Maternal Zinc Supplementation Enhanced Skeletal Muscle Development Through Increasing Protein Synthesis and Inhibiting Protein Degradation of Their Offspring

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Abstract Previous studies proved that maternal zinc supplementation had no significant effect on body weight (BW) of the offspring, but the effects of maternal zinc supplementation on skeletal muscle development of the offspring are poorly defined. Here, broiler breeders at 46 weeks old were allocated into three treatments with six replicates of 40 hens each and fed with diets supplemented with zinc from $ZnSO_4$ at 0 (group Zn/C), 50 mg/kg (group Zn/L), and 300 mg/kg (group Zn/H) respectively for 6 weeks. The male offspring from each dietary treatment were divided into seven cages of ten birds each and fed with a commercial diet with supplemental zinc from $ZnSO_4$ at 20 mg/kg. Results indicated that with the increase of zinc supplementation in hen's diet, the zinc levels were significantly elevated ($P<0.05$) in the egg yolk. Compared with the control group, the breast muscle yield and muscle fiber width were significantly ($P<0.05$) higher and larger in the broilers from group Zn/H at 2 and 5 weeks post-hatch, the phosphorylation of AKT at serine 473 residue (Ser 473), mammalian target of rapamycin (mTOR) at serine 2448 residue (Ser 2448), and FOXO at serine 256 residue (Ser 256) in skeletal muscles of the birds from various dietary treatments at two different age post-hatch were significantly ($P<0.05$) increased. The phosphorylation of mTOR and FOXO was usually related to protein synthesis and degradation. In conclusion, supplemental zinc into the breeders' diet could increase protein synthesis and decrease protein degradation, which, in turn, enhance breast muscle development of the offspring.

Keywords Zinc · Protein synthesis · Protein degradation · Skeletal muscle · Offspring

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Introduction

Skeletal muscle of the animals is the most important tissue from the perspective of animal production and has a lower priority in nutrients partitioning compared with other tissues and organs in response to the challenges the fetus faces during the development, rendering it particularly vulnerable to nutrient deficiency [1]. It is well known that inheritance, environment, maternal nutrition, and other factors have influences on development of the skeletal muscle of the fetus. In mammals, if the fetus cannot obtain nutrients required for development, not only the fetal growth and development but also the newborn offspring's performance could be compromised [2]; therefore, maternal nutrition is important for fetal growth and offspring development [3, 4]. Although birds and mammals are collectively referred to as the amniota, the embryonic development of the chickens is different from the mammals, which is in the shelled eggs outside of the hen's body. Maternal nutrition has an influence on the avian embryonic development through deposition of nutrients in eggs, which is modulated by the nutrients delivery from breeders' blood [5].

The development of skeletal muscle in poultry contained two processes: hyperplasia and hypertrophy. The trait of hyperplasia refers to the number increasing of myoblast completed during the embryonic stage, while the hypertrophy is characterized by the enlargement of muscle fibers during the process of muscle growth post-hatch [6], so the number of myofiber has been set during the hatch [6], and the development of skeletal muscles post-hatch are mainly determined by the increase in muscle fiber diameter [7], which is modulated by the balance between protein synthesis and protein degradation [6]. Only if the rate of protein synthesis is higher than that of protein degradation, the post-natal size of muscle fiber could be increased [6]. The mammalian target of rapamycin (mTOR) signaling pathway is critical for nutrient-stimulated

muscle growth [8, 9], so the mTOR plays a significant role in post-natal development of the skeletal muscle during the changing of maternal nutrition [7]. Protein degradation is also important for the regulation of muscle mass [7]; therefore, the ubiquitin-proteasome system (UPS) may influence the post-natal development of the skeletal muscle by modulating the protein degradation [10].

Zinc, an essential trace element, plays a critical role in the embryo [11] and post-natal [12, 13] development. Zinc was also found to be a signaling molecule at the cellular level, including the regulation of protein kinase phosphorylation [14]. Recent studies have shown that zinc plays a role in the stimulation of mTOR in vivo and in vitro. McClung et al. [15] found that AKT (Ser 473) phosphorylation in skeletal muscles from the mice (C57BL/6J) refeeding a diet with supplemental zinc at 300 mg/kg for 3 and 6 h was significantly increased compared to the fasting group. Tang and Shay [16] stated that the treatments of preadipocytes and adipocytes with up to 200 μmol/L zinc led to a significant increase in Ser 473 phosphorylation of AKT. Barthel et al. [17] reported that zinc has an effect on the activation of AKT, which results in modulation of FOXO family transcription factors. So far, little information about the influence of maternal zinc nutritional status on the skeletal muscle development of the offspring is available. Hence, the objective of the present study was to evaluate the effects of maternal zinc supplementation on the development of the offspring's skeletal muscles and the possible role of mTOR signaling pathway in which the ubiquitin-proteasome system is involved.

Material and Methods

Broiler Breeder Husbandry

All procedures used in the present experiment were approved by the Institutional Animal Care and Use Committee of China Agricultural University. A total of 720 Ross 308 broiler breeding hens at age of 46 weeks were allocated into three treatments with six replicates of 40 hens each and were fed with diets containing zinc at different levels, i.e., (1) Zn/C group (control) was fed with basal diet without supplemental zinc, (2) Zn/L group was fed with supplemental zinc (as $ZnSO_4$) at 50 mg/kg of the diet, and (3) Zn/H group was fed with supplemental zinc (as $ZnSO_4$) at 300 mg/kg of the diet. A corn-soybean meal basal diet (Table 1) was formulated to meet the nutrient requirements of broiler breeders according to the National Research Council [18] except zinc. The content of zinc in diets was 33.52, 82.97, and 324.68 mg/kg respectively based on the actual analysis. All diets were iso-energetic and iso-nitrogenous. All breeders were housed in a completely enclosed, ventilated, conventional caged breeder house in which the light regimen was 16 light (L):8 dark (D).

Table 1 Composition of diets of broiler breeders

	CON	Zn/L	Zn/H
Ingredient (%)			
Corn	63.35	63.35	63.35
Soybean meal	24.40	24.40	24.40
Limestone	8.20	8.20	8.20
Soybean oil	2.00	2.00	2.00
DL-methionine	0.10	0.10	0.10
Choline chloride	0.12	0.12	0.12
Dicalcium phosphate	0.96	0.96	0.96
Sodium chloride	0.35	0.35	0.35
flavomycin	0.015	0.015	0.015
Antioxidants	0.03	0.03	0.03
Phytase	0.04	0.04	0.04
Mycotoxin antidote	0.10	0.10	0.10
Trace mineral premix[a]	0.30	0.30	0.30
Vitamin premix[b]	0.035	0.035	0.035
Total	100	100	100
Nutrient and energy concentration ((calculated except for zinc))			
Metabolizable energy (MJ/kg)	11.76	11.76	11.76
Crude protein (%)	17.30	17.30	17.30
Calcium (%)	3.20	3.20	3.20
Available phosphorus (%)	0.30	0.30	0.30
Methionine (%)	0.38	0.38	0.38
Lysine (%)	0.85	0.85	0.85
Zinc (mg/kg)	33.52	82.97	324.68

[a] Trace mineral premix provided the following from either inorganic or organic sources (per kg of diet): Cu 8 mg, Mn 60 mg, Se 0.3 mg, Fe 80 mg, and I 0.7 mg

[b] Vitamin premix provided the following (per kg of diet): vitamin A 15,000 IU, vitamin D_3 3,000 IU, vitamin E 22.50 IU, vitamin K_3 mg, vitamin B_1 3 mg, vitamin B_2 8 mg, vitamin B_6 6 mg, vitamin B_{12} 0.03 mg, pantothenate acid 17.64 mg, niacin 44 mg, folic acid 1.49 mg, and biotin 0.15 mg

Before the start of the experiment, all hens were fed with a basal diet for 2 weeks to deplete body reserves of zinc. During the 6-week experiment, each female breeder was allotted 155 g of feed at 7:00 a.m. every day. Male breeders were caged and given a commercial diet. Hens were artificially inseminated, and hatching eggs laid during the 53rd week of age were incubated at 37.5 °C in a humidified incubator.

Broiler Chicks Husbandry

The collected offspring of the broiler breeders from each treatment was vent sexing (venting) and divided into seven cages of ten birds each. All hatchlings obtained from a local hatchery and reared in an environmentally controlled room were fed with a commercial diet (Table 2) with supplemental zinc at 20 mg of $ZnSO_4$/kg of diet ad libitum. The temperature

Table 2 Composition of diets of broiler chickens

	0–21 days	22–42 days
Ingredient (%)		
Corn	53.23	60.75
Soybean meal	38.69	32.00
Soybean oil	3.70	3.26
Dicalcium phosphate	1.98	1.69
Limestone	1.05	1.08
Sodium chloride	0.35	0.35
DL-methionine	0.18	0.12
Choline chloride, 50 %	0.30	0.25
Antioxidants	0.03	0.03
L-lysine	0.04	0.02
Aureomycin, 15 %	0.10	0.10
Colistin sulfate, 10 %	0.03	0.03
Trace mineral premix[a]	0.30	0.30
Vitamin premix[b]	0.02	0.02
Total	100.0	100.0
Nutrient and energy concentration ((calculated except for zinc))		
Metabolizable energy (MJ/kg)	12.35	12.56
Crude protein (%)	21.00	19.00
Calcium (%)	0.98	0.90
Available phosphorus (%)	0.45	0.40
Methionine (%)	0.50	0.40
Lysine (%)	1.15	1.00
Zinc (mg/kg)	56.79	46.61

[a] Trace mineral premix provided the following from either inorganic or organic sources (per kg of diet): Cu 8 mg, Mn 90 mg, Se 0.3 mg, Fe 80 mg, and I 0.7 mg

[b] Vitamin premix provided the following (per kilogram of diet): vitamin A 12,500 IU, vitamin D_3 2,500 IU, vitamin E 30 IU, vitamin K 2.65 mg, vitamin B_1 2 mg, vitamin B_2 6 mg, vitamin B_6 4 mg, vitamin B_{12} 0.025 mg, pantothenate acid 12 mg, niacin 50 mg, folic acid 1.25 mg, and biotin 0.0325 mg

was maintained at 34 °C on day 1 and decreased by 2~4 °C each week until 22 °C on day 21. The light regimen was 23L:1D. On days 14 and 35, BW and feed consumption were measured for each cage in order to calculate the feed conversion ratio (FCR) (kg of feed consumed/kg of live BW).

Zinc Analysis

After the broiler breeders were fed with the experimental diets for 6 weeks, the eggs from each treatment were collected to analyze the zinc concentration in yolk and albumen. The diet sample (1–2 g), freeze-dried egg yolk, and albumen were heated in silica crucibles under (550±15 °C) for 16 h, and the residue was dissolved in 10 mL mineral analysis grade nitric acid (6 mol/L) for Zn analysis in a flame atomic absorption spectrophotometer after its dilution to a certain volume. In

the egg yolk samples, Zn was determined by the flame atomic absorption spectrophotometer after 100-fold dilution. In this case, the Zn standards were prepared from a 1 mg/ml Zn nitrate standard solution. All tubes were soaked in hydrochloric acid (10 % v/v) for 12 h and rinsed with doubly distilled water [12, 13, 19].

Breast Muscle Measurements

On days 14 and 35 post-hatch, the chicks were sacrificed by exsanguination after being weighed, then the skin was immediately removed from the breast region, a sample of the pectoralis major muscle (PMM) was obtained by dissecting 0.5 cm of the muscle fibers, and the length of about 2.5 cm following the orientation of the muscle fibers. To prevent muscle contraction, the ends of the muscle fibers were fixed to wooden applicator sticks by surgical thread prior to removal. One to two grams of fresh sample was taken from the left PMM, cooled down in liquid nitrogen, and stored at −70 °C for further analysis. The harvested breast muscles were also weighed, and the relative weight was calculated based on live BW. The sample of serially sectioned PMM was fixed in 4 % paraformaldehyde, dehydrated with increasing concentrations of ethanol, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE) stains as previously described by Velleman and Nestor [20] to measure muscle fiber width. The images of the stained muscle sections were captured by an Olympus XI 70 microscope equipped with an Olympus Magna Fire digital camera and analyzed with Image-Proplus 6.0.

Western Blotting

Polyclonal anti-mTOR, anti-AKT, and anti-FOXO1 antibodies as well as phospho-specific antibodies for mTOR (Ser 2448), AKT (Ser 473), and FOXO1 (Ser 256) were purchased from Cell Signaling Technology.

An amount of 100 mg of each muscle sample was homogenized in a polytron homogenizer and sonicated on ice in RIPA buffer (CW2333, CWBIO Ltd., Beijing) and then centrifuged at 10,000g for 15 min at 4 °C. Protein concentration was determined using Pierce™ BCA protein assay kit (CW0014, CWBIO Ltd., Beijing). Each muscle homogenate was mixed with 5×sample loading buffer and boiled at 100 °C for 10 min. The same amount of protein from each muscle sample (30 μg) was loaded and electrophoresed in running buffer on a 7.5–12 % Tris–glycine SDS-polyacrylamide gel. Following electrophoresis, proteins were transferred to methanol presoaked PVDF membrane (Huaxingbochuang Bio-technology Center, Beijing) in a transfer buffer. After the membrane was blocked in a blocking solution containing 5 % milk in TBS/T buffer for 1 h, the membrane was incubated overnight in primary antibodies (1:1000) at 4 °C. Then,

the membrane was washed with 10 ml of TBS/T three times for 5 min each time. After being washed, the membrane was incubated with anti-rabbit (or mouse) IgG-conjugated horse-radish-peroxidase (1:2000) (CW0103 and CW0102, CWBIO Ltd., Beijing) for 1 h. Following the same aforementioned washing steps, the membrane was reacted with ECL-Plus chemiluminescent reagent (Huaxingbochuang Biotechnology Center, Beijing) and exposed to film. The film was captured and analyzed with Image J software [7, 21].

Statistical Analysis

All data were analyzed by one-way ANOVA using the SPSS version 17.0 program. A post hoc Duncan's multiple range test was used to separate the means that significantly differ at $P < 0.05$.

Results

Growth and Muscle Development of the Offspring

The data showed that there was no difference between treatments of the offspring regarding BW, feed intake, and FCR during 1–14 and 1–35 days post-hatch (Table 3).

On day 14 post-hatch, the breast muscle yield of the offspring from Zn/H group was tended to be higher ($P = 0.092$) than the control group, while there was no apparent difference between Zn/L and Zn/C groups (Fig. 1a). The muscle fiber width of the offspring from Zn/H group was larger than that of the control group ($P < 0.05$; Fig. 1b), but not from Zn/L group.

On day 35 post-hatch, the breast muscle yield of the offspring from the Zn/H group was significantly higher ($P < 0.05$) than the control group, and no difference was observed in the Zn/L group in reference to the same parameter (Fig. 2a). The

muscle fiber width of the offspring from Zn/H and Zn/L groups was significantly larger ($P < 0.05$) than that of the control group, but there was no difference between Zn/H and Zn/L groups (Fig. 2b).

Zinc Concentration in Yolk and Albumen of the Hen's Eggs

The zinc concentration in the yolk from Zn/L and Zn/H groups was significantly higher ($P < 0.05$) than the control group, and the Zn/H group had higher zinc content than Zn/L group (Fig. 3). In contrast, there was no difference among treatments in zinc concentration of egg albumen (data not shown).

AKT, mTOR, FOXO, and Their Phosphorylation in the Skeletal Muscle of the Offspring

On day 14 post-hatch, compared with the control group, there was no difference in the content of AKT (Fig. 4) and FOXO (Fig. 5) in PMM of the offspring either from Zn/L or Zn/H group. Significant upregulation ($P < 0.05$) in phosphorylation of AKT (Fig. 4) at Ser 473 and FOXO (Fig. 5) at Ser 256 in PMM of the offspring from Zn/L and Zn/H groups compared with the control group.

On day 35 post-hatch, there were no differences in the content of mTOR (Fig. 6) and FOXO (Fig. 7) in PMM of the offspring from breeders fed with zinc supplementation compared with the control group. Nevertheless, the phosphorylation of mTOR (Fig. 6) at Ser 2448 and FOXO (Fig. 7) at Ser 256 in PMM of the offspring was significantly ($P < 0.05$) increased in Zn/L and Zn/H groups than the control group.

Table 3 Effect of maternal zinc nutritional status on body weight, food intake, and FCR of the offspring during 1–14 and 1–35 days

	CON	Zn/L	Zn/H	SEM	P value
0–14 days					
Body weight, g	373.31	388.70	384.77	9.43	0.804
Food intake, g	380.52	388.63	381.99	13.12	0.968
FCR	1.17	1.11	1.14	0.01	0.074
0–35 days					
Body weight, g	2137.86	2036.43	2110.00	22.18	0.157
Food intake, g	3287.79	3088.18	3241.06	36.21	0.054
FCR	1.58	1.55	1.57	0.01	0.760

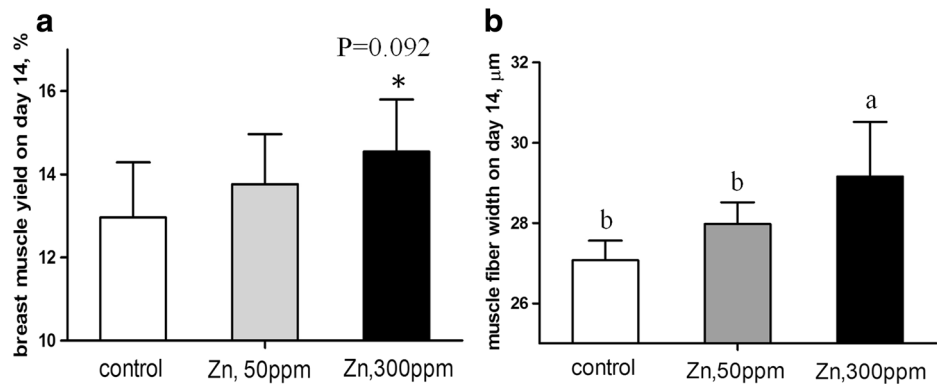Data are means, $n = 7$

Discussion

The results of current study demonstrate that maternal zinc supplementation is beneficial for post-natal skeletal muscle development through increasing protein synthesis and inhibiting protein degradation of the skeletal muscle of the offspring. Zinc is an important trace mineral for the growth and development of animals [22], being an essential element for enzyme systems and involved in protein synthesis, carbo-hydrate metabolism, and many other biochemical reactions [23]. Hence, zinc deficiency universally influences nucleic acid biosynthesis, amino acid utilization and protein synthesis, and results in growth retardation [24]. The deposition of zinc in the egg yolk was increased by the treatment of maternal zinc supplementation, which resulted in the better development of the skeletal muscle of the offspring.

Fig. 1 Breast muscle yield and muscle fiber width of the offspring on day 14 post-hatch. The breast muscle yield (*n*=7 per group) and muscle fiber width (*n*=6 per group) of the offspring on day 14 post-hatch from control (*white bars*), Zn/L (*gray bars*), and Zn/H (*black bars*) group. Values are means±SD. **a**, **b** Means with different letter differ significantly, *P*<0.05

## Maternal Zinc Supplementation Increased the Deposition of Zinc in the Egg Yolk

With the increase of zinc supplementation into the hen's diet, the zinc level in the egg yolk was significantly elevated, but no change was observed in the zinc concentration of the egg albumen. In agreement with the previous study [25] in which the zinc concentration in the whole egg was significantly elevated by high level of zinc supplementation into the broiler breeders' diet, no report is found to confirm the observed difference between the egg albumen and yolk. There are two routes for hens to deposit minerals into the eggs: one is from the ovary to the ovum yolk and the other is from the oviduct to the albumen, shell, and shell membranes of the eggs [26]. Egg yolk, an important place for nutrition storage, provides essential nutrients for the development of the chicken embryo. Previous studies suggest that zinc was consumed by E17, the amount of zinc in the yolk was low and the embryo consumed little in the last days of incubation [23]. The increased deposition of zinc in the egg yolk leads to higher mineral consumption by the embryo, which is beneficial for the chicken embryo growth, and development of critical tissues of the offspring, such as the skeletal muscle.

Maternal Zinc Supplementation Enhanced the Skeletal Muscle Development of the Offspring

The present study showed that maternal zinc supplementation increased the breast muscle yield of the offspring, and the muscle fiber width of the offspring was also significantly increased by zinc supplementation into the hen's diet. Previous studies suggested that the development of skeletal muscles post-hatch is mainly determined by the increase in muscle fiber diameter [7]; this indicated that the promotion of muscle growth of the offspring is due to the enlargement of muscle fiber. In poultry production, the skeletal muscle is the most important tissue and has a lower priority in nutrient partitioning compared with other tissues and organs in response to the challenges the fetus faces during the development [1]. Zinc is important for the embryo [11] and post-natal [12, 13] development; the muscle fiber width of the offspring was increased by the more zinc consumption by the embryo through the increased deposition of zinc in the egg yolk.

In the present study, maternal zinc supplementation had no effect on BW and FCR of the offspring during 1–14 and 1–35 days post-hatch. This result is in agreement with earlier work of Stahl et al. [27] who reported that the offspring from

Fig. 2 Breast muscle yield and muscle fiber width of the offspring on day 35 post-hatch. The breast muscle yield (*n*=7 per group) and muscle fiber width (*n*=6 per group) of the offspring on day 35 post-hatch from control (*white bars*), Zn/L (*gray bars*), and Zn/H (*black bars*) group. Values are means±SD. **a**, **b** Means with different letter differ significantly, *P*<0.05
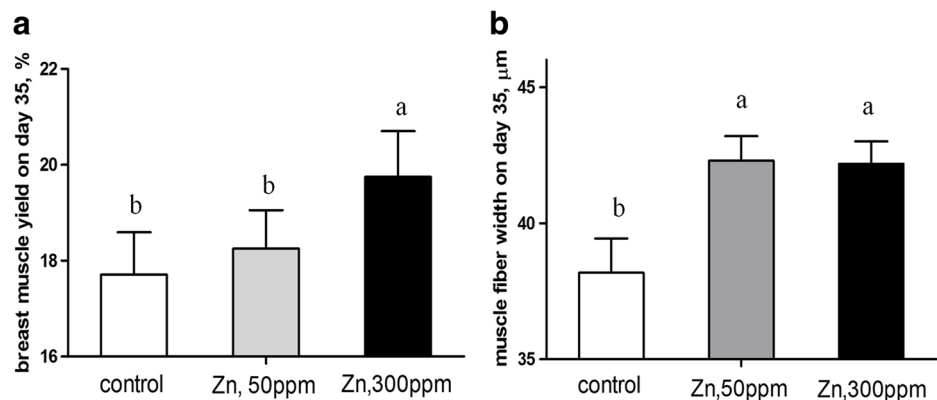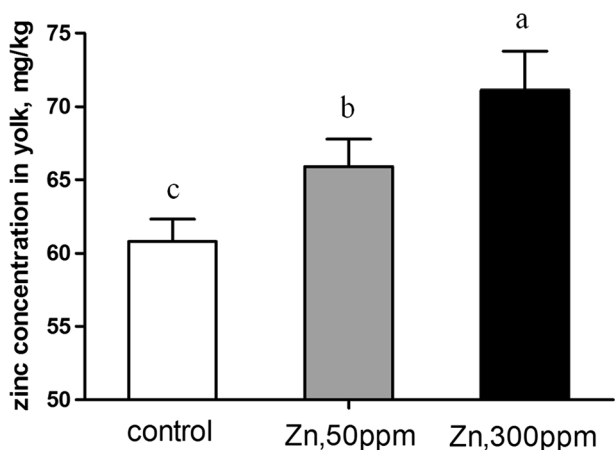
Fig. 3 Zinc concentration in egg yolk. The zinc concentration in egg yolk laid by the broiler breeders fed with control (*white bars*), Zn/L (*gray bars*), and Zn/H (*black bars*) diets for 6 weeks. Values are means±SD (*n*=12 per group). **a**, **b** Means with different letter differ significantly, *P*<0.05

the broiler breeders fed with soybean meal diet (zinc at 28 or 34 mg/kg) without zinc supplementation exhibited the same performance as the offspring from the broiler breeders supplemented with zinc up to 40 mg/kg of the diet for 3 weeks post-hatch. Additional experiments showed that the broiler breeders fed with different supplementation of zinc at 0, 20, 200, and 2000 mg/kg of the diet did not influence the BW of the offspring at age of 3 weeks post-hatch compared with
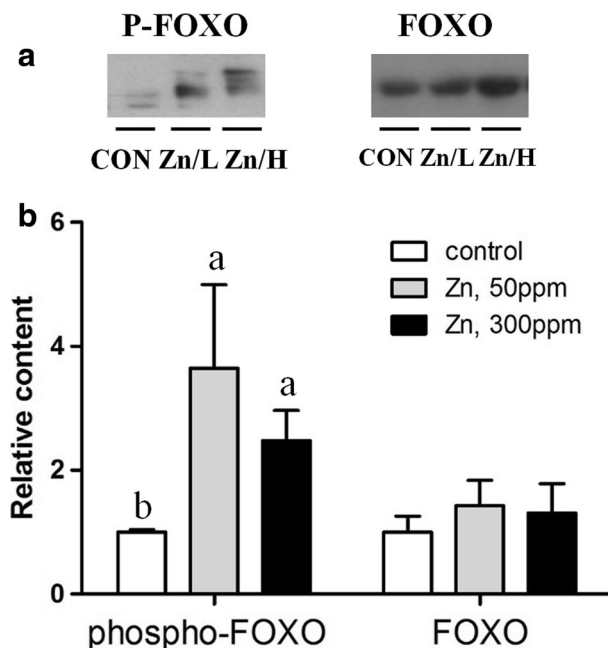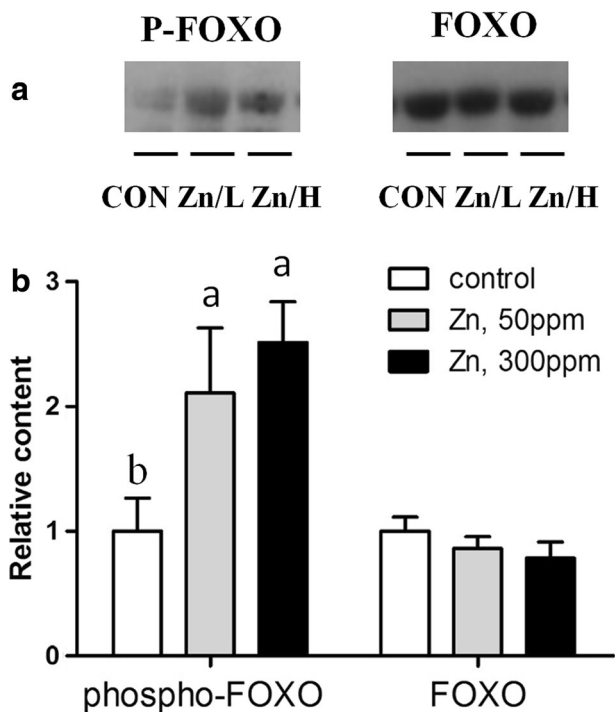

Fig. 5 Protein expression of phospho-FOXO and FOXO in the PMM of the offspring on day 14 post-hatch. Phospho-FOXO and FOXO in the PMM of the offspring on day 14 post-hatch from control (*white bars*), Zn/L (*gray bars*), and Zn/H (*black bars*) groups (*n*=4 per group). **a** Representative Western blots. **b** Values are means±SD. **a**, **b** Means with different letter differ significantly, *P*<0.05
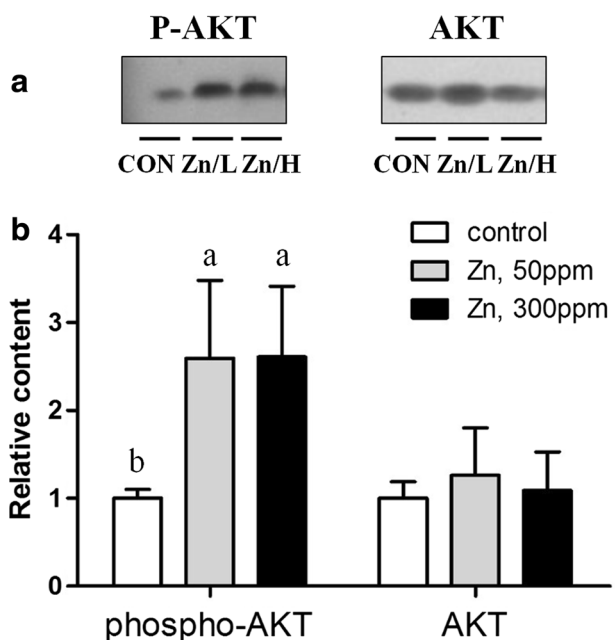
Fig. 4 Protein expression of phospho-AKT and AKT in the PMM of the offspring on day 14 post-hatch. Phospho-AKT and AKT in the PMM of the offspring on day 14 post-hatch from control (*white bars*), Zn/L (*gray bars*), and Zn/H (*black bars*) groups (*n*=4 per group). **a** Representative Western blots. **b** Values are means±SD. **a**, **b** Means with different letter differ significantly, *P*<0.05
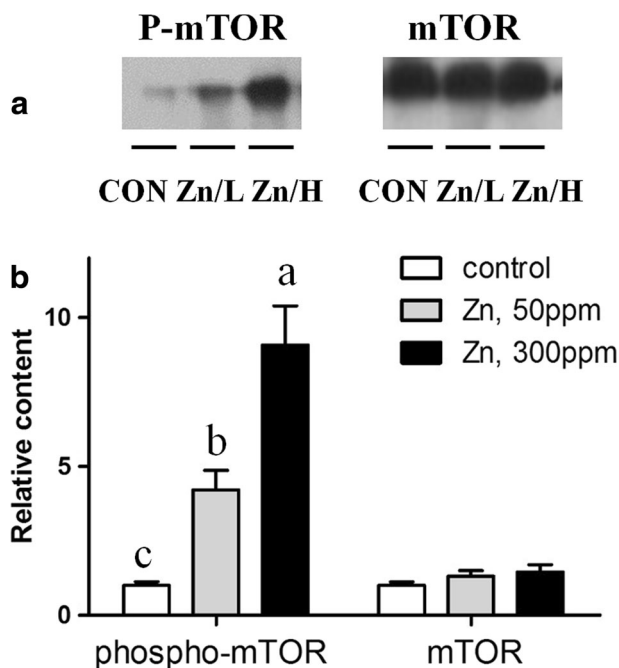
Fig. 6 Protein expression of phospho-mTOR and mTOR in the PMM of the offspring on day 35 post-hatch. Phospho-mTOR and mTOR in the PMM of the offspring on day 35 post-hatch from control (*white bars*), Zn/L (*gray bars*), and Zn/H (*black bars*) groups (*n*=4 per group). **a** Representative Western blots. **b** Values are means±SD. **a**, **b** Means with different letter differ significantly, *P*<0.05

Fig. 7 Protein expression of phospho-FOXO and FOXO in the PMM of the offspring on day 35 post-hatch. Phospho-FOXO and FOXO in the PMM of the offspring on day 35 post-hatch from control (*white bars*), Zn/L (*gray bars*), and Zn/H (*black bars*) groups (*n*=4 per group). **a** Representative Western blots. **b** Values are means±SD. a, b Means with different letter differ significantly, *P*<0.05

those fed with corn and soybean meal-based diets [28]. Kidd et al. [29, 30] also reported that zinc supplementation in hen's diet had no impact on growth of the offspring.

Maternal Zinc Supplementation Promoted Protein Synthesis in the Skeletal Muscle of the Offspring

The skeletal muscle hypertrophy is not a simple process of fusion of satellite cell nuclei and muscle fiber, which requires lower rate of protein degradation than that of protein synthesis [6]. In the present study, it was shown that the muscle fiber width of the offspring was enlarged by the treatment of zinc supplementation into the hen's diet. Bodine et al. [8] reported that the activation of the AKT/mTOR pathway is related to the regulation of the size of skeletal muscle fiber. AKT, a serine/threonine kinase, could be activated in response to different nutrients and growth factors and leads to the activation of its downstream kinase known as mTOR [8, 31]. A large quantity of researches also reported that mTOR is an important kinase involved in integrating lots of growth signals, and the activation of mTOR is beneficial for protein synthesis [32, 33]. The activity of AKT and mTOR is facilitated by phosphorylation at Ser 473 [8] and Ser 2448 [34]. The results of the present study showed that the phosphorylation of AKT at Ser 473 (day 14) and mTOR at Ser 2448 (day 35) in the breast muscle of the

offspring was significantly increased by zinc supplementation into the hen's diet and indicated that the AKT/mTOR pathway was activated. The protein synthesis in the skeletal muscle of the offspring could be enhanced through maternal zinc supplementation and resulted in the enlargement of the muscle fiber width.

Maternal Zinc Supplementation Inhibited Protein Degradation in the Skeletal Muscle of the Offspring

AKT/mTOR pathway plays a key role in protein synthesis, while activation of the ubiquitin-proteasome system is responsible for the protein degradation [35]. AKT/mTOR pathway inhibits the expression of the key genes downstream FOXO in the ubiquitin-proteasome system through inactivating FOXO factors [36, 37]. In the present study, the phosphorylation of FOXO at Ser 256 in the skeletal muscle of the offspring on 14 and 35 days post-hatch was significantly increased by the maternal zinc supplementation. The FOXO transcription factors were excluded from the nucleus when phosphorylated [33], which indicated that the activation of FOXO was downregulated, and the ubiquitin-proteasome system was inhibited. The inhibition of protein degradation in the skeletal muscle of the offspring by the zinc supplementation into the hens' diet is also beneficial for enlargement of the muscle fiber width of the offspring.

Conclusion

In conclusion, the breast muscle yield and the muscle fiber width of the offspring became higher and larger due to maternal zinc supplementation, with the partial molecular mechanism could be the upregulation of AKT/mTOR pathway and downregulation of the ubiquitin-proteasome system in the skeletal muscle of the chicks post-hatch. Inorganic zinc supplementation into the broiler breeders' diet has a long-term influence on the post-hatch skeletal muscle development and breast muscle yields, which is beneficial for poultry production.

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