

## Effect of single or combined supplementation of zinc and probiotics on muscle and bone characteristics and haematobiochemical profile in broilers

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**Abstract:** The study was conducted to elucidate the effect of a single or combined supplementation of zinc sulphate monohydrate ( $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ ) and a probiotic (Protexin<sup>®</sup>) on the pectoral muscle, tibial bone and serum biochemistry in broilers. Day-old chicks ( $n = 192$ ) were divided into: Control (basal diet), Zn30 (basal diet +  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$  30 mg/kg feed), Zn60 (basal diet +  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$  60 mg/kg feed), Pro (basal diet + Protexin<sup>®</sup> 0.1 g/kg feed), Com30 (basal diet +  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$  30 mg/kg feed + Protexin<sup>®</sup> 0.1 g/kg feed) and Com60 (basal diet +  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$  60 mg/kg feed + Protexin<sup>®</sup> 0.1 g/kg feed). The birds were slaughtered on the 42<sup>nd</sup> day and blood was collected to determine the cortisol, cholesterol and zinc concentrations. The pectoral muscle was selected for the assessment of the pH and water holding capacity (WHC) along with the histological sections. After defleshing, tibial bone measurements were also taken. Statistical analysis revealed a significant ( $P < 0.05$ ) increase in the ultimate pH (pHu), WHC and muscle fascicle diameter in the Zn30, Zn60, Pro, Com30 and Com60 groups compared to the control group. Myofibre area showed a significant increase ( $P > 0.05$ ) in the Com30 and Com60 groups against the control group. The medullary canal diameter of the tibia was smaller ( $P > 0.05$ ) in the Zn60, Pro, Com30 and Com60 groups than in the control group. The tibiotarsal index was greater ( $P > 0.05$ ) in the Zn60, Pro, Com30 and Com60 groups. The serum cholesterol was decreased ( $P > 0.05$ ) in the Pro and Com30 groups when compared to the control group. It is concluded that zinc sulfate monohydrate and probiotic supplementation individually or in combination positively affected the histological characteristics of the muscle, tibial bone strength and haematobiochemical indicators.

**Keywords:** zinc sulphate monohydrate; Protexin<sup>®</sup>; meat; tibia; cholesterol

Consumers are increasingly aware of the importance of meat quality and demand high quality poultry meat attainable through proper

selection, meat handling and cooking. The quality of the meat depends on various factors including the ultimate pH, water holding capacity and

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fibre characteristic traits (Rehfeldt and Kuhn 2006; Shah et al. 2019a). The fibre characteristic traits are the fibre size, fibre cross-sectional area and total number of fibres (Joo et al. 2013; Shah et al. 2019b). The muscle mass is mainly composed of muscle fibres; therefore, muscle mass is generally determined by the number and size of the muscle fibres (Rehfeldt et al. 1999; Alam et al. 2019). The tenderness of the meat correlates to the muscle fibre thickness (Genchev et al. 2008). Also, the storage and processing quality of the meat depends on the pH values and the water holding capacity. The early decline of the pH *post mortem* leads to a decreased water holding capacity (WHC) of the muscle that ultimately causes a pale coloration (Owens et al. 2000). The decrease in WHC may compromise the processing of the meat by-products (Garcia et al. 2010).

The tibia plays a key role in supporting the broilers' weight and the bone is an important source of minerals required for metabolic needs. The bone strength can be checked through the bone length, weight and breaking strength (Sahraei et al. 2012).

Nutritional supplements are used to improve meat quality and bone development to some extent. These nutritional supplements are trace minerals, such as selenium (Se) and zinc (Zn), and also probiotics (Khan et al. 2014; Chand et al. 2019). Zn plays an important role in improving the quality of the meat (Naz et al. 2016). It increases the water-holding capacity of the muscle through an increase in the ultimate pH (Liu et al. 2011). It has an effect on the tibia through increasing the thickness of its wall, the tibiotarsal index, the medullary canal diameter and the ash content (Sahraei et al. 2012). It further enhances the plasma Zn (Mohanna and Nys 1999; Khan et al. 2011) and cholesterol concentration (Al-Daraji and Amen 2011; Salim et al. 2012) in broiler chickens. Similarly, probiotics have been documented to increase the breast muscle size, improve the meat quality (Zhang et al. 2005; Khan and Naz 2013) and wall thickness of the tibial and the tibiotarsal index as well as the bone ash percentage in broilers (Mutus et al. 2006). It also decreases the blood cholesterol and cortisol concentration in broilers (Al-Kassie et al. 2008; Mansoub 2010). Protexin<sup>®</sup> (Probiotics International Ltd, Somerset, UK) is a multi-species probiotic containing bacterial strains of *Lactobacillus plantarum* ( $1.89 \times 10^{10}$  cfu/kg), *Lactobacillus acidophilus* ( $3.09 \times 10^{10}$  cfu/kg), *Lactobacillus delbrueckii* sub-

sp. *Bulgaricus* ( $3.09 \times 10^{10}$  cfu/kg), *Bifidobacterium bifidum* ( $3.00 \times 10^{10}$  cfu/kg), *Lactobacillus rhamnosus* ( $3.09 \times 10^{10}$  cfu/kg), *Enterococcus faecium* ( $8.85 \times 10^{10}$  cfu/kg), *Streptococcus salivarius* subsp. *Thermophilus* ( $6.15 \times 10^{10}$  cfu/kg) and the yeasts *Aspergillus oryzae* ( $7.98 \times 10^9$  cfu/kg) and *Candida pintolopesii* ( $7.98 \times 10^9$  cfu/kg) (Khan et al. 2013). It is hypothesised that a combined supplementation of Zn and a probiotic (Protexin<sup>®</sup>) may have a more pronounced effect on the muscle characteristics and bone strength of broilers. Since limited reports are available on the effect of a single or combined supplement of zinc and probiotics on the muscle and bone development, the present study is focused on the effect of the single or combined supplementation of zinc and probiotic (Protexin<sup>®</sup>) on the muscle quality, bone development and serum biochemistry of broilers.

## MATERIAL AND METHODS

The procedures followed in this experiment were performed after proper approval from the ethical review committee, the University of Veterinary and Animal Sciences Lahore, Pakistan.

### Study design and dietary plan

The one hundred and ninety-two (192) day-old broiler chicks (Cobb 500) used in this study were purchased from a local hatchery (Pakistan Birds, 14-km Multan road Lahore, Punjab). They were randomly assigned to six groups comprised of: Control (basal diet), Zn30 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 30 mg/kg feed), Zn60 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 60 mg/kg feed), Pro (basal diet + Protexin<sup>®</sup> 0.1 g/kg feed), Com30 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 30 mg/kg feed + Protexin<sup>®</sup> 0.1 g/kg feed) and Com60 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 60 mg/kg feed + Protexin<sup>®</sup> 0.1 g/kg feed). Each group consisted of four replicates (8 birds per replicate). The birds were reared under standard management conditions in an environmentally controlled shed for 42 days at the University of Veterinary and Animal Sciences Lahore, Pakistan (Shah et al. 2019a). The temperature of 35 °C was maintained from the 1<sup>st</sup> day and decreased by 3 °C per week for three weeks, i.e., day 21 and maintained at 26 °C till the end of experiment (Shah et al. 2019b).

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The birds were fed on a corn-based basal feed. The basal diet was formulated using National Research Council (1994) guidelines. The composition of the basal diet is presented in Table 1. Two dietary phases, starter (days 0–21) and grower (days 22–42) were adopted. The feed and water were provided *ad libitum*.

## pH of muscle

At day 42, two birds from each replicate (8 birds/group) were randomly selected. The birds were

killed by cervical dislocation. The right superficial pectoral muscle was selected for the determination of the pH. The initial pH (pHi) was measured by a pH meter (Portable meat pH meter HI 99163; Hanna, Padova, Italy) with a conventional electrode probe through inserting it 1 cm deep into the superficial pectoral muscle (Shah et al. 2019b). For the ultimate pH (pHu), the piece of muscle was stored at 4 °C for 24 h and then the pHu was recorded. The pHu was noted in the same manner as the pHi.

## Water holding capacity (WHC)

For the water holding capacity (WHC), an approximate 25 g piece from the left superficial muscle was selected. The muscle piece was wrapped in a non-absorbable inflated bag, hung in a tightly packed bottle and then kept at 4 °C for 24 hours. Thereafter, the muscle piece was removed from the bottle, wiped with tissue paper, weighed using an electronic balance (Uniblock UX320; Shimadzu, Shiga, Japan) and the WHC was calculated (Rasmussen and Andersson 1996).

Table 1. The feed ingredients and composition of the starter and grower diets for the broilers

Feed ingredients (%)	Starter	Grower
Corn	40.15	57.57
Rice broken	15.0	–
Rice polish	–	4.00
Wheat bran	1.34	–
Soya meal	11.54	9.60
Sunflower meal	12.00	13.00
Canola meal	9.00	5.00
Rapeseed meal	5.00	7.60
Guar meal	1.00	–
Molasses	2.00	–
Dicalcium phosphate	1.73	1.96
Premix*	1.00	1.00
Sodium chloride	0.21	0.21
Sodium bicarbonate	0.03	0.065
Proximate composition (%)		
Crude protein	19.6	18.5
Crude fibre	1.26	1.80
Crude fat	2.16	2.35
Total ash	5.77	5.40
Calculated apparent metabolizable energy (Kcal/kg)	2 750	2 850

\*Vitamin mineral premix (each kg contained): K, 70 g; Ca, 195 g; Mg, 6 g; Na, 18 g; Zn, 2 837 mg; Cu, 400 mg; Fe, 2 000 mg; Se, 8 mg; I, 40 mg; Mn, 1 200 mg; Co, 20 mg; vitamin D3, 80 000 IU; vitamin A, 200 000 IU; vitamin K3, 34 mg; vitamin E, 1 072 IU; Thiamine, 35 mg; Riboflavin, 135 mg; Ascorbic acid, 1 300 mg; vitamin B6, 100 mg; Niacin, 1 340 mg; vitamin B12, 670 µg; folic acid, 34 mg; and biotin, 3 350 µg

## Muscle histomorphometry

For the muscle histomorphometry, one cm<sup>3</sup> of the muscle piece from the left superficial pectoral muscle was selected and preserved in a 10% neutral buffered formalin solution. The tissues were processed through the paraffin embedding technique (Bancroft et al. 2013). Microscopic sections (5 µm) were obtained using a semi-automatic rotary microtome (AEM 450; Amos Scientific, Melbourne, Australia) and stained with Haematoxylin and Eosin (H&E).

The muscle fibre diameter was determined from the cross-section of the H&E stained microscopic slides. The muscle fibre diameter was calculated at × 100 by the Morphometry Program (Progress Capture Pro 2.7.7.; Labomed, Culver City, CA, USA). Five muscle fibres, each from three random fascicles of the microscopic slides, were selected and the average was considered as the fibre diameter. Similarly, the diameter of the fascicle at × 40 was measured (Saleem 2012). The cross-sectional area of the muscle fibre was measured from the diameter of the muscle fibre. The muscle fibre number per unit area (muscle fibres/mm<sup>2</sup>) was calculated.

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### Bone characteristics

The left tibia bone was separated, boiled at 100 °C for ten minutes and defleshed. The bones were then air-dried and weighed using a digital balance. The length, outer diameter and medullary canal diameter were measured using a digital Calliper. The wall thickness was determined by subtraction of the medullary canal diameter from the diaphysis diameter. The bone weight/length index, Robusticity index and tibiotarsal index were determined using the following formulae, respectively (Kocabagli 2001):

$$\text{Weight/length index} = \frac{\text{weight (mg)}}{\text{length (mm)}} \quad (1)$$

$$\text{Robusticity index} = \frac{\text{bone length (mm)}}{\text{cube root of bone weight (mg)}} \quad (2)$$

$$\text{Tibiotarsal index} = \frac{(\text{diaphysis diameter} - \text{medullary canal diameter})}{\text{diaphysis diameter}} \times 100 \quad (3)$$

### Serum biochemical indicators

For the serum cortisol, cholesterol and Zn determination, the blood was collected and centrifuged at 3 000 g for 10 min to collect the serum. The serum was stored at –40 °C for further analysis. The serum cortisol was analysed using a commercially available ELISA (Enzyme-linked immunosorbent assay) kit (Monobind 3625-300; Monobind Inc., Lake Forest, CA, USA) and the serum cholesterol was determined using a commercially available kit (Human GmbH, Wiesbaden, Germany). The serum Zn concentration was measured using an atomic absorption spectrophotometer (Z-8230 Polarized Zeeman; Hitachi Inc., Tokyo, Japan).

### Statistical analysis

The results obtained were expressed as a mean  $\pm$  SEM. To compare the means, a one-way ANOVA (analysis of variance) was used through the statistical software (SPSS Inc., v20; Chicago, IL, USA).

The group differences were compared by Tukey's post hoc test. The differences were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Muscle physical and histomorphological characteristics

The effect of ZnSO<sub>4</sub>·H<sub>2</sub>O and Protexin® on the superficial pectoral muscle is presented in Table 2. The pH<sub>i</sub> of the muscle did not differ ( $P > 0.05$ ) among the groups under study. A significantly ( $P < 0.05$ ) higher ultimate pH (pH<sub>u</sub>) occurred in the control birds than in all the other groups. The water holding capacity (WHC) expressed in terms of the drip loss of the muscle was higher ( $P < 0.05$ ) in all the groups except for the control group. However, it did not vary among the Zn30, Zn60, Pro, Com30 and Com60 groups. The higher pH<sub>u</sub> of the muscle in the Zn-supplemented groups may be attributed to the antioxidant effect of Zn (Aksu et al. 2005). The increase in the pH<sub>u</sub> that was observed in the Pro group is in line with other researchers (Aksu et al. 2005) who reported an increased pH value in breast meat in broiler chickens with a probiotic (*Saccharomyces cerevisiae*) supplementation. The higher WHC in the Zn supplemented groups may be linked with the higher pH<sub>u</sub> as the muscle pH has a positive correlation to the WHC (Qiao et al. 2001). The present results regarding WHC are in line with the findings in earlier studies (Yang et al. 2011). They reported that Zn increased the WHC of the breast meat in broiler chickens. The findings in the present study regarding the WHC agree with those reported in a previous study (Ali 2010) which stated that the probiotic *Bacillus subtilis* increased the WHC in broiler chickens.

The muscle fibre diameter (MFD) of the superficial pectoral muscle in the control group was smaller ( $P < 0.05$ ) than all the other groups. The cross-sectional area (CSA) of the muscle fibre was found to be greater ( $P > 0.05$ ) in the Com30 and Com60 than the control group. A larger ( $P < 0.05$ ) muscle fascicle diameter was observed in the Zn30, Zn60, Pro, Com30 and Com60 groups in relationship to the control group. The CSA of the muscle fascicle did not vary ( $P > 0.05$ ) among the studied groups. The muscle fibre number was recorded as significantly ( $P > 0.05$ ) higher in the control birds compared to all the other groups

<https://doi.org/10.17221/152/2019-VETMED>Table 2. The effects of Zinc sulphate monohydrate ( $ZnSO_4 \cdot H_2O$ ) and Protexin® on the superficial pectoral muscle of the broilers (mean  $\pm$  SEM)

Parameters	Groups*						P-value
	Control	Zn30	Zn60	Pro	Com30	Com60	
pHi	6.34 $\pm$ 0.06	6.31 $\pm$ 0.12	6.43 $\pm$ .06	6.41 $\pm$ 0.07	6.42 $\pm$ 0.04	6.41 $\pm$ .003	0.25
pHu	5.99 $\pm$ .013 <sup>b</sup>	6.06 $\pm$ 0.04 <sup>a</sup>	6.11 $\pm$ 0.03 <sup>a</sup>	6.09 $\pm$ 0.07 <sup>a</sup>	6.13 $\pm$ 0.06 <sup>a</sup>	6.12 <sup>a</sup> $\pm$ 0.04	< 0.01
Drip loss (%)	3.7 $\pm$ 0.29 <sup>a</sup>	3.0 $\pm$ 0.24 <sup>b</sup>	2.5 $\pm$ 0.63 <sup>b</sup>	2.5 $\pm$ 0.57 <sup>b</sup>	2.4 $\pm$ 0.85 <sup>b</sup>	2.4 $\pm$ 0.66 <sup>b</sup>	< 0.01
MFD ( $\mu$ m)	41.7 $\pm$ 2.02 <sup>b</sup>	51.8 $\pm$ 2.17 <sup>a</sup>	52.3 $\pm$ 2.50 <sup>a</sup>	52.9 $\pm$ 2.39 <sup>a</sup>	54.7 $\pm$ 1.97 <sup>a</sup>	54.9 $\pm$ 1.94 <sup>a</sup>	< 0.01
MFCSA ( $\mu$ m <sup>2</sup> )	1 445.3 $\pm$ 547.50 <sup>b</sup>	1 947.3 $\pm$ 681.84 <sup>ab</sup>	2 243.2 $\pm$ 849.77 <sup>ab</sup>	2 214.3 $\pm$ 815.23 <sup>ab</sup>	2 372.7 $\pm$ 697.29 <sup>a</sup>	2 389.5 $\pm$ 647.61 <sup>a</sup>	0.03
MFSD (mm)	0.6 $\pm$ 0.06 <sup>b</sup>	0.9 $\pm$ 0.03 <sup>a</sup>	1.0 $\pm$ 0.13 <sup>a</sup>	1.0 $\pm$ 0.05 <sup>a</sup>	1.0 $\pm$ 0.04 <sup>a</sup>	1.1 $\pm$ 0.12 <sup>a</sup>	0.01
MFSCSA (mm <sup>2</sup> )	0.3 $\pm$ 0.28	0.7 $\pm$ 0.16	0.9 $\pm$ 1.02	0.7 $\pm$ 0.28	0.7 $\pm$ 0.28	0.9 $\pm$ 1.02	0.12
MFN/mm <sup>2</sup>	448.6 $\pm$ 42.95 <sup>a</sup>	348.7 $\pm$ 25.34 <sup>b</sup>	335.8 $\pm$ 12.83 <sup>b</sup>	289.6 $\pm$ 12.75 <sup>b</sup>	298.3 $\pm$ 15.77 <sup>b</sup>	290.5 $\pm$ 19.74 <sup>b</sup>	< 0.01

The values in the rows with the different superscript are significantly different at  $P < 0.05$

\*Control (basal diet), Zn30 (basal diet +  $ZnSO_4 \cdot H_2O$  30 mg/kg feed), Zn60 (basal diet +  $ZnSO_4 \cdot H_2O$  60 mg/kg feed), Pro (basal diet + Protexin® 0.1 g/kg feed), Com30 (basal diet +  $ZnSO_4 \cdot H_2O$  30 mg/kg feed + Protexin® 0.1 g/kg feed) and Com60 (basal diet +  $ZnSO_4 \cdot H_2O$  60 mg/kg feed + Protexin® 0.1 g/kg feed)

MFCSA = muscle fibre cross sectional area; MFD = muscle fibre diameter; MFN = muscle fibre number; MFSCSA = muscle fascicle cross sectional area; MFSD = muscle fascicle diameter; pHi = initial pH; pHu = ultimate pH

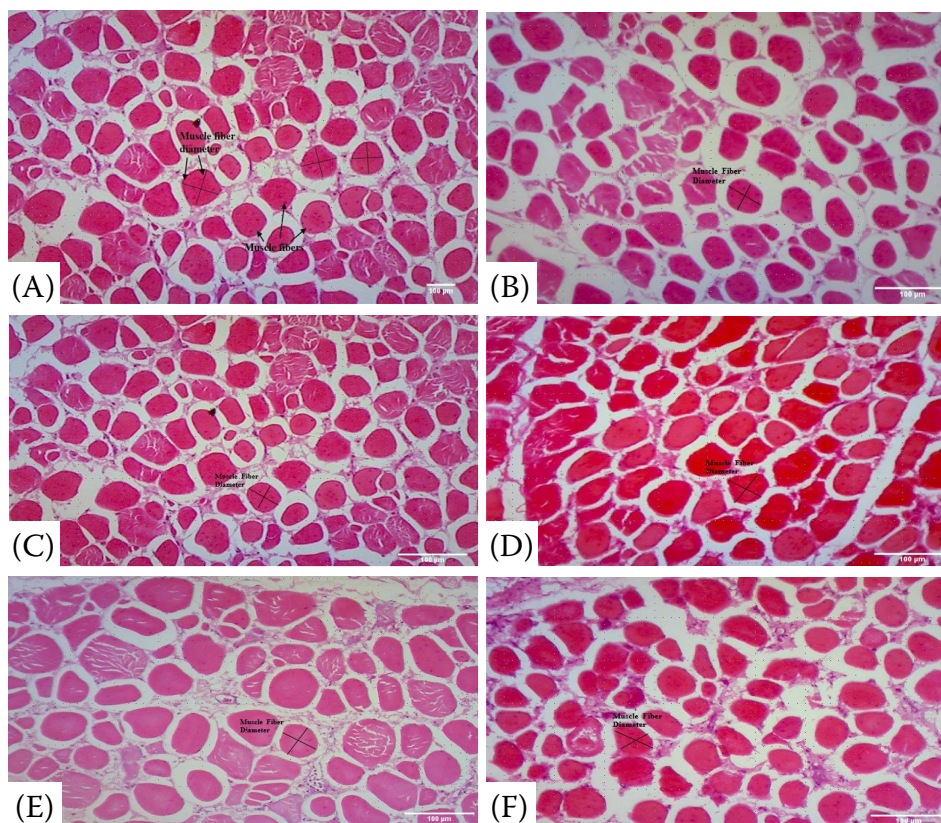


Figure 1. The cross-sectional histomicrograph of the superficial pectoral muscle of the different groups representing the muscle fibre diameter at  $\times 100$  (Haematoxylin and Eosin stain)

Group description: A: Control (basal diet), B: Zn30 (basal diet +  $ZnSO_4 \cdot H_2O$  30 mg/kg feed), C: Zn60 (basal diet +  $ZnSO_4 \cdot H_2O$  60 mg/kg feed), D: Pro (basal diet + Protexin® 0.1 g/kg feed), E: Com30 (basal diet +  $ZnSO_4 \cdot H_2O$  30 mg/kg feed + Protexin® 0.1 g/kg feed) F: Com60 (basal diet +  $ZnSO_4 \cdot H_2O$  60 mg/kg feed + Protexin® 0.1 g/kg feed)

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under study (Figure 1). In the Zn30 and Zn60 groups, the increased diameter may be associated with Zn which is involved in the synthesis of protein, thus resulting in the hypertrophy of the myocytes, ultimately leading to the build-up and enhanced development of the muscles. The increased muscle fibre diameter in the Pro group may be attributed to the probiotic supplementation having a growth-promoting effect. The muscle fibre cross-sectional area was higher in the Com30 and Com60 groups when compared to the control birds which might be due to the synergistic effect of Zn and Protexin®. The muscle fascicle diameter was enhanced in all the groups of Zn30, Zn60, Pro, Com30 and Com60 except for the control one. The increased muscle fascicle diameter in the Zn30, Zn60, Pro, Com30 and Com60 groups might be correlated with the increased muscle fibre diameters in these groups as the muscle fascicle diameter has a positive correlation to the muscle fibre diameter. The higher number of muscle fibres/unit area were lower in the control group compared to all the other groups. The increased number of muscle fibres/unit area in the control group may be linked to the packing density of the higher number of muscle fibres having small diameters within a unit area.

**Bone characteristics**

The supplementation of Zn and probiotics is known to improve the bone characteristics. In chicken meat, leg disorders occur due to rapid bone growth. Severe disorders impair the walking ability, and result in starvation and dehydration, thus increasing mortality (Mutus et al. 2006). The statistical analysis revealed that a single or combined supplementation of ZnSO<sub>4</sub>·H<sub>2</sub>O and Protexin® did not affect any of the bone characteristics including the weight, length, W/L (weight to length) index, tibiotarsal index and robusticity index of the tibia bone (Table 3). The length increased (*P* > 0.05) in the Pro and Com30 group when compared to the control group. The results about the aforementioned parameter were in parallel with the findings in the previous study (Vahdatpour et al. 2014) reporting an increased length of the tibia bone in Japanese quails with a Protexin® supplementation. The increased tibia length may be due to the probiotics as they are involved in the synthesis of vitamins (Hancock and Viola 2001). Vitamins K, D and C are involved in calcium metabolism and are essential for the bone formation (Weber 1999). The increased length in the Com30 group may be

Table 3. The effect of ZnSO<sub>4</sub>·H<sub>2</sub>O and Protexin® on the tibia bone characteristics of the broilers (mean ± SEM)

Parameters	Groups*						P-value
	Control	Zn30	Zn60	Pro	Com30	Com60	
Weight (g)	6.22 ± 0.16	6.33 ± 0.39	6.41 ± 0.37	7.05 ± 0.29	7.47 ± 0.26	6.67 ± 0.28	0.04
Length (mm)	95.92 ± 0.84 <sup>b</sup>	95.43 ± 0.93 <sup>b</sup>	97.85 ± 0.86 <sup>ab</sup>	100.35 ± 0.94 <sup>a</sup>	99.84 ± 0.98 <sup>a</sup>	98.29 ± 0.64 <sup>ab</sup>	< 0.01
Wall thickness (mm)	1.66 ± 0.29 <sup>b</sup>	2.07 ± 0.30 <sup>b</sup>	4.41 ± 0.26 <sup>a</sup>	4.69 ± 0.24 <sup>a</sup>	4.81 ± 0.47 <sup>a</sup>	4.12 ± 0.38 <sup>a</sup>	< 0.01
Diameter (mm)	6.69 ± 0.31 <sup>c</sup>	7.17 ± 0.26 <sup>bc</sup>	8.25 ± 0.19 <sup>a</sup>	8.22 ± 0.21 <sup>a</sup>	8.58 ± 0.24 <sup>a</sup>	7.83 ± 0.18 <sup>ab</sup>	< 0.01
Med canal diameter (mm)	5.02 ± 0.12 <sup>a</sup>	5.11 ± 0.87 <sup>a</sup>	3.84 ± 0.25 <sup>b</sup>	3.54 ± 0.13 <sup>b</sup>	3.77 ± 0.40 <sup>b</sup>	3.72 ± 0.28 <sup>b</sup>	< 0.01
W/L index	64.89 ± 1.70	66.19 ± 3.85	65.50 ± 3.75	70.22 ± 2.53	74.89 ± 2.56	67.89 ± 2.62	0.17
Tibiotarsal index	23.79 ± 3.69 <sup>b</sup>	28.02 ± 3.31 <sup>b</sup>	53.39 ± 2.67 <sup>a</sup>	56.79 ± 1.89 <sup>a</sup>	55.73 ± 4.53 <sup>a</sup>	52.21 ± 4.27 <sup>a</sup>	< 0.01
Robusticity Index	5.22 ± 0.06	5.19 ± 0.09	5.30 ± 0.12	5.24 ± 0.05	5.12 ± 0.07	5.23 ± 0.06	0.69

The values in the rows with the different superscript are significantly different at *P* < 0.05

\*Control (basal diet), Zn30 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 30 mg/kg feed), Zn60 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 60 mg/kg feed), Pro (basal diet + Protexin® 0.1 g/kg feed), Com30 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 30 mg/kg feed + Protexin® 0.1 g/kg feed) and Com60 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 60 mg/kg feed + Protexin® 0.1 g/kg feed)

due to the synergistic effect of the Zn with the probiotics. The diaphysis diameter showed a significant increase ( $P < 0.05$ ) in the Zn60, Pro, Com30 and Com60 groups than the control group. The results in the current study were contrary to the findings reported by other researchers (Mutus et al. 2006; Sahraei et al. 2012). They reported no effect of the Zn and probiotics on the diaphysis diameter of the tibia bone in broilers. The greater diaphysis diameter in the Com30 and Com60 group in the current study may be attributed to the combined effect of the Zn and probiotics. However, we did not find any study in the literature regarding the combined supplementation of Zn and probiotics on the tibia bone to compare and discuss our results. The medullary canal diameter was lower ( $P > 0.05$ ) in the Zn60, Pro, Com30 and Com60 group than in the control group. The results in the present study are in agreement with the previous studies (Mutus et al. 2006; Sahraei et al. 2012). The decreased medullary canal diameter with an individual or combined supplementation can be related to the increased wall thickness of the tibia bone as proven in the current study. In the present study, the tibiotarsal index was greater with the individual or combined supplementation of Zn and probiotics that suggests the better mineral deposition and development of the bone as reported in an earlier study (Hancock and Viola 2001).

### Serum biochemical profile

The effect of ZnSO<sub>4</sub>·H<sub>2</sub>O and Protexin® on the serum biochemical indicators is presented in Table 4. The results show that a single or combined supple-

mentation of ZnSO<sub>4</sub>·H<sub>2</sub>O and Protexin® had no effect on the cortisol concentration among the groups in the broiler chickens.

The serum cholesterol concentration decreased in the birds fed with the probiotics alone or in combination with Zn30 (30 mg/kg), whereas the cholesterol concentration increased with the Zn60 (60 mg/kg) supplementation. Similar findings regarding the cholesterol have been reported in earlier studies (Mansoub 2010; Al-Daraji and Amen 2011). The decreased cholesterol concentration with the probiotic supplementation in the present study can be related to *L. acidophilus* being an active ingredient of Protexin® that causes the deconjugation of bile acids (glycolic and taurocholic acids) in anaerobic conditions. The deconjugated acids are not able to emulsify and absorb the fatty acids in the small intestine as conjugated acids and, consequently, the cholesterol absorption is prohibited which leads to the decreased cholesterol concentration in the blood (Mansoub 2010). The increased concentration of cholesterol with the dietary Zn supplementation (60 mg/kg) can be attributed to the factors that affect the plasma cholesterol concentration feedback control (Al-Daraji and Amen 2011). The supplementation of Zn (60 mg/kg) increased the concentration of the serum Zn in the current study. The findings about the aforementioned variable were comparable with an earlier study (Mohanna and Nys 1999). They reported an increased plasma Zn concentration with increased concentrations of the dietary Zn supplementation in the broilers. In conclusion, the present study has shown that zinc sulphate and probiotic (Protexin®) exert favourable effects on the pecto-

Table 4. The effect of ZnSO<sub>4</sub>·H<sub>2</sub>O and Protexin® on the serum biochemical indicators of the broilers (mean ± SEM)

Parameters	Groups*						P-value
	Control	Zn30	Zn60	Pro	Com30	Com60	
Cortisol (µg/dl)	0.47 ± 0.08	0.69 ± 0.12	0.61 ± 0.10	0.53 ± 0.10	0.47 ± 0.06	0.47 ± 0.05	0.37
Cholesterol (mg/dl)	134.1 ± 4.82 <sup>bc</sup>	135.9 ± 3.43 <sup>b</sup>	154.1 ± 3.29 <sup>a</sup>	112.7 ± 3.90 <sup>d</sup>	116.0 ± 4.40 <sup>d</sup>	116.9 ± 5.1 <sup>cd</sup>	< 0.01
Zinc (mg/l)	9.87 ± 0.44 <sup>b</sup>	12.0 ± 0.75 <sup>ab</sup>	14.5 ± 0.56 <sup>a</sup>	9.75 ± 0.61 <sup>b</sup>	11.00 ± 0.86 <sup>b</sup>	12.25 ± 0.64 <sup>ab</sup>	< 0.01

The values in the rows with the different superscript are significantly different at  $P < 0.05$

\*Control (basal diet), Zn30 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 30 mg/kg feed), Zn60 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 60 mg/kg feed), Pro (basal diet + Protexin® 0.1 g/kg feed), Com30 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 30 mg/kg feed + Protexin® 0.1 g/kg feed) and Com60 (basal diet + ZnSO<sub>4</sub>·H<sub>2</sub>O 60 mg/kg feed + Protexin® 0.1 g/kg feed)

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ral muscle characteristics as well as the tibia bone characteristics and the serum biochemical profile of broilers.

### Conflict of interest

The authors declare no conflict of interest.

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