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Effect of an Organic Trace Mineral Premix on the Semen Quality, Testicular Morphology and Gene Expression Related to Testosterone Synthesis of Male Broiler Breeders

■**Author(s)**

Shan T[I] and Dai P[I]
Zhu P[II]
Chen L[I]
Wu W[II]
Li Y[I]
Li C[I]

[I] College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China
[II] Jiangsu Lihua Animal Husbandry Stock Co., LTD, Changzhou 213168, China

Shan T and Dai P contributed equally to this work

■**Mail Address**

Corresponding author e-mail address
Chunmei Li
College of Animal Science & Technology
Nanjing Agricultural University
No.1 Weigang, Nanjing, Jiangsu, PR.China 210095
Tel: +86-25-84395971
Email: chunmeili@njau.edu.cn

■**Keywords**

Organic trace minerals; San Huang roosters; semen; testes.

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ABSTRACT

In order to investigate the effect of organic trace minerals premix (OTM) on the reproductive performance of breeder roosters, a total of 240 San Huang roosters (23 weeks of age) were randomly divided into two treatments with six replicates of 20 roosters each. The first group (n = 120) was fed a basal diet containing an inorganic trace minerals premix (ITM) and the other group (n = 120) was fed the basal diet in which ITM was replaced by OTM. The experiment period was 22 weeks. Semen from one randomly-selected rooster per replicate was collected two weeks after the beginning of the experiment and other 10 times every two weeks. Another rooster per replicate was randomly selected at 30, 35, and 45 weeks of age, and sacrificed. Results showed that OTM did not affect relative organ weights. There was a significant increase in semen parameters in OTM group (p<0.05), such as semen volume, semen density, and semen motility from 31 to 35 weeks. OTM-fed roosters presented higher serum testosterone levels at 45 weeks of age, as well as higher testicular mRNA expression of the genes 3-beta dehydrogenase 2 (*HSD3B2*) and cytochrome P450 17A1 (*CYP17A1*) in the OTM-fed group at 45 weeks of age compared with those fed ITM (p<0.05). Considering the results of the present study, it was concluded that feeding organic instead of inorganic trace minerals to male broilers breeders improves semen quality, which may be attributed to their better testicular development and higher expression of enzymes related to testosterone synthesis.

INTRODUCTION

Trace minerals are daily required for poultry growth and production, and are important intermediates in physiological and biochemical processes (Leeson *et al.,* 2005). Three main types of trace mineral supplements are added to animal feeds: inorganic metal elements, such as ferrous (Fe) sulfate, zinc (Zn) oxide, and cupric chloride; primary simple organic compounds, like Fe citric acid and Zn gluconate; and organic trace minerals (OTM), such as copper glycine (Cu), iron glycine, and Zn methionine, among others (Saeid *et al.,* 2013).

Organic trace minerals are chelates, formed by ionic bond or covalent bonds between the ligand (amino acids, small peptides, proteins, organic acids, and polysaccharide derivatives) and the metallic element. Organic trace minerals present better rate of absorption and utilization, good chemical stability, higher biological potency, and delay the antagonism among different minerals (Spears, 1996) compared with inorganic and simple organic trace minerals.

Lipid peroxidation is lethal to the sperm cells. The antioxidant glutathione peroxidase (GSH-Px), containing selenium (Se) in its activity center, which can improve GSH-Px catalytic activity directly, plays a crucial role in protecting sperm from oxidative damage (Mohammad

& Moslemi, 2011). Selenium deficiency may slow down the development of the testis and epididymis, decreasing sperm density in semen, and increasing the production of abnormal sperm cells (Ahsan *et al.,* 2014; Graupner *et al.,* 2015).

Zinc is closely related with the antibacterial property of the seminal fluid. In addition, Zn plays a role in the stabilization of the sperm membrane, preventing its degradation (Taniguchi *et al.,* 2007). The early development of the prostate requires high Zn levels, and its deficiency delays the development of secondary sexual characteristics, and reduces testosterone serum levels (Xu *et al.,* 2015; Kothari & Chaudhari, 2016).

Copper is required to maintain the stability of the luteotropic hormone (LH) and the follicle-stimulating hormone (FSH) in the serum. In addition, it enhances prostate capacity to secrete testosterone by improving the binding capacity of prostaglandin E2 (PGE2) to its receptor in the prostate (Rajeswari & Swaminathan 2014; Wang *et al.,* 2014). PEG2 can regulate the synthesis of LH through copper, increasing LH serum levels, thereby inducing testosterone secretion to promote the differentiation and maturation of sperm cells (Sakumoto *et al.,* 2014).

High concentration of manganese (Mn) chloride in diet can affect the development of testes, impairing spermatogenesis, interfering with sperm cell growth and development, and resulting in the production of abnormal sperm cells (Gu & Hecht 1996). In addition, serum Mn excess induces significant changes in testes histology and physiology, and cause seminiferous tubule degeneration (Gabrielsen & Tanrikut, 2016; McGough & Jardine, 2017).

Most previous studies evaluated the effects of single inorganic element on the male reproductive system, and there is limited research on the effects of the dietary addition of OTM on the reproductive performance of male broiler breeders.

In the present study, rooster of the San Huang Chinese chicken breed were fed with Zn, Mn, Cu, and Fe chelated with glycine, and Se yeast to determine their effect on semen quality, tissue accumulation, testicular morphology, serum testosterone level, and the gene expression of testosterone synthesis-related enzymes.

MATERIALS AND METHODS

Birds, diets and experimental design

The experiments were carried out on a farm belonging to Jiangsu Lihua Animal Husbandry Stock Co, Ltd., Jiangsu, China. The San Huang roosters were housed in a commercial broiler breeder farm, and fed according to the Chicken Feed Regulations of Lihua. Roosters were used to semen collection every two days.

Birds were housed in individual cages and maintained under uniform management conditions (15 h light/day, 20-30 °C). Water was provided *ad libitum*. The following feed allowances were supplied, according to age: 129 g per bird from 23 to 26 weeks, 121 g per bird from 27 to 34 weeks, and 131 g per bird from 35 to 45 weeks of age.

Two hundred and forty San Huang roosters with 23 weeks of age were randomly divided into two treatments, with six replicates of 20 birds each. In the control treatment, the basal diet (Table 1) was supplemented with 100% inorganic trace minerals (ITM) at levels of 55, 60, 5.5, 70, and 0.15 mg of Zn, Mn, Cu, Fe, and Se per kg of diet, respectively, in the form of sulfate, except for Se, which was added as sodium selenite. The inorganic compounds contained 34.5% Zn, 31.8% Mn, 25% Cu, 30% Fe, and 0.45% Se, according to the products' specifications. In the second treatment, the basal diet did not contain ITM and was supplemented with 100% OTM, at the levels of 45, 49, 4.5, 58, and 0.13 mg of Zn, Mn, Cu, Fe, and Se per kg of diet, respectively, as methionine chelates,

Table 1 – Composition of the basal diet.

Item	Composition, %
Ingredients	
Corn	63.91
Soybean meal	9.60
Rice bran	23.57
Limestone	1.62
Liquid methionine	0.04
Choline chloride	0.13
Sodium chloride	0.30
Dicalcium phosphate	0.66
Vitamin premix[1]	0.05
Minerals premix[2]	0.12
Calculated composition	
Metabolizable energy, kcal/kg	2,76
Crude protein	12.98
Methionine	0.28
Lysine	0.58
Calcium	0.81
Total phosphorus	0.80

[1] Provided per kilogram of diet: vitamin A, 6,000 IU; cholecalciferol, 2,500 IU; vitamin E, 40 mg; menadione, 1.5 mg; thiamine, 3 mg; riboflavin, 12 mg; pyridoxine, 6 mg; vitamin B_{12}, 35 µg; pantothenic acid, 10 mg; niacin, 60 mg; folic acid, 0.8 mg; biotin, 0.225 mg.

[2] Provided per kilogram of diet: copper, 5.5 mg; iron, 70 mg; zinc, 55 mg; manganese, 60 mg; iodine, 0.4 mg; selenium, 0.15 mg.

except for glycine-Fe and Se-yeast. The organic trace mineral product specifications are 16% Zn, 13% Mn, 15% Cu, 17% Fe, and 0.2% Se. The OTM were purchased from Novus International Trade Co, Ltd. (Shanghai, China).

The basal diet was based on corn and soybean meal basal and formulated to contain adequate levels of all nutrients, as recommended by the National Research Council (NRC, 1994). The experimental period was 22 weeks. One rooster was randomly selected per replicate for semen collection. Semen was collected for the first time 2 weekss after the beginning of the treatments, and 10 times thereafter in 2-week intervals (at 27, 29, 31, 33, 35, 37, 39, 41, 43, and 45 weeks of age). One rooster per replicate was randomly selected at three time points (30, 35, and 45 weeks of age) and sacrificed for blood and organ collection.

Sample collection

Blood samples were collected in plastic tubes and centrifuged at 1369.6 (×g) for 15 min at 4 °C. The serum was separated and stored at -20 °C until assayed for testosterone levels. Internal organs (heart, liver and spleen) and reproductive organs (testis and epididymis) were excised and immediately weighed. A subsample from each testis sample was fixed in 4% paraformaldehyde and processed for histological examination; the remaining testes were stored in liquid nitrogen for RT-PCR.

Determination of the five evaluated trace minerals concentrations in the feed, serum and testis

Diet and testis samples (0.5 g), as well as blood samples (0.5 mL) were digested with nitric acid (75%) and perchloric acid (25%) overnight at 25 °C. After the solution became faint yellow, the samples were placed in a digestion System. After digestion, the solution was removed to 25-mL volumetric flask, then levels of the five supplemented trace minerals (Zn, Mn, Cu, Fe, and Zn) were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima 2100 DV, USA). The results were determined against the standard curve of each element.

Semen quality analysis

The semen was collected by dorso-abdominal massage (Burrows & Quinn, 1937). A semen sample was placed into a sterile stoppered graduated measuring glass, and its volume was immediately measured and recorded. After collection, the sample was incubated at 37 °C until analysis. A 10-µL sample was diluted (1:20) in hydrosaline solution (3%, wt/vol; NaCl) to determine sperm concentration using hemocytometer. Sperm motility was visually assessed in at least 10 microscopic fields, and the percentage of moving sperm relative to non-motile cells was calculated. Sperm morphology was assessed in 300 sperm cells per air-dried sample.

Testis histology

The testis samples fixed in formaldehyde were dehydrated in graded ethanol series, cleared in xylene, and embedded in paraffin wax. Sections (5-µm thickness) were then cut and were with hematoxylin and eosin (HE). The stained sections were mounted on slides and examined under light microscopy (400 ×).

Radioimmunoassay

The serum concentrations of testosterone were determined by the Nanjing General Hospital of the Nanjing Military Command using a 125 I-labeled radioligand double-antibody RIA kit. The commercial RIA kit was purchased from Beijing Beifang Institute of Biotechnology. The intra- and inter-assay coefficients of variation were less than 10% for testosterone.

Reverse transcription-polymerase chain reaction

Total RNA was extracted from the tissue using a RNeasy mini kit (Qiagen, China) according to the manufacturer's instructions. The concentration and purity of the isolated total RNA was determined spectrophotometrically at 260 and 280 nm with a Nanodrop 8000 (Thermo Fisher Scientific, Wilmington, USA). The total RNA (1 µg) was reverse transcribed to cDNA with an Omniscript Reverse Transcription kit (Takara, Japan) with Oligo-dT primers (Takara, Japan) according to the manufacturer's instructions. The target fragments were quantified by real-time PCR using a QuantiTectTMSYBR Green PCR Kit (F Hoffmann-La Roche Ltd., Switzerland) with 100 ng of the cDNA template. The gene expression data were normalized to β-actin expression. The specific primer sets, designed using Primer 5 Plus program, are described in Table 2. For the quantification of real-time PCR results, the threshold cycle Ct was determined for each reaction. Ct values for each gene of interest were normalized to the housekeeping gene; PCR amplification efficiencies were taken into account by amplifying various amounts of target cDNA for each reaction. Normalized values were used to calculate the degree of induction or inhibition expressed as a "fold difference" compared with normalized control values.

Therefore, all of the data were statistically analyzed as "fold induction" between treated and control animals.

Table 2 – Primers for mRNA analyses.

Genes	Sequence (5' - 3')	Product size, bp
β-actin	F: TGCTGTGTTCCCATCTATCG R:TTGGTGACAATACCGTGTTCA	150
CYP17A1	F: TCTGCTCCCTCTGCTTCAA R: AGGTCCCTCACAGTGTCCC	250
HSD3B2	F:TCCTCACATGAGCTACGCAGAC R: CAGGTGGCGGTTGGTTGA	178
CYP19A1	F: TGTTCCATCACGCTATTT R: GATTCTTGTTTGGGCTTC	238
HSD17B4	F: TTTGCCATGAGACCTGTA R: GTCTATCTCAGTGTTCCCTC	247

CYP17A1 = cytochrome P450 17A1, *HSD3B2* = hydroxysteroid 3-beta dehydrogenase 2, *CYP19A1* = cytochrome P450 19A1, *HSD17B4* = hydroxysteroid 17-beta dehydrogenase 4.

Statistical analysis

All data are presented as mean ± standard error of the mean (SEM) and were analyzed by one-way analysis of variance (ANOVA). Statistical analysis was performed using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant when p value was less than 0.05.

RESULTS

Semen quality

As shown in Table 3, semen volume increased in the OTM group both from 31 to 35 weeks and from 37 to 45 weeks, compared with the ITM group ($p<0.01$). Higher sperm density ($p<0.001$) and motility ($p<0.01$) were determined in the OTM group compared with the ITM group from 31 to 35 weeks. Lower abnormal sperm morphology was detected in the OTM group than in the ITM group from 25 to 45 weeks ($p<0.05$).

Body weight and relative organ weights

Body weight and relative organ weights at 3-time points were shown in Table 4. Body weight and relative organ weights were not significantly different between groups as determined from 30 to 45 weeks at 3-time points.

Trace minerals concentrations in the diet, serum and testis

The trace minerals levels were evaluated in the diet, and in the serum and testis of the birds fed OTM or ITM at 30, 35, and 45 weeks of age (Table 5).

The OTM diet presented lower Mn and Zn levels ($p<0.001$) than the ITM diet, but the levels of the other evaluated trace minerals (Cu, Fe, Se) were not different. Testicular Zn level at 45 weeks of age were higher ($p<0.05$) in the OTM group compared with the ITM group. In 30-week-old birds, serum Fe level was lower in the OTM group compared with the ITM group ($p<0.01$), but not at the other evaluated ages. Serum Mn levels in the OTM group were higher than in the ITM group at 45 weeks ($p<0.05$).

Testicular histology

Figure 1 shows the testicular histology of roosters evaluated at 30, 35, and 45 weeks of age. As shown in Fig. 1, the testicular histology was not different

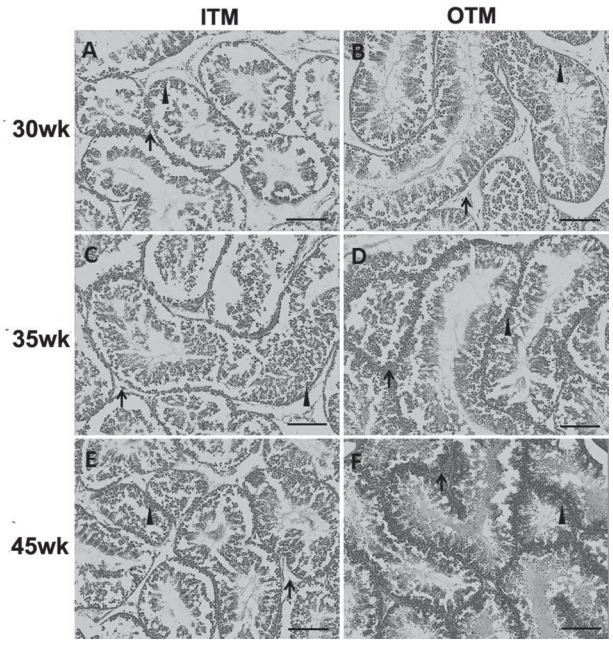

Figure 1 – Effects of dietary organic trace minerals premix (OTM) on spermatogonia and Leydig cells of the testes of male broiler breeders at 30 (A and B), 35 (C and D) and 45 (E and F) weeks of age. Sections were stained with hematoxylin and eosin (HE). Bar = 100 µm. Arrowhead shows spermatogonia and primary spermatocyte cells. Arrow shows Leydig cells (400 ×).

Table 3 – Effect of dietary organic trace minerals premix on semen quality.

Semen Parameters	25 – 29 weeks		31 – 35 weeks		37– 45 weeks	
	ITM	OTM	ITM	OTM	ITM	OTM
Volume, mL	0.40 ± 0.24	0.47 ± 0.18	0.37 ± 0.14	0.52 ± 0.12**	0.34 ± 0.08	0.54 ± 0.10***
Density, $\times 10^9$/mL	3.90 ± 0.65	4.45 ± 1.01	3.74 ± 0.66	4.76 ± 0.52***	3.84 ± 0.45	4.61 ± 0.40
Motility, %	80.50 ± 9.84	85.42 ± 3.96	82.00 ± 6.21	87.50 ±3.53**	84.33 ±4.57	86.50 ± 2.53
Abnormal morphology, %	8.05 ± 0.75	5.91 ± 0.68*	9.73 ± 0.85	6.72 ± 0.45**	8.67 ± 0.55	6.62 ± 0.35**

ITM = inorganic trace minerals premix; OTM = organic trace minerals premix. The values shown are the mean ± SEM of 6 animals per group. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table 4 – Effect of dietary organic trace minerals premix on body weight and the relative organ weight.

	30 weeks		35 weeks		45 weeks	
Item	ITM	OTM	ITM	OTM	ITM	OTM
Body weight, kg	4.41 ± 0.07	4.15 ± 0.07	4.56 ± 0.12	4.35 ± 0.13	4.44 ± 0.11	4.25 ± 0.14
Heart, g	22.49 ± 0.56	20.34 ± 0.61	23.61 ± 0.64	21.16 ± 0.85	21.13 ± 0.71	21.14 ± 0.45
Relative Heart (%)	0.51 ± 0.01	0.49 ± 0.01	0.52 ± 0.02	0.49 ± 0.03	0.48 ± 0.01	0.49 ± 0.09
Liver, g	66.01 ± 1.50	58.39 ± 3.30	45.54 ± 2.11	47.26 ± 2.98	43.11 ± 3.63	47.53 ± 3.90
Relative Liver (%)	1.46 ± 0.05	1.40 ± 0.07	1.00 ± 0.05	1.08 ± 0.05	0.96 ± 0.05	1.11 ± 0.06
Spleen, g	4.18 ± 0.24	3.89 ± 0.15	3.71 ± 0.11	4.06 ± 0.21	4.46 ± 0.46	4.43 ± 0.29
Relative Spleen (%)	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.01
Testis, g	24.63 ± 1.87	24.29 ± 1.72	23.02 ± 1.47	21.02 ± 2.56	19.46 ± 1.97	20.73 ± 2.45
Relative Testis (%)	0.55 ± 0.05	0.59 ± 0.04	0.51 ± 0.04	0.49 ± 0.06	0.44 ± 0.04	0.49 ± 0.05

ITM = inorganic trace minerals premix; OTM = organic trace minerals premix. The values shown are the mean ± SEM of 6 animals per group. The relative organ weights were calculated as the organ weights divided by BW (g/g BW).

between ITM- and OTM-fed roosters at 30 and 35 weeks of age. The seminiferous epithelium was tighter in the OTM group than in the ITM group at 45 weeks of age, and the number of spermatogonia, primary spermatocytes and Leydig cells were higher in the OTM group compared with the ITM group.

Serum testosterone concentrations and mRNA expression levels of enzymes related to testosterone synthesis

Figure 2 shows the serum testosterone levels of 30-, 35-, and 45-week-old roosters. Those fed OTM presented higher testosterone levels at 35 ($p<0.05$) and 45 ($p<0.01$) weeks of age than those fed ITM, but not at 30 weeks of age.

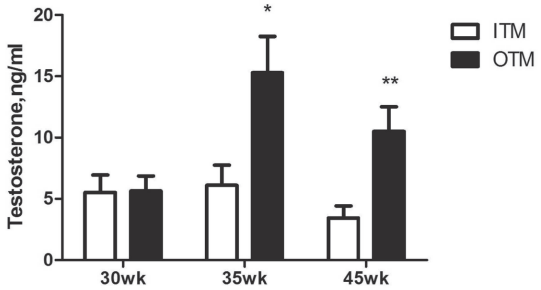

Figure 2 – Effect of dietary organic trace minerals premix (OTM) on serum testosterone levels of roosters at 30, 35, 45 weeks of age. The values shown are the mean ± SEM of 6 animals per treatment. *$p<0.05$, **$p<0.01$. ITM = inorganic trace minerals premix.

Figure 3 presents the mRNA expression levels of enzymes related to testosterone synthesis in the testes of 30-, 35-, and 45-week-old roosters. Higher mRNA

Table 5 – The concentrations of 5 trace minerals in fodder, serum and testis.

		30 weeks		35 weeks		45 weeks	
	Item	ITM	OTM	ITM	OTM	ITM	OTM
Zn	Diet, μg/g of DM	122.10 ± 4.27	82.60 ± 2.26***				
	Serum, μg/mL	8.92 ± 0.54	12.19 ± 2.47	8.13 ± 1.16	5.37 ±1.60	4.95 ± 0.53	6.04 ± 0.46
	Testis, μg/g of FW	14.13 ± 0.66	14.50 ± 0.55	18.06 ± 1.37	16.43 ± 0.21	17.62 ± 0.92	22.40 ± 0.79*
Mn	Diet, μg/g of DM	144.10 ± 5.81	87.14 ± 0.54***				
	Serum, μg/mL	0.76 ± 0.18	0.58 ± 0.15	0.45 ± 0.11	0.38 ± 0.14	0.54 ± 0.07	1.09 ± 0.18*
	Testis, μg/g of FW	0.37 ± 0.02	0.34 ± 0.01	0.38 ± 0.02	0.36 ± 0.01	0.39 ± 0.01	0.67 ± 0.13
Cu	Diet, μg/g of DM	9.98 ± 1.38	9.57 ± 2.91				
	Serum, μg/mL	2.23 ± 0.13	1.87 ± 0.08	1.94 ± 0.17	2.15 ± 0.18	2.04 ± 0.19	2.24 ± 0.12
	Testis, μg/g of FW	0.15 ± 0.03	0.18 ± 0.06	0.13 ± 0.03	0.10 ± 0.03	0.20 ± 0.04	0.19 ± 0.03
Fe	Diet, μg/g of DM	192.60 ± 16.00	171.20 ± 11.26				
	Serum, μg/mL	55.21 ± 3.05	36.48 ± 2.13**	32.47 ± 4.75	41.69 ± 3.89	25.78 ± 1.78	26.74 ± 1.56
	Testis, μg/g of FW	31.21 ± 2.98	25.42 ± 1.98	19.38 ± 1.51	21.08 ± 4.26	28.27 ± 3.99	28.48 ± 3.64
Se	Diet, μg/g of DM	0.25 ± 0.06	0.28 ± 0.08				
	Serum, μg/mL	2.90 ± 0.60	2.05 ± 0.57	0.62 ± 0.21	1.41 ± 0.21	1.96 ± 0.50	3.83 ± 0.78
	Testis, μg/g of FW	1.05 ± 0.19	0.96 ± 0.41	0.69 ± 0.28	0.81 ± 0.51	0.99 ± 0.45	1.21 ± 0.52

ITM = inorganic trace minerals premix; OTM = organic trace minerals premix; DM = dry matter; FW = fresh weight (the weight of tissue used for detection). The values shown are the mean ± SEM of 6 animals per group. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

expression levels of the *HSD3B2* and *CYP17A1* genes were determined in the OTM-fed roosters compared with those fed ITM at 45 weeks of age ($p<0.05$) (Fig. 3A and 3B), but not at younger ages (30 and 35 weeks of age). In contrast with the ITM-fed birds, the mRNA expression level of the *HSD17B4* gene in the OTM-fed birds was higher at 30 ($p<0.01$) and 35 ($p<0.05$) weeks of age, but lower at 45 ($p<0.05$) weeks of age (Fig. 3C). The mRNA expression of the *CYP19A1* gene was lower in the OTM-fed birds at 30 weeks of age ($p<0.001$), and higher at 35 and 45 weeks of age ($p<0.01$) than in those fed ITM (Fig 3D).

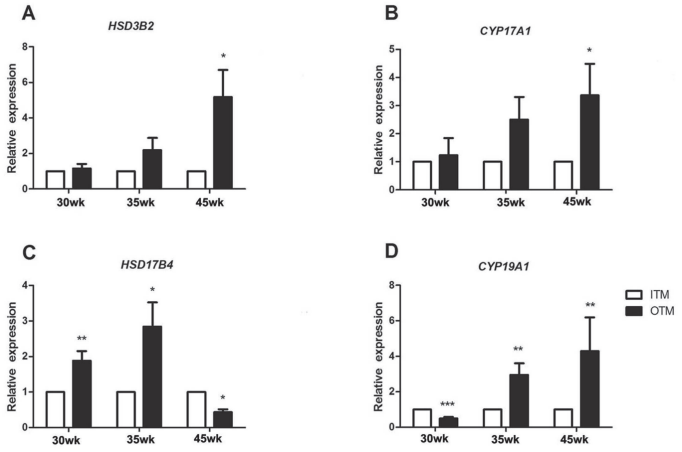

Figure 3 – Effect of dietary organic trace minerals premix (OTM) on the mRNA expression level of the genes hydroxysteroid 3-beta dehydrogenase 2 (*HSD3B2*), cytochrome P450 17A1 (CYP17A1), hydroxysteroid 17-beta dehydrogenase 4 (HSD17B4) and cytochrome P450 19A1 (CYP19A1) in the testes of roosters at 30, 35, 45 weeks of age. The values shown are mean ± SEM of 6 animals per group. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

DISCUSSION

This is the first report that focuses on the effects of the OTM on semen quality and testosterone synthesis in San Huang breeder roosters. The present study demonstrated that OTM had a positive impact on the growth of closed-packed spermatogonia and Leydig cells, and improved the semen quality of roosters from 31 to 35 weeks of age. At 45 weeks of age, roosters fed OTM presented significantly higher serum testosterone levels, as well as higher mRNA expression levels of the *HSD3B2* and *CYP17A1* genes in the testes.

Several markers are used to evaluate semen quality, such as semen volume, sperm density, sperm motility, and abnormal morphology. In the mammalian studies, trace minerals have been shown to be essential for improving semen quality. There is a positive correlation between OTM and high semen quality in broilers (Abdallah *et al.,* 2009; Atig *et al.,* 2013). The absence of Zn or Mn may suppress enzyme systems required for sperm motility and sperm count in rats (Yamaguchi *et al.,* 2009; Babaei & Abshenas, 2013). The trace elements of Zn, Mn, and Se enhance spermatogenesis and improve semen quality in broiler breeders (Barber *et al.,* 2005). It was reported that the supplementation of OTM, including Zn, Cu, Co, and Mn, improved the semen quality of bulls, even after freezing, compared with ITM (Rowe *et al.,* 2013; Rowe *et al.,* 2014). In the present study, the OTM-fed roosters presented better semen parameters, such as semen volume, density, motility, and number of normal sperm during the entire experimental period compared with those fed ITM, indicating that OTM improved semen quality.

Dietary organic mineral sources increase the intestinal absorption of trace elements as they do not form indigestible complexes with dietary antinutrient compounds (Świątkiewicz *et al.,* 2014). Due to their high bioavailability, OTM supplementation of broiler diets is an efficient method to reduce trace mineral excretion in the environment (Bao *et al.,* 2007). In the present study, the levels of the five trace minerals were higher in the ITM diet than in the OTM diet. However, both ITM- and OTM-fed roosters presented similar serum and testicular levels of these trace minerals, indicating that the organic forms had higher bioavailability and absorption rate than the inorganic forms.

A previous study reported that feeding high OTM levels increased pregnancy rate to artificial insemination of beef cows compared with ITM either at low or high levels (Stanton *et al.,* 2000). Angus heifers fed OTM presented a higher number of fertilized oocytes than those fed ITM (Lamb *et al.,* 2008). Feeding OTM benefitted sow reproductive performance compared with ITM (Peters & Mahan, 2008). In present study, the seminiferous epithelium was tighter in the OTM-fed roosters, which is consistent with mentioned previous studies showing that feeding OTM may improve reproductive performance.

Testosterone plays a vital role in the development of the testis, epididymis, an prostate, and on semen quality (Murashima *et al.,* 2015). In the present study, dietary OTM increased serum testosterone levels. In order to investigate the potential mechanisms underlying the OTM-induced testosterone increase, the mRNA expression of enzymes related to the testosterone biosynthesis were measured. The *HSD3B2*, *CYP17A1*, hydroxysteroid 17-beta dehydrogenase 4 (*HSD17B4*) genes are known to encode enzymes involved in biosynthesis of testosterone (Taniguchi *et al.,* 2007; Pierce *et al.,* 2010; Rabbani *et al.,* 2012). Cytochrome P450 19A1 (*CYP19A1*) converts androstenedione

to estrone and testosterone to estradiol (Hosono *et al.,* 2011). In the present study, the higher mRNA expression of the *HSD3B2*, *CYP17A1* and *HSD17B4* genes possibly indicates an increase in the activity of the corresponding enzymes, which accounts for the increase in the serum testosterone levels of the roosters fed OTM. Moreover, the testicular seminiferous epithelium may benefit from the elevated testosterone levels.

CONCLUSIONS

In summary, feeding male broiler breeders with organic trace minerals promoted better semen quality, as identified by its physical traits, compared with inorganic trace minerals. This result is possibly due to the increase in testosterone synthesis in OTM-fed roosters. The present experiment demonstrates that supplementing OTM to the basal diet is beneficial to the male broiler breeder reproductive system, and provides further evidence of the advantages of supplementing trace minerals in organic forms.

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