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Maternal Selenium Supplementation Enhanced Skeletal Muscle Development Through Increasing Protein Synthesis and SelW mRNA Levels of their Offspring

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Abstract

The present study aimed to investigate the influence of maternal selenium supplementation on the skeletal muscle development of the offspring. A total of 720 Ross 308 broiler breeders at 24-week-old were allocated into 3 treatments with 6 replicates of 40 hens each and fed with 0 mg/kg-(group Se/C), 0.5 mg/kg organic-(group Se/O), and 0.5 mg/kg inorganic-(group Se/I) selenium, respectively for 8 weeks. The male offspring from each nutritional treatment were divided and housed into 8 cages of 12 birds each and fed with a commercial diet supplemented with selenium from Na_2SeO_3 at 0.15 mg/kg. Results showed that Se/O group had the highest selenium deposition ($P < 0.05$) in the egg yolk and albumen. Furthermore, maternal selenium supplementation promoted breast muscle yield; increased serum insulin and IGF-I concentration; upregulated AKT, mammalian target of rapamycin (mTOR), P70S6K, Myf5, MyoD, MyoG, and SelW mRNA levels; and improved the phosphorylation of AKT at Serine 473 residue, mTOR at Serine 2448 residue, and FOXO at Serine 256 residue in skeletal muscles of the offspring. In contrast, the hens' diet supplemented with selenium could result in reduction of uric acid level in serum and downregulation of Atrogin-1 and MuRF1 mRNA levels in the skeletal muscle of the offspring. Additionally, no significant effect on the skeletal muscle development post-hatch was observed between organic and inorganic selenium supplementation. In conclusion, maternal organic selenium supplementation improved selenium deposition in egg; however, no significant effect has been detected on the breast muscle development of the offspring of broiler breeder compared with inorganic selenium supplementation.

Keywords Organic selenium · Inorganic selenium · Protein metabolism · Skeletal muscle · Offspring

Introduction

In poultry, maternal nutrition affects performance and health of the offspring short termly and long termly during embryonic and post-hatch development [1] through transferring all nutrients required by the developing offspring from the dam via the egg [2]. Trace element, which is undoubtedly considered to be the indispensable part of maternal nutrition, is critical for physiology and metabolism of the progeny [3]. Selenium, an essential trace element, plays an important role in protecting against gizzard myopathy and skeletal muscle degeneration and increasing reproductive performance in poultry [4–6]. More and more evidence have indicated that maternal selenium supplementation has influence on embryo viability, hatchability, and growth of the progeny [1, 7, 8] through increasing selenium deposition in the yolk and the albumen as well as the shell and the shell membranes of the egg [9].

Skeletal muscle, which is the most important tissue from the perspective of animal production, is easily affected by maternal nutrition [10]. The skeletal muscle development in poultry contained two processes: hyperplasia and hypertrophy [11]. The number of myofiber has been set during the process of hyperplasia [11], while the hypertrophy is characterized by the enlargement of muscle fibers during the process of muscle growth post-hatch [11, 12], which not only needs to incorporate more satellite cell nuclei into a muscle fiber but also needs to modulate the balance between protein synthesis and degradation [11]. The proliferation and differentiation of satellite cells were characterized by the expression of members of the myogenic regulatory factors (MRFs) contained MyoD, Myf5, and MyoG [13–15]. The protein synthesis and protein degradation were respectively modulated by the mammalian target

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of rapamycin (mTOR) signaling pathway [16, 17] and ubiquitin-proteasome pathway [18] which are critical for nutrient-stimulated muscle growth of the offspring.

Selenium exerted biological functions through incorporation selenocysteine (Sec) into various selenoproteins [19]. Up to now, 25 selenoproteins in mammalian [20] and 23 selenoproteins in chicken [21] have been identified. The selenoprotein W (SelW), the first selenoprotein to be linked to muscular disorders [22], is primarily expressed in skeletal muscle during normal selenium supplementation, while its expression was notably downregulated under Se-deficient conditions [23, 24]. The gene expression of SelW was activated by the binding of MyoD to a specific E-box during early skeletal muscle differentiation [25]. Numerous studies have demonstrated that selenium was an insulin mimic [26], which could activate the insulin signaling cascade [27] and regulate the activation of phosphoinositide 3-kinase (PI3K) and AKT [28]. So far, little information about the influence of maternal selenium 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 selenium supplementation on the development of the offspring's skeletal muscles and the possible regulatory mechanism by SelW, the mTOR signaling pathway, and the ubiquitin-proteasome system.

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 24 weeks were allocated into three treatments with 6 replicates of 40 hens each and were fed with diets containing selenium at different levels, i.e., (1) the Se/C group (control) was fed with basal diet without supplemental selenium; (2) the Se/O group was fed with supplemental organic selenium (as selenium yeast) at 0.5 mg/kg of the diet; and (3) the Se/I group was fed with supplemental inorganic selenium (as Na_2SeO_3) at 0.5 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 [29] except selenium. The content of selenium in diets was 0.04, 0.53, and 0.54 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). Before the start of the experiment, all hens were fed with a basal diet for 3 weeks to deplete body reserves of selenium. During the 8-week experiment, each female breeder was allotted 160 g of feed at 6:00 a.m. every day. 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.

Table 1 Composition of diets of broiler breeders

Ingredient (%)	CON	Se/O	Se/I
Corn	63.314	63.314	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	0.12	0.12	0.12
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
Nutrient and energy concentration (calculated except for selenium)			
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
Selenium (mg/kg)	0.04	0.53	0.54

[a] Trace mineral premix provided the following from either inorganic or organic sources (per kilogram of diet): Cu 8 mg, Mn 60 mg, Zn 50 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

Broiler Chick Husbandry

The collected offspring of the broiler breeders from each treatment were vent sexing (venting). The male offspring were divided into 8 cages of 12 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 selenium at 0.15 mg of Na_2SeO_3/kg of *diet* ad libitum. The temperature was maintained at 34 °C on day 1 and decreased by 2~4 °C each week till 22 °C on day 21. The light regimen was 23 L/1 D. On days 21 and 42, body weight (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).

Table 2 Composition of diets of broiler chickens

Ingredient (%)	1–21 days	22–42 days
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 selenium)		
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
Selenium (mg/kg)	0.21	0.21

[a] Trace mineral premix provided the following from either inorganic or organic sources (per kilogram of diet): Cu 8 mg, Mn 90 mg, Zn 70 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

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, 1–2 g of fresh sample was taken from left pectoralis major muscle (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 a live BW.

Selenium Analysis

The selenium assay of all samples was performed with the fluorometric method as described previously by Li and Wang [30]. Briefly, 1-mL serum or 1- to 2-g diet sample, freeze-dried egg yolk, and albumen were dissolved with 8 mL of HNO_3 and 2 mL of $HClO_4$ to a 100-mL triangular flask and maintained at room temperature for 12 h. Then, the flasks were heated on the galvanothermy board until the appearance of white fumes. After cooling, 5 mL of 6 mol/L hydrochloric acid were added to the solution and heated again until the end point. At the same time, ultrapure water and a certified reference material for Se (GBW 08551 pork liver, Food Detection Science Institute, Ministry of Commerce, Beijing, China) were digested by the same method, which were served as the blank and the standard control, respectively. After digestion, 5-mL EDTA was added to the solution, the pH adjusted to 1.5–2.0 with ammonia and hydrochloric acid, 5 mL of 0.1% DAN solution added in the dark room, and the solution heated at 50 °C for 30 min. 4,5-Benzopiaseleno was extracted and the Se content was determined by fluoro-spectrophotometry.

Serum Measurement

The blood sample was drawn from a wing vein of all thechickens after a 12-h feed withdrawal using a syringe within 30 s and stored in tubes. Serum was obtained after centrifugation at 400 g for 10 min and was stored at − 20 °C for further analysis. Serum concentration of uric acid was measured spectrophotometrically with commercial diagnostic kits (Beijing Huangying institute of Biological Technology). Serum insulin and IGF-I were measure by radioimmunoassay as previously described [31]. All samples were included in the same assay to avoid interassay variability, the intraassay coefficient of variation of two measurements were 6.9 and 7.0%.

mRNA Levels

Total RNA was isolated from 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 a 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 an 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, MyoD,

Myf5, MyoG, and SelW are listed in Table 3. All samples were run in duplicates, and primers were designed spanning an intron to avoid genomic DNA contamination [32]. The comparative cycle threshold (CT) method (2−ΔΔCT) was used to quantitate mRNA expression according to Livak and Schmittgen [33].

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 protein was extracted from 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). The membrane was blocked, incubated in primary antibodies, washed, incubated with anti-rabbit IgG-conjugated horseradish-peroxidase, washed again, reacted with ECL-Plus chemiluminescent reagent, and exposed to film as previously described [34]. The film was captured and analyzed with the ImageJ 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

Selenium Concentration in Serum of Broiler Breeders and in Yolk and Albumen of the Hen's Eggs

After all broiler breeders were fed with a basal diet for 3 weeks, there was no significant difference in the selenium concentration in serum from different group (Fig. 1a), However, the selenium concentration in serum of hens from the Se/O and Se/I groups was significantly higher ($P < 0.05$) at the end of the experiment, and the Se/O group had higher selenium concentration than the Se/I group (Fig. 1b). At the same time, the same result was found in selenium concentration in egg yolk and albumen (Fig. 2a, b).

Growth and Muscle Development of the Offspring

The data showed that there was no difference between treatments of the offspring regarding BW and feed intake during 1–21 days, and feed intake and FCR during 1–42 days post-

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

Gene	GenBank no.	Orientation	Primer sequence (5′ → 3′)
GAPDH	NM_204305	Forward	GGTGAAAGTCGGAGTCAACGG
		Reverse	CGATGAAGGGATCATTGATGGC
AKT	AF039943	Forward	CATTCCCGCCATTATGAATGAAGTA
		Reverse	CTTGTAGCCAATGAATGTGCCATC
mTOR	XM_4l7614	Forward	GAAGTCCTGCGCGAGCATAAG
		Reverse	TTTGTGTCCATCAGCCTCCAGT
p70S6K	NM_001030721.1	Forward	ATTCGATCACCTCGCAGATTCATAG
		Reverse	AGTATTTGATGCGCTGGCAGAAG
Atrogin-1	NM_001030956	Forward	CACGGAAGGAGCAGTATGGT
		Reverse	AGGTCTCTGGGTTGTTGGCT
MuRF1	XM_424369	Forward	CGACATCTACAAGCAGGAGT
		Reverse	TGAGCACCGAAGACCTT
MyoD	NM_204214	Forward	ATCACCAAATGACCCAAAGC
		Reverse	GGGAACAGGGACTCCCTTCA
Myf5	NM_001030363	Forward	TGAGGAACGCCATCAGGT
		Reverse	GCGAGTCCGCCATCACAT
MyoG	NM_204184	Forward	GGAGGCTGAAGAAGGTGAA
		Reverse	TGCTGGTTGAGGCTGCTGA
SelW	NM_001166327.1	Forward	TGGTGTGGGTCTGCTTTACG
		Reverse	CCAAAGCTGGAAGGTGCAA

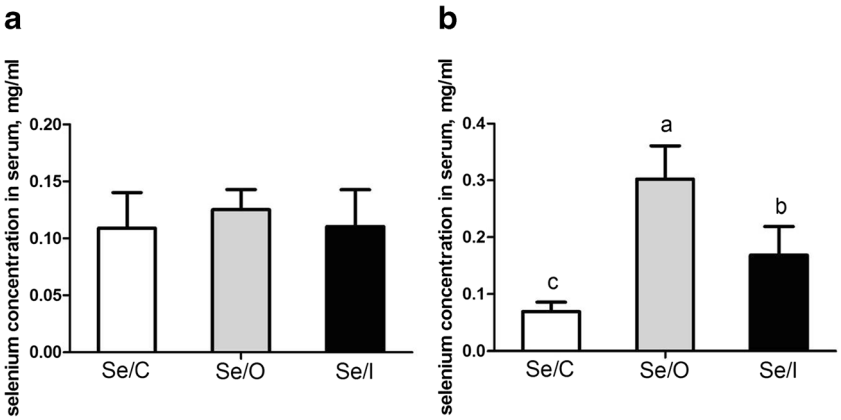

Fig. 1 Selenium concentration in serum of broiler breeders. The selenium concentration in serum of broiler breeders fed with basal diets for 3 weeks (**a**) and fed with Se/C (white bars), Se/O (gray bars), and Se/I (black bars) diets for 8 weeks (**b**). Values are means ± SD ($n = 12$ per group). [a, b] Means with different letters differ significantly, $P < 0.05$

hatch (Table 4), but the FCR of the offspring during 1–21 days from the Se/O and Se/I group was tender to be decreased compared with the Se/C group ($P = 0.061$), the body weight of the offspring from the Se/O and Se/I group was significantly higher than the Se/C group during 1–42 days ($P < 0.05$).

On day 14 post-hatch, the breast muscle yield of the offspring from the Se/I group was tended to be higher ($P = 0.084$) than the Se/C group, while there was no apparent difference between the Se/O and Se/C groups (Fig. 3a). On day 35 post-hatch, the breast muscle yield of the offspring from the Se/O and Se/I group was significantly higher ($P < 0.05$) than the Se/C group (Fig. 3b), while there was no apparent difference between the Se/O and Se/I groups (Fig. 3b).

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

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

On day 35 post-hatch, compared with the Se/C group, there was no apparent difference in uric acid and insulin (Table 5) concentration in serum of the offspring from the Se/O and Se/I group. On the contrast, the IGF-I concentration in serum of the offspring from the Se/O and Se/I group was significantly higher ($P < 0.05$) than the Se/C group (Table 5).

AKT, mTOR, P70S6K, Atrogin-1, and MuRF1 mRNA Level in the Skeletal Muscle of the Offspring

On day 14 post-hatch, the mRNA levels of mTOR, Myf5, MyoD, MyoG, and SelW in PMM of the offspring were significantly upregulated ($P < 0.05$) from the Se/O and Se/I group than the Se/C group (Table 6), while significant upregulation ($P < 0.05$) in mRNA levels of Myf5, MyoD, MyoG, and SelW in PMM of the offspring from the Se/O groups compared with the Se/I group (Table 6). On the contrast, the expression levels of Atrogin-1 and MuRF1 in PMM of the offspring from the Se/O and Se/I groups were significantly lower ($P < 0.05$) than the Se/C group (Table 6). However, there was no difference in the gene expression of AKT and P70S6K in PMM of the offspring.

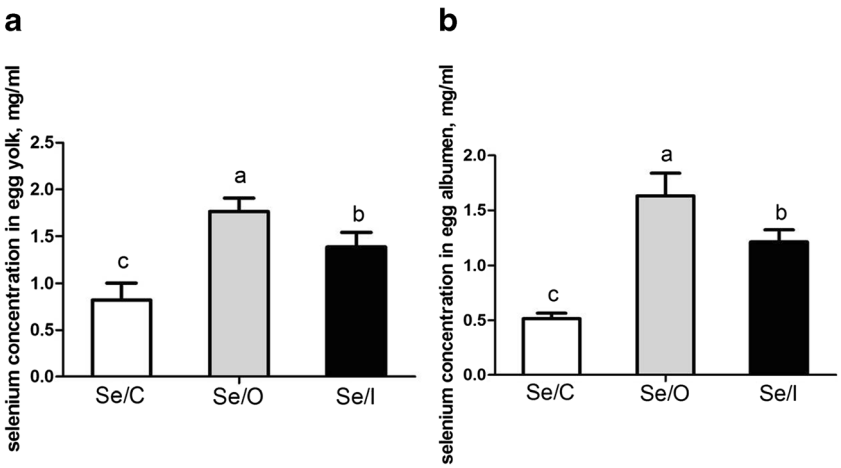

Fig. 2 Selenium concentration in egg yolk and albumen. The selenium concentration in egg yolk (**a**) and albumen (**b**) laid by the broiler breeders fed with Se/C (white bars), Se/O (gray bars), and Se/I (black bars) diets for 8 weeks. Values are means ± SD ($n = 12$ per group). [a, b] Means with different letters differ significantly, $P < 0.05$

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

	CON	Se/O	Se/I	SEM	*P* value
1–21 days					
Body weight (g)	767.59	762.52	796.34	6.88	0.091
Food intake (g)	1055.34	1034.48	1066.50	8.34	0.294
FCR	1.38	1.34	1.34	0.01	0.061
1–42 days					
Body weight (g)	2513.59[b]	2628.27[a]	2687.16[a]	19.67	$P < 0.001$
Food intake (g)	4537.58	4529.06	4614.75	24.73	0.305
FCR	1.76	1.73	1.72	0.001	0.264

Data are means, $n = 8$

On day 35 post-hatch, the gene expression of P70S6K, Myf5, MyoD, MyoG, and SelW in PMM of the offspring from the Se/O and Se/I group was higher ($P < 0.05$) than the Se/C group (Table 6), while significant upregulation ($P < 0.05$) in mRNA levels of Myf5, MyoD, MyoG, and SelW in PMM of the offspring from the Se/O groups compared with the Se/I group (Table 6). On the contrast, compared with the Se/C group, the mRNA levels of Atrogin-1 ($P < 0.05$) and MuRF1 ($P < 0.05$) in PMM of the offspring from the Se/O and Se/I groups were significantly downregulated (Table 6). However, there was no difference in gene expression of AKT and mTOR in PMM of the offspring.

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

On day 14 post-hatch, compared with the Se/C group, there was no difference in the content of AKT (Fig. 4), mTOR (Fig. 5), and FOXO (Fig. 6) in PMM of the offspring either from the Se/O or Se/I group. Significant upregulation ($P < 0.05$) in phosphorylation of AKT at Ser 473 (Fig. 4), mTOR at Ser 2448 (Fig. 5), and FOXO at Ser 256 (Fig. 6) in PMM of the offspring from the Se/O and Se/I groups compared with the Se/C group.

On day 35 post-hatch, there were no differences in the content of AKT (Fig. 7), mTOR (Fig. 8), and FOXO (Fig. 9) in PMM of the offspring from breeders fed with selenium supplementation compared with the Se/C group. Nevertheless, the phosphorylation of AKT at Ser 473 (Fig. 7), mTOR at Ser 2448 (Fig. 8), and FOXO at Ser 256(Fig. 9) in PMM of the offspring was significantly ($P < 0.05$) increased in the Se/O and Se/I groups than the Se/C group.

Discussion

The results of current study indicated that maternal selenium supplementation enhanced post-hatch development of skeletal muscle through upregulation of gene expression of selenoprotein, activating protein synthesis, and inhibiting protein degradation of the skeletal muscle of the offspring. Selenium has been recognized as an essential trace element, which is beneficial for maintaining of health, growth, and physiological functions of broilers [35]. Selenium is an essential element for glutathione peroxidase to protect the cell against free radicals [36]. Selenium was also as an insulin mimic [26] to activate the insulin signaling cascade [27] and regulate protein metabolism [36] which is conducive for skeletal muscle development. The deposition of selenium in the egg yolk and albumen was increased by the treatment of maternal selenium supplementation, which resulted in the better development of the skeletal muscle of the offspring.

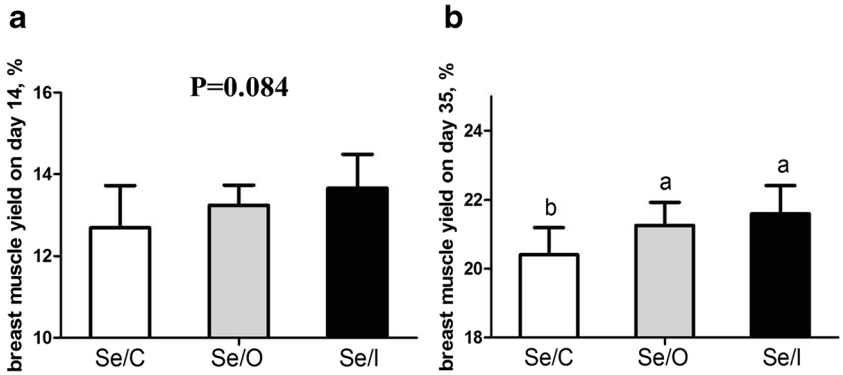

Fig. 3 Breast muscle yield of the offspring on days 14 and 35 post-hatch. The breast muscle yield ($n = 16$ per group) of the offspring on days 14 (**a**) and 35 (**b**) post-hatch from Se/C (white bars), Se/O (gray bars), and Se/I (black bars) groups. Values are means ± SD. [a, b] Means with different letters differ significantly, $P < 0.05$

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

	CON	Se/O	Se/I	SEM	*P* value
Day 14					
UA (μmol/L)	373.09^{a}	253.63^{b}	263.70^{b}	18.62	0.006
INS (μIU/mL)	12.26^{b}	15.86^{a}	13.98^{ab}	0.58	0.031
IGF-1 (ng/mL)	169.68	213.23	196.94	8.72	0.116
Day 35					
UA (μmol/L)	250.32	174.17	213.94	15.14	0.128
INS (μIU/mL)	10.59	12.08	11.88	0.31	0.105
IGF-1 (ng/mL)	147.43^{b}	187.57^{a}	184.80^{a}	7.24	0.031

Data are means, $n = 8$

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

	CON	Se/O	Se/I	SEM	*P* value
Day 14					
AKT	1.00	1.08	1.03	0.03	0.507
mTOR	1.00^{b}	1.34^{a}	1.25^{a}	0.04	0.001
P70S6K	1.00	1.10	1.03	0.02	0.262
Atroign-1	1.00^{a}	0.47^{b}	0.45^{b}	0.06	$P < 0.001$
MuRF1	1.00^{a}	0.50^{b}	0.48^{b}	0.07	0.001
Myf5	1.00^{c}	3.36^{a}	2.17^{b}	0.23	$P < 0.001$
MyoD	1.00^{c}	2.61^{a}	2.05^{b}	0.17	$P < 0.001$
MyoG	1.00^{c}	2.82^{a}	1.83^{b}	0.18	$P < 0.001$
SelW	1.00^{c}	1.53^{a}	1.30^{b}	0.06	$P < 0.001$
Day 35					
AKT	1.00	1.22	1.25	0.08	0.379
mTOR	1.00	1.23	1.32	0.08	0.238
P70S6K	1.00^{b}	1.32^{a}	1.43^{a}	0.06	0.005
Atroign-1	1.00^{a}	0.43^{b}	0.50^{b}	0.06	$P < 0.001$
MuRF1	1.00^{a}	0.17^{b}	0.20^{b}	0.09	$P < 0.001$
Myf5	1.00^{c}	6.38^{a}	4.11^{b}	0.62	$P < 0.001$
MyoD	1.00^{c}	4.45^{a}	2.95^{b}	0.33	$P < 0.001$
MyoG	1.00^{c}	5.67^{a}	2.88^{b}	0.50	$P < 0.001$
SelW	1.00^{c}	3.28^{a}	2.19^{b}	0.23	$P < 0.001$

Maternal Organic Selenium Supplementation Increased the Deposition of Selenium in Serum of Broiler Breeders and in the Eggs

The selenium level in the serum of broiler breeders and in the egg yolk and albumen was significantly elevated by the treatment of maternal selenium supplementation, while the serum and egg from the Se/O group had more selenium deposition compared with the Se/I group. In agreement with the previous study [37–40], in which the selenium concentration of serum and egg from laying hens was significantly increased when they were fed diets supplemented with selenium yeast rather than sodium selenite. Therefore, maternal organic supplementation is more effective for selenium deposition into eggs, which provides more selenium for embryo to utilize and is beneficial for the development of skeletal muscle of the offspring.

Maternal Selenium Supplementation Enhanced the Skeletal Muscle Development of the Offspring

In agreement with earlier work [5, 41], our present study found that the FCR of the broiler chickens during 1–21 days post-hatch was tended to be decreased by maternal selenium supplementation, and the body weight of the offspring during 1–42 days was significantly increased by maternal selenium supplementation. In our knowledge, selenium is beneficial for skeletal muscle development through activating the insulin signaling cascade and regulating protein metabolism [27, 36], these results suggested maternal selenium supplementation resulted in the better development of the skeletal muscle of the offspring. Our present study also found that the breast muscle yield of the offspring on day 35 was significantly improved by maternal selenium supplementation. However, there was no difference between the Se/O and the Se/I group in the growth performance and skeletal muscle development of the offspring. Selenium is an important trace element for embryo and post-natal development [42]. The increased deposition of selenium in the egg leads to higher mineral utilization by the embryo, which is beneficial for skeletal muscle development of the offspring. This result also suggested that different selenium sources just affected the deposition of selenium in egg, but had no influence of the development of skeletal muscle of the offspring.

In present study, the uric acid concentration in serum of broiler chickens on day 14 post-hatch was significantly decreased by maternal selenium supplementation, but the insulin (day 14) and IGF-I (day 35) concentration in serum of the offspring was significantly increased by maternal selenium supplementation. In poultry, uric acid is the end product of protein metabolism [31]. The lower concentration of uric acid in serum from the Se/O and Se/I group of the offspring suggested that the protein degradation was inhibited in chickens. Insulin and IGF-1 are the important growth stimulating factor, which activated protein synthesis [43] and inhibited protein degradation [44] through regulating the activity of AKT. The increased concentration of insulin and IGF-1 in serum of the offspring suggested that the protein synthesis was activated and the protein degradation was inhibited. Therefore, the all results in serum indicated that maternal selenium supplementation is beneficial for post-hatch development of skeletal muscle.

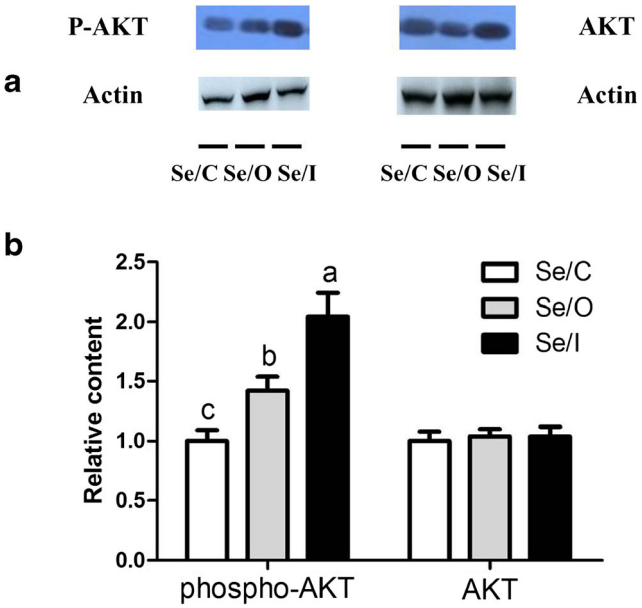

Fig. 4 Protein expression of phospho-AKT and AKT in the pectoralis major muscle of the offspring on day 14 post-hatch from Se/C (white bars), Se/O (gray bars), and Se/I (black bars) groups ($n = 4$ per group). **a**) AKT phosphorylation. Representative Western blots; **b**) Blots were quantified and ratio phospho-AKT/AKT was determined, values are means ± SD. [a, b] Means with different letters differ significantly, $P < 0.05$

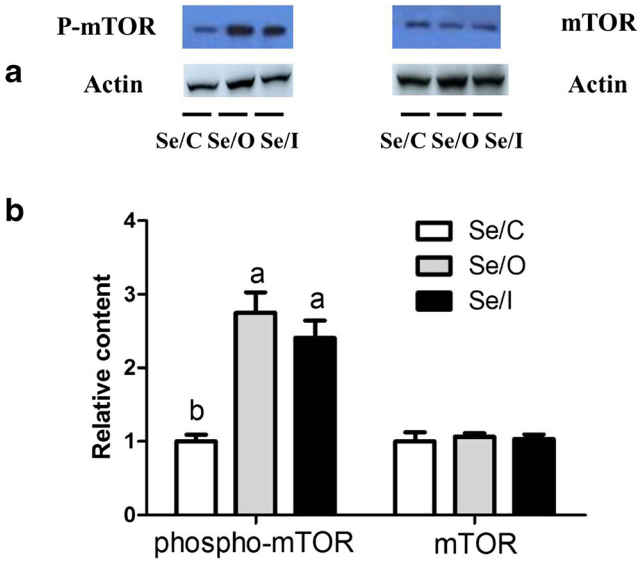

Fig. 5 Protein expression of phospho-mTOR and mTOR in the pectoralis major muscle of the offspring on day 14 post-hatch from Se/C (white bars), Se/O (gray bars), and Se/I (black bars) groups ($n = 4$ per group). **a**) mTOR phosphorylation. Representative Western blots; **b**) Blots were quantified and ratio phospho-mTOR/mTOR was determined, values are means ± SD. [a, b] Means with different letters differ significantly, $P < 0.05$

The Molecular Mechanism of the Influence of Maternal Selenium Supplementation on the Skeletal Muscle Development of the Offspring

To our knowledge, the myofiber number has been set during hatch, while the skeletal muscle development post-hatch is characterized by the enlargement of muscle fibers [11]. The process involves not only the process of the incorporation of more satellite cell nuclei into muscle fiber alone but also the maintenance of the balance between protein synthesis and degradation [11]. The proliferation and differentiation of satellite cells are regulated by the myogenic regulatory factors (MRFs) contained MyoD, Myf5, and MyoG [13–15]. The protein synthesis and protein degradation are, respectively, modulated by the mammalian target of rapamycin (mTOR) signaling pathway [45, 46] and ubiquitin-proteasome pathway [47]. Only if the rate of protein synthesis is higher than that of protein degradation, the post-hatch development of the skeletal muscle could be enhanced [11]. Therefore, the mRNA and protein expression of myogenic regulatory factors and the two signaling pathways reflected the skeletal muscle development of the offspring.

In present study, the gene expression of Myf5, MyoD, and MyoG in the breast muscle of the offspring on days 14 and 35 was significantly upregulated by maternal selenium supplementation. The myogenic regulatory factors (MRFs) are critical for the proliferation and differentiation of satellite cells [14], the quiescent satellite cells were activated to incorporate into muscle fibers. However, the present study also showed that the mRNA level of SelW in the breast muscle of the offspring on days 14 and 35 was significantly increased by maternal selenium supplementation. Only one selenoprotein was reported to be linked to muscular disorders, which is selenoprotein W. The selenoprotein W was the first selenoprotein observed to regulate early skeletal muscle differentiation through binding of MyoD to a specific E-box [22, 25]. Therefore, the upregulation mRNA levels of Myf5, MyoD, and MyoG incorporates more satellite cells into muscle fibers and activate the gene expression of SelW in skeletal muscle, which is beneficial for skeletal muscle development of the offspring.

The AKT/mTOR/p70S6K pathway could be activated through integrating a large quantity of different nutrients and growth factors to result in protein synthesis [48]. Previous study also showed that the mTOR pathway was activated during the process of hypertrophy [49]. The present study showed that the gene expression of mTOR (day 14), P70S6K (day 35), in the breast muscle of the offspring was significantly upregulated, and the phosphorylation of AKT at Ser 473 and mTOR at Ser 2448 in the PMM of the offspring on days 14 and 35 was significantly increased by maternal selenium supplementation, which suggested that the AKT/mTOR pathway was activated. Muscle Atrophy F-box (MAFbx, also called Atrogin-1) and Muscle Ring Finger-1 (MurRF-1) are two most important genes in the ubiquitin-proteasome pathway [48], and its mRNA levels are significantly upregulated during skeletal muscle atrophy [50]. AKT could downregulate gene expression of Atrogin-1 and MuRF1 through increasing the phosphorylation the FOXO [50], which was deactivated due to its phosphorylation [48]. In present study, the mRNA levels of Atrogin-1 and MuRF1 in

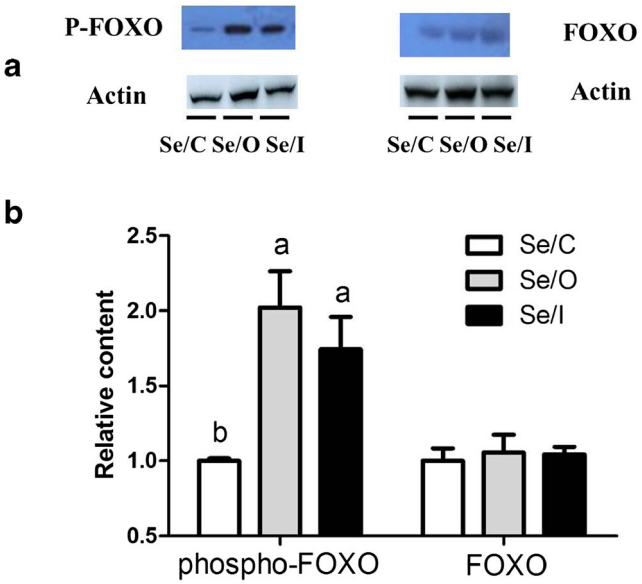

Fig. 6 Protein expression of phospho-FOXO and FOXO in the pectoralis major muscle of the offspring on day 14 post-hatch from Se/C (white bars), Se/O (gray bars), and Se/I (black bars) groups ($n = 4$ per group). **a**) FOXO phosphorylation. Representative Western blots; **b**) Blots were quantified and ratio phospho-FOXO/FOXO was determined, values are means ± SD. [a, b] Means with different letters differ significantly, $P < 0.05$

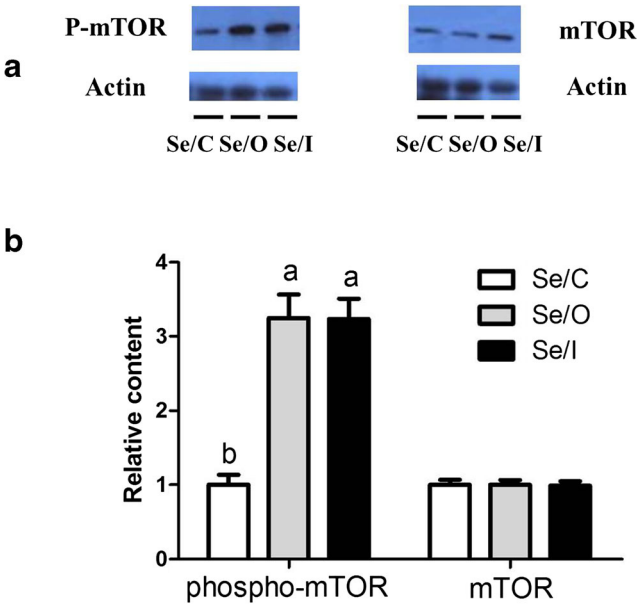

Fig. 8 Protein expression of phospho-mTOR and mTOR in the pectoralis major muscle of the offspring on day35 post-hatch from Se/C (white bars), Se/O (gray bars), and Se/I (black bars) groups ($n = 4$ per group). **a**) mTOR phosphorylation. Representative Western blots; **b**) Blots were quantified and ratio phospho-mTOR/mTOR was determined, values are means ± SD. [a, b] Means with different letters differ significantly, $P < 0.05$

breast muscle on days 14 and 35 post-hatch were significantly downregulated by maternal selenium supplementation, and the phosphorylation of FOXO at Ser 256 in the PMM of the offspring on days 14 and 35 was significantly enhanced by maternal selenium supplementation, which demonstrated that the ubiquitin-proteasome pathway was inhibited. Therefore, these results indicated that maternal selenium supplementation could promote the protein synthesis and inhibit the protein degradation in skeletal muscle of the offspring from transcriptional and translational levels.

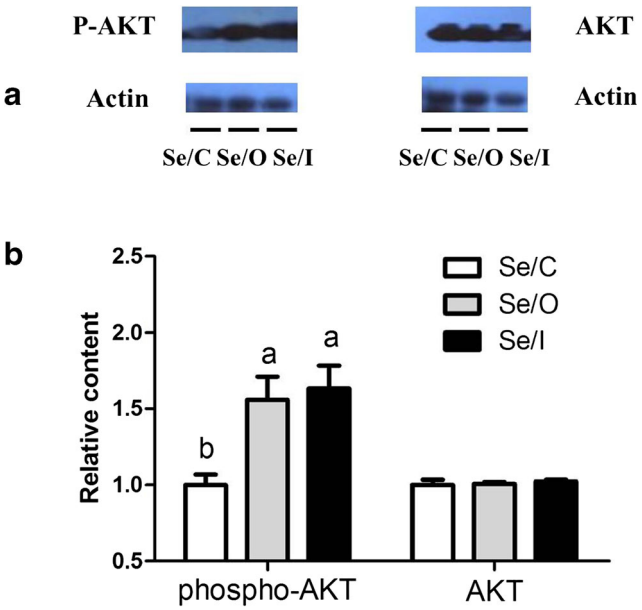

Fig. 7 Protein expression of phospho-AKT and AKT in the pectoralis major muscle of the offspring on day 35 post-hatch from Se/C (white bars), Se/O (gray bars), and Se/I (black bars) groups ($n = 4$ per group). **a**) AKT phosphorylation. Representative Western blots; **b**) Blots were quantified and ratio phospho-AKT/AKT was determined, values are means ± SD. [a, b] Means with different letters differ significantly, $P < 0.05$

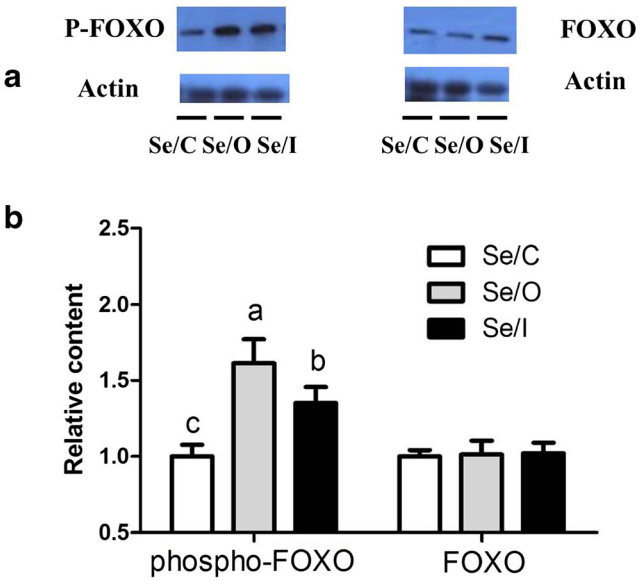

Fig. 9 Protein expression of phospho-FOXO and FOXO in the pectoralis major muscle of the offspring on day 35 post-hatch from Se/C (white bars), Se/O (gray bars), and Se/I (black bars) groups ($n = 4$ per group). **a**) FOXO phosphorylation. Representative Western blots; **b**) Blots were quantified and ratio phospho-FOXO/FOXO was determined, values are means ± SD. [a, b] Means with different letters differ significantly, $P < 0.05$

Conclusion

In conclusion, maternal selenium supplementation increased selenium concentration in egg yolk and albumen, and organic selenium had more selenium deposition than inorganic selenium. Both selenium sources supplementation into the broiler breeders' diet improved breast muscle yield of the offspring, which was modulated by upregulation of Myf5, MyoD, MyoG, and SelW gene expression; activation of AKT/mTOR pathway; and deactivation of the ubiquitin-proteasome system in the skeletal muscle of the chicks post-hatch. Therefore, maternal organic selenium supplementation had a better effect in selenium deposition in egg than inorganic selenium, but has no difference on skeletal muscle development of the offspring.

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