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Effect of Zinc Amino Acid Complexes on Growth Performance, Tissue Zinc Concentration, and Muscle Development of Broilers

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Abstract

The present study aimed to evaluate the effects of zinc amino acid complexes on growth performance, tissue zinc concentration, and muscle development in broilers. A total of 504 day-old male arbor acres broilers were randomly divided into seven treatments (fed with a basal diet or a basal diet supplemented with 120 mg kg^{-1} Zn as $ZnSO_4$, 30, 60, 90 or 120 mg kg^{-1} Zn as ZnN, or 30 mg kg^{-1} Zn as ZnA separately). Each group had six replicates, with 12 birds per replicate. The results showed that the addition of 60 mg kg^{-1} ZnN significantly increased ($P < 0.05$) the average daily gain (ADG) and breast muscle percentage of broilers. Zinc concentration of ZnN and ZnA added groups were higher than ($P < 0.05$) that in the Zn sulfate group under the same addition dose. Except for the 30 mg kg^{-1} ZnN group, the muscle fiber diameter and cross-sectional area (CSA) were significantly increased ($P < 0.05$) in the ZnN addition groups. Compared with the basal diet group, adding ZnN significantly increased ($P < 0.05$) the expression of MTOR, MYOD, and MYOG at day 21 and decreased ($P < 0.05$) the expression of Atrogin-1. The expression levels of AKT, MTOR, P70S6K, and MYOD were increased at day 42, while the expression levels of MuRF1 and Atrogin-1 were decreased. Adhesion, backbone regulation of actin, MAPK, mTOR, and AMPK were significantly enriched as indicated by KEGG pathway enrichment analysis. In conclusion, zinc amino acid complexes could improve growth performance, tissue zinc concentration, and regulate breast muscle development.

Keywords Zinc amino acid complexes · Performance · Tissue zinc concentration · Muscle development · Broiler

Introduction

Zinc (Zn) is an essential trace mineral for livestock that serves as a cofactor of various enzymes and transcription factors in the body [1, 2]. Dietary zinc supplementation has been revealed to improve broilers' growth performance, meat quality, and immune response [3, 4]. Excess minerals are usually provided in commercial broiler production to maximize their performance. The recommendation of the Cobb Broiler Performance and Nutrition Supplement [5] for Zn in the diet was 100 mg/kg, which was 2.5 fold than NRC (1994) [6] at 40 mg/kg for poultry. However, due to the low absorption and bio-availability of inorganic minerals, a resultant increase of minerals in excreta raised concerns about their detrimental effect on the environment [7, 8]. Therefore, finding alternatives to inorganic minerals is essential to improve growth performance and reduce mineral excretion.

The organic trace elements were gradually used in animal feed because they have a higher absorption rate and bio-availability than inorganic trace minerals [9, 10]. In organic zinc sources, zinc is bound to different ligands, including glycinates, amino acid complexes, polysaccharide complexes, and propionates [11]. Zinc amino acid complexes have been reported to improve growth performance, response to pathogens, and reproduction [12]. Given the increased bioavailability of organic sources, it is a promising alternative to replace inorganic zinc additives below industry standard and reduce zinc excretion without dampening the broiler's growth performance. However, different ligands sources, like feather meal or soybean protein, water

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solubility [9], and chelating strength [13], affected the outcome of zinc amino acid complexes in feed. ZnN is a new source of amino acid organic trace element supply. It is produced by the enzymatic digestion of defatted soy protein by complex proteases to form dipeptides and tripeptides, which are then chelated with zinc ions. The chelation rate of ZnN is greater than or equal to 98%, and the chelation strength Q_f value is 637. This supplement can provide animals with trace elements that are easier to absorb and utilize in a form that is closer to nature.

In addition, studies have shown that the addition of zinc amino acid complexes can improve the antioxidant capacity, meat color, and water retention of muscles, thus improving their meat quality [14–16], but the possible mechanisms regarding the regulation of zinc on muscle development remain to be investigated. Therefore, the aim of this study was to investigate the effect of dietary supplementation with zinc amino acid complex on muscle development in broilers and its potential mechanisms.

Materials and Methods

Experimental Design, Animals, and Diets

A total of 504 day-old male arbor acres (AA) broilers were randomly divided into seven treatments (fed with a basal diet or a basal diet supplemented with 120 mg kg^{-1} Zn as ZnS, 30, 60, 90 or 120 mg kg^{-1} Zn as ZnN, or 30 mg kg^{-1} Zn as ZnA separately). Each group had six replicates, with 12 birds per replicate. ZnS is zinc sulfate, containing 34.5% zinc. ZnN and ZnA were zinc amino acid complexes provided by the Beijing Deyuanshun Biotechnology Co. Ltd. ZnN, named Numine®-Zn, is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio. In contrast, ZnA is zinc chelated with methionine from hydrolyzed feather meal. The zinc content of ZnN and ZnA are 15% and 12%, respectively. The experiment lasted for 42 days. Birds were fed mash diets to meet the nutrient requirements according to AA broiler recommendations (Table 1) during the starter (1–21 days) and grower (22–42 days) periods, respectively. The analyzed Zn levels in the feed are shown in Table 2. All chicks were raised following the AA broiler management guidelines. The environment was kept at 34 °C for the first week, gradually dropping to 24 °C from the fourth week onwards. Feed and water were provided ad libitum during the whole period.

Sample Collection

At 21 and 42 days, one male broiler from each replicate was randomly selected for slaughter performance measurement, and another was selected for meat quality measurement and tissue sampling. The breast muscle, thigh muscle, tibia, pancreas, liver, and jejunum were removed and stored in a

Table 1 Composition and nutrient levels of basal diets (as-fed basis)

Items	Starter 1–21 d	Grower 22–42 d
Diet composition, %		
Corn	56.51	62.21
Soybean meal	35.30	29.20
Limestone	1.52	1.60
Soybean oil	4.50	4.90
Calcium phosphate	1.00	1.00
L-Lysine	0.35	0.30
Methionine	0.16	0.13
L-Threonine	0.06	0.06
Salt	0.30	0.30
Premix[1]	0.30	0.30
Total	100.00	100.00
Nutrient levels[2]		
ME MJ/kg	12.72	13.02
CP, %	20.65	18.28
Lys, %	1.27	1.09
Met, %	0.47	0.41
Ca, %	0.90	0.91
TP, %	0.54	0.52

[1]Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 2500 IU; vitamin E, 20.0 IU; vitamin K_3, 3.0 mg; vitamin B_1, 3.0 mg; vitamin B_2, 8.0 mg; vitamin B_6, 7.0 mg; vitamin B_{12}, 0.03 mg; pantothenic acid, 20.0 mg; nicotinic acid, 50.0 mg; biotin, 0.1 mg; folic acid, 1.5 mg; iron, 60 mg; copper, 17.5 mg; iodine, 1.5 mg; selenium, 0.3 mg; manganese, 124 mg

[2]The nutrient levels were calculated values

Table 2 Analyzed zinc (Zn) concentrations in experimental diets

Treatment	Supplemented Zn, mg/kg	Analyzed Zn contents, mg/kg	
		d 0 to 21	d 22 to 42
CON	0	35.21	38.25
ZnN-30	30	68.35	69.75
ZnN-60	60	96.95	98.50
ZnN-90	90	126.40	133.87
ZnN-120	120	155.49	159.00
ZnS	120	157.28	158.25
ZnA	30	67.56	70.75

ZnS is $ZnSO_4 \cdot H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups, basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4 \cdot H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

freezer at − 20 °C immediately to determine tissue zinc concentration. The right breast muscle was removed and fixed with 4% formaldehyde solution for morphometry. Another molecular sample of breast muscle was stored at − 80 °C for quantitative real-time polymerase chain reaction (qRT-PCR) analysis.

Growth Performance

Male broilers in each replicate were weighed at days 0, 21, and 42 after a 12-h fast to calculate the average daily gain (ADG). The difference between the offered and residual feed for each replicated at each period was recorded to estimate the average daily feed intake (ADFI). Based on ADG and ADFI data, the feed-to-gain ratio (F: G) was calculated.

Measurement of Zinc Concentration

Zinc content in the tissues and feed was determined according to the methods of Meng et al. [17]. Each tissue sample of approximately 0.5–3 g (accurate to 0.001 g) was weighed, and it was digested with 10 mL of nitric acid and perchloric acid mixture (9:1) overnight. Then, the mixture was heated until the digestive liquid became colorless and transparent or slightly yellow, accompanied by white smoke. After cooling, ultrapure water was used to set the volume to a constant value, and the sample was mixed well before measurement. Zinc levels were determined using an A3 flame atomic absorption spectrometer (A3F, Persee, Beijing, China).

Slaughter Performance

At the end of the experiment (42 days of age), the selected birds from each treatment were processed to calculate the total evisceration rate, half evisceration rate, slaughter rate, breast muscle rate, thigh muscle rate, and abdominal fat rate of broilers.

Meat Quality

The meat samples were taken from each broiler's left breast and thigh muscles. Meat quality were determined reference to Xie et al. [18]. The pH value of the breast and thigh muscles was measured using a pH meter (Mettler Toledo, Zurich, Switzerland) 45 min and 24 h after slaughter during storage at 4 °C. Other meat quality indexes were determined within 45 min after euthanasia. The luminance (L^*), redness (a^*), and yellowness (b^*) of the thigh muscle were measured using a colorimeter (Minolta, Tokyo, Japan). The shear force was measured using a digital tenderness meter (C-LM3B, Tenovo, Beijing, China). The drip loss was measured using a pressure gravimetric method [19].

Muscle Fiber Characteristics

Muscle fiber morphological analyses were carried out similarly to the procedures described by Shah M et al. [20, 21]. For morphometric analysis, the breast muscle of birds was cut and preserved in 4% paraformaldehyde before being dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The muscle fibers' diameter and cross-sectional area (CSA) were observed with an Olympus AX70 microscope (Olympus Corporation, Tokyo, Japan). All morphological parameters were measured using the ImageJ Software Package (National Institutes of Health, Bethesda, MD, USA).

RNA Isolation and Real-time Quantitative PCR

The total RNA of the breast muscle was isolated using the AG RNAex Pro reagent (Accurate Bioengineering Co., Ltd., Hunan, China). The concentration and purity of extracted RNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). All primers were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). The primer information of serine/threonine kinase (AKT), mammalian target protein of rapamycin (MTOR), ribosomal protein S6 kinase 1 (P70S6K), myogenic determining factor (MYOD), myogenin (MYOG), muscle ring finger 1 (MuRF1), muscle atrophy f-box (Atrogin-1), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and β-actin genes are listed in Table 3. Quantitative RT-PCR reactions amplified total cDNA in a LightCycler 384 system with SYBR Green I Master. The mRNA levels were calculated using the $2^{-\Delta\Delta CT}$ method.

Transcriptome

The total RNA of breast muscles quality was measured by a quantitative NanoDrop 2000 spectrophotometer using 1.5% agarose gel electrophoresis. After qualified RNA detection, the library was constructed with TruSeq Stranded mRNA LT RNA library preparation kit (Illumina, San Diego, USA). The library quality was then evaluated using the NanoPhotometer, Qubit2.0 Fluorometer and Agilent 2100 bioanalyzer and then sequenced using Illumina HiSeq™ 2500 by the Genedenovo Biotechnology Co., Ltd. The FastQC (v0.11.4) [22] was used to check the raw data quality. The rRNA mapped reads were removed using the Bowtie2 (Version 2.2.8) [23] tool. HISAT2-2.2.4 software [24] was used to align the clean reads to the reference genome. Sample repeatability was tested via principal component analysis. Then, RNA differential expression analysis was performed by the DESeq2 [25] software between two different groups. The genes with the parameter of $P < 0.1$ and $|\log2FC| > 1.5$ were considered differentially expressed genes. Kyoto

Table 3 Sequence of primers for real-time PCR

Genes	Primer sequence (5′→3′)	GenBank no	Product size (bp)
β-actin	F:GCCCTGGCACCTAGCACAATG R:CTCCTGCTTGCTGATCCACATCTG	NM_205518.2	172
AKT	F:CATTCCCGCCATTATGAATGAAGTA R:CTTGTAGCCAATGAATGTGCCATC	AF039943	130
MTOR	F:GAAGTCCTGCGCGAGCATAAG R:TTTGTGTCCATCAGCCTCCAGT	XM_4l7614	92
P70S6K	F:ATTCGATCACCTCGCAGATTCATAG R: AGTATTTGATGCGCTGGCAGAAG	NM_001030721	111
MYOD	F:ATCACCAAATGACCCAAAGC R:GGGAACAGGGACTCCCTTCA	NM_204214	149
MYOG	F:GGAGGCTGAAGAAGGTGAA R:TGCTGGTTGAGGCTGCTGA	NM_204184	151
MuRF1	F:CGACATCTACAAGCAGGAGT R:TGAGCACCGAAGACCTT	XM_424369	163
Atrogin-1	F:CACGGAAGGAGCAGTATGGT R:AGGTCTCTGGGTTGTTGGCT	NM_001030956	124
CAT	F:GTTGGCGGTAGGAGTCTGGTCT R:GTGGTCAAGGCATCTGGCTTCTG	NM_001031215.1	182
SOD	F:TTGTCTGATGGAGATCATGGCTTC R:TGCTTGCCTTCAGGATTAAAGTGA	NM_205064.2	98
GPX	F:CAAAGTTGCGGTCAGTGGA R:AGAGTCCCAGGCCTTTACTA	NM_001163245.2	136

β-actin beta, *AKT* serine/threonine kinase, *MTOR* mammalian target protein of rapamycin, *P70S6K* ribosomal protein S6 kinase 1, *MYOD* myogenic determining factor, *MYOG* myogenin, *MuRF1* muscle ring finger 1, *Atrogin-1* muscle atrophy f-box, *CAT* catalase, *SOD* superoxide dismutase, *GPX* glutathione peroxidase, *F* forward, *R* reverse

Encyclopedia of Genes and Genomes (KEGG) is the major public pathway–related database. Pathway enrichment analysis identified significantly enriched metabolic pathways or signal transduction pathways in DEGs compared with the whole genome background.

Statistical Analysis

All statistical analyses were performed using the SPSS (25.0, Chicago, IL,USA) software, and values are shown as means ± SD. Significant differences among different treatment groups were evaluated by one-way analysis of variance (ANOVA) followed by the Duncan's multiple-range test. Then, the linear and quadratic effects of ZnN levels were analyzed using regression analysis in SPSS 25. $P < 0.05$ was considered statistically significant.

Results

Growth Performance

There were no significant differences ($P > 0.05$) in ADG, ADFI, and F:G among all treatments at the starter stage. During the grower phase, the ADG of broilers from the 30, 60, or 90 mg kg^{-1} ZnN supplemented groups was higher than ($P < 0.05$) the CON group, while the addition of ZnS, ZnA, or 120 mg kg^{-1} ZnN did not affect ($P > 0.05$) broiler ADG compared to the CON group. However, only the dosage of 60 or 90 mg kg^{-1} supplemented in the diet significantly increased ($P < 0.05$) the ADG of the broiler throughout the whole feeding trial. Additionally, supplementation with ZnN, ZnS, or ZnA all reduced ($P < 0.05$) the F:G during the grower phase and the whole experimental period relative to the CON group, while there were no detectable differences ($P > 0.05$) among ZnN, ZnS, or ZnA groups (Table 4).

Tissue Zinc Concentration

ZnN supplementation increased ($P < 0.05$) the zinc concentration in all analyzed tissue linearly at 21 days and 42 days. Except for the 30 mg kg^{-1} ZnN group, other ZnN groups showed a significant increase ($P < 0.05$) in tissue zinc content compared to the CON group. Moreover, compared with the ZnS group, dietary supplementation with 120 mg kg^{-1} ZnN improved ($P < 0.05$) the zinc deposition in the pancreas at 21 days and in the pancreas, tibia, and jejunum at 42 days. Meanwhile, broilers in the ZnA group had a higher

Table 4 Effect of dietary supplementation with zinc amino acid complexes on growth performance of broilers

Item	Group							*P*-value		
	CON	ZnN-30	ZnN-60	ZnN-90	ZnN-120	ZnS	ZnA	ANOVA	Linear	Quadratic
Starter (1–21 d)										
FBW (g)	738.18 ± 19.38	756.97 ± 32.81	755.28 ± 19.66	757.64 ± 40.60	748.61 ± 38.16	753.80 ± 20.53	753.66 ± 33.23	0.953	0.545	0.344
ADG (g)	32.89 ± 0.92	33.78 ± 1.56	33.71 ± 0.94	33.82 ± 1.94	33.39 ± 1.82	33.63 ± 0.98	33.63 ± 1.58	0.953	0.544	0.344
ADFI (g)	49.27 ± 1.82	50.03 ± 1.56	48.77 ± 0.28	49.55 ± 0.96	48.07 ± 0.95	50.93 ± 1.97	48.85 ± 1.97	0.085	0.257	0.163
F/G	1.49 ± 0.06	1.49 ± 0.09	1.45 ± 0.04	1.47 ± 0.06	1.43 ± 0.07	1.52 ± 0.02	1.43 ± 0.06	0.135	0.157	0.661
Grower (22–42 d)										
FBW (g)	2579.83 ± 48.32c	2736.27 ± 57.00abc	2781.25 ± 198.23ab	2809.26 ± 63.12a	2631.04 ± 129.55bc	2628.39 ± 154.01bc	2691.23 ± 120.72abc	0.019	0.105	0.002
ADG (g)	87.04 ± 3.47c	94.67 ± 3.72ab	96.48 ± 9.80ab	97.70 ± 1.86a	89.64 ± 5.00bc	89.79 ± 6.85bc	92.27 ± 4.39abc	0.018	0.110	0.002
ADFI (g)	152.32 ± 9.83	156.13 ± 3.95	152.00 ± 5.29	155.77 ± 9.03	152.25 ± 5.12	151.27 ± 7.92	150.37 ± 7.93	0.790	0.888	0.504
F/G	1.83 ± 0.15a	1.65 ± 0.06b	1.55 ± 0.14b	1.60 ± 0.10b	1.61 ± 0.07b	1.63 ± 0.11b	1.63 ± 0.04b	0.012	0.004	0.028
Whole period (1–42 d)										
ADG (g)	60.30 ± 1.15c	64.01 ± 1.36abc	65.09 ± 4.72ab	65.76 ± 1.50a	61.51 ± 3.09bc	61.45 ± 3.67bc	62.95 ± 2.88abc	0.019	0.105	0.002
ADFI (g)	99.47 ± 3.11	103.45 ± 2.66	100.42 ± 2.59	102.66 ± 4.13	99.96 ± 3.27	100.63 ± 3.94	100.31 ± 3.85	0.419	0.787	0.429
F/G	1.73 ± 0.09a	1.62 ± 0.05b	1.54 ± 0.08b	1.56 ± 0.09b	1.56 ± 0.06b	1.60 ± 0.08b	1.58 ± 0.03b	0.002	< 0.001	0.057

a–c: Means within a row with different letters are significantly different ($P < 0.05$) by one-way ANOVA. Linear and quadratic refer to statistical analyses performed with CON, ZnN-30, ZnN-60, ZnN-90, and ZnN-120 groups. ZnS is $ZnSO_4 \cdot H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups, basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4 \cdot H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

FBW final body weight, *ADG* average daily gain, *ADFI* average daily feed intake, *F/G* feed-to-gain ratio

($P < 0.05$) zinc content in the tibia at 21 days and in the breast muscle at 42 days than broilers in the ZnN-30 group, but there were no significant differences ($P > 0.05$) in other tissue zinc contents between the two groups (Table 5).

Slaughtering Performance

There were no significant differences ($P > 0.05$) in dressing percentage, full evisceration rate, half evisceration rate, thigh percentage, and abdominal fat percentage of broilers among treatments. In contrast, the breast muscle percentage of broilers in the ZnN-60 and ZnA groups significantly increased ($P < 0.05$) at 42 days of age compared with the CON group (Table 6).

Meat Quality

There were no significant differences ($P > 0.05$) observed in b^* value, pH_{45min}, pH_{24h}, shear force, or drip loss percentage in breast muscle and thigh muscle of broilers at 42 days. However, compared with the CON group, dietary addition of ZnA or 60 mg kg^{-1}, 90 mg kg^{-1}, and 120 mg kg^{-1} ZnN significantly decreased ($P < 0.05$) the L^* value of breast and pressure loss percentage of the thigh, while supplemented with ZnA, other than ZnN and ZnS, improved ($P < 0.05$) the a^* value of breast. Also, ZnA or 30 mg kg^{-1}, 60 mg kg^{-1}, and 120 mg kg^{-1} ZnN supplementation decreased ($P < 0.05$) the L^* value of the thigh at 42 days (Table 7).

Breast Muscle Antioxidant

The CAT and GPX expression levels showed no significant differences ($P > 0.05$) among treatments on days 21 and 42. At 21 days of age, both dietary ZnN and ZnA treatments, but not ZnS, upregulated ($P < 0.05$) the expression of SOD in the breast muscles relative to the CON group. However, only 60 mg kg^{-1}, 90 mg kg^{-1}, or 120 mg kg^{-1}, not 30 mg kg^{-1}, ZnN supplementation improved ($P < 0.05$) the SOD expression at 42 days (Table 8).

Muscle Fiber Characteristics in The Breast

Compared with the CON group, except for the ZnN-30 group, the diameter of the breast muscle fibers of the broilers in other groups was significantly increased ($P < 0.05$) at day 21. At day 42, the diameter and cross-sectional area (CSA) of muscle fibers in all treated groups were significantly increased ($P < 0.05$) compared with the CON. In addition, compared with the ZnS group, the diameter of myofibers in ZnN-30, ZnN-60, ZnN-90, and ZnA groups was significantly increased ($P < 0.05$), and the CSA of myofibers inZnN-60 and ZnN-90 group were also significantly increased ($P < 0.05$) and showed a quadratic effect (Table 9; Fig. 1).

Expression Levels of Muscle Fiber Relative Genes in the Breast

Compared with the CON group, dietary ZnN treatments upregulated the MYOD and MYOG expression in breast muscle at day 21, but only dietary addition of 60 mg kg^{-1}, 90 mg kg^{-1}, and 120 mg kg^{-1} of ZnN improved ($P < 0.05$) the MTOR expression in breast muscle. Broilers fed the diet with 120 mg kg^{-1} ZnN had a higher ($P < 0.05$) expression of MYOG than broilers in the ZnS group. Furthermore, there was no significant difference ($P > 0.05$) in gene expression for AKT, P70S6K, and MuRF1 in the breast muscle among treatments at 21 days of age. At day 42, ZnN treatments significantly increased ($P < 0.05$) the expression of AKT, MTOR, P70S6K, and MYOD and decreased ($P < 0.05$) MuRF1 and Atrogin-1 expression compared to the CON group. There was no significant difference ($P > 0.05$) in MTOR, P70S6K, MuRF1, and Atrogin-1 among the ZnN groups, ZnS group, and ZnA group. However, compared with the ZnS group, dietary ZnN treatments, except the ZnN-30 group, improved ($P < 0.05$) AKT expression, while only 60 or 90 mg kg^{-1} ZnN in the diet increased P70S6K expression (Table 10).

Transcriptome of the Breast Muscles

Breast muscle samples from CON, ZnS, and ZnN-60 were classified according to principal component analysis (PCA). As can be seen from the figure, there are differences between the CON, ZnS, and ZnN-60 groups (Figs. 2 and 3).

Genes screened for $P < 0.1$ and $|\log2FC| > 1.5$ were significantly differentiated, as seen from the volcano plot. In the starter stage, ZnS vs. CON, ZnN-60 vs. CON, and ZnN-60 vs. ZnS, there were 1944, 1772, and 1494 differential genes, respectively, of which 1772, 1166, and 634 differential genes were upregulated and 172, 328, and 2015 were downregulated. In the grower stage, ZnS vs. CON, ZnN-60 vs. CON, and ZnN-60 vs. ZnS, there were 857, 2354, and 1717 differential genes, respectively, of which 666, 2101, and 1295 differential genes were upregulated and 191, 253, and 422 were downregulated. KEGG pathway analysis revealed that signaling pathways such as adhesion, regulation of actin cytoskeleton, biosynthesis of amino acids, growth hormone synthesis, MAPK, mTOR, Hedge, and AMPK signaling pathways were significantly enriched (Figs. 2 and 3).

Table 5 Effect of dietary supplementation with zinc amino acid complexes on tissue zinc concentration of broilers (mg kg^{-1})

Item	Group							*P*-value		
	CON	ZnN-30	ZnN-60	ZnN-90	ZnN-120	ZnS	ZnA	ANOVA	Linear	Quadratic
21 d										
Breast muscle	7.45 ± 0.49c	7.66 ± 0.41c	9.29 ± 0.64b	9.75 ± 0.71ab	10.15 ± 0.56a	9.74 ± 0.78ab	8.11 ± 0.39c	< 0.001	< 0.001	0.208
Thigh muscle	17.59 ± 1.52c	17.73 ± 0.53bc	18.72 ± 1.21a	19.18 ± 0.52a	19.32 ± 0.86a	18.52 ± 0.54ab	17.74 ± 0.64bc	< 0.001	< 0.001	0.545
Liver	29.93 ± 2.19d	35.09 ± 5.64 cd	48.53 ± 4.62b	51.07 ± 7.25ab	57.55 ± 6.57a	52.00 ± 3.34ab	36.72 ± 5.62c	< 0.001	< 0.001	0.151
Pancreas	33.99 ± 2.22f	37.72 ± 5.53ef	53.04 ± 4.38d	66.74 ± 4.39c	78.12 ± 4.13a	72.92 ± 3.38b	40.32 ± 1.44e	< 0.001	< 0.001	< 0.001
Tibia	222.04 ± 16.94c	244.14 ± 9.43c	283.76 ± 18.72b	318.80 ± 15.34a	342.30 ± 25.11a	326.05 ± 16.69a	276.32 ± 25.91b	< 0.001	< 0.001	0.042
Jejunum	50.01 ± 3.25c	52.81 ± 6.60bc	57.55 ± 3.84a	60.64 ± 7.36a	58.17 ± 7.23ab	58.95 ± 2.64ab	54.17 ± 5.05abc	0.021	0.004	0.532
42 d										
Breast muscle	7.51 ± 0.58d	8.00 ± 0.40c	9.12 ± 0.72ab	9.42 ± 0.52a	9.70 ± 0.38a	9.52 ± 0.37a	8.69 ± 0.57b	< 0.001	< 0.001	0.515
Thigh muscle	15.72 ± 1.07c	16.30 ± 0.52bc	17.18 ± 0.63ab	17.13 ± 0.56ab	17.39 ± 1.02a	17.15 ± 0.82ab	16.19 ± 0.51bc	0.004	< 0.001	0.582
Liver	19.95 ± 2.26d	22.37 ± 2.24d	27.99 ± 3.42c	31.35 ± 2.51bc	35.86 ± 3.31a	32.31 ± 3.27ab	24.20 ± 1.94d	< 0.001	< 0.001	0.046
Pancreas	31.70 ± 4.18e	40.22 ± 3.50d	47.66 ± 6.24bc	54.04 ± 6.16b	65.25 ± 5.54a	50.69 ± 7.86b	42.47 ± 2.46 cd	< 0.001	< 0.001	0.035
Tibia	160.96 ± 11.48e	171.55 ± 12.15de	185.97 ± 10.06d	196.28 ± 13.27 cd	237.33 ± 17.03a	219.63 ± 11.09b	173.50 ± 13.77de	< 0.001	< 0.001	< 0.001
Jejunum	27.39 ± 2.16d	28.58 ± 3.59d	35.72 ± 2.64bc	36.91 ± 6.62b	51.81 ± 6.20a	34.77 ± 2.47bc	30.25 ± 4.47 cd	< 0.001	< 0.001	< 0.001

a–e: Means within a row with different letters are significantly different ($P < 0.05$) by one-way ANOVA. Linear and quadratic refer to statistical analyses performed with CON, ZnN-30, ZnN-60, ZnN-90, and ZnN-120 groups. ZnS is $ZnSO_4 \cdot H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups, basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4 \cdot H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

Table 6 Effect of dietary supplementation with zinc amino acid complexes on slaughter performance of broilers

Item	Group							*P*-value		
	CON	ZnN-30	ZnN-60	ZnN-90	ZnN-120	ZnS	ZnA	ANOVA	Linear	Quadratic
Dressing percentage, %	91.00 ± 3.15	91.54 ± 1.05	91.81 ± 1.07	91.92 ± 1.08	90.18 ± 4.10	91.27 ± 1.26	90.62 ± 1.43	0.802	0.799	0.247
Full evisceration rate, %	82.75 ± 3.34	82.95 ± 1.34	82.93 ± 1.26	83.57 ± 1.48	80.84 ± 4.58	82.55 ± 1.40	81.56 ± 1.70	0.536	0.449	0.245
Half evisceration rate, %	72.53 ± 3.20	72.71 ± 1.17	72.54 ± 1.92	71.96 ± 1.54	69.66 ± 4.90	71.39 ± 1.41	70.27 ± 2.12	0.299	0.127	0.180
Breast percentage, %	28.01 ± 2.26b	27.97 ± 0.81b	31.09 ± 1.28a	30.01 ± 2.69ab	29.59 ± 1.97ab	29.79 ± 1.73ab	31.00 ± 1.45a	0.027	0.039	0.384
Thigh percentage, %	22.03 ± 1.43	20.27 ± 1.80	21.39 ± 1.95	21.35 ± 1.63	21.32 ± 1.47	21.27 ± 2.33	21.20 ± 1.27	0.774	0.679	0.245
Abdominal fat yield, %	1.53 ± 0.34	1.88 ± 0.34	1.43 ± 0.48	1.92 ± 0.32	1.55 ± 0.36	1.53 ± 0.47	1.47 ± 0.26	0.163	0.742	0.332

a–b: Means within a row with different letters are significantly different ($P < 0.05$) by one-way ANOVA. Linear and quadratic refer to statistical analyses performed with CON, ZnN-30, ZnN-60, ZnN-90, and ZnN-120 groups. ZnS is $ZnSO_4{\cdot}H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups, basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4{\cdot}H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

Discussion

Growth performance is generally regarded as a primary indicator for evaluating the nutritional requirements of broilers and is highly valued by producers [26]. In the present study, ZnN supplementation showed a beneficial effect by increasing ADG and reducing F:G ratio in the diet. Consistent with our results, previous studies demonstrated that adding various forms of zinc to broiler diets can effectively increase ADG and reduce F:G ratio in broilers [4, 12]. The reason may be that zinc is a part of several enzymes involved in the metabolism of protein, fat, carbohydrates, and nucleic acids and has physiological functions to promote growth [1, 2].

The source and level of zinc in the diet may directly affect the deposition of zinc in broilers' tissues. Our study findings were that the amount of organic zinc deposition was higher than that of inorganic zinc at the same addition dose, and the contents of zinc in the tissues of broilers increased linearly with the ZnN addition level. In addition, in response to zinc deposition in all tissues, tibial zinc deposition was the largest, followed by the pancreas, indicating that tibial zinc content is a better indicator for evaluating zinc bioavailability. This result is consistent with the reports of Hu et al. and Kong et al., that organic zinc is more favorable for deposition than inorganic zinc in vivo, and tibial zinc content is a sensitive indicator to evaluate zinc bioavailability [27, 28]. The reason may be that amino acid chelated trace elements are mainly absorbed by the amino acid or peptide transport system, characterized by high transport speed, low energy consumption, and difficult carrier saturation [10].

The color of meat is the intuitive performance of muscle. Buyers are more motivated to consume vividly colored meat products. The change in a^* value is mainly determined by the content and presence of pigments (myoglobin and hemoglobin) in the muscle, with a^* value proportional to meat quality and b^* and L^* values inversely proportional to meat quality. Our results showed that dietary supplementation of ZnN increased the a^* value of thigh muscle and decreased the L^* value of breast and thigh muscle. The reason may be that it can reduce muscle oxidation and the conversion of red myoglobin to brown myoglobin, which improves flesh color [29]. In addition, zinc is a cofactor of Cu,Zn-superoxide dismutase (Cu/ZnSOD), which can enhance the body's ability to clear ROS by increasing the activity of SOD. In the present study, the supplementation of ZnN could increase the expression of SOD in breast muscle, improve their antioxidant capacity, and thus maintain meat color. Other studies have proved that the hydrolysis and oxidation of proteins can directly affect the water-holding capacity of muscle tissue [30]. Our results

Table 7 Effect of dietary supplementation with zinc amino acid complexes on meat quality of breast muscle of broilers

Item	Group							*P*-value		
	CON	ZnN-30	ZnN-60	ZnN-90	ZnN-120	ZnS	ZnA	ANOVA	Linear	Quadratic
Breast										
*L**	59.00 ± 3.30a	55.59 ± 2.36ab	53.73 ± 1.77b	54.05 ± 1.88b	54.69 ± 3.25b	57.31 ± 3.02ab	54.43 ± 3.32b	0.034	0.002	0.048
a*	16.85 ± 3.10bc	14.67 ± 2.17c	18.00 ± 4.10bc	20.70 ± 3.83ab	19.93 ± 2.39ab	20.33 ± 2.95ab	22.25 ± 2.03a	0.002	0.014	0.188
b*	10.62 ± 1.58	9.30 ± 1.24	9.05 ± 0.96	11.08 ± 1.91	10.86 ± 1.70	11.52 ± 2.73	11.99 ± 1.85	0.061	0.446	0.030
$pH_{45\ min}$	6.67 ± 0.53	6.62 ± 0.28	6.61 ± 0.14	6.81 ± 0.22	6.84 ± 0.13	6.99 ± 0.34	6.65 ± 0.24	0.247	0.229	0.286
$pH_{24\ h}$	6.14 ± 0.06	6.19 ± 0.19	6.07 ± 0.22	6.08 ± 0.09	6.05 ± 0.15	6.08 ± 0.06	6.04 ± 0.14	0.533	0.176	0.636
Shear force, *N*	24.05 ± 3.37	22.57 ± 1.98	24.33 ± 3.74	25.33 ± 4.23	22.37 ± 5.88	22.22 ± 2.83	23.50 ± 4.19	0.783	0.887	0.758
Drip loss, %	15.54 ± 0.05	13.13 ± 0.03	13.99 ± 0.04	17.04 ± 0.05	11.60 ± 0.05	18.03 ± 0.08	13.40 ± 0.07	0.475	0.465	0.723
Pressure loss, %	22.45 ± 0.03	24.87 ± 0.10	26.75 ± 0.07	23.03 ± 0.07	26.28 ± 0.08	30.21 ± 0.08	22.76 ± 0.13	0.690	0.515	0.712
Thigh										
*L**	58.85 ± 3.67a	53.65 ± 3.37b	52.74 ± 1.00b	55.50 ± 2.35ab	54.34 ± 1.15b	55.93 ± 4.44ab	53.17 ± 3.23b	0.019	0.011	0.006
a*	15.70 ± 3.53b	15.39 ± 2.28b	19.82 ± 3.49a	19.89 ± 1.89a	20.57 ± 2.65a	18.32 ± 4.10ab	20.90 ± 3.64a	0.016	0.001	0.689
b*	10.28 ± 1.88	11.13 ± 0.82	9.49 ± 1.60	11.40 ± 1.42	11.10 ± 1.49	10.54 ± 1.21	12.43 ± 2.15	0.076	0.333	0.638
$pH_{45\ min}$	6.72 ± 0.46	6.69 ± 0.26	6.62 ± 0.14	6.76 ± 0.17	6.90 ± 0.15	6.97 ± 0.32	6.65 ± 0.07	0.184	0.278	0.166
$pH_{24\ h}$	6.20 ± 0.10	6.28 ± 0.20	6.18 ± 0.12	6.18 ± 0.12	6.19 ± 0.15	6.32 ± 0.17	6.13 ± 0.11	0.326	0.546	0.612
Shear force, *N*	34.40 ± 5.78	33.93 ± 8.51	36.42 ± 8.21	36.52 ± 7.06	32.76 ± 5.51	30.31 ± 5.88	32.73 ± 7.28	0.732	0.98	0.526
Drip loss, %	11.93 ± 0.06	17.77 ± 0.07	17.07 ± 0.08	18.79 ± 0.06	16.30 ± 0.08	12.82 ± 0.04	10.53 ± 0.03	0.181	0.208	0.233
Pressure loss, %	34.92 ± 0.07a	29.57 ± 0.09ab	19.99 ± 0.05b	22.88 ± 0.13b	19.19 ± 0.07b	30.34 ± 0.05ab	19.29 ± 0.0b	0.016	0.529	0.390

a–c: Means within a row with different letters are significantly different ($P < 0.05$) by one-way ANOVA. Linear and quadratic refer to statistical analyses performed with CON, ZnN-30, ZnN-60, ZnN-90, and ZnN-120 groups. a*, redness; b*, yellowness; *L**, lightness. ZnS is $ZnSO_4 \cdot H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups, basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4 \cdot H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

Table 8 Effect of dietary supplementation with zinc amino acid complexes on antioxidant genes in breast muscle of broilers

Item	Group							*P*-value		
	CON	ZnN-30	ZnN-60	ZnN-90	ZnN-120	ZnS	ZnA	ANOVA	Linear	Quadratic
21 d										
CAT	1.00 ± 0.09	1.44 ± 0.35	1.29 ± 0.23	1.11 ± 0.12	1.20 ± 0.19	1.21 ± 0.19	1.13 ± 0.15	0.104	0.509	0.038
SOD	1.00 ± 0.12c	1.38 ± 0.11ab	1.55 ± 0.17a	1.42 ± 0.15ab	1.39 ± 0.30ab	1.18 ± 0.11bc	1.59 ± 0.31a	0.002	0.014	0.004
GPX	1.00 ± 0.13	1.03 ± 0.15	1.21 ± 0.08	1.21 ± 0.17	1.16 ± 0.12	1.15 ± 0.16	1.18 ± 0.09	0.125	0.016	0.441
42 d										
CAT	1.00 ± 0.29	1.24 ± 0.26	1.34 ± 0.25	1.13 ± 0.14	1.16 ± 0.26	1.29 ± 0.27	1.26 ± 0.16	0.464	0.567	0.260
SOD	1.00 ± 0.23c	1.17 ± 0.12abc	1.44 ± 0.27ab	1.44 ± 0.16ab	1.37 ± 0.24ab	1.12 ± 0.22bc	1.47 ± 0.21a	0.015	0.001	0.194
GPX	1.00 ± 0.16	1.28 ± 0.21	1.24 ± 0.22	1.28 ± 0.17	1.19 ± 0.16	1.19 ± 0.16	1.32 ± 0.21	0.202	0.045	0.246

a–c: Means within a row with different letters are significantly different ($P < 0.05$) by one-way ANOVA. Linear and quadratic refer to statistical analyses performed with CON, ZnN-30, ZnN-60, ZnN-90, and ZnN-120 groups. ZnS is $ZnSO_4 \cdot H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups, basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4 \cdot H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

CAT catalase, *SOD* superoxide dismutase, *GPX* glutathione peroxidase

Table 9 Effect of dietary supplementation with zinc amino acid complexes on muscle fiber morphology in breast muscle of broilers

Item	Group							*P*-value		
	CON	ZnN-30	ZnN-60	ZnN-90	ZnN-120	ZnS	ZnA	ANOVA	Linear	Quadratic
21 d										
Diameter (μm)	21.29 ± 0.51c	21.77 ± 0.51bc	23.04 ± 0.92a	22.95 ± 1.08a	23.14 ± 0.64a	22.73 ± 0.97ab	22.94 ± 1.16a	0.003	< 0.001	0.620
CSA (μm^2)	821.08 ± 39.54	839.92 ± 35.35	869.54 ± 57.58	892.81 ± 40.96	882.35 ± 42.53	847.35 ± 14.04	877.6 ± 51.47	0.068	0.004	0.768
42 d										
Diameter (μm)	32.58 ± 0.98d	36.31 ± 0.57ab	36.83 ± 1.13a	36.96 ± 0.45a	35.71 ± 0.58bc	35.24 ± 0.75c	36.68 ± 0.33a	< 0.001	< 0.001	< 0.001
CSA (μm^2)	1224.41 ± 10.33d	1374.4 ± 65.07bc	1423.66 ± 32.93ab	1494.12 ± 64.15a	1343.39 ± 114.80bc	1313.07 ± 56.34c	1355.54 ± 71.41bc	< 0.001	< 0.001	< 0.001

a–d: Means within a row with different letters are significantly different ($P < 0.05$) by one-way ANOVA. Linear and quadratic refer to statistical analyses performed with CON, ZnN-30, ZnN-60, ZnN-90, and ZnN-120 groups. ZnS is $ZnSO_4 \cdot H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups, basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4 \cdot H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

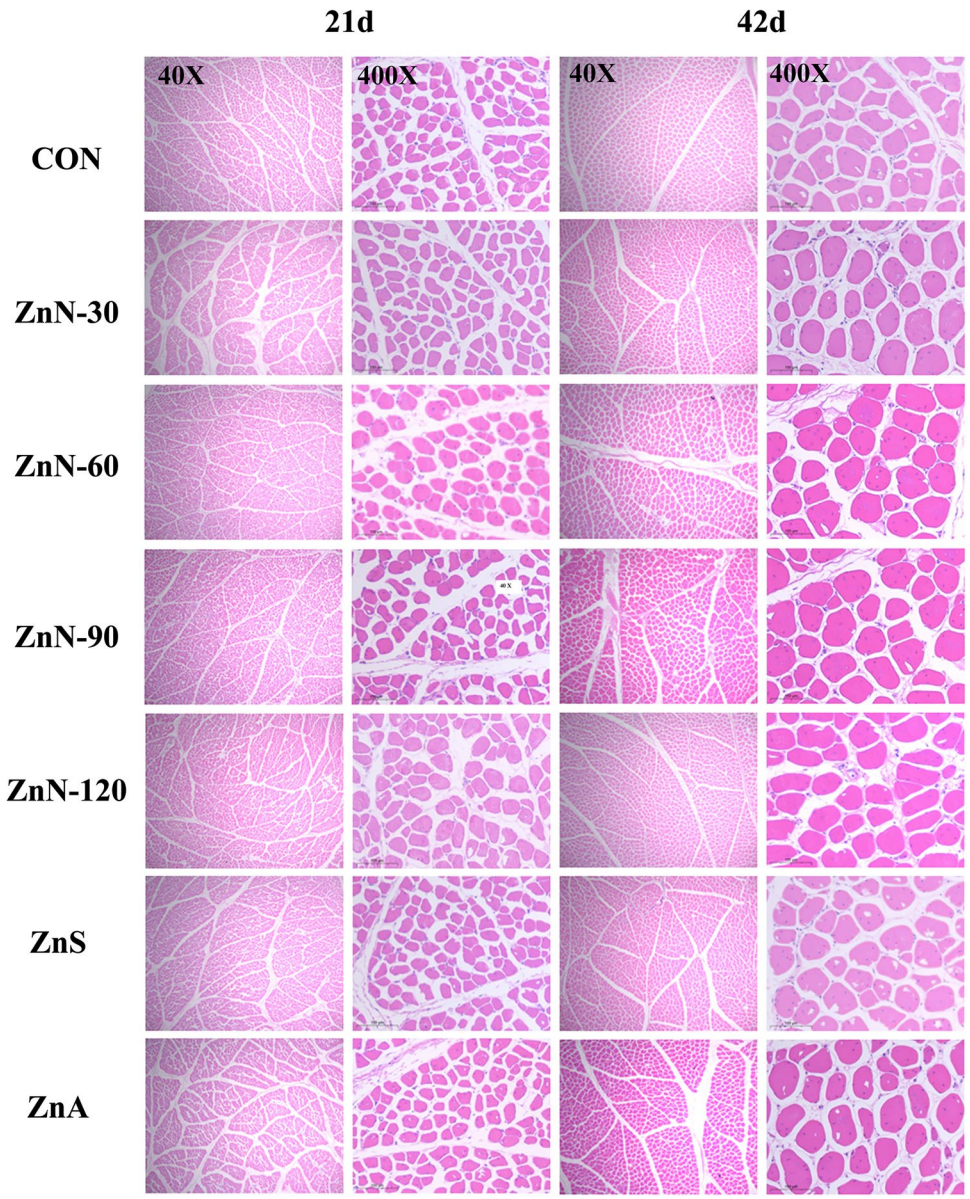

Fig. 1 Hematoxylin and eosin staining of breast muscle of broilers at 21 and 42 days of age (40× and 400×). ZnS is $ZnSO_4 \cdot H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups; basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4 \cdot H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

showed that ZnN could reduce protein loss by reducing the pressure loss percentage of the thigh. Similarly, Piotr Sałek [31] found that zinc methionine chelate can improve meat color, increase muscle retention water, reduce drip loss, and thus, improve meat quality.

Meat production performance is one of the critical indicators to evaluate the economic value of poultry. In the present study, ZnN supplementation in the diet could increase broilers' ADG and breast muscle percentage. Therefore, we speculated that ZnN might achieve the above experimental results by improving the skeletal muscle development of broilers. Similar studies also found that zinc supplementation in broiler diets can improve the slaughter rate and breast muscle rate of broilers [9, 32], which may be related to zinc promoting skeletal muscle development by activating insulin signaling cascade and regulating protein metabolism [27, 33]. Skeletal muscle growth can be divided into two stages: proliferation and hypertrophy. Proliferation refers to the increase in the number of myoblasts during the embryonic period, which proliferate and differentiate into multinuclear muscle tubes and then form muscle fibers [34]. Hypertrophy refers to the enlargement of muscle fibers when the number of muscle fibers is unchanged, and the enlargement of muscle fibers is determined by the diameter of muscle fibers [35]. In the present study, adding ZnN to the diet increased the breast muscle fiber diameter and CSA of broilers. This result is similar to Gao et al., that adding zinc to the maternal diet can increase the yield and width of muscle fibers of offspring, indicating that zinc can promote the development of breast muscle by promoting the development of muscle fibers [36].

Table 10 Effect of dietary supplementation with zinc amino acid complexes on breast muscle development genes in broilers

Item	Group							*P*-value		
	CON	ZnN-30	ZnN-60	ZnN-90	ZnN-120	ZnS	ZnA	ANOVA	Linear	Quadratic
21 d										
AKT	1.00 ± 0.10	1.01 ± 0.09	1.13 ± 0.19	1.04 ± 0.18	1.19 ± 0.29	1.14 ± 0.20	1.20 ± 0.21	0.439	0.136	0.569
MTOR	1.00 ± 0.22b	1.30 ± 0.28ab	1.47 ± 0.25a	1.53 ± 0.21a	1.51 ± 0.28a	1.39 ± 0.24a	1.45 ± 0.25a	0.027	0.001	0.250
P70S6K	1.00 ± 0.14	1.18 ± 0.23	1.32 ± 0.15	1.11 ± 0.25	1.21 ± 0.19	1.10 ± 0.08	1.23 ± 0.26	0.743	0.860	0.842
MYOD	1.00 ± 0.20b	1.44 ± 0.31a	1.67 ± 0.11a	1.41 ± 0.16a	1.47 ± 0.24a	1.35 ± 0.19a	1.44 ± 0.28a	0.005	0.002	0.005
MYOG	1.00 ± 0.14c	1.37 ± 0.22ab	1.44 ± 0.22ab	1.44 ± 0.22ab	1.66 ± 0.29a	1.24 ± 0.24bc	1.30 ± 0.22bc	0.008	< 0.001	0.631
MuRF1	1.00 ± 0.17	0.71 ± 0.14	0.74 ± 0.13	0.75 ± 0.18	0.67 ± 0.15	0.68 ± 0.25	0.69 ± 0.20	0.165	0.011	0.160
Atrogin-1	1.00 ± 0.10a	0.66 ± 0.15bc	0.55 ± 0.11c	0.51 ± 0.19c	0.58 ± 0.14c	0.80 ± 0.20b	0.51 ± 0.18c	< 0.001	< 0.001	0.004
42 d										
AKT	1.00 ± 0.14c	1.28 ± 0.22ab	1.43 ± 0.20a	1.49 ± 0.19a	1.41 ± 0.23a	1.15 ± 0.18bc	1.44 ± 0.17a	0.003	0.001	0.073
MTOR	1.00 ± 0.23b	1.34 ± 0.27a	1.50 ± 0.18a	1.53 ± 0.21a	1.32 ± 0.19a	1.25 ± 0.14ab	1.41 ± 0.25a	0.012	0.007	0.010
P70S6K	1.00 ± 0.23c	1.37 ± 0.25ab	1.49 ± 0.17a	1.55 ± 0.15a	1.38 ± 0.27ab	1.16 ± 0.22bc	1.41 ± 0.06ab	0.003	0.003	0.018
MYOD	1.00 ± 0.16c	1.30 ± 0.16abc	1.47 ± 0.20ab	1.62 ± 0.28a	1.52 ± 0.29ab	1.24 ± 0.22bc	1.59 ± 0.30ab	0.006	< 0.001	0.090
MYOG	1.00 ± 0.21	1.17 ± 0.12	1.33 ± 0.11	1.34 ± 0.19	1.18 ± 0.27	1.22 ± 0.11	1.30 ± 0.12	0.075	0.035	0.070
MuRF1	1.00 ± 0.25a	0.57 ± 0.25b	0.45 ± 0.17b	0.51 ± 0.16b	0.60 ± 0.17b	0.61 ± 0.14b	0.48 ± 0.11b	0.001	0.001	0.004
Atrogin-1	1.00 ± 0.24a	0.53 ± 0.18b	0.54 ± 0.18b	0.48 ± 0.20b	0.52 ± 0.15b	0.73 ± 0.12b	0.53 ± 0.08b	0.005	0.001	0.008

a–c: Means within a row with different letters are significantly different ($P < 0.05$) by one-way ANOVA. Linear and quadratic refer to statistical analyses performed with CON, ZnN-30, ZnN-60, ZnN-90, and ZnN-120 groups. ZnS is $ZnSO_4 \cdot H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups, basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4 \cdot H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

AKT serine/threonine kinase, *MTOR* mammalian target protein of rapamycin, *P70S6K* ribosomal protein S6 kinase 1, *MYOD* myogenic determining factor, *MYOG* myogenin, *MuRF1* muscle ring finger 1, *Atrogin-1* muscle atrophy f-box

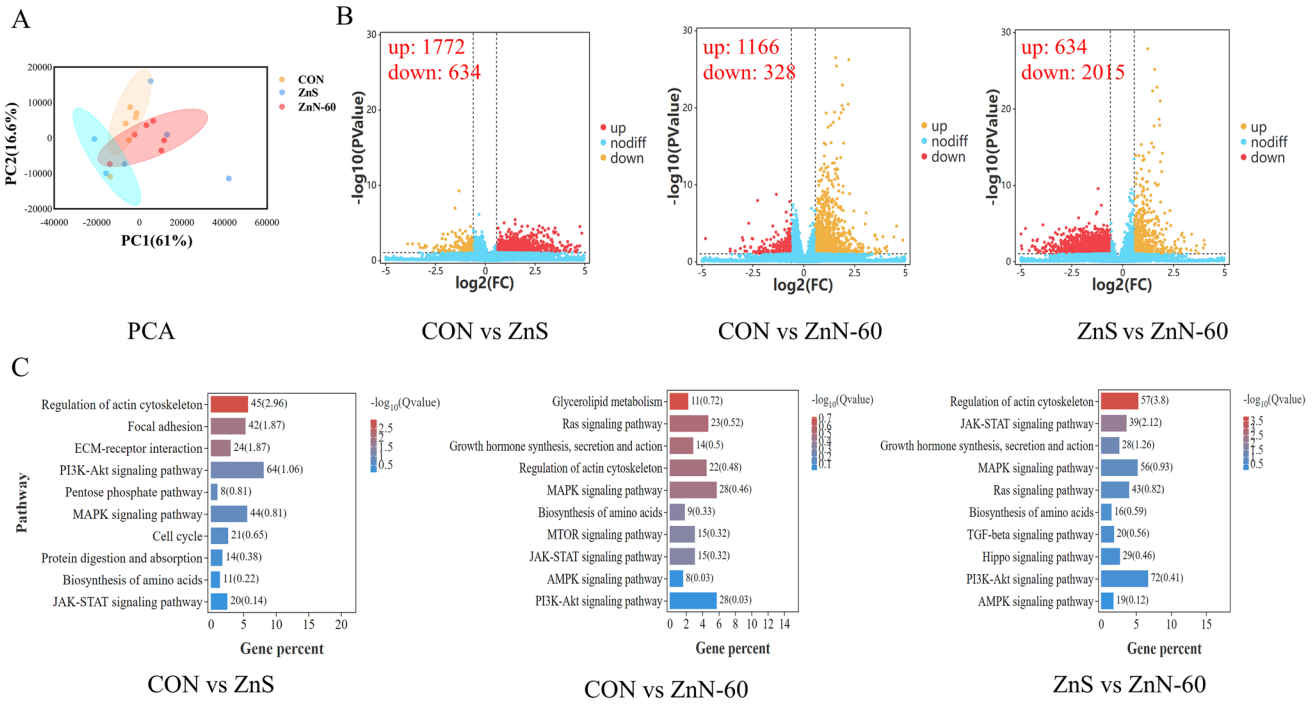

Fig. 2 Effect of different dietary zinc sources on breast muscle transcriptome of 21-day-old breast muscle. **A** PCA cluster analysis of transcriptome samples; **B** volcano plot of differential genes; **C** KEGG pathways enriched by differential genes. ZnS is $ZnSO_4 \cdot H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups, basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4 \cdot H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

The growth and differentiation of skeletal muscle cells are regulated by various factors, among which myogenic regulatory factors (MRFs) play a critical role in muscle development. The MRF family includes myogenic differentiation antigen (MYOD) and myogenic cytokine (MYOG), Myf5 and MRF4. Among them, MYOD and Myf5 regulate the development and differentiation from satellite cells to myoblasts, promoting the proliferation of myoblasts [37]. MYOG and MRF4 are expressed in the late stage of muscle development and are involved in regulating the formation of muscle fibers, which can jointly regulate the differentiation of muscle cells [38, 39]. In the present study, adding ZnN to the diet increased the expression levels of MYOD and MYOG, and promoted the development and regeneration of skeletal muscle. Muscle development is also a net result of protein synthesis and catabolism, and the growth and development of skeletal muscle can be better reflected through the deposition state of muscle tissue protein [40]. Postnatal muscle fiber size increases only when the rate of protein synthesis is higher than the rate of protein degradation [34]. Among them, mammalian target protein of rapamycin (mTOR) signaling pathway [41] and ubiquitin proteasome system (UPS) [42] are critical pathways for regulating protein synthesis and degradation, respectively, and are essential for muscle growth and development. Akt is a serine/threonine kinase that can be activated by different nutrients and growth factors and leads to the activation of its downstream kinase mTOR [41, 43]. Activation of the mTOR pathway and its downstream target gene P70S6K is necessary to regulate skeletal muscle fiber size to activate protein synthesis [41, 44]. Trendelenburg [45] demonstrated that the Akt/mTOR/p70S6K pathway mediated myoblast differentiation and hypertrophy in myotubule promoted muscle growth. In the present study, we found that addition of ZnN increased the expression levels of AKT, MTOR, and P70S6K, indicating that ZnN can promote protein synthesis through Akt/mTOR/p70S6K pathway.

Meanwhile, protein degradation is also essential for regulating muscle mass [35]. UPS is a vital protein degradation system in eukaryotes, and studies have shown that it plays an important role in the development of muscle atrophy [46, 47]. Atrogin-1 and MuRF1 are the most characteristic E3 ubiquitin ligases in skeletal muscle, mediating protein ubiquitination and targeting it for 26S proteasome degradation. Thus, UPS may affect skeletal muscle development by regulating protein degradation [48]. Previous studies of skeletal muscle in different animals have shown that mRNA levels of Atrogin-1 and MuRF1 are significantly increased, while the muscle weight

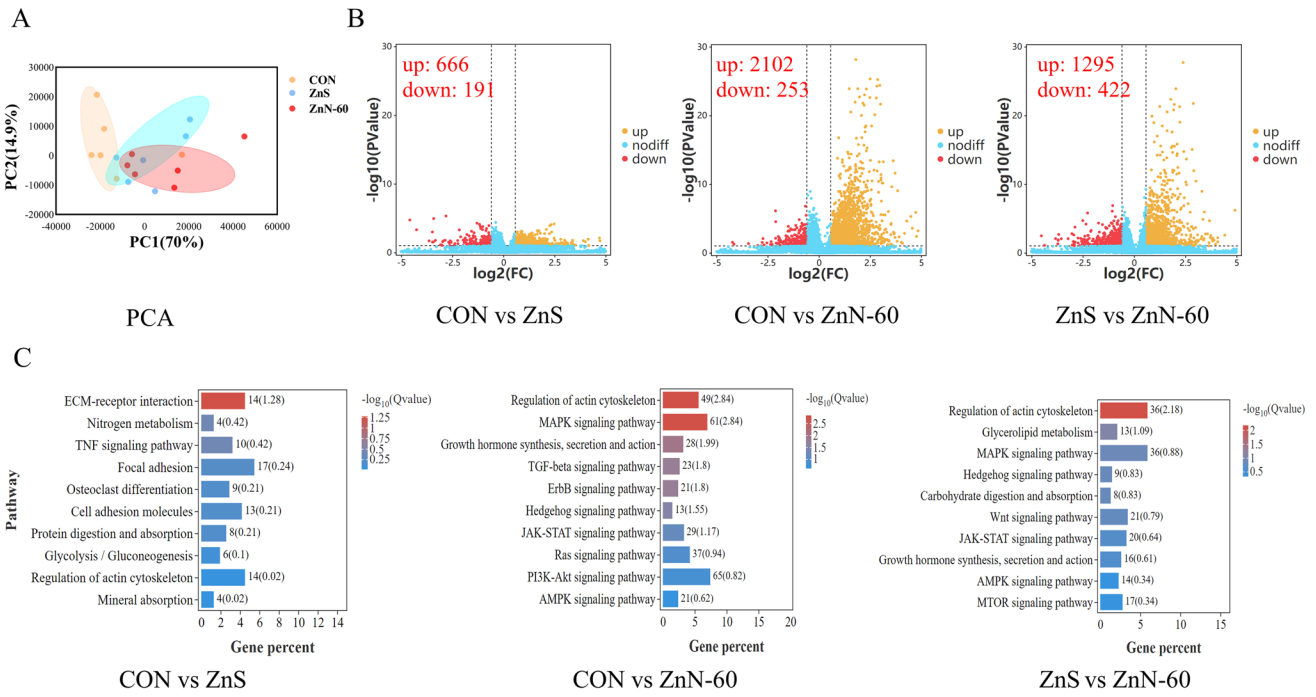

Fig. 3 Effect of different dietary zinc sources on breast muscle transcriptome of 42-day-old breast muscle. **A** PCA cluster analysis of transcriptome samples; **B** volcano plot of differential genes; **C** KEGG pathways enriched by differential genes. ZnS is $ZnSO_4 \cdot H_2O$; ZnN is zinc chelated with a variety of amino acids (including methionine, glycine, and threonine) from enzymatic hydrolyzed soy protein in a 1:1 molar ratio; ZnA is zinc chelated with methionine from hydrolyzed feather meal. CON group, basal diet; ZnN-30, 60, 90, and 120 groups, basal diet with 30, 60, 90, and 120 mg zinc kg^{-1} added as ZnN; ZnS group, basal diet with 120 mg zinc kg^{-1} added as $ZnSO_4 \cdot H_2O$; ZnA group, basal diet with 30 mg zinc kg^{-1} added as ZnA

of animals is significantly decreased [49, 50]. In the present study, we found that the addition of ZnN decreased the expression levels of Atrogin-1 and MuRF1. Therefore, ZnN may promote breast muscle development by regulating the Akt/mTOR/p70S6k and UPS pathways to promote protein synthesis rather than decomposition. However, Akt/mTOR/p70S6K are protein kinases whose expression levels may require further study by western blotting phosphorylated protein levels or other molecular assays.

To further explore the underlying mechanism of ZnN on muscle growth and meat quality, we conducted the transcriptome analysis on the breast muscle of broilers. KEGG pathway enrichment analysis revealed that signaling pathways such as adhesion, backbone regulation of actin, biosynthesis of amino acids, MAPK, MTOR, and AMPK were significantly enriched. Actin is involved in contraction and myoblast differentiation [51]. MTOR is a vital regulator of animal growth and plays an important regulatory role in the proliferation, growth, and differentiation of cells [52]. Our results indicated that adding ZnN to the diet could increase the expression of MTOR in breast muscle. Mitogen-activated protein kinase (MAPK) signaling pathway regulates multiple cellular processes such as cell division, differentiation, and release of inflammatory mediators [53]. It induces protein synthesis and promotes skeletal muscle growth and hypertrophy [54]. Studies have shown that ZIP7-mediated intracellular zinc release can promote the participation of pathways such as MAPK, mTOR, and PI3K-Akt in cell growth and proliferation [55]. Adenosine 5′-monophosphate activated protein kinase (AMPK) is an important kinase that regulates energy homeostasis, and can provide energy for skeletal muscle growth and differentiation [56]. The MAPK and mTOR pathways are interdependent during muscle growth regulation [57], and the signaling pathways related to muscle development and energy metabolism interact to jointly regulate muscle growth and development.

In conclusion, zinc amino acid complexes could improve growth performance, tissue zinc concentration, and regulate breast muscle development.

Author Contribution Gang Zuo and Zehe Song contributed to the conception of the study. Mengmeng Ma and Liwei Li performed experiment, collected and analyzed data, and wrote the paper. Xi He, Jian Xiao, and Junlie Chen contributed significantly to analysis and manuscript preparation. Zehe Song supervised the study and reviewed the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval Experiments were carried out following the ethical guidelines of Hunan Agricultural University for the care and use of laboratory animals and approved by the animal ethics committee of Hunan Agricultural University (HAU ACC 2022189).

Competing Interests The authors declare no competing interests.

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