




Comparative Study of Different Maternal Zinc Resource Supplementation on Performance and Breast Muscle Development of their Offspring

Jing Gao¹ · Wei Nie¹  · Kun Xing¹ · Yuming Guo¹

Received: 6 June 2018 / Accepted: 5 September 2018 / Published online: 29 September 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Maternal zinc supplementation has a pivotal role in enhancing breast muscle development of the offspring. What is poorly defined is the impact of supplemental zinc from different sources on the offspring. Broiler breeders at 24-week-old were randomly divided into three treatments with six replicates of 40 hens each and respectively fed for 8 weeks with supplemental 0-(group Zn/C), 100 mg/kg organic-(group Zn/O), and 100 mg/kg inorganic-(group Zn/I) zinc. The male offspring from each nutritional treatment were allocated into eight cages of 14 birds each, and a commercial diet supplemented with zinc from ZnSO₄ at 20 mg/kg was fed to the offsprings. Results showed that eggs from Zn/O group had the highest zinc deposition ($P < 0.05$). Furthermore, maternal zinc supplementation promoted breast muscle yield; increased serum insulin and IGF-I concentration; upregulated AKT, mTOR, and P70S6K mRNA levels; and improved the phosphorylation of AKT at Serine 473 residue, mTOR at Serine 2448 residue, and FOXO at Serine 256 residue in the breast muscles of the offspring. In contrast, hens' diet supplemented with zinc could result in downregulation of atrogin-1 and MuRF1 mRNA levels in the breast muscle of the offspring. Additionally, no significant effect on breast muscle development post-hatch was observed between organic and inorganic zinc supplementation. In conclusion, maternal organic zinc supplementation improved zinc deposition in egg; however, no significant difference was detected in breast muscle development of the offspring of broiler breeder between organic and inorganic zinc supplementation.

Keywords Organic zinc · Inorganic zinc · Protein metabolism · Breast muscle · Offspring

Introduction

Maternal nutrition affects fetal development with long-term consequences on postnatal growth of the offspring [1]. The deficiency of trace element could cause metabolic disorders [2, 3]. Therefore, researchers have paid more attention to the studies of association between maternal trace element nutrition and postnatal growth of the offspring [4]. Zinc, an essential trace element, plays a critical role in enzyme systems and is involved in protein synthesis, carbohydrate metabolism, and many other biochemical reactions [5]. The influence of maternal zinc on postnatal development occurred via deposition of zinc in egg, which was modulated by zinc delivery from

breeders' blood [6]. Our previous study reported that zinc supplemented in broiler breeders' diet could increase the zinc deposition in egg yolk, which had a long-term influence on the skeletal muscle development post-hatch [7].

The skeletal muscle is the most important tissue from the perspective of animal production [8]. The post-hatch growth of the skeletal muscle is mainly determined by increasing muscle fiber diameter [8], which needs higher rate of protein synthesis than protein degradation [9]. Further, the mammalian target of rapamycin (mTOR) signaling pathway [10, 11] and ubiquitin-proteasome pathway [12] are critical for nutrient-stimulated muscle growth of the offspring through modulating protein synthesis and degradation, respectively [8]. Recent studies have found that zinc is a signaling molecule that regulates the protein kinase phosphorylation [13], including the stimulation of mTOR activity [14, 15]. Gao et al. [7] showed that maternal zinc supplementation could activate the AKT/mTOR signaling pathway and inhibit the ubiquitin-proteasome system in the chicks' skeletal muscle development post-hatch.

✉ Wei Nie
niewei@cau.edu.cn

¹ State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, No. 2 Yuanmingyuan West Road, Haidian District 100193, Beijing, China

Supplemental zinc in broiler diets has two forms: inorganic form, such as zinc sulfate, zinc chloride, and zinc oxide; organic form includes zinc that is bonded to amino acids, proteins, or carbohydrates [16]. Inorganic zinc is commonly added in animal diets in the feed industry [17]; however, the use of organic zinc has been increased during the last 20 years [18]. With respect to bioavailability of organic and inorganic zinc, the reported results are inconsistent [18]. Some researchers have documented that organic zinc from a zinc-amino acid complex has higher bioavailability than that of inorganic sources [19, 20]; in contrast, other researchers have indicated that negligible differences have been reported concerning the bioavailability between organic and inorganic zinc [21]. So far, however, there has been little research about the influence of organic and inorganic zinc supplementation in hens' diets on the breast muscle development of the offspring. Therefore, this study attempts to evaluate the effects of maternal organic and inorganic zinc supplementation on the development of the breast muscles in broiler breeder chicken.

Materials and Methods

Dietary Treatments and Animal Care

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 24 weeks were allocated into three treatments with six replicates of 40 hens each and were fed with diets containing organic and inorganic zinc at different levels, i.e., (1) Zn/C group (control) was fed with basal diet without supplemental zinc; (2) Zn/O group was fed with supplemental organic zinc (as methionine hydroxy analog-chelated Zn) at 100 mg/kg of the diet; and (3) Zn/I group was fed with supplemental zinc (as ZnSO₄) at 100 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 [22] with the exception of zinc. The content of zinc in diets was 30.37, 134.65, and 133.58 mg/kg respectively based on the actual analysis. All diets were iso-energetic, iso-nitrogenous, and iso-methionine. All breeders were housed in a completely enclosed, ventilated, and conventional caged-breeder house in which the light regimen was 16 Light (L): 8 Dark (D). Before the start of the experiment, all hens were fed with a basal diet for 3 weeks to deplete body reserves of zinc. During the 8-week experiment, each female breeder was allotted 160 g of feed at 6:00 AM every day; the laying rate and egg weight were recorded. Male breeders were caged and given a commercial diet. Hens were artificially inseminated, and hatching

eggs laid during the 34th week of age were incubated at 37.8 °C in a humidified incubator.

The male offspring from each treatment were divided into eight cages of 14 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 from ZnSO₄/kg of diet ad libitum. The temperature 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 day 21 and 42, body weight (BW) and feed consumption for each bird were measured for each cage in order to calculate the feed conversion ratio (FCR) (kg of feed consumed/kg of live BW).

Breast Muscle Measurements

On day 14 and 35 post-hatch, the chicks were sacrificed by exsanguination after being weighed, then the skin was immediately removed from the breast region, 1–2 g of fresh sample was taken from the left pectoralis major muscle (PMM), quick-frozen 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 a live BW.

Zinc Analysis

After the broiler breeders were fed with the experiment diets for 8 weeks, the two eggs during the 34th week of age were randomly chosen from each replicate to detect the zinc concentration. The egg yolk and albumen were separated, weighed, sampled, and freeze-dried. The diet, freeze-dried egg yolk and albumen samples 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 [7]. The left tibia was dried at 105 °C for 24 h after removing the skin, muscle, and soft tissue. Then, it was soaked in anhydrous ethanol for 48 h and extracted in a Soxhlet apparatus with anhydrous diethyl ether for 12 h. After the tibia was dried again (105 °C, 10 h), the fat-free dry tibia was heated in silica crucibles under (550 ± 15) °C for 18 h, and the residue was dissolved in 10 mL mineral analysis grade nitric acid (6 mol/L) for zinc analysis with a flame atomic absorption spectrophotometer (Savant AA, GBC, Australia) after its dilution to a certain volume. The egg yolk and tibia samples were analyzed after ten-fold dilution. In this case, the zinc standards were prepared from a 1-mg/mL zinc nitrate standard solution. All tubes were soaked in hydrochloric acid (10% v/v) for 12 h and rinsed with double distilled water [23, 24].

Table 1 Composition of diets of broiler breeders

Ingredient (%)	CON	Zn/O	Zn/I
Corn	63.314	63.344	63.314
Soybean meal	25.00	25.00	25.00
Limestone	8.20	8.20	8.20
Soybean oil	1.50	1.50	1.50
DL-methionine	0.09	0.09	0.09
MHA	0.03	0	0.03
Choline chloride	0.12	0.12	0.12
Dicalcium phosphate	0.90	0.90	0.90
Sodium chloride	0.35	0.35	0.35
Flavomycin	0.015	0.015	0.015
Antioxidants	0.03	0.03	0.03
Phytase	0.016	0.016	0.016
Mycotoxin antidote	0.1	0.1	0.1
Trace mineral premix ^a	0.3	0.3	0.3
Vitamin premix ^b	0.035	0.035	0.035
Total	100	100	100
Zinc source and supplementation level	0	100 mg/kg methionine hydroxy analog-chelated Zn	100 mg/kg ZnSO ₄
Nutrient and energy concentration (calculated except for zinc)			
Metabolizable energy (MJ/kg)	11.55	11.55	11.55
Crude protein (%)	16.10	16.10	16.10
Calcium (%)	3.42	3.42	3.42
Available phosphorus (%)	0.29	0.29	0.29
Methionine (%)	0.36	0.36	0.36
Lysine (%)	0.82	0.82	0.82
Zinc (mg/kg)	30.37	134.65	133.58

^a Trace mineral premix provided the following from either inorganic or organic sources (per kilogram 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 kilogram of diet): vitamin A 15,000 IU, vitamin D₃ 3000 IU, vitamin E 22.50 IU, vitamin K 3 mg, vitamin B₁ 3 mg, vitamin B₂ 8 mg, vitamin B₆ 6 mg, vitamin B₁₂ 0.03 mg, pantothenate acid 17.64 mg, niacin 44 mg, folic acid 1.49 mg, and biotin 0.15 mg

Serum Measurement

Serum uric acid was measured spectrophotometrically with commercial diagnostic kits (Beijing Huangying institute of Biological Technology, China). Serum insulin and IGF-I concentration were measured by radioimmunoassay as previously described [25]. All samples were included in the same assay to avoid interassay variability, and the intraassay coefficient of variation of two measurements was 6.9 and 7.0%.

mRNA Levels

Total RNA was isolated from the PMM using Trizol (Invitrogen, San Diego, CA, USA), its purity and concentration were respectively determined by biophotometer (Eppendorf, Hamburg, Germany) and agarose-gel electrophoresis. Then, reverse transcription was carried out using the PrimeScript RT reagent Kit (10 µL) consisted of 500 ng

total RNA, 2 µL 5 × PrimeScript RT Master Mix (RR036A, TaKaRa Biotechnology, Co., Ltd., Dalian, P. R. China). After reverse transcription, cDNA was amplified on the ABI 7500 Real-Time PCR System using SYBR Premix Ex Taq II in a 20 µL PCR reaction, including SYBR green master mix (RR420A, TaKaRa Biotechnology, Co., Ltd., Dalian, P. R., China) and 0.1 µmol/L of each specific primer (Sangon Biological Engineering Technology & Service Co., Ltd., Shanghai, P. R., China). Each cycle consisted of denaturation at 95 °C for 10 s, annealing at 95 °C for 5 s, and extension at 60 °C for 34 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified as an internal control to normalize the differences in individual samples. The primer sequences for chicken AKT, mTOR, P70S6K, Atrogin-1, and MuRF1 are listed in Table 3. All samples were run in duplicates, and primers were designed spanning an intron to avoid genomic DNA contamination [26]. The comparative cycle threshold (CT) method ($2^{-\Delta\Delta CT}$) was

Table 2 Composition of diets of broiler chickens

Ingredient (%)	1-21d	22-42d
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)	52.96	49.38

^a Trace mineral premix provided the following from either inorganic or organic sources (per kilogram 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 D₃ 2500 IU, vitamin E 30 IU, vitamin K 2.65 mg, vitamin B₁ 2 mg, vitamin B₂ 6 mg, vitamin B₆ 4 mg, vitamin B₁₂ 0.025 mg, pantothenate acid 12 mg, niacin 50 mg, folic acid 1.25 mg, and biotin 0.0325 mg

used to quantitate mRNA expression according to Livak and Schmittgen [27].

Table 3 Primers used for Q-PCR of the target and reference genes

Gene	GenBank no.	Orientation	Primer sequence(5' → 3')	Product size (bp)
GAPDH	NM_204305	Forward	ggtgaaagtcggatcaacgg	244
		Reverse	cgatgaaggatcattgatggc	
AKT	AF039943	Forward	cattcccgcattatgaatgaagta	130
		Reverse	ctgtagccaatgaatgtgccatc	
mTOR	XM_417614	Forward	gaagtctctgcccagcagataag	92
		Reverse	tttgttccatcagcctccagt	
p70S6K	NM_001030721	Forward	atcgcacacctcgcagattcatag	111
		Reverse	agtatttgatgcctggcagaag	
Atrogin-1	NM_001030956	Forward	cacggaaggagcagatgtgt	124
		Reverse	aggtctctgggttttgct	
MuRF1	XM_424369	Forward	cgacatctacaagcaggagt	163
		Reverse	tgagcaccgaagacct	

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.

The total protein was extracted from the breast muscle using RIPA buffer (CW2333, CWBIO Ltd., Beijing), and the 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 electrophoresed and transferred to methanol presoaked PVDF membrane (Huaxingbochuang biotechnology center, Beijing, China). The membrane was blocked, incubated in primary antibodies, washed, and incubated with anti-rabbit IgG-conjugated horseradish-peroxidase. Then, it was washed again, reacted with ECL-Plus chemiluminescent reagent, and exposed to film as previously described [7, 8, 15]. The film was captured and analyzed with Image J software.

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

Laying Rate and Egg Weight of Broiler Breeders

The laying rate of broiler breeders from the Zn/O group was significantly increased in the third ($P = 0.028$) and fourth ($P =$

0.050) weeks (Fig. 1), but no detectable differences were found in other time periods. There was no notable difference in egg weight from broiler breeders between the Zn/O and Zn/I groups (data not shown).

Growth and Muscle Development of the Offspring

The different treatments exerted no considerable effects on the BW, feed intake, and FCR during 1–21 and 1–42 days post-hatch of the offsprings (Table 4).

The breast muscle yield of the offspring from the Zn/O and Zn/I groups was significantly increased ($P < 0.05$) compared to Zn/C group on day 14 post-hatch, (Fig. 2(a)); at the same time, there was no notable difference between the Zn/O and Zn/I groups (Fig. 2(a)). The offspring from the Zn/I group had significantly higher breast muscle yield ($P < 0.05$) than the Zn/C group on day 35 post-hatch, and there was no difference been observed in the Zn/O group regarding to the same parameter (Fig. 2(b)).

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

The Zn/O and Zn/I groups got notably higher yolk zinc concentration ($P < 0.05$) than the control group, and the higher zinc content in yolk was observed in Zn/O group compared to the Zn/I group ($p < 0.05$) (Fig. 3(a)). At the same time, the same result was found in zinc concentration of the egg albumen (Fig. 3(b)).

The zinc concentration in tibia of the offspring from the Zn/O and Zn/I groups was distinctly higher than the control group

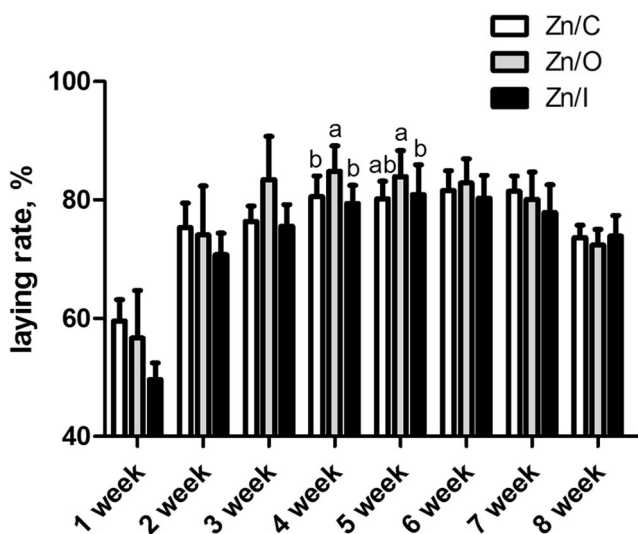


Fig. 1 Laying rate of broiler breeders. The laying rate ($n = 6$ per group) of broiler breeders on the whole period of the experiment from Zn/C (white bars), Zn/O (gray bars), and Zn/I (black bars) group. Values are means \pm SD. ^{a,b} Means with different letters differ significantly, $P < 0.05$

Table 4 Effect of maternal zinc nutritional status on body weight, food intake, FCR of the offspring during 1–21 days and 1–42 days

	CON	Zn/O	Zn/I	SEM	<i>P</i> value
1–21 days					
Body weight (g)	801.04	806.73	809.96	7.83	0.903
Food intake (g)	1056.72	1069.41	1067.90	12.45	0.909
FCR	1.33	1.33	1.32	0.01	0.752
1–42 days					
Body weight (g)	2532.38	2530.33	2545.50	41.86	0.988
Food intake (g)	4407.24	4364.53	4353.34	53.14	0.916
FCR	1.745	1.726	1.715	0.01	0.554

Data are means, $n = 8$

on day 14 ($P < 0.05$) and 35 ($P < 0.05$) post-hatch (Fig. 4(a, b)), and there was no detectable difference between the Zn/O and Zn/I groups.

UA, INS, and IGF-I Concentration in Serum of the Offspring

On day 14 post-hatch, the serum uric acid concentration of the offspring from the Zn/O and Zn/I groups was significantly lower ($P < 0.05$) than the control group (Table 5). However, compared with the control group, the serum insulin (Table 5) concentration of the offspring from the Zn/O and Zn/I groups was significantly increased ($P < 0.05$). There was no difference in serum IGF-I concentration of the offspring between the Zn/O and Zn/I groups.

On day 35 post-hatch, compared with the control group, the serum uric acid (Table 5) concentration of the offspring from the Zn/O and Zn/I groups was significantly decreased ($P < 0.05$). On the contrast, the insulin ($P < 0.05$) and IGF-I ($P < 0.05$) concentration in serum of the offspring from the Zn/O and Zn/I groups was significantly higher than the control group (Table 5).

mRNA Expression in the Breast Muscle of the Offspring

On day 14 post-hatch, the mRNA levels of AKT, mTOR, and P70S6K in the PMM of the offspring were significantly up-regulated ($P < 0.05$) from the Zn/O group than the control group (Table 6), whereas significant upregulation ($P < 0.05$) of mRNA levels of AKT and P70S6K in the PMM of the offspring from the Zn/I group compared with the control group (Table 6) was observed. On the other hand, the mRNA levels of atrogin-1 and MuRF1 in the PMM of the offspring from the Zn/O and Zn/I groups were significantly lower ($P < 0.05$) than the control group (Table 6).

On day 35 post-hatch, the levels of mTOR ($P = 0.074$) and P70S6K ($P = 0.071$) in the PMM of the offspring from the Zn/

Fig. 2 Breast muscle yield of the offspring on day 14 and 35 post-hatch. The breast muscle yield ($n = 16$ per group) of the offspring on day 14 and 35 post-hatch from Zn/C (white bars), Zn/O (gray bars), and Zn/I (black bars) group. Values are means \pm SD. ^{a,b} Means with different letters differ significantly, $P < 0.05$

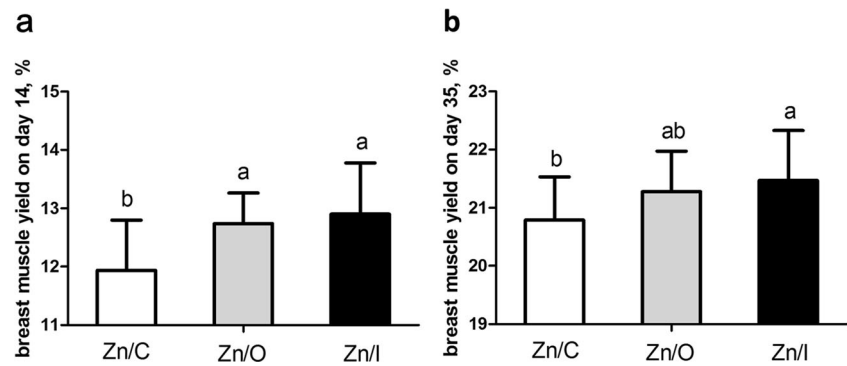
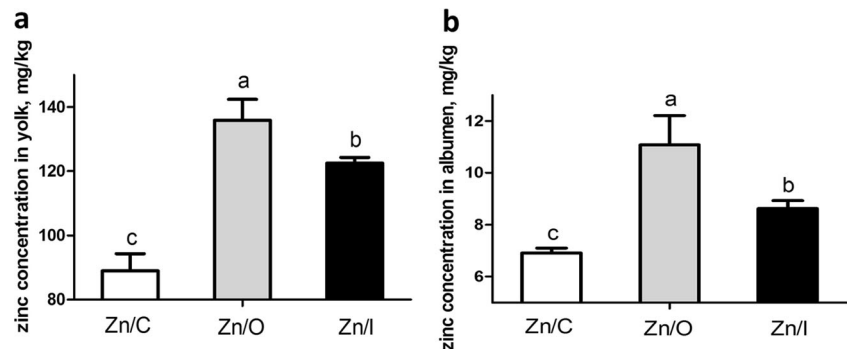


Fig. 3 Zinc concentration in egg yolk and albumen. The zinc concentration in egg yolk and albumen laid by the broiler breeders fed with Zn/C (white bars), Zn/O (gray bars), and Zn/I (black bars) diets for 8 weeks. Values are means \pm SD ($n = 12$ per group). ^{a,b} Means with different letters differ significantly, $P < 0.05$



O group were tended to be higher than the control group (Table 6). While, compared with the control group, the mRNA levels of atrogin-1 ($P < 0.05$) and MuRF1 ($P < 0.05$) in the PMM of the offspring from the Zn/O and Zn/I groups were sharply downregulated (Table 6).

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

The data showed that no significant difference was observed in the content of AKT (Fig. 5), mTOR (Fig. 6), and FOXO (Fig. 7) in the PMM of the offspring between different treatments on day 14 post-hatch. However, the offspring from the

Zn/O and Zn/I groups had increased levels of phosphorylation of AKT at Ser 473 (Fig. 5), mTOR at Ser 2448 (Fig. 6), and FOXO at Ser 256 (Fig. 7) in the PMM than those in the control group ($P < 0.05$).

No notable differences in the content of AKT (Fig. 8), mTOR (Fig. 9), and FOXO (Fig. 10) in the PMM of the offspring from broiler breeders fed with zinc supplementation were found between the different diet treatments. While, the offspring from the Zn/O and Zn/I groups got apparently higher phosphorylation of AKT at Ser 473 (Fig. 8), mTOR at Ser 2448 (Fig. 9), and FOXO at Ser 256 (Fig. 10) in the PMM compared with the control group.

Fig. 4 Zinc concentration in the tibia of the offspring on day 14 and 35 post-hatch. The zinc concentration in the tibia of the offspring on day 14 and 35 post-hatch from Zn/C (white bars), Zn/O (gray bars), and Zn/I (black bars) group. Values are means \pm SD ($n = 8$ per group). ^{a,b} Means with different letters differ significantly, $P < 0.05$

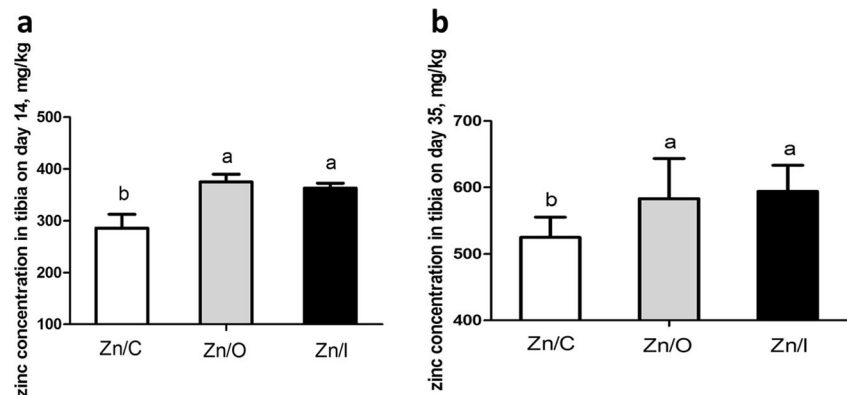


Table 5 Effect of maternal zinc nutritional status on uric acid, insulin, and IGF-I concentration in serum of the offspring on day 14 and 35 post-hatch

	CON	Zn/O	Zn/I	SEM	<i>P</i> value
Day 14					
UA ($\mu\text{mol/L}$)	342.86 ^a	216.25 ^b	184.78 ^b	17.34	<i>P</i> < 0.001
INS ($\mu\text{IU/mL}$)	10.99 ^b	14.18 ^a	13.87 ^a	0.48	0.005
IGF-1 (ng/mL)	173.73	198.63	191.20	6.12	0.242
Day 35					
UA ($\mu\text{mol/L}$)	229.35 ^a	139.78 ^b	161.95 ^b	14.61	0.025
INS ($\mu\text{IU/mL}$)	11.78 ^b	13.90 ^a	13.73 ^a	0.37	0.031
IGF-1 (ng/mL)	169.55 ^b	214.99 ^a	201.97 ^a	5.65	0.001

Data are means, *n* = 8

^{a,b} Means with different letters differ significantly, *P* < 0.05

Discussion

Organic Zinc Supplementation Increased Laying Rate and Zinc Deposition in the Egg

Maternal organic zinc supplementation has a significant influence on the laying rate in the third and fourth weeks. However, there is still very little scientific understanding of the effect of maternal organic zinc supplementation on their offspring. Trindade Neto et al. [28] found that different chelated zinc levels (137, 309, and 655 mg/kg) had no marked effect on the laying rate of hens at 24–36 weeks and 48–60 weeks. In contrast to earlier findings, however, Khajaren et al. [29] illustrated that layers fed with organic zinc resulted in their egg production improvements. Recently, our study showed that the laying rate of hens was instantly and significantly increased with the

Table 6 Effect of maternal zinc nutritional status on mRNA levels in the PMM of the offspring on day 14 and 35 post-hatch

	CON	Zn/O	Zn/I	SEM	<i>P</i> value
Day 14					
AKT	1.00 ^b	1.60 ^a	1.33 ^a	0.08	0.003
mTOR	1.00 ^b	1.37 ^a	1.19 ^{ab}	0.06	0.028
p70S6K	1.00 ^b	1.72 ^a	1.61 ^a	0.11	0.006
Atroign-1	1.00 ^a	0.29 ^b	0.35 ^b	0.08	<i>P</i> < 0.001
MuRF1	1.00 ^a	0.27 ^b	0.33 ^b	0.08	<i>P</i> < 0.001
Day 35					
AKT	1.00	1.50	1.27	0.12	0.253
mTOR	1.00	1.69	1.32	0.13	0.074
p70S6K	1.00	1.59	1.29	0.11	0.071
Atroign-1	1.00 ^a	0.66 ^b	0.56 ^b	0.06	0.001
MuRF1	1.00 ^a	0.53 ^b	0.54 ^b	0.08	0.022

Data are means, *n* = 8

^{a,b} Means with different letters differ significantly, *P* < 0.05

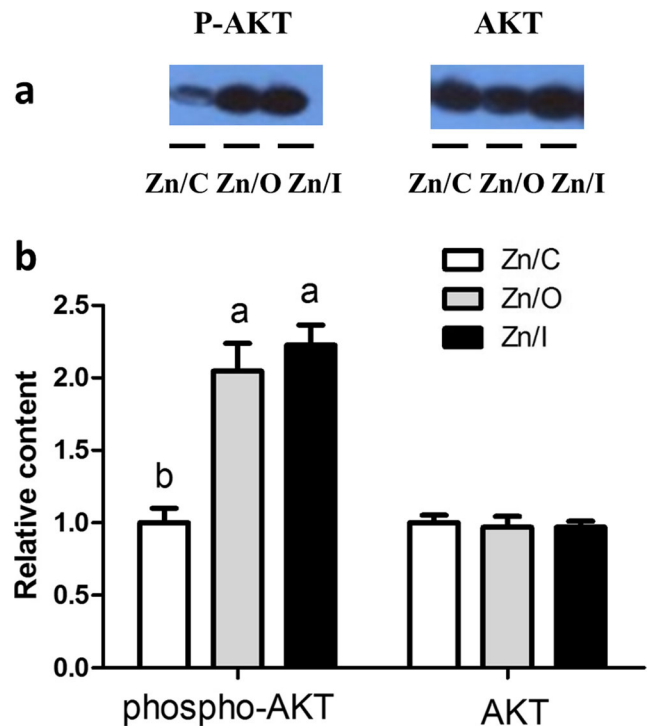


Fig. 5 Protein expression of Phospho-AKT and AKT in the pectoralis major muscle of the offspring on day 14 post-hatch. Phospho-AKT and AKT in the pectoralis major muscle of the offspring on day 14 post-hatch from Zn/C (white bars), Zn/O (gray bars), and Zn/I (black bars) group (*n* = 4 per group). (a) Representative Western blots; (b) values are means \pm SD. ^{a,b} Means with different letters differ significantly, *P* < 0.05

supplementation of organic zinc in hen's diet; however, it had no influence on laying rate during the remaining experimental period.

Yair and Uni have shown that the 0.6 mg/egg organic Zn, 0.96 mg/egg organic Fe, 0.36 mg/egg organic Mn, and 0.018 mg/egg organic Cu were enriched by in ovo feeding methodology on E17 were benefit for the embryo development [30]. Our current study indicating that the egg yolk and albumen zinc concentration was elevated when hen's diet was supplemented with 100 mg/kg methionine hydroxy analog-chelated Zn and ZnSO₄. Therefore, the embryo has more zinc to utilize due to the increased deposition of zinc in the egg yolk and albumen, which is beneficial for the development of the skeletal muscle of the offspring [7]. Our results also showed that the egg yolk and albumen from the Zn/O group had more zinc deposition compared with the Zn/I group. In the same view with our current study, Guo et al. [31] reported that the egg yolk zinc concentration of laying hens was significantly increased when the hens' diet was supplemented with organic zinc rather than inorganic zinc. Additionally, the complex of metal ion bound to a single amino acid may increase metal accumulation in birds [19]. Therefore, it is possible that

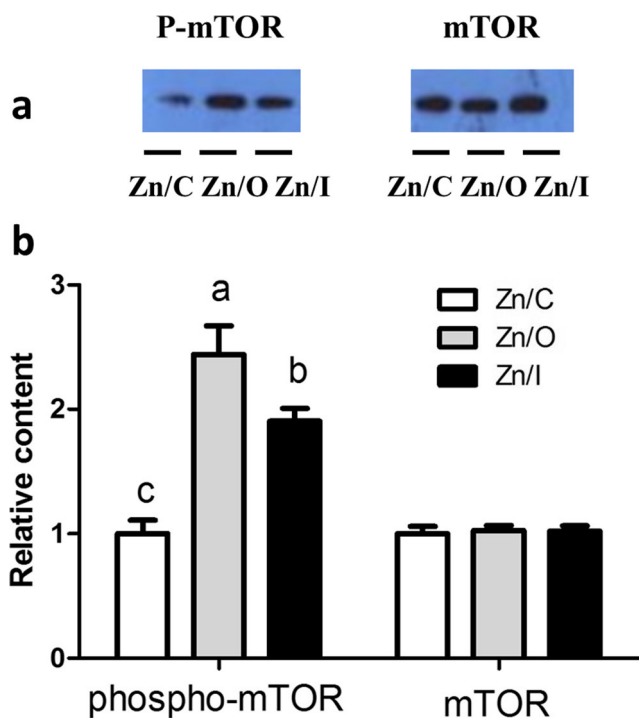


Fig. 6 Protein expression of Phospho-mTOR and mTOR in the pectoralis major muscle of the offspring on day 14 post-hatch. Phospho-mTOR and mTOR in the pectoralis major muscle of the offspring on day 14 post-hatch from Zn/C (white bars), Zn/O (gray bars), and Zn/I (black bars) group ($n = 4$ per group). (a) Representative Western blots; (b) values are means \pm SD. ^{a,b} Means with different letters differ significantly, $P < 0.05$

zinc from a zinc-amino acid complex is more available to chicks than zinc from inorganic sources [19].

Maternal Zinc Supplementation Enhanced the Breast Muscle Development of the Offspring

In agreement with earlier work [7, 32, 33], our present study found that there was no significant effect on BW, food intake, and FCR of the offspring during 1–21 and 1–42 days post-hatch by zinc supplemented in hens' diet. Our present study also found that the breast muscle yield of the offspring was sharply increased by maternal zinc supplementation, which further supported our previous study [7]. Zinc is important for postnatal development [24] due to its crucial role in the metabolism of proteins, carbohydrates, and fat in the body. Therefore, there was a consensus among researchers that zinc deposition in egg yolk and albumen reinforced the development of the breast muscle of the offspring [7, 30]. However, there was no detectable difference between the Zn/O and Zn/I groups in the development of the breast muscle of the offspring. This result suggested that different zinc sources only affect the deposition of zinc in eggs; however, they have no influence on the development of the breast muscle of the offspring.

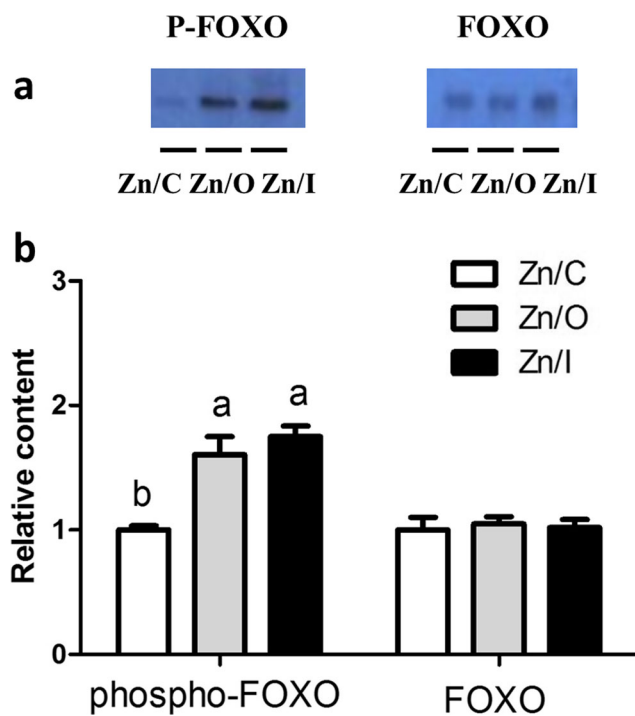


Fig. 7 Protein expression of Phospho-FOXO and FOXO in the pectoralis major muscle of the offspring on day 14 post-hatch. Phospho-FOXO and FOXO in the pectoralis major muscle of the offspring on day 14 post-hatch from Zn/C (white bars), Zn/O (gray bars), and Zn/I (black bars) group ($n = 4$ per group). (a) Representative Western blots; (b) values are means \pm SD. ^{a,b} Means with different letters differ significantly, $P < 0.05$

In present study, the uric acid concentration in serum of the offspring was significantly decreased by maternal zinc supplementation on day 14 and 35. By contrast, the insulin (on day 14 and 35) and IGF-I (on day 35) concentrations in serum of the offspring were significantly increased by zinc supplementation into the hen's diet. Uric acid is the end product of protein metabolism in poultry [25]. The decreased concentration of uric acid in serum of the offspring suggested that the protein degradation was inhibited in bird. Insulin and IGF-I, the important growth factors, control the protein metabolism through AKT/mTOR and AKT/FOXO signaling transduction pathways [34]. Therefore, the increased serum concentration of insulin and IGF-I of the offspring indicated that the protein synthesis was enhanced and its degradation was inhibited, which were beneficial for post-hatch development of the breast muscle.

Maternal Zinc Supplementation Affected Protein Metabolism in the Breast Muscle of the Offspring

Hypertrophy is the primary process of the post-hatch development of the breast muscle, which is modulated by the balance between protein synthesis and degradation [9]. By augmenting protein synthesis at a higher rate than

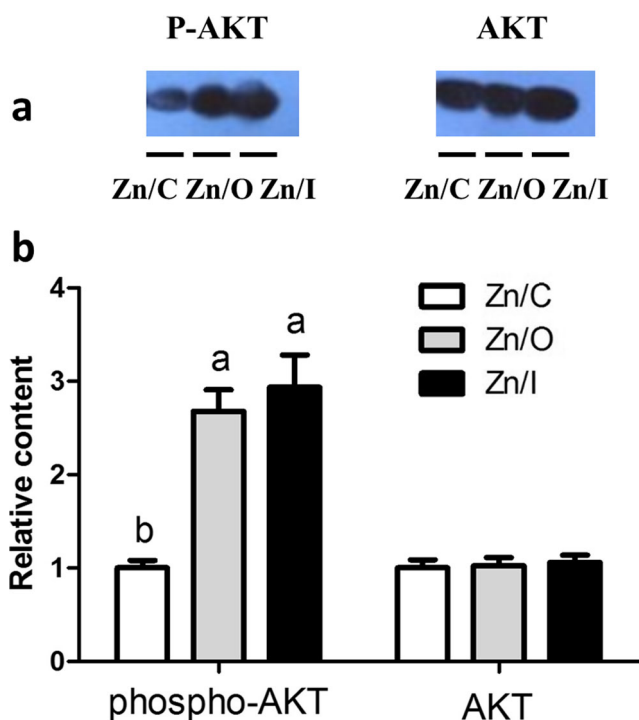


Fig. 8 Protein expression of Phospho-AKT and AKT in the pectoralis major muscle of the offspring on day 35 post-hatch. Phospho-AKT and AKT in the pectoralis major muscle of the offspring on day 35 post-hatch from Zn/C (white bars), Zn/O (gray bars), and Zn/I (black bars) group ($n = 4$ per group). (a) Representative Western blots; (b) values are means \pm SD. ^{a,b} Means with different letters differ significantly, $P < 0.05$

protein degradation, the post-hatch breast muscle development could be heightened [9]. Mammalian target of rapamycin (mTOR) [10] and ubiquitin-proteasome system (UPS) [12] were important in molecular signaling pathways for protein synthesis and degradation, respectively. Therefore, the development of the breast muscle of the offspring was controlled by gene and protein expression of these two signaling pathways.

Further, AKT, a serine/threonine kinase, could activate its downstream kinase by different nutrients and growth factors [10, 35]. mTOR, an important regulator, could integrate a variety of growth signals for protein synthesis [36]. Ribosomal p70S6 kinase was necessary for muscle fibers to achieve its normal size [37]. Therefore, the activation of the AKT/mTOR/ p70S6K pathways is a crucial regulator of the skeletal muscle development of the offspring. In present study, the mRNA levels of AKT, mTOR, and P70S6K in the breast muscle of the offspring were significantly upregulated by zinc supplementation into the hen's diet. The phosphorylation of AKT at Ser 473 and mTOR at Ser 2448 in the PMM of the offspring on day 14 and 35 was significantly increased by maternal zinc supplementation which resulted in the activation of AKT/mTOR pathways. The gene and protein expression demonstrated that maternal zinc supplementation could

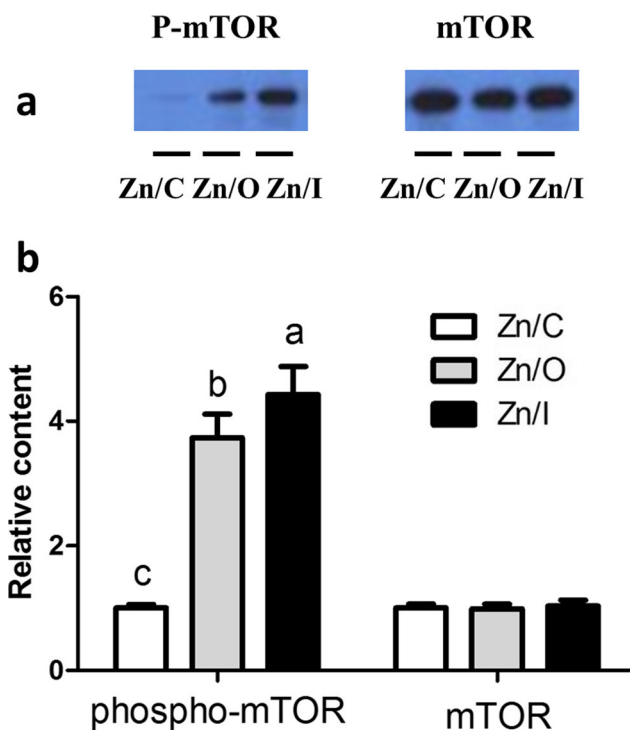


Fig. 9 Protein expression of Phospho-mTOR and mTOR in the pectoralis major muscle of the offspring on day 35 post-hatch. Phospho-mTOR and mTOR in the pectoralis major muscle of the offspring on day 35 post-hatch from Zn/C (white bars), Zn/O (gray bars), and Zn/I (black bars) group ($n = 4$ per group). (a) Representative Western blots; (b) values are means \pm SD. ^{a,b} Means with different letters differ significantly, $P < 0.05$

promote the protein synthesis in the skeletal muscle of the offspring.

Moreover, the increased protein degradation was primarily caused by the activation of the ubiquitin-proteasome pathway [12]. Both muscle atrophy F box (MAFbx, also called atrogin-1) and muscle ring finger-1 (MurRF-1) were shown to encode E3 ubiquitin ligases, which were an indispensable part of the ubiquitin-proteasome pathway [36]. Previous studies on skeletal muscle of different animals demonstrated that the mRNA levels of atrogin-1 and MuRF1 were significantly increased, which preceded the onset of muscle weight loss [38]. AKT could inhibit the upregulation of atrogin-1 and MuRF1 through regulating the FOXO family of transcription factors [38], which was deactivated due to its phosphorylation [36]. The present data showed that the gene expression of atrogin-1 and MuRF1 in the breast muscle of the offspring on day 14 and 35 was significantly downregulated by maternal zinc supplementation. The phosphorylation of FOXO at Ser 256 in the PMM of the offspring on day 14 and 35 was significantly increased by zinc supplementation into the hen's diet, which indicated that AKT could inhibit atrogin-1 and MuRF1 gene expression by phosphorylation of FOXO family. Therefore, these results demonstrated that maternal zinc supplementation could inhibit the protein degradation in the breast muscle of the offspring.

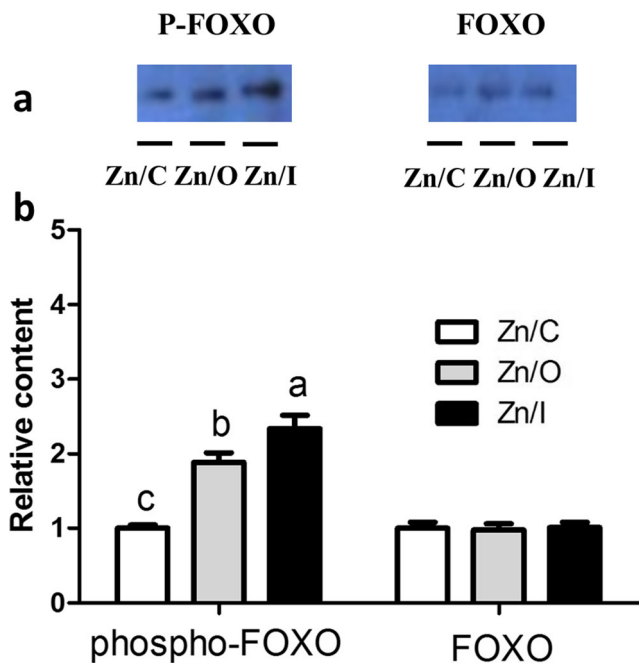


Fig. 10 Protein expression of Phospho-FOXO and FOXO in the pectoralis major muscle of the offspring on day 35 post-hatch. Phospho-FOXO and FOXO in the pectoralis major muscle of the offspring on day 35 post-hatch from Zn/C (white bars), Zn/O (gray bars), and Zn/I (black bars) group ($n = 4$ per group). **(a)** Representative Western blots; **(b)** values are means \pm SD. ^{a,b} Means with different letters differ significantly, $P < 0.05$

Conclusion

Maternal organic zinc supplementation increased more zinc concentration in egg. Both zinc source supplementation into the broiler breeders' diet improved the breast muscle yield of the offspring, which could be due to the activation of AKT/mTOR pathways and deactivation of the ubiquitin-proteasome system in the breast muscle of the chick post-hatch. However, there was no significant difference on the breast muscle development of the offspring compared between organic and inorganic zinc supplementation.

Author Contribution The contributions of the authors are as follows: J. G. and W. N. were responsible for carrying out and the experiments; J. G. W. N. and K. X. were responsible for data collection and analysis and writing the manuscript; and Y. M. G. designed and supervised the study and revised the manuscript.

Funding Information This work was supported by China Agriculture Research System program (CARS-42-G13).

Compliance with Ethical Standards

Ethics Approval and Consent to Participate All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of China Agricultural University and performed in accordance with the Guidelines for Experimental Animals of the Ministry of Science and Technology (Beijing, China).

Conflict of Interest The authors declare that they have no conflict of interest.

References

- Zhu MJ, Ford SP, Means WJ, Hess BW, Nathanielsz PW, Du M (2006) Maternal nutrient restriction affects properties of skeletal muscle in offspring. *J Physiol* 575(1):241–250
- Kidd MT (2007) A treatise on chicken dam nutrition that impacts on progeny. *Worlds Poultry Sci J* 59(4):475–494
- Angel R (2007) Metabolic disorders: limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. *J Appl Poult Res* 16(1):138–149
- Jou MY, Philipps AF, Lönnerdal B (2010) Maternal zinc deficiency in rats affects growth and glucose metabolism in the offspring by inducing insulin resistance postnatally. *J Nutr* 140(9):1621–1627
- Salim HM, Jo C, Lee BD (2008) Zinc in broiler feeding and nutrition. *Avian Biology Res* 1(1):5–18
- Sun Q, Guo Y, Ma S, Yuan J, An S, Li J (2012) Dietary mineral sources altered lipid and antioxidant profiles in broiler breeders and posthatch growth of their offsprings. *Biol Trace Elem Res* 145(3):318–324
- Gao J, Lv Z, Li C, Yue Y, Zhao X, Wang F, Guo Y (2014) Maternal zinc supplementation enhanced skeletal muscle development through increasing protein synthesis and inhibiting protein degradation of their offspring. *Biol Trace Elem Res* 162(1–3):309–316
- Zhu MJ, Ford SP, Nathanielsz PW, Du M (2004) Effect of maternal nutrient restriction in sheep on the development of fetal skeletal muscle. *Biol Reprod* 71(6):1968–1973
- Velleman SG (2007) Muscle development in the embryo and hatching. *Poult Sci* 86(5):1050–1054
- Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, Zlotchenko E, Scrimgeour A, Lawrence JC, Glass DJ (2001) Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* 3(11):1014–1019
- Pallafacchina G, Calabria E, Serrano AL, Kalhovde JM, Schiaffino S (2002) A protein kinase B-dependent and rapamycin-sensitive pathway controls skeletal muscle growth but not fiber type specification. *Proc Natl Acad Sci U S A* 99(14):9213–9218
- Jagoe RT, Goldberg AL (2001) What do we really know about the ubiquitin-proteasome pathway in muscle atrophy? *Curr Opin Clin Nutr Metab Care* 4(3):183–190
- Huang JS, Mukherjee JJ, Chung T, Crilly KS, Kiss Z (2010) Extracellular calcium stimulates DNA synthesis in synergism with zinc, insulin and insulin-like growth factor I in fibroblasts. *FEBS J* 266(3):943–951
- Barthel A, Ostrakhovitch EA, Walter PL, Kampkötter A, Klotz LO (2007) Stimulation of phosphoinositide 3-kinase/Akt signaling by copper and zinc ions: mechanisms and consequences. *Arch Biochem Biophys* 463(2):175–182
- McClung JP, Tarr TN, Barnes BR, Scrimgeour AG, Young AJ (2007) Effect of supplemental dietary zinc on the mammalian target of rapamycin (mTOR) signaling pathway in skeletal muscle and liver from post-absorptive mice. *Biol Trace Elem Res* 118(1):65–76
- Star L, Jd VDK, Rapp C, Ward TL (2012) Bioavailability of organic and inorganic zinc sources in male broilers. *Poult Sci* 91(12):3115–3120
- Sobhanirad S, Naserian AA (2012) Effects of high dietary zinc concentration and zinc sources on hematology and biochemistry of blood serum in Holstein dairy cows. *Animal Feed Sci Technol* 177(3–4):242–246
- Huang YL, Lu L, Li SF, Luo XG, Liu B (2009) Relative bioavailabilities of organic zinc sources with different chelation strengths

- for broilers fed a conventional corn-soybean meal diet. *J Anim Sci* 87(6):2038–2046
19. Wedekind K (1992) Methodology for assessing zinc bioavailability: efficacy for ZnMet, zinc sulfate and zinc oxide. *Janimsci* 70:178–187
 20. Salim HM, Lee HR, Cheorun J, Lee SK, Lee BD (2010) Effect of sources and levels of zinc on the tissue mineral concentration and carcass quality of broilers. *Avian Biology Research* 3 (1):-
 21. Ammerman CB, Baker DH, Lewis AJ (1995) Bioavailability of nutrients for animals: amino acids, minerals, and vitamins. (12): 753–754
 22. Dale N (1994) National research council nutrient requirements of poultry - ninth revised edition (1994). *J Appl Poult Res* 3(1):101–101
 23. Kechrid Z, Hamdi M, Nazıroğlu M, Flores-Arce M (2012) Vitamin D supplementation modulates blood and tissue zinc, liver glutathione and blood biochemical parameters in diabetic rats on a zinc-deficient diet. *Biol Trace Elem Res* 148(3):371–377
 24. Fatmi W, Kechrid Z, Nazıroğlu M, Flores-Arce M (2013) Selenium supplementation modulates zinc levels and antioxidant values in blood and tissues of diabetic rats fed zinc-deficient diet. *Biol Trace Elem Res* 152(2):243–250
 25. Song ZG, Zhang XH, Zhu LX, Jiao HC, Lin H (2011) Dexamethasone alters the expression of genes related to the growth of skeletal muscle in chickens (*Gallus gallus domesticus*). *J Mol Endocrinol* 46(3):217–225
 26. Wen C, Wu P, Chen Y, Wang T, Zhou Y (2014) Methionine improves the performance and breast muscle growth of broilers with lower hatching weight by altering the expression of genes associated with the insulin-like growth factor-I signalling pathway. *Br J Nutr* 111(2):201–206
 27. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C(T)}$ method. *Methods* 25(4):402–408
 28. Trindade Neto MA, Pacheco BHC, Albuquerque R, Schammas EA, Rodriguez-Lecompte JC (2011) Dietary effects of chelated zinc supplementation and lysine levels in ISA Brown laying hens on early and late performance, and egg quality. *Poult Sci* 90:2837–2844
 29. Khajarem J, Khajarem S, Rapp CJ et al (2002) Effect of zinc and manganese amino acid complexed (Availa-Z/M) on layer production and egg quality. *Poult Sci* 81(Suppl. 1):120
 30. Yair R, Uni Z (2011) Content and uptake of minerals in the yolk of broiler embryos during incubation and effect of nutrient enrichment. *Poult Sci* 90(7):1523–1531
 31. Guo YM, Yang R, Yuan J et al (2002) Effect of AvailaZn and ZnSO₄ on laying hen performance and egg quality. *Poult Sci* 81(Suppl. 1):40
 32. Stahl JL, Greger JL, Cook ME (1990) Breeding-hen and progeny performance when hens are fed excessive dietary zinc. *Poult Sci* 69(2):259–263
 33. Kidd MT, Anthony NB, Lee SR (1992) Progeny performance when dams and chicks are fed supplemental zinc. *Poult Sci* 71(7):1201–1206
 34. Tesseraud S, Métayer-Coustard S, Boussaid S, Crochet S, Audouin E, Derouet M, Seiliez I (2007) Insulin and amino acid availability regulate atrogen-1 in avian QT6 cells. *Biochem Biophys Res Commun* 357(1):181–186
 35. Sakamoto K, Aschenbach WG, Hirshman MF, Goodyear LJ (2003) Akt signaling in skeletal muscle: regulation by exercise and passive stretch. *Ajp Endocrinology Metabolism* 285(5): E1081–E1088
 36. Glass DJ (2005) Skeletal muscle hypertrophy and atrophy signaling pathways. *Int J Biochem Cell Biol* 37(10):1974–1984
 37. Ohanna M, Sobering AK, Lapointe T, Lorenzo L, Praud C, Petroulakis E, Sonenberg N, Kelly PA, Sotiropoulos A, Pende M (2005) Atrophy of S6K1(−/−) skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control. *Nat Cell Biol* 7(3): 286–294
 38. Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, Walsh K, Schiaffino S, Lecker SH, Goldberg AL (2004) Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogen-1 and cause skeletal muscle atrophy. *Cell* 117(3):399–412