

Influence of L-selenomethionine on pigeon breeder's reproductive performance and squab's growth and muscle development

HAOPENG LU¹, YUNYAN HUANG¹, JIASHUI LIANG², SHIHONG WU²,
GUOLONG ZHANG¹, ANJIE CHEN¹, XIAOYU ZHOU¹, XINTING LAI¹,
LVYING YANG¹, YUXI LIANG¹, XUEKANG XIA³, SHUO ZHOU^{1*}, YAYAN LIANG^{1*}

¹College of Animal Science and Technology, Zhongkai University of Agriculture and Engineering, Guangzhou, Guangdong, P.R. China

²Huizhou Huabao Feed Co., Ltd., Huizhou, Guangdong, P.R. China

³Guangdong Juyuanxing Agricultural Development Co., Ltd., Shaoguan, Guangdong, P.R. China

Haopeng Lu and Yunyan Huang contributed equally to this work

*Corresponding authors: shuozhou@zju.edu.cn; sandy130130@163.com

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Abstract: This study evaluated the effects of dietary L-selenomethionine supplementation on breeding pigeons and their squabs. Forty-eight pairs of breeding pigeons were divided into two groups: a control group receiving a basal diet and an experimental group receiving the same diet supplemented with 0.1 mg/kg selenium as L-selenomethionine. Both groups were fed for 45 days following a “2 + 4” breeding pattern. Compared with the control group, experimental males showed greater weight loss (77.8 vs 55.8 g, +39.4%, $P < 0.05$), while females showed a trend towards reduced loss ($P = 0.097$). Squabs in the experimental group exhibited significantly higher nest and individual weights at Day 14 and Day 21 ($P < 0.05$), alongside reduced breast muscle fibre area and diameter ($P < 0.01$). Two-way ANOVA revealed that L-selenomethionine significantly increased plasma T-SOD activity in breeders (treatment main effect, $P = 0.003$), which remained significant after FDR correction ($q = 0.014$). Additionally, L-selenomethionine reduced MDA content in squab leg muscle and upregulated GPX1/2 mRNA expression in muscle ($P < 0.05$), although these changes were only nominally significant after FDR correction ($q > 0.05$). In conclusion, dietary supplementation with 0.1 mg/kg L-selenomethionine promoted squab growth and altered muscle fibre morphology, with no significant adverse effects detected on reproductive performance; however, the biological significance of these changes, particularly the molecular and antioxidant responses, warrants further investigation.

Keywords: gene expression; muscle fibre; performance; RT-qPCR; selenium

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Edible pigeons (*Columba livia domestica*) were domesticated as early as 2 500 BCE. Once a royal delicacy in ancient Egypt, pigeon meat is now a daily staple in over 60 countries, valued for its high protein, low fat, and abundant n-3 polyunsaturated fatty acids (EPA and DHA) (Liu et al. 2025; Zheng et al. 2025). Currently, China's annual production of squabs stands at approximately 804 million birds, accounting for more than 80% of global production. Major production regions, including Guangdong, Jiangsu, and Xinjiang, have established comprehensive industrial chains spanning from breeding stock development to cold-chain distribution of fresh products. However, breeding is still largely based on conventional cages with limited space (approximately 0.8 m² per pair) (Lan et al. 2025). Currently, the “2 + 4” breeding model (where one pair of parent pigeons raises 4 squabs) has emerged as the standard production method (Peng et al. 2023). This approach directly increases breeding density within limited cage space while nearly doubling the feeding frequency and energy expenditure of breeding pigeons compared with natural rearing of two squabs. Prolonged high physiological stress triggers excessive accumulation of free radicals, thereby disrupting the antioxidant defence system of breeding pigeons and significantly intensifying oxidative stress (Xie et al. 2023). Consequently, the transfer of maternal antibodies and antioxidants to squabs diminishes, compromising their intestinal barrier integrity. This reduction ultimately leads to increased morbidity and mortality rates (Chang et al. 2018). As such, the pigeon-breeding industry is actively exploring nutritional interventions that align with high-yield brooding models, such as the inclusion of functional feed additives. This approach aims to alleviate oxidative stress in parent pigeons and reduce morbidity among squabs caused by high brood sizes, thereby enhancing overall production efficiency.

Selenium can be classified into 3 major categories: inorganic, organic, and nano forms. It protects cells from oxidative stress by enhancing the activity of antioxidant enzymes, such as glutathione peroxidase (GSH-Px) (Tao et al. 2023). Fu et al. (2023) and Liang et al. (2023) demonstrated that supplementing the diets of breeding pigeons with 0.3 mg/kg of sodium selenite (selenium content), yeast-derived selenium, or chitosan-nanoparticle selenium enhances antioxidant capacity in both muscle and intestinal tissues of squabs, while also

improving meat quality and regulating intestinal morphological development. However, different selenium sources vary considerably in bioavailability and tissue-deposition efficiency. Chen et al. (2024) demonstrated that, at equivalent doses, L-selenomethionine exhibited significantly higher selenium absorption and conversion efficiency in egg and hen serum compared with selenium-enriched yeast, nano-selenium, and the control group. Similarly, Delezie et al. (2014) observed that, within the 0.1–0.5 mg/kg dosage range, the selenium deposition efficiency in eggs from L-selenomethionine supplementation was 1.65 times greater than that of selenium yeast and 4.9 times greater than that of sodium selenite. Furthermore, Tona et al. (2022) reported that eggs laid by hens fed selenomethionine significantly reduced incubation time while improving hatchability, feed conversion ratio, haemoglobin levels, and other physiological indicators. As an organic selenium source, L-selenomethionine is directly incorporated into body proteins in place of methionine, providing a metabolically active reservoir of selenium that can be mobilised during periods of increased physiological demand (Surai et al. 2018). This characteristic makes it particularly attractive for breeding pigeons under the “2 + 4” high-yield model, where sustained antioxidant support is critical. However, to date, no studies have evaluated the effects of L-selenomethionine supplementation on reproductive performance, squab growth, or muscle development in pigeons. Therefore, this study investigates the effects of L-selenomethionine supplementation on the reproductive performance of breeding pigeons, the growth performance of squabs, and breast muscle development, aiming to provide reliable data references and scientific support for further research, development, and application of L-selenomethionine in meat pigeon feed production. The central hypothesis is that dietary L-selenomethionine supplementation will improve the reproductive performance of breeding pigeons and promote squab growth by enhancing antioxidant capacity and modulating muscle fibre development.

MATERIAL AND METHODS

Experimental design

The study selected 48 pairs of 52-week-old American King pigeons with similar body weight

and reproductive history. The birds were randomly assigned to two dietary treatments (control and L-selenomethionine), each with four replicate blocks. Within each block, there were six cages, and each cage housed one breeding pair. Each pair raised four squabs according to the “2 + 4” pattern. The individual cage was considered the experimental unit for reproductive performance, whilst the nest was the experimental unit for squab growth traits. The control group received a basal diet of 55.3% whole grain + 44.7% pellet feed, whereas the experimental group was fed the basal diet supplemented with an additional 0.1 mg/kg of L-selenomethionine. The experimental cycle lasted 45 days, comprising a 7-day pre-test phase (to acclimatise birds to the experimental conditions), a 17-day incubation phase, and a 21-day nursing phase. The experimental animals and facilities were provided by Guangdong Juyuanxing Pigeon Industry Co., Ltd. The nutritional composition of the daily diet is presented in Table 1. All animal procedures were approved by the Institutional Animal Care and Use

Committee of Zhongkai University of Agriculture and Engineering (Approval No. ZK2024-009). The study was conducted in accordance with the guidelines for the care and use of laboratory animals and complied with Chinese national legislation on animal experimentation.

Animal care and management

This study was conducted from July to September 2024. The pigeon loft was semi-open and naturally ventilated, and the environmental conditions reflected natural seasonal variation, with ambient temperature ranging from 25 °C to 35 °C and relative humidity from 55% to 73% during the trial. Environmental conditions were recorded daily; no significant differences in temperature or humidity were observed among the four replicate blocks, nor between the control and experimental groups, as all cages were evenly distributed across the loft and managed under identical conditions. Each pair of breeding pigeons was housed individually in a separate cage, provided with ample food and drinking water, and supplemented with mineral grit (5% salt, 35% grit, and 60% oyster shell chips). The loft was designed with an open-style, 3-tier vertical cage system, with one pair housed per cage. Throughout the experimental phase, all experimental pigeons had free access to feed and water, with a daily 16-hour photophase maintained. The loft was ventilated on all sides, and regular cleaning (droppings were removed every 3–4 days, and the area where droppings had been was disinfected using lime powder) and disinfection of droppings were performed. Daily observations and records were maintained to monitor the pigeons' feeding behaviour and health status.

Sample collection and processing

Breeding pigeon weight and reproductive performance record. During the experimental phase, feed was provided daily at 7:00 a.m., 11:30 a.m., and 3:00 p.m. to ensure adequate supply. On the evening of Day 1 of the lactation phase and the evening before Day 21 of the lactation phase, the breeding pigeons were fasted for 12 h, starting at 7:00 p.m. Their body weights were then measured to calculate the changes in weight. Throughout the experimental phase, the egg production rate, fertilisation rate, and hatchability were monitored and recorded. The egg production rate was calculated as the number of breeding pigeons laying

Table 1. Composition and nutritional levels of pigeon diets

Feed composition ¹	Ratio (%)	Nutritional status	Content (%)
Corn	20.7	dry matter	87.7
Sorghum	10.5	crude protein	14.9
Wheat	8.4	metabolic energy/(MJ/kg) ³	12.6
Peas	15.7	crude fat	2.96
Pellet feed ²	44.7	crude ash	3.45
		calcium	0.59
Total	100.0	total phosphorus	0.42

¹The experimental diet consisted of 55.3% whole grain + 44.7% pellet feed. ²The pelleted feed was purchased from Huabao Feed Co., Ltd., Huizhou, Guangdong, P.R. China. The pelleted feed contained 4% premix, which provided the following per kilogram: vitamin A, 14 200 IU; vitamin D, 3 300 IU; vitamin E, 22.50 mg; vitamin K, 3.90 mg; vitamin B₁, 3.00 mg; vitamin B₂, 9.60 mg; vitamin B₆, 6.00 mg; niacin, 60.00 mg; calcium pantothenate, 18.00 mg; folic acid, 1.50 mg; vitamin B₁₂, 0.03 mg; biotin, 0.06 mg; copper, 6.00 mg; iron, 60.00 mg; zinc, 58.00 mg; manganese, 65.00 mg; iodine, 0.35 mg; selenium, 0.24 mg. ³The metabolisable energy was a calculated value, while the other values were measured. L-selenomethionine was purchased from Sichuan Xinyimei Biotechnology Co., Ltd.

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eggs during the experimental phase divided by the total number of breeding pigeons. The fertilisation rate was determined as the number of fertilised eggs during the experimental phase divided by the number of eggs incubated. Hatchability was recorded as the number of fledglings hatched during the experimental phase divided by the number of eggs incubated.

Growth performance records of squab pigeon.

On Days 6, 13, and 20 of the lactation phase, any remaining feed was removed at 7:00 p.m., followed by a 12-hour fasting phase. On Days 1, 7, 14, and 21 of the lactation phase, the squabs were weighed as a litter at 7:00 a.m. to assess growth performance.

Biochemical plasma marker testing for breeding pigeons. 21 days after lactation, 8 pairs of breeding pigeons were randomly selected from different replicates within each treatment group. After a fasting phase of 12 h, blood samples were collected from the wing vein of each pigeon and transferred into vacuum tubes containing an anticoagulant. These tubes were then centrifuged at 3 000 rpm for 10 min at 4 °C. The resulting plasma was collected and stored at –80 °C.

Biochemical indicator testing of squab pigeon muscle. For squab tissue analyses, one squab was randomly selected from each of eight distinct nests per treatment group ($n = 8$ nests per group), with nests distributed across different replicate blocks. After a 12-h fasting period, the squabs were euthanised, and samples of breast and leg muscle were collected immediately. A portion of each muscle sample was fixed in 4% paraformaldehyde for histomorphological observation, and the remaining tissue was snap-frozen in liquid nitrogen and stored at –80 °C for subsequent biochemical and qPCR analyses.

Approximately 1 g of breast and leg muscle tissue was collected from identical anatomical sites on slaughtered squabs and immediately placed in liquid nitrogen for subsequent experimental analyses. Testing was conducted using a kit provided by the Nanjing Jiancheng Biological Engineering Research Institute to measure the following indicators: H_2O_2 , MDA, T-SOD, T-AOC, CAT, and GSH-Px. All experiments were carried out following the manufacturer's instructions (Jiancheng Bioengineering Institute, Nanjing, P.R. China).

Chest muscle quality assessment of squabs. Muscle samples were collected from identical sites on the breast muscles of squabs. Meat colour of the breast muscle was assessed using a fully automated

colourimeter (TS7030; ThreeNH, P.R. China) according to the CIE 1977 standard (CIE 1977), and the lightness (L^*), redness (a^*), and yellowness (b^*) values were recorded. Approximately 10 g of breast muscle tissue was collected, ensuring consistency in the sampling site. Excess surface moisture was gently removed with absorbent paper. The initial weight (W1) was accurately measured and recorded. Each breast muscle sample was then tied with cotton thread and placed in a resealable plastic bag to prevent contact between the sample tissue and the bag wall. The tied breast muscle samples were then kept in a refrigerator at 4 °C for 24 hours. Subsequently, the samples were removed, the surface moisture was blotted with absorbent paper, reweighed, and the final weight (W2) was recorded. The percentage of drip loss was calculated using this formula:

$$\text{Drip loss (\%)} = (W1 - W2)/W1 \times 100 \quad (1)$$

Morphological analysis. Approximately 1 cm³ of muscle tissue was harvested from the mid-belly of the left pectoralis major muscle (breast) and the mid-belly of the left biceps femoris muscle (leg) of different squabs. The tissue was placed in 4% formalin for preservation and subjected to haematoxylin and eosin (H&E) staining following the method described by Agazzi et al. (2020). Subsequently, the prepared breast muscle sections of pigeons were examined under a 20× optical microscope. Five random fields of view were selected per section, and 20 muscle fibres were randomly chosen from each field. The cross-sectional area measurements and diameter calculations were performed using ImageJ software.

Real-time quantitative PCR. Total RNA was extracted from the breast and leg muscle tissues of squabs using Trizol reagent. Subsequently, the concentration and purity (A_{260}/A_{280}) of the RNA samples were quantitatively assessed using a NanoDrop 2000 microvolume spectrophotometer to ensure that the RNA quality met the requirements for subsequent analyses. The extracted RNA was reverse transcribed into cDNA according to the TaKaRa reverse transcription instructions and stored at –20 °C for later use. Relative expression levels of the target genes and control gene were determined using the PowerUp™ SYBR™ Green Master Mix kit (Transgen Biotech, P.R. China) following its protocol. To measure the relative ex-

pression levels of superoxide dismutase 1 (SOD1), superoxide dismutase 2 (SOD2), glutathione peroxidase 1 (GPX1), glutathione peroxidase 2 (GPX2), glutathione peroxidase 3 (GPX3), glutathione peroxidase 4 (GPX4), and catalase (CAT) in the breast and leg muscles, primers were obtained from Tao et al. (2023). The primer sequences are listed in Table 2.

Statistical analysis

Data were initially processed using Microsoft Excel and analysed using SPSS v26.0. Normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. For comparisons between the control and L-selenomethionine groups, Welch's two-sample *t*-test (two-tailed) was applied, as it does not assume equal variances. For plasma biochemical parameters in male and female pigeons, two-way ANOVA was employed with sex and treatment as fixed factors, followed by Benjamini-Hochberg false discovery rate (FDR) correction for multiple comparisons.

For all squab traits measured at the individual level, the nest served as the independent experimental unit: eight squabs per group were selected from eight distinct nests (one squab per nest) across

different replicate blocks, ensuring that no pseudoreplication was involved.

To control the false discovery rate due to multiple comparisons, the Benjamini–Hochberg procedure was applied separately to each group of related variables (plasma markers, muscle biochemical indices, and qPCR data). Growth performance and reproductive traits were not subjected to FDR correction, as these represent distinct biological categories. Adjusted *q*-values are reported alongside nominal *P*-values; $q < 0.05$ was considered statistically significant, and $P < 0.05$ with $q \geq 0.05$ is reported as nominally significant. Data are expressed as means $\pm$ SD.

RESULTS

Assessment of weight loss and reproductive performance in breeder pigeons. Table 3 indicates that, compared with the control group, male pigeons in the experimental group exhibit significantly greater weight loss during the 1–21 day lactation phase, with an average loss of 77.8 g versus 55.8 g in the control group – representing a 39.4% increase ($P < 0.05$). Female pigeons show a trend towards reduced weight loss (37.1 g vs 60.9 g, a 39.1% decrease, $P = 0.097$). No significant differences were observed between groups for body weight on Day 1 or Day 21, healthy squab rate per nest, fertilisation rate, or hatchability ($P > 0.05$).

Assessment of antioxidant capacity in plasma of breeder pigeons. As shown in Table 4, two-way ANOVA revealed that dietary L-selenomethionine supplementation had no significant effects on plasma CAT, T-AOC or MDA in breeding pigeons (gender main effect, treatment main effect, and their interaction, all $P > 0.05$). For GSH-Px, a significant gender main effect was observed ($P = 0.048$), whereas the treatment main effect ($P = 0.064$) and the interaction ($P = 0.711$) were not significant. For T-SOD, the treatment main effect was significant ($P = 0.003$) and remained significant after FDR correction ($q = 0.014$), while the gender main effect and the interaction were not significant ($P > 0.05$). These results indicate that L-selenomethionine significantly increased plasma T-SOD activity in breeding pigeons, with consistent effects in both sexes.

Analysis of growth performance in squabs. Table 5 shows that the experimental group has

Table 2. qPCR primers and sequences

Gene name	Primer sequence (5'→3')	Product length (bp)
SOD1	F: ACTGCTAAAGGAGGTGTGGC	163
	R: GAGGTCCCGCATTTCAGTT	
SOD2	F: CGTGACTTTGGTTCCTTCGC	122
	R: GCTGCTATTTGTAGGCGTCC	
GPX1	F: GCTTGGTTTTCTCGCAGGTG	157
	R: GCCACTTCCCCAGCATAGT	
GPX2	F: TTTGGAGCCCAACACATCACG	164
	R: TGAAATCCACCTTCTCCCCCT	
GPX3	F: CCTTTTCTTGGCTGGGCTTG	128
	R: GGATGTACTCGTCCCCATCG	
GPX4	F: TCCCTGGAGAAGTACCGGGG	117
	R: CTTCTCAGCGTATCTGGCGT	
GAPDH	F: TCCTCCACCTTTGATGCG	138
	R: GTGCCTGGCTCACTCCTT	
CAT	F: TGCACTGTTGTTTCCGTCCT	125
	R: CCCACAGGGATGAGAGGGTA	

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Table 3. Weight of breeding pigeons and reproductive performance

Item	Lactation phase				Weight loss during lactation (1–21 days)/g				Reproductive performance of breeder pigeons			
	male pigeon weight/g		female pigeon weight/g		Day 1	Day 21	male pigeon	female pigeon	total weight loss	healthy squab rate per nest/%	fertilisation rate (%)	hatch rate (%)
	Day 1	Day 21	Day 1	Day 21								
Control	594 ± 43	555 ± 56	567 ± 68	550 ± 81	55.8 ± 35	60.9 ± 45	82.1 ± 56.1	96.9 ± 8.45	80.0 ± 24.9	93.3 ± 17.2		
Experimental	598 ± 58	532 ± 46	559 ± 61	547 ± 61	77.8 ± 22	37.1 ± 20	88.1 ± 39.2	98.9 ± 5.10	78.3 ± 28.4	91.7 ± 18.9		
SEM	7.47	7.59	9.30	10.3	5.54	7.18	7.55	0.01	0.03	0.02		
<i>P</i> -value	0.81	0.13	0.69	0.87	0.04	0.09	0.69	0.30	0.81	0.72		

P < 0.05 indicates significant difference, *P* > 0.05 indicates no significant difference, *n* = 24 nests/group

Table 4. Plasma antioxidant capacity indicators in breeding pigeons

Item	CAT (U/ml)	GSH-Px (U/ml)	T-AOC (U/ml)	MDA (mmol/l)	T-SOD (U/ml)
Male pigeon	control	488 ± 9.34	6.38 ± 2.62	4.95 ± 1.88	7.26 ± 4.95
	experimental	5.05 ± 1.25	6.18 ± 2.95	6.23 ± 2.58	14.17 ± 1.99
Female pigeon	control	430 ± 2.29	3.59 ± 1.02	4.64 ± 1.71	7.58 ± 1.03
	experimental	358 ± 68.30	4.60 ± 0.30	5.60 ± 0.98	14.13 ± 3.69
<i>P</i> -value	gender	0.05	0.09	0.68	0.93
	treatment	0.06	0.77	0.36	0.003
	interaction	0.73	0.62	0.89	0.92
	<i>q</i> -value	0.92	0.92	0.59	0.014

P < 0.05 indicates significant difference, *P* < 0.01 indicates highly significant difference, *P* > 0.05 indicates no significant difference, *n* = 8 animals/group. The *q*-value refers to the *P*-value adjusted for the false discovery rate (FDR)

Table 5. Growth performance of squab pigeon

Item	Average nest weight/g				Average weekly weight gain/g				Average individual weight/g			
	Day 1	Day 7	Day 14	Day 21	the first week	the second week	the third week	total weight gain	Day 1	Day 7	Day 14	Day 21
Control	59.6 ± 3.55	587 ± 90	1 200 ± 194	1 701 ± 168	527 ± 91	613 ± 165	547 ± 178	1 640 ± 172	14.90 ± 0.89	146 ± 22	300 ± 48	436 ± 30
Experimental	59.2 ± 3.70	618 ± 83	1 356 ± 129	1 800 ± 77	558 ± 83	738 ± 89	443 ± 88	1 740 ± 79	14.81 ± 0.94	154 ± 20	339 ± 32	450 ± 19
SEM	0.13	3.24	26.75	20.23	13.08	21.39	21.75	20.30	0.14	3.25	6.69	5.06
<i>P</i> -value	0.46	0.14	0.01	0.02	0.23	0.01	0.02	0.02	0.45	0.14	0.01	0.02

P < 0.05 indicates significant difference, *P* < 0.01 indicates highly significant difference, *P* > 0.05 indicates no significant difference, *n* = 24 nests/group

significantly higher nest weights at Day 14 (1 356 vs 1 200 g, a 13.0% increase, $P < 0.01$) and Day 21 (1 800 vs 1 701 g, a 5.8% increase, $P < 0.05$). Nest weight gains during the second week (738 vs 613 g, a 20.4% increase, $P < 0.01$) and the third week (443 vs 547 g, a 19.0% decrease, $P < 0.05$) are also significantly different. Average individual weights are significantly higher at Day 14 (339 vs 300 g, a 13.0% increase, $P < 0.01$) and Day 21 (450 vs 436 g, a 3.2% increase, $P < 0.05$). No significant differences were observed in nest weight at Day 1, individual weight at Day 1 or Day 7, or weight gain during the first week ($P > 0.05$).

Assessment of breast muscle quality in squab.

Table 6 shows that, compared with the control group, dietary L-selenomethionine supplementation had no significant effect on the L^* , a^* , b^* , and drip loss of the breast muscle in squabs ($P > 0.05$).

Observation of muscle fibres in squab. Figures 1 and 2 show that dietary L-selenomethionine supplementation significantly reduced the cross-sectional area and diameter of breast muscle fibres in squabs ($P < 0.01$), with no significant effect on leg muscle fibre morphology ($P > 0.05$).

Assessment of antioxidant capacity in pigeon squab muscle. Table 7 indicates that dietary L-se-

Table 6. Breast muscle quality of squabs

Item	L^*	a^*	b^*	Drip loss/%
Control	40.3 ± 1.65	15.9 ± 1.26	13.3 ± 1.00	2.35 ± 0.79
Experimental	41.0 ± 2.17	16.2 ± 0.62	13.3 ± 0.67	2.51 ± 0.64
SEM	0.47	0.24	0.20	0.17
P -value	0.44	0.60	0.88	0.66

$P < 0.05$ indicates significant difference, $P < 0.01$ indicates highly significant difference, $P > 0.05$ indicates no significant difference, $n = 8$ animals/group

a^* = redness; L^* = lightness; b^* = yellowness

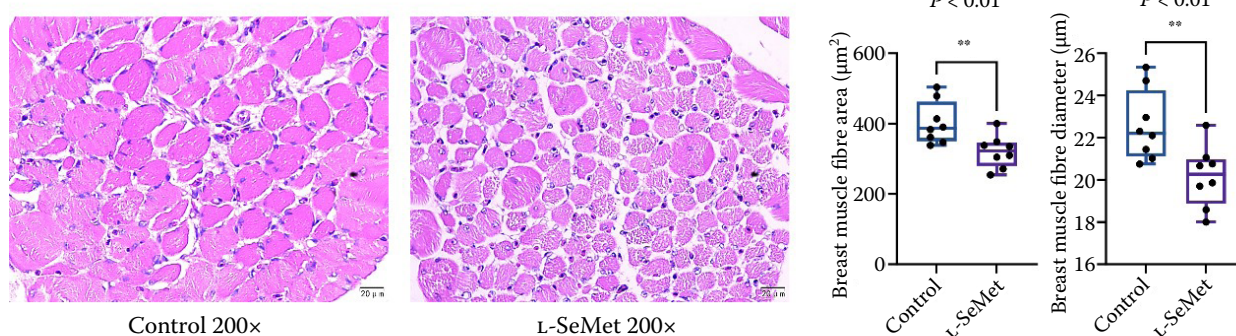


Figure 1. Morphology and quantitative analysis of squab pigeon breast muscle fibres ($n = 8$ animals/group)

L-SeMet = L-selenomethionine

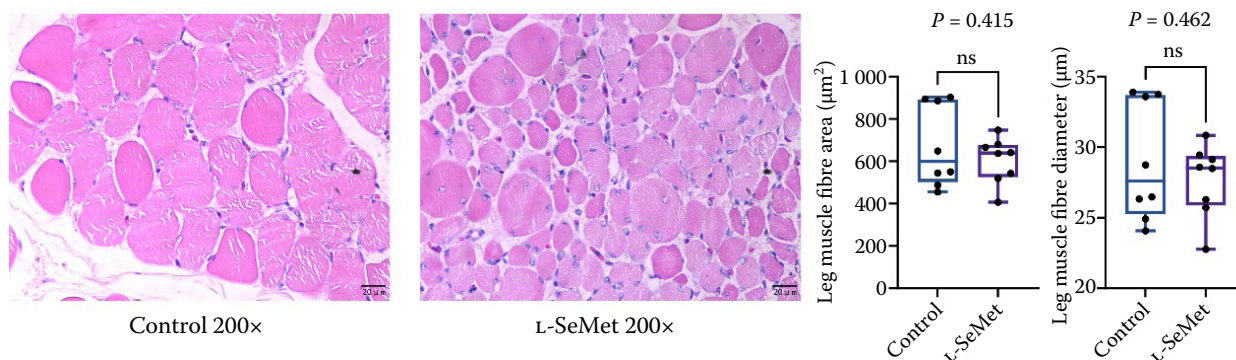


Figure 2. Morphology and quantitative analysis of squab pigeon leg muscle fibres ($n = 8$ animals/group)

L-SeMet = L-selenomethionine; ns = not significant

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Table 7. Antioxidant stress indicators in squab breast muscle

Item	CAT (U/mg protein)	H ₂ O ₂ (mmol/mg protein)	GSH-Px (U/mg protein)	T-AOC (U/mg protein)	MDA (mmol/mg protein)	T-SOD (U/mg protein)
Control	9.09 ± 1.85	7.40 ± 2.73	110 ± 52.9	2.81 ± 1.04	5.22 ± 2.06	6.55 ± 2.00
Experimental	8.49 ± 1.95	6.94 ± 6.46	112 ± 56.6	3.50 ± 1.96	5.13 ± 1.77	5.26 ± 1.45
SEM	0.57	1.43	17.00	0.39	0.48	0.47
<i>P</i> -value	0.26	0.88	0.96	0.43	0.93	0.18
<i>q</i> -value	0.78	0.96	0.96	0.86	0.96	0.96

P > 0.05 indicates no significant difference, *n* = 8 animals/group. The *q*-value refers to the FDR-adjusted *P*-value (Benjamini–Hochberg); *q* < 0.05 was considered statistically significant

CAT = catalase; GSH-Px = glutathione peroxidase; H₂O₂ = hydrogen peroxide; MDA = malondialdehyde; T-AOC = total antioxidant capacity; T-SOD = total superoxide dismutase

lenomethionine supplementation had no significant effect on any of the antioxidant parameters measured in breast muscle (CAT, H₂O₂, GSH-Px, T-AOC, MDA, or T-SOD; *P* > 0.05).

Table 8 indicates that dietary L-selenomethionine supplementation significantly reduced MDA content in the leg muscle of squabs (5.27 vs

9.91 mmol/mg protein, a 46.8% reduction, *P* < 0.05, *q* = 0.18), though this difference is nominally significant after FDR correction (*q* > 0.05). No significant effects were observed for CAT, H₂O₂, GSH-Px, T-AOC, or T-SOD in leg muscle (*P* > 0.05).

Expression of selenoprotein genes in pigeon squab muscle. Figure 3 shows that dietary L-se-

Table 8. Antioxidant stress indicators in squab leg muscle

Item	CAT (U/mg protein)	H ₂ O ₂ (mmol/mg protein)	GSH-Px (U/mg protein)	T-AOC (U/mg protein)	MDA (mmol/mg protein)	T-SOD (U/mg protein)
Control	8.96 ± 2.48	4.52 ± 1.59	117 ± 26.2	3.54 ± 1.40	9.91 ± 2.67	9.95 ± 1.89
Experimental	9.48 ± 2.44	3.76 ± 1.34	119 ± 26.2	4.03 ± 2.04	5.27 ± 1.42	10.21 ± 3.94
SEM	0.61	0.40	7.95	0.45	1.14	0.85
<i>P</i> -value	0.68	0.37	0.77	0.61	0.03	0.88
<i>q</i> -value	0.88	0.88	0.92	0.88	0.18	0.88

P < 0.05 indicates significant difference, *P* > 0.05 indicates no significant difference, *n* = 8 animals/group. The *q*-value refers to the FDR-adjusted *P*-value (Benjamini–Hochberg); *q* < 0.05 was considered statistically significant

CAT = catalase; GSH-Px = glutathione peroxidase; H₂O₂ = hydrogen peroxide; MDA = malondialdehyde; T-AOC = total antioxidant capacity; T-SOD = total superoxide dismutase

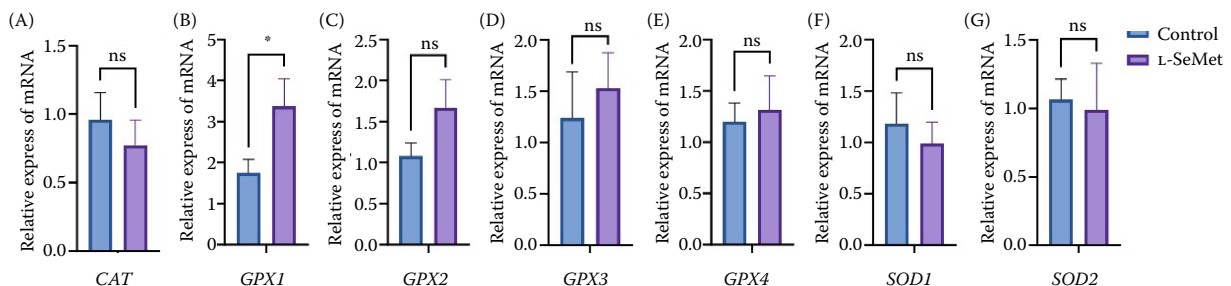


Figure 3. Gene expression of selenoprotein in squab pigeon breast muscle

(A) CAT; (B) GPX1; (C) GPX2; (D) GPX3; (E) GPX4; (F) SOD1; (G) SOD2

**P* < 0.05; ns, *P* > 0.05. Data are presented as means ± SD. After Benjamini–Hochberg FDR correction for multiple comparisons across the seven genes, no genes remained significant at *q* < 0.05; GPX1 was nominally significant (*P* = 0.04, *q* = 0.30) (*n* = 8 animals/group)

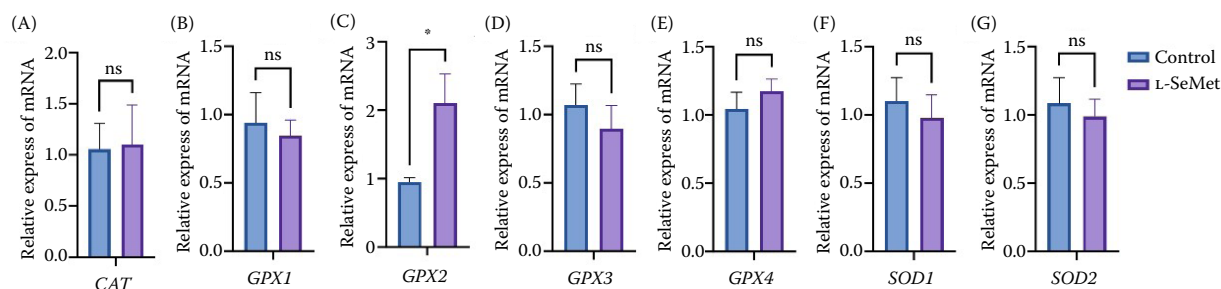


Figure 4. Gene expression of selenoprotein in squab pigeon leg muscle

(A) CAT; (B) GPX1; (C) GPX2; (D) GPX3; (E) GPX4; (F) SOD1; (G) SOD2

* $P < 0.05$; ns, $P > 0.05$. Data are presented as means $\pm$ SD. After Benjamini–Hochberg FDR correction for multiple comparisons across the seven genes, no genes remained significant at $q < 0.05$; GPX2 was nominally significant ($P = 0.038$, $q = 0.26$) ($n = 8$ animals/group)

lenomethionine supplementation significantly increased GPX1 mRNA expression in breast muscle (3.37 vs 1.753, a 48.0% increase, $P = 0.04$) (Figure 3B). However, after FDR correction, this difference did not reach the adjusted significance threshold ($q = 0.30$) and is therefore considered nominally significant. No significant effects were observed for CAT, GPX2, GPX3, GPX4, SOD1, or SOD2 ($P > 0.05$) (Figure 3A,C–G). As shown in Figure 4, dietary L-selenomethionine supplementation significantly increased GPX2 mRNA expression in leg muscle (2.106 vs 0.95, a 54.9% increase, $P = 0.038$) (Figure 4C). However, after FDR correction, this difference did not reach the adjusted significance threshold ($q = 0.26$) and is therefore considered nominally significant. No significant effects were observed for CAT, GPX1, GPX3, GPX4, SOD1, or SOD2 ($P > 0.05$) (Figure 4A–B,D–G).

DISCUSSION

Changes in body weight directly reflect the growth status of livestock and poultry and serve as key indicators of reproductive performance. Wang et al. (2023a) reported that male and female pigeons perform distinct roles during the reproductive cycle: females primarily undertake incubation duties (approximately 19 h per day), whereas males increase their contribution to feeding from 50% to 90% between Days 4 and 28 of the nursing phase, resulting in greater energy expenditure among males. The present study results indicate that dietary L-selenomethionine supplementation significantly increased weight loss in male pigeons between Days 1 and 21 of the nursing phase, sug-

gesting an increased feeding burden. Although female pigeons showed no statistically significant weight loss difference, their average weight loss decreased by 39.1%. This suggests L-selenomethionine may offer potential benefits in mitigating energy depletion in females. Peng et al. (2023) reported that reducing weight loss in breeding pigeons during the reproductive cycle facilitates a more rapid transition to the subsequent breeding cycle. Although individual male pigeons exhibited significant weight loss, the overall weight loss in the experimental group did not differ significantly from the control group. Contrasting this with the weight loss trend in female pigeons, this suggests L-selenomethionine may regulate energy allocation through sex-specific mechanisms, potentially influencing the timing of breeding pigeons' entry into the next reproductive cycle. Nevertheless, despite the significantly greater weight loss in experimental-group males compared with controls, no differences were detected in healthy chick rate, fertilisation rate, or hatchability between groups in this study. This indicates that the degree of male weight loss remained within reasonable physiological thresholds. However, confirmation through additional experimental data is required to determine whether this weight loss affects the long-term reproductive performance of breeding pigeons.

Plasma oxidative stress levels serve as a crucial biochemical indicator for assessing livestock and poultry health, encompassing antioxidants such as MDA, GSH-Px, and T-SOD. T-SOD is a key component of the body's antioxidant enzyme system, and its activity directly reflects the organism's ability to scavenge free radicals. Long et al. (2022) found that supplementing diets with 0.25–2 mg/kg

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of L-selenomethionine resulted in a linear increase in plasma T-SOD activity in laying hens, with the highest activity observed in the 1 mg/kg group. Jiang et al. (2009) found that dietary supplementation with 0.225 mg/kg of L-selenomethionine significantly enhanced plasma antioxidant enzyme activities (T-AOC, GPX, and T-SOD) in male broiler chickens. Xia et al. (2022) reported that selenium supplementation in the diets of female ducks and ducklings increased hepatic T-SOD activity and reduced MDA concentrations in duckling livers. The results of this study showed that adding 0.1 mg/kg (selenium content) of L-selenomethionine significantly increased plasma T-SOD activity in both male and female pigeons, which is consistent with the results of previous studies. However, other antioxidant indicators, including CAT, GSH-Px, T-AOC, and MDA, showed no significant changes in either male or female pigeons. This discrepancy may be attributed to the relatively low supplemental dosage (0.1 mg/kg) and species differences.

Assessing differences in growth performance among young poultry can be achieved by recording and analysing changes in body weight over a defined growth cycle, which also provides the most direct measure of additive efficacy. Anizoba et al. (2024) demonstrated that dietary supplementation with 1.50 mg/kg selenium yeast significantly increased final body weight, body weight gain and feed conversion ratio in broilers. The results of the present study showed that dietary supplementation with 0.1 mg/kg L-selenomethionine significantly increased the live weight of squabs at marketing, which is similar to the findings of the above-mentioned studies.

The breast and leg muscles are the most important meat-producing parts of poultry, and their meat quality directly determines economic value and consumer experience (Sun et al. 2026). Wei et al. (2024) conducted a meta-analysis of 846 publications and indicated that selenium supplementation can significantly improve the meat quality of broiler breast muscle, as well as shear force and pH value, but has no significant effect on cooking loss, water holding capacity, colour, or the meat quality of leg muscle. The results of this study showed that adding selenomethionine to the diet significantly reduced the area and diameter of breast muscle fibres in squabs, but had no significant effect on meat colour or drip loss of breast

muscle, or on the area and diameter of leg muscle fibres, which is consistent with the findings of the aforementioned meta-analysis. The reduction in muscle fibre diameter observed in the present study may be linked to the improved antioxidant status and enhanced protein synthesis signalling induced by L-selenomethionine. Recent evidence indicates that selenomethionine promotes skeletal muscle growth through the PI3K-AKT-mTOR signalling pathway, facilitating myoblast proliferation and protein synthesis (Zhang et al. 2023). In addition, dietary selenium has been shown to promote myofibre hypertrophy by reducing endoplasmic reticulum stress and enhancing protein deposition (Wang et al. 2023b). In the present study, the increased plasma T-SOD activity in breeder pigeons, together with the decreased MDA content and upregulated GPX1/2 expression in squab muscle, suggests an attenuated oxidative burden, which may reduce oxidative damage to myofibrillar proteins and, in combination with enhanced anabolic signalling, contribute to the altered muscle fibre morphology. However, since fibre-type composition, protein turnover-related markers, and total fibre number were not evaluated in the present study, this interpretation remains speculative and warrants further investigation.

It should also be noted that the present assessment of meat quality was limited to colour and drip loss; other important technological traits, such as pH, cooking loss, and shear force, were not measured, which constrains a comprehensive evaluation of overall meat quality.

Enhancing the antioxidant capacity of poultry muscle contributes to improved health, enhanced meat quality, and extended shelf life (Bien et al. 2023). Jiang et al. (2009) reported that supplementing yellow-feathered broiler diets with 0.225 mg/kg of L-selenomethionine significantly increased muscle GSH-Px, T-AOC, and T-SOD activity while reducing the MDA level. Fu et al. (2023) demonstrated that supplementing breeding pigeon diets with 0.3 mg/kg of inorganic selenium (selenium-equivalent), yeast-derived selenium, or chitosan-nanoparticle selenium significantly increased the T-AOC and T-SOD levels in squab muscle while reducing the MDA level. However, different selenium sources had no significant effect on muscle GSH activity. The present study showed that supplementing the diet of breeding pigeons with 0.1 mg/kg L-selenomethionine reduced MDA levels

in the leg muscles of squabs, but this difference was only nominally significant before correction and did not reach the adjusted significance threshold. This trend is directionally consistent with previous results. While the previous studies showed varying effects of different selenium sources on enhancing the activity of antioxidant enzyme systems in poultry, all treatments improved muscle antioxidant capacity. Nevertheless, in this study, the addition of 0.1 mg/kg L-selenomethionine had no significant effect on the antioxidant enzyme systems in the breast and leg muscles of squabs. This discrepancy may be related to factors such as the experimental animals, the dosage of the additive, and bioavailability efficiency (Surai and Fisinin 2014; Xu et al. 2025). Further experimental data are warranted to verify and support the underlying reasons.

The antioxidant enzyme system in animals comprises 3 major components: CAT, GPXs, and SOD. CAT rapidly degrades hydrogen peroxide, preventing its accumulation and subsequent oxidative damage to cells. GPXs, containing selenocysteine, are key enzymes in antioxidant defence that reduce peroxides. SOD catalyses the dismutation of superoxide anion radicals into oxygen and hydrogen peroxide, thereby reducing their toxicity. Lei et al. (2026) demonstrated that supplementing yellow catfish diets with inorganic selenium and selenomethionine significantly increased the expression levels of selenoproteins (GPXs) and antioxidant genes (SOD and CAT). However, excessive selenium intake may cause muscle tissue damage. The present study showed that dietary L-selenomethionine supplementation in breeding pigeons upregulated GPX1 mRNA expression in squab breast muscle and GPX2 mRNA expression in squab leg muscle, but these changes were only nominally significant before correction and did not remain significant after FDR adjustment. These changes represent suggestive trends rather than definitive effects, and warrant confirmation in future studies. No significant effects were observed for other GPX isoforms, or for CAT and SOD gene expression. These findings contrast with those of previous studies. Accumulating evidence indicates that selenium metabolism primarily occurs in the liver, where it is metabolised and stored. Liver selenoproteins have preferential access to selenium, and inadequate supplementation may impair selenium absorption and utilisation in other tissues (Brandt-Kjelsen et al. 2017). Other studies

have reported that selenoproteins in organs such as the heart, liver, and kidneys are higher than those in muscle tissue. This disparity may affect selenium utilisation in the muscle, thereby influencing the expression of selenoprotein genes (Gawor et al. 2020). Therefore, the lack of significant effects on the gene expression of other antioxidant enzyme systems in squab muscle observed in this study may be related to the aforementioned reasons. Further experimental verification is warranted to support this conclusion.

CONCLUSION

In conclusion, dietary supplementation with 0.1 mg/kg L-selenomethionine promoted squab growth and altered muscle fibre morphology, with no significant adverse effects on reproductive performance detected. The additive significantly increased plasma T-SOD activity in breeders. However, the observed reductions in MDA content in leg muscle and upregulation of GPX1/2 mRNA expression in muscle were only nominally significant and should be interpreted with caution. Because total fibre number and total muscle cross-sectional area were not quantified, the reduced muscle fibre diameter cannot be interpreted as improved muscle development, and its biological significance remains uncertain. Furthermore, given that only a single concentration was tested, dose–response studies are needed to systematically evaluate the physiological and biochemical effects of graded L-selenomethionine supplementation levels in breeding pigeons and squabs.

Conflicts of interest

The authors declare no conflict of interest.

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