Effects of Supplemental Dietary Zinc-Methionine on the Reproductive Performance and Plasma Levels of Estrogen and Progesterone of Yuehuang Broiler Breeders

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Abstract: At 14 weeks of age, 567 female broiler breeders were housed in two step battery cages and fed either I) a corn-soybean (CSB) diet with no supplement of Zn or Methionine (control). II) a CSB diet supplemented with ZnO and no Methionine (Met). III) a CSB diet supplemented with Met and no Zn. IV) a CSB diet supplemented with Zn-Met N. R. C. values. V) a CSB diet supplemented with Zn-Met (low level). VI) a CSB diet supplemented with Zn-Met (med. level) and VII) a CSB diet supplemented with Zn-Met (high level). These diets contained 30.09, 70.09, 30.09, 70.09, 78.69, 97.19 and 134.20 mg Zn of one kilogram diet, respectively. The Met values were 0.28%, 0.28%, 0.36%, 0.36%, 0.37%, 0.38% and 0.38%, respectively. Supplemental Zn of ZnO or Zn-Met in CSB resulted in differences in egg mass, fertility, hatchability, egg production and plasma levels of estrogen and progesterone. In all cases groups II, IV and V with levels of 70.09, 70.09 and 78.69 mg/kg Zn and 0.28%, 0.36% and 0.37% Met respectively showed significant increase ($P < 0.05$), compared to the control group. However, levels of Zn-Met higher than 78.69 mg/kg Zn and 0.37% Met did not result in any increase in hatchability, fertility, egg production and plasma concentrations of estrogen and progesterone.

Key words: zinc; methionine; broiler breeder; reproductive performance; estrogen; progesterone
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The role of Zn in living systems is attributed primarily to its association with proteins, especially as a functional component of metalloenzymes. Carbonic anhydrase was a Zn dependent enzyme. Zn has numerous biological roles, including protein metabolism, DNA synthesis, cell division and multiplication. Improving the status of trace minerals such as Zn, Cu, Mn and Co can enhance reproductive performance. Despite its role in living systems, the literature reveals an uncertainty as to the value of Zn methionine (Met) products (ZP) in poultry diets. Waibel et. al.[1] and Kidd et. al.[2] reported that ZP in diets resulted in overall beneficial responses in turkey growth and egg production under laboratory conditions. Sanford[3] reported improved broiler breeder performance from ZP fortification of broiler diets. Kienholz et. al.[4] examined the effects of Zn-Met on stressed laying hens and reported that Zn-Met was beneficial to hens during low-Ca stress and during the recovery period following that stress. In another study Zinpro Co[5] reported that layers fed (Availa Zn-zinc amino acid complex and Availa Mn-manganese amino acid complex) under increased temperature and humidity showed increased egg production, increased egg specific gravity and improved egg and shell mass. Broiler chicks fed ZINPRO Zn Met, MANPRO Mn Met, Availa Zn zinc amino acid complex and Availa Mn manganese amino acid complex showed significantly reduced mortality rates without any adverse effect on body mass, feed consumption or feed conversion[6]. Stahl et. al.[7] examined the effect of Zn Met supplementation on the performance of mature broiler breeders, and reported that fertility and hatchability of eggs from birds fed the Zn-supplemented diets did not alter egg mass, chick mass, egg production, livability, and humoral or cellular immunity when compared to the control-fed birds. In a similar experiment, Sandoval et. al.[8] showed that Zn supplementation, from inorganic sources, of a corn soybean meal diet containing 28 g/kg of diet did not improve the production or reproduction of single comb white leghorn (SCWL) hens. Some reports have shown improved performance in chicken hens fed Zn Met products[9], but others have shown that ZP was not beneficial

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to chicken hens[10, 11]. In spite of the fact that Zn supplementation in broiler breeder diet has been the focus of many studies, no attention has been given to the Yuehuang broiler breeder hen. Therefore the objective of this study was to determine the effects of feeding various levels of Zn Met on the reproductive performance and plasma concentration of estrogen and progesterone of Yuehuang broiler breeder chickens.

1 Materials and methods

1.1 Animals and husbandry

A total of 567 Yuehuang broiler breeder females of the South China Agricultural University Experimental Poultry Farm at 14 weeks of age were used in the study. The experimental design consisted of seven dietary treatments or groups, assigned randomly to each group were three replications. Experimental units consisted of 27 broiler breeder hens. Three breeders were housed in each wired cages in two step batteries, with cage dimensions of 76.2 cm × 137.1 cm in a room with adequate ventilation and lighting (14 h light and 10 h darkness). The broiler breeders were weighed and assigned to experimental units such that no significant differences in average body mass existed among experimental units.

1.2 Diet

Breeders were given free access to feed (daily feed intake noted) and water throughout the experiment. Breeders were fed the experimental diets for 2-weeks equilibration phase followed by 18 weeks of data collection. Tab. 1 shows the basal breeder diet used by the poultry farm.

Tab. 1 Basal breeder diet

w(corn)/%	w(soybean meal)/%	w(fish meal)/%	w($CaCO_3$)/%	w(salt)/%	w(choline)/%	w($CaHPO_4$)	w(coarse limestone)	w(Met)/%	w(Vit.)[1)]/%
63.01	20.00	3.00	8.08	0.15	0.20	1.20	4.00	0.00	0.05

w(trace mineral mix)[2)]/%	w(non-nutritive ingredients)[3)]/%	w(variable ingredients)/%	analyzed nutrients					
			ME/(MJ·kg^{-1})	w(CP)/%	w(Ca)/%	w(P)/%	w(Met)	w(Zn)/(mg·kg^{-1})
0.05	0.12	0.04	12.20	16.00	3.25	0.65	0.28	30.09

1) provided the following per kilogram of diet: Vitamin A, 8 818 IU; cholecalciferol, 2 756 IU; Vitamin E, 22 IU; Vitamin K_3, 1 362 mg; Vitamin B_{12}, 12 mg; riboflavin, 600 mg; niacin, 30 000 mg; pantothenic acid, 10 000 mg; folic acid, 750 mg; pyridoxine hydrochloride, 1 215 mg; biotin, 100 mg; 2) provided the following per kilogram of diet: Mn, 67.5 mg; Fe, 20 mg; Cu, 2 mg; I, 1.25 mg; Se, 3 mg. 3) Non-nutritive ingredients as percentage of the diet: Hygromycin-8, gentian violet

At 14 weeks of age broiler breeders were fed either: Ⅰ) Corn-soybean (CSB) diet with no supplement of Zn or Met. Ⅱ) CSB diet supplemented with ZnO and no Met. Ⅲ) CSB diet supplemented with Met and no Zn. Ⅳ) CSB diet supplemented with Zn-Met using the N. R. C. values. Ⅴ) CSB diet supplemented with Zn-Met (low level). Ⅵ) CSB diet supplemented with Zn-Met (med. Level). Ⅶ) CSB diet supplemented with Zn-Met (high level). Tab. 2 shows the amount of Zn and Met supplemented to basal breeder diet.

Tab. 2 Amount of Zn and Met supplemented to the basal breeder diets

treatment	diet[1)]	w(Zn)/(mg·kg^{-1})[2)]	w(Met)/%[3)]
Ⅰ) control	CBS no Zn or Met added	0.00	0.00
Ⅱ) ZnO	CSB & ZnO	40.00	0.00
Ⅲ) Met	CSB & Met	0.00	0.08
Ⅳ) N. R. C.	CSB Zn-Met	40.00	0.08
Ⅴ) Zn-Met (low level)	CSB Zn-Met	48.60	0.09
Ⅵ) Zn-Met (med. level)	CSB Zn-Met	67.10	0.10
Ⅶ) Zn-Met (high level)	CSB Zn-Met	104.11	0.10

1) Corn-soybean (CSB) meal diet; 2) Control level of Zn in CSB=30.09 mg/kg; 3) Control level of Met in CSB=0.280%

The above diets contained Zn 30.09, 70.09, 30.09, 70.09, 78.69, 97.19 and 134.20 mg/kg of diet, respectively. The Met values were 0.28%, 0.28%, 0.36%, 0.36%, 0.37%, 0.38% and 0.38%, respectively.

Records were kept of daily feed intake, daily oviposition/cage, individual egg mass and daily mortality/cage evaluated.

Eggs were collected for incubation from the 7 dietary treatments, after breeders had received diets for 3, 6, 9, 12, 15 and 18 weeks for six hatches. Upon collection, eggs were identified by marking treatment group number as well as the cage number over air cell.

A random sample of 648, 928, 1 121, 1 133, 962, 949 and 1 077 eggs for treatment groups Ⅰ through Ⅶ were set for hatches Ⅰ through Ⅵ. Eggs were collected once daily and stored at 16.7 ℃ and 60% relative humidity. Eggs were cleaned prior to settings. Fertility of eggs was determined by candling and break-out of un-hatched eggs. Fertile eggs were further classified as early or late mortality and used to evaluate the embryonic state at which mortality occurred. Percentages of hatchablility, embryonic mortality, dead chicks, infertile eggs and normal chicks were evaluated.

1.3 Analysis of blood hormone concentration

From the above experiment A total of 96 broiler breeders were randomly selected in the investigation of plasma concentration of estrogen and progesterone during the study.

The experimental design consisted of the seven dietary treatments or groups. Assigned randomly to group Ⅱ, Ⅲ, Ⅳ, Ⅴ, Ⅵ were 12 breeders and 24 breeders for the control group. All other management practices (housing, feeding etc.) remain the same as in the first experiment.

Blood samples (2—3 mL) were collected by direct venipuncture when broiler breeders were 16 and 34 weeks of age respectively.

The heparin zed blood were centrifuged within 3 min at 600 g at 4 ℃ for 15 min. The plasma was collected and stored at −20 ℃ for determination of progesterone and estrogen. The two hormones were analyzed by radioimmunoassay. The plasma concentration of progesterone and estradiol—17 beta were determined.

1.4 Statistical analysis

The results of the experiments were analyzed by means of ANOVA as a randomized block design. The computer program SPSS V. 10.0.1 (27 Oct. 1999) was used to calculate the ANOVA.

2 Results and discussion

2.1 Effect of feeding broiler breeders a CSB diet supplemented with Zn Met on the reproductive performance of Yuehuang broiler breeders

Tab. 3 and 4 summarizes the performance data collected. The addition of supplemental Zn or Zn-Met resulted in differences in fertility, hatchability, daily feed consumption, average egg mass, hen-day production and mortality. Compared with the control, addition of supplemental Zn level of either 70.09 mg/kg feed (ZnO) or 70.09 mg/kg feed (Zn-Met) (N.R.C. values) or 78.69 mg/kg feed (Zn-Met lower level) resulted in increase ($P < 0.05$) mean percentage fertility, hatchability, average egg mass, average daily feed consumption, mortality and

Tab. 3 Effect of feeding broiler breeders a corn soybean diet supplemented with Zn Met on fertility and hatching performance[1)]

treatment	egg set no.	fertility /%	hatchability /%	embryonic mortality /% early	embryonic mortality /% late
Ⅰ) control	648	80.58 ± 1.74^{a}	60.41 ± 3.75^{a}	4.65 ± 1.10	13.82 ± 3.08
Ⅱ) ZnO	928	84.60 ± 1.16^{c}	67.73 ± 2.91^{d}	5.25 ± 1.58	12.43 ± 1.76
Ⅲ) Met	1 121	80.34 ± 1.06^{a}	61.71 ± 3.16^{a}	7.13 ± 0.81	14.45 ± 2.37
Ⅳ) N.R.C.	1 133	84.84 ± 1.08^{c}	68.03 ± 3.00^{d}	6.90 ± 0.96	13.55 ± 1.60
Ⅴ) Zn-Met (low level)	962	85.59 ± 1.78^{c}	68.14 ± 5.18^{d}	5.05 ± 0.85	11.42 ± 2.49
Ⅵ) Zn-Met (med. Level)	949	81.71 ± 2.97^{b}	63.62 ± 5.56^{c}	5.38 ± 0.88	12.70 ± 6.97
Ⅶ) Zn-Met (high level)	1 077	80.60 ± 2.26^{a}	63.74 ± 4.02^{c}	7.43 ± 1.26	11.42 ± 2.49

1) Mean ± SE followed by the same letter within a column are not significantly different from each other ($P < 0.05$)

Tab. 4 Effects of feeding different levels of Zn on food consumption, egg mass percentage hen-day and percentage mortality[1)]

treatment	average daily feed con. /($g \cdot d^{-1}$)	average egg mass/g	hen-day production/%	mortality /%
Ⅰ) control	76.70 ± 1.01^{b}	35.94 ± 0.12(1 394)^{ab}	21.27 ± 3.68^{a}	13.05 ± 0.13^{a}
Ⅱ) ZnO	71.13 ± 0.84^{b}	37.64 ± 0.11(2 159)^{b}	37.64 ± 4.54^{ab}	12.81 ± 0.17^{ab}
Ⅲ) Met	72.88 ± 1.23^{a}	36.09 ± 0.18(2 273)^{ab}	34.18 ± 5.30^{ab}	14.28 ± 0.14^{a}
Ⅳ) N.R.C.	81.22 ± 1.58^{c}	38.30 ± 1.79(2 284)^{b}	38.40 ± 6.03^{b}	16.81 ± 0.12^{b}
Ⅴ) Zn-Met (low level)	80.93 ± 1.20^{c}	38.44 ± 0.28(1 957)^{b}	38.23 ± 5.01^{b}	16.75 ± 0.17^{a}
Ⅵ) Zn-Met (med. level)	72.13 ± 1.28^{a}	36.06 ± 0.11(2 021)^{ab}	34.08 ± 4.98^{ab}	17.99 ± 0.15^{ab}
Ⅶ) Zn-Met (high level)	78.37 ± 0.99^{bc}	35.91 ± 0.11(2 227)^{ab}	32.10 ± 5.10^{ab}	12.20 ± 0.19^{a}

1) Mean ± SE followed by the same letter within a column are not significantly different from each other ($P < 0.05$)

hen-day production compared with the control. Although the percentage mortality was significant, it could be attributed mainly to heat stress considering the time when the study was conducted (May-September).

Fertility and hatchability tended to drop significantly as the Zn-Met level exceeded 78.69 mg/kg and 0.37%.

Egg production was calculated based on percentage hen-day. Egg production was higher in treatment Ⅱ, Ⅳ and Ⅴ but drop sharply when the Zn-Met level exceeded 78.69 mg/kg Zn and 0.37% Met.

Feed intake was found to increase in groups Ⅳ and Ⅴ and the palatability of feed in groups Ⅳ and Ⅴ compared to other groups. Early and late embryonic mortalities were not significantly different ($P>0.05$).

Average egg mass remained significantly higher in group Ⅱ, Ⅳ and Ⅴ. Laying hens fed isolated soybean meal based diets containing 10 mg/kg had impaired hatchability and egg production unless diets were supplemented with Zn. Many studies have shown the detrimental effects of Zn deficiency on hatchability. Stahl et. al.[12] observed that a CSB diet containing 78 mg/kg improved fertility in broiler breeders when compared with a CSB diet containing 28 mg/kg. Supplemental Zn in CSB diet had significant effect on hatchability, fertility, hen-day egg yield and egg mass ($P<0.05$) compared with the control group, however at levels higher than 78.69 mg/kg feed all the above mentioned parameters decreases significantly ($P<0.05$). It is important to note that embryonic mortality is not significantly ($P>0.05$) affected by supplemental Zn or Zn-Met. However, more embryonic death were observed in the later stage of incubation (late embryonic mortality). Percentage hen-day production were high for groups Ⅱ, Ⅳ and Ⅴ compared to other groups, this can be attributed mainly to the role of Zn in the promotion of reproduction and bioavailability of organic Zn to the breeders. Studies has also shown that Zn in the diet can produce positive as well as negative effects depending on the level used. For example Bartov[13] studied the use of diets containing high levels of Zn for controlling early growth in female broiler chicks and noted that Zn supplementation can be used as an efficient tool for controlling early growth of young broiler breeder chicks. In an experiment conducted by Batal et. al.[14] to investigate the relative bioavailability of supplemental Zn in inorganic sources for broiler chicks revealed that bioavailability estimates were similar when Zn was supplemented to diets at high or low concentrations. In a similar study Sandoval et. al.[15] found that bioavailability of supplemental Zn organic sources was more than that of inorganic sources. The minimal bioavailable Zn requirement for chicks 1 to 3 weeks of age fed a soy concentrate diet was estimated at 22.4 mg/kg feed[16].

The present study agrees with the work of the authors[7,17] in which they noted that Zn supplementation in a CSB or MCSB diet for hatchability and fertility in young broiler breeders was not required above 72 and 83 mg/kg of diet, in CSB meal or milo CSB respectively.

2.2 Effect of feeding broiler breeders a CSB diet supplemented with Zn Met on the plasma progesterone and estrogen levels of Yuehuang broiler breeders

The results showed that in all the treatments studied in which ovulation occurred, a consistent pattern in the levels of both hormones was observed (Tab. 5). Thus the level of progesterone in plasma increased 2 folds in all treatments to reach a maximum values of 4.34 and 4.29

Tab. 5 Effect of Zn-Met on the plasma progesterone (P_4) and estrogen (E_2) concentrations of broiler breeder1)

treatment	$\rho(P_4)/(ng \cdot mL^{-1})$		$\rho(E_2)/(pg \cdot mL^{-1})$	
	16 weeks of age	34 weeks of age	16 weeks of age	34 weeks of age
Ⅰ) control	$0.83\pm0.14(16)^{a}$	$1.69\pm0.57(0.16)^{a}$	$116.14\pm10.64(18)^{ab}$	$156.48\pm10.06(18)^{a}$
Ⅱ) ZnO	$1.33\pm0.51(9)^{b}$	$3.62\pm0.81(9)^{c}$	$169.41\pm12.82(12)^{bc}$	$172.66\pm11.81(12)^{b}$
Ⅲ) Met	$1.58\pm0.50(11)^{b}$	$2.34\pm0.55(11)^{ab}$	$159.70\pm15.70(12)^{b}$	$162.55\pm13.88(12)^{b}$
Ⅳ) N.R.C.	$2.52\pm0.99(8)^{ab}$	$4.34\pm1.28(8)^{d}$	$202.87\pm19.87(12)^{c}$	$234.45\pm15.61(12)^{c}$
Ⅴ) Zn-Met (low level)	$2.53\pm0.68(9)^{ab}$	$4.29\pm0.97(9)^{d}$	$197.16\pm17.94(12)^{a}$	$220.15\pm14.31(12)^{c}$
Ⅵ) Zn-Met (med. level)	$2.53\pm1.33(9)^{ab}$	$3.56\pm1.09(9)^{c}$	$189.18\pm15.33(10)^{a}$	$195.23\pm13.35(10)^{d}$
Ⅶ) Zn-Met (high level)	$2.10\pm0.53(9)^{ab}$	$3.20\pm1.09(11)^{c}$	$166.23\pm9.22(9)^{c}$	$188.23\pm10.44(9)^{d}$

1) Mean±SE followed by the same letter within a column are not significantly different from each other ($P<0.05$); the datas between parentheses were the number of samples analyzed

ng/mL for groups Ⅳ and Ⅴ respectively at the end of the experiment. Group Ⅱ plasma concentrations of progesterone was 3.62 ng/mL, this value being next to that of group Ⅳ and Ⅴ. In all these 3 groups (Ⅱ, Ⅳ, and Ⅴ) the level of Zn or Zn-Met were 70.09, 70.09 and 78.69 mg/kg Zn and 0.28%, 0.38%, 0.38% Met respectively. However, at levels higher than 78.69 for example group Ⅵ and Ⅶ with Zn-Met levels of 97.19, 134.2 mg/kg Zn and 0.38%, 0.38% Met respectively, the plasma concentrations of progesterone tended to decrease significantly. In a similar way the plasma concentrations of estrogen increased significantly ($P < 0.05$) at the end of the experiment in all treatment groups (Tab. 5). The highest plasma concentrations of estrogen were observed in treatment groups Ⅳ and Ⅴ which were 234.45 and 220.15 pg/mL respectively. However, this was not the case with group Ⅵ and Ⅶ which had lower plasma concentrations of estrogen, although the Zn-Met levels (97.19 and 134.2 mg/kg Zn and 0.38%, 0.38% respectively) were higher than those in group Ⅳ and Ⅴ.

The concentrations of progesterone were significantly ($P < 0.05$) greater in treatment groups Ⅱ, Ⅳ and Ⅴ than the base line concentration of the control group (Tab. 5). The changes in plasma concentrations of estrogen were similar to those of progesterone.

The present study suggests that Zn or Zn-Met at a certain level (70.09—78.69 mg/kg feed) has the tendency to increase the plasma concentrations of estrogen and progesterone, thereby increasing ovulation rate. Johnson et. al.[18] reported that both estrogen and progesterone reach highest plasma concentrations 6—4 h before ovulation.

In conclusion, these experiments report an enhancement of reproductive performance in Yuehuang broiler breeders receiving supplemental Zn-Met. The increase in reproductive performance was observed in egg production, fertility, hatchability, egg mass, blood plasma concentration of estrogen and progesterone. The consistent increase in blood plasma concentration of estrogen and progesterone indicates a possibility to increase ovulation rate, however there is a need for more studies to relate this increase to ovulation and ovulation rate.

It was also reported that higher levels of Zn-Met (97 mg/kg, 0.38%) may not necessarily enhance reproductive performance in agreement with published data.

The results show that Zn supplementation in a CSB diet for enhancement of reproductive performance in young Yuehuang broiler breeders is not required above 79 mg/kg of diet.

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日粮中添加蛋氨酸锌对粤黄鸡繁殖性能和血浆中雌激素和孕酮水平的影响

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摘要: 将 567 只 14 周龄的粤黄母鸡分为 7 个组饲养于双层的种鸡笼中, 各组饲以不同的日粮: Ⅰ)对照组, 饲喂无外源添加锌和蛋氨酸的玉米—豆粕型基础日粮; Ⅱ)饲喂添加氧化锌的相同基础日粮; Ⅲ)饲喂添加蛋氨酸的相同基础日粮; Ⅳ)饲喂按 N. R. C 标准配制的基础日粮; Ⅴ)饲喂添加低剂量蛋氨酸锌的基础日粮; Ⅵ)饲喂添加中剂量蛋氨酸锌的基础日粮; Ⅶ)饲喂添加高剂量蛋氨酸锌的基础日粮. 上述日粮中锌的水平分别为: 30.09, 70.09, 30.09, 70.09, 78.69, 97.19 和 134.20 mg/kg. 蛋氨酸的水平分别为: 0.28%, 0.28%, 0.36%, 0.36%, 0.37%, 0.38%和 0.38%. 实验表明: 日粮中添加外源性的锌和蛋氨酸可影响母鸡的蛋质量、受精率、孵化率和血浆中孕酮和雌激素的水平, 其中, 锌水平分别为 70.09, 70.09 和 78.69 mg/kg; 蛋氨酸的水平分别为 0.28%, 0.36%和 0.37%的第Ⅱ、第Ⅳ和第Ⅴ组, 上述指标均显著高于对照组, 但日粮中 Zn 和蛋氨酸的量高于 78.69 mg/kg 和 0.37%水平, 对种鸡的受精率、孵化率、产蛋量以及血浆中孕酮和雌激素均无促进作用.

关键词: 锌; 蛋氨酸; 肉种鸡; 繁殖性能; 雌激素; 孕酮

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