	*0.592*	*0.393*	*0.962*
VH: CD	7.63[b]	8.89[a]	7.79[b]	8.28[ab]	***0.16***	*0.356*	*0.003*	*0.010*

[a-c] different superscript within a row indicate significantly difference ($P \leq 0.05$)
[1]Treatments, Zn40 = Diet containing 40 mg/kg supplemental zinc-methionine, ZnP40 = Diet containing 40 mg/kg supplemental zinc-methionine + 1 mg/kg Mannon Oligo Saccharides (MOS), Zn80 = Diet containing 80 mg/kg supplemental zinc-methionine, ZnP80 = Diet containing 40 mg/kg supplemental zinc-methionine + 1 mg/kg Mannon Oligo Saccharides (MOS)
[2]ZL= Zinc-methionine Level (40mg *vs.* 80mg); PP= Prebiotic Presence [not added (-) *vs.* added (+)], ZL× PP = Zinc-methionine Level × Prebiotic Presence

Table 5. Solitary and combined effect of dietary zinc-methionine and prebiotic on immunity and livability in broilers

	Treatments[1]							
	Zn40		Zn80			[2]P-value		
Parameters	Zn40	ZnP40	Zn80	ZnP80	***SEM***	ZL	PP	ZL × PP
Spleen (g)	2.37^{c}	2.98^{a}	2.20^{bc}	2.68^{ab}	***0.09***	*0.075*	*0.001*	*0.003*
Bursa (g)	1.78^{c}	2.21^{a}	1.83^{bc}	2.05^{ab}	***0.06***	*0.468*	*0.001*	*0.006*
Titer 20 d	5.14^{b}	6.33^{a}	5.57^{b}	6.34^{a}	***0.15***	*0.143*	*0.001*	*0.000*
Titer 35 d	6.70^{b}	7.43^{a}	6.60^{b}	7.61^{a}	***0.14***	*0.824*	*0.000*	*0.002*
Livability	99.00	99.00	99.50	99.50	***0.25***	*0.372*	*1.000*	*1.000*

[a-c] different superscript within a row indicate significantly difference ($P \leq 0.05$)

[1]Treatments, Zn40 = Diet containing 40 mg/kg supplemental zinc-methionine, ZnP40 = Diet containing 40 mg/kg supplemental zinc-methionine + 1 mg/kg Mannon Oligo Saccharides (MOS), Zn80 = Diet containing 80 mg/kg supplemental zinc-methionine, ZnP80 = Diet containing 40 mg/kg supplemental zinc-methionine + 1 mg/kg Mannon Oligo Saccharides (MOS)

[2]ZL= Zinc-methionine