

Effects of replacing fishmeal with Antarctic krill meal on growth and reproductive performance of *Litopenaeus vannamei* broodstock

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Research Article

Keywords: *Litopenaeus vannamei* broodstock, Antarctic krill meal, Growth performance, Reproductive performance

Posted Date: April 1st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9095966/v1>

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Additional Declarations: No competing interests reported.

Abstract

This study evaluated the effects of replacing fishmeal with Antarctic krill meal (AKM) on the growth and reproductive performance of *Litopenaeus vannamei* broodstock. Six isonitrogenous and isolipidic diets were formulated by replacing fishmeal with different proportions of AKM (0% (M1, control), 20% (M2), 40% (M3), 60% (M4), 80% (M5), and 100% (M6)) and fed to *L. vannamei* broodstock (initial weight: 27.95 ± 0.23 g) for 70 days. Results showed: (1) The weight gain rate (WGR) and specific growth rate (SGR) of the M6 group were significantly lower than the control group ($P < 0.05$). (2) The gonadosomatic index (GSI) of the M2 and M3 groups was significantly higher than the control group ($P < 0.05$). (3) AKM significantly increased triglyceride (TG), total cholesterol (TC), and estradiol (E_2) levels in hemolymph, hepatopancreas, and gonads, elevated progesterone (PROG) in hemolymph, and increased vitellogenin (VTG) in hemolymph and gonads while decreasing VTG in the hepatopancreas ($P < 0.05$). (4) An appropriate replacement level of krill meal significantly reduced gonad-inhibiting hormone (GIH) in the eyestalk ($P < 0.05$), with no significant effect on molting-inhibiting hormone (MIH) ($P > 0.05$). (5) Gene expression analysis revealed that krill meal significantly upregulated ovarian development-related genes (*vtg*, *vtgr*, *vasa*, *e75*, *rxr*) and steroidogenesis pathway genes (*star*, *3 β -hsd*, *17 β -hsd*) ($P < 0.05$). Polynomial regression analysis of WGR and GSI indicated that the optimal dietary replacement level of fishmeal by AKM was 27.22%-41.78% under the present experimental conditions.

1. Introduction

With the rapid development of the global aquaculture industry, the Pacific white shrimp (*Litopenaeus vannamei*) has emerged as one of the most economically significant crustacean species worldwide, owing to its fast growth rate, strong adaptability, and substantial market demand (Huang et al., 2022). The Pacific white shrimp is cultivated worldwide, with major production centers located along the coasts and in inland freshwater or saline-alkaline environments of Latin America, India, and East and Southeast Asia. Global aquaculture production of this species reached 6.8 million metric tons in 2022, making it the highest-yielding farmed aquatic species that year (FAO, 2024; Sui et al., 2024). Broodstock cultivation represents a critical link in the shrimp farming supply chain, where growth performance and reproductive efficiency directly influence seed quality and farming profitability (Wang et al., 2022). However, under intensive farming conditions, broodstock frequently encounter challenges such as inadequate nutritional supply and impaired gonadal development, leading to reproductive impairments including reduced spawning output and egg quality deterioration (Anand et al., 2023; Vo et al., 2024; Ye et al., 2023). Moreover, the industry's heavy reliance on fishmeal poses challenges for sustainability and cost. Consequently, optimizing broodstock feed formulations while identifying sustainable alternative protein sources have become pivotal research directions in aquatic nutrition.

Antarctic krill (*Euphausia superba*), a vital marine biological resource, is rich in bioactive compounds including high-quality protein (60%-70%), essential amino acids, astaxanthin, and minerals (Lin et al., 2024). Notably, AKM has been widely adopted in aquafeeds due to its high protein content and natural attractant properties (Kaur et al., 2022). Antarctic krill meal has been widely investigated as a functional

ingredient in aquaculture feeds. Yang (2020) found that adding 10% to 20% krill meal to the feed of silver pomfret (*Pampus argenteus*) could effectively improve the growth and metabolic indicators and non-specific immune levels of the experimental group (initial weight: 131.88 ± 4.25 g), and also promote the development of the gonads and reproductive capacity. In a study on European sea bass (*Dicentrarchus labrax*) with an initial weight of 22.54 ± 0.3 g, Torrecillas et al. (2021) confirmed that the 30% to 50% fish meal replacement scheme had significant advantages in maintaining liver health and promoting growth, especially in low fish meal-low fat feed systems. Wei et al. used 6.59% to 32.93% and 8.55% to 57.02% Antarctic krill meal to replace fish meal in the feed of large yellow croaker (*Larimichthys crocea*) with an initial weight of 30.27 ± 0.06 g and rainbow trout (*Oncorhynchus mykiss*) with an initial weight of 102.06 ± 0.04 g, respectively. This significantly improved the feeding efficiency and growth rate, and also optimized the muscle nutrient composition and promoted pigment deposition (2019a, 2019b). 20% replacement could significantly enhance the reproductive performance of female Chinese mitten crabs (*Eriocheir sinensis*) (initial weight: 145.38 ± 8.01 g) and improve the quality of embryos (Tan et al., 2025). Moreover, the incorporation of Antarctic krill meal as a sustainable protein source offers a viable alternative to fishmeal in aquafeeds, thereby reducing pressure on wild fish stocks and supporting more sustainable aquaculture practices (Wang et al., 2024). Nevertheless, research on the application of AKM as a fishmeal replacer in *L. vannamei* broodstock cultivation remains limited. There is a particular lack of systematic studies elucidating the effects of graded fishmeal replacement levels on gonadal development, which is essential for determining the optimal replacement ratio and identifying potential adverse effects of excessive replacement (Huang et al., 2024).

Therefore, this study evaluated the potential of AKM as a fishmeal replacement in diets for *L. vannamei* broodstock.. Through systematic replacement of fishmeal with graded levels of krill meal, we comprehensively assess its impacts on broodstock growth performance, reproductive parameters, and underlying physiological mechanisms. Our findings will provide a scientific basis for optimizing broodstock feed formulations with a reduced fishmeal footprint, thereby promoting the sustainable development of *L. vannamei* aquaculture.

2. Materials and methods

2.1 Experimental diets

All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, as recommended by the National Institutes of Health. The experimental protocols were approved by the Animal Ethics Committee of Guangdong Ocean University (protocol code GDOU-IACUC-2023-A0228 dated Feb 2023).

Based on white fish meal and wheat gluten meal as primary protein sources, and fish oil combined with soybean lecithin as lipid sources, a basal diet (M1) was formulated. AKM was then used to replace white fish meal at graded levels, generating five experimental diets (M2–M6) formulated to be isonitrogenous

(51% crude protein) and isolipidic (11% crude lipid). Methionine was supplemented to ensure amino acid balance across all diets (Table 1), with complete amino acid profiles detailed in Table 2.

The ingredients were accurately weighed and premixed according to Table 1 after being pulverized and passed through an 80-mesh sieve (180 µm). Fish oil and soybean lecithin were subsequently incorporated, followed by manual disaggregation and secondary homogenization through a 60-mesh sieve (250 µm). After adding appropriate amount of water and re-mixing, the mash was pelleted into 2-mm diameter granules using the F-26 type twin-screw extruder (South China University of Technology). Diets were air-dried in a climate-controlled room until moisture content reached approximately 10%, then vacuum-sealed in laminated bags and stored at −20°C.

Table 1. Ingredient composition and nutrient contents of the experimental diets (% dry matter)

Ingredients (%)	Experimental diets					
	M1	M2	M3	M4	M5	M6
White fish meal	45.00	36.00	27.00	18.00	9.00	0.00
Antarctic krill meal	0.00	10.60	21.20	31.80	42.40	52.90
Wheat gluten	10.00	10.00	10.00	10.00	10.00	10.00
Shrimp meal	10.00	10.00	10.00	10.00	10.00	10.00
Squid meal	7.00	7.00	7.00	7.00	7.00	7.00
Pig liver powder	5.00	5.00	5.00	5.00	5.00	5.00
Beer yeast	2.00	2.00	2.00	2.00	2.00	2.00
α -Starch	7.00	6.16	5.26	4.52	3.63	2.85
Fish oil	7.00	6.20	5.40	4.50	3.70	2.90
Soy lecithin	1.50	1.50	1.50	1.50	1.50	1.50
Choline chloride	0.30	0.30	0.30	0.30	0.30	0.30
Compound premix ^a	1.00	1.00	1.00	1.00	1.00	1.00
CaH ₂ PO ₄	1.50	1.50	1.50	1.50	1.50	1.50
CMC-Na ^b	1.00	1.00	1.00	1.00	1.00	1.00
Astaxanthin	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin C	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol	0.50	0.50	0.50	0.50	0.50	0.50
Ethoxyquin	0.10	0.10	0.10	0.10	0.10	0.10
Met	0.00	0.04	0.14	0.18	0.27	0.35
Total	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition ^c						
Crude protein	51.37	51.26	51.90	51.30	51.61	51.21
Crude lipid	11.42	11.74	11.66	11.19	11.67	11.68
Moisture	8.92	9.54	10.39	10.42	10.98	11.41

^a Compound premix was obtained from Qingdao Master Biotechnology Co, Ltd (Qingdao, China).

^b Carboxymethylcellulose sodium.

^c Measured value.

Table 2. Amino acid composition of experimental feeds (% dry matter)

Amino acid	Experimental diets					
	M1	M2	M3	M4	M5	M6
NEAA ^a						
Ala	1.91	2.11	2.17	2.31	2.42	2.55
Asp	3.31	3.55	3.56	3.67	3.77	3.78
Tyr	1.32	1.39	1.36	1.41	1.40	1.40
Ser	1.43	1.58	1.67	1.78	1.83	1.97
Glu	6.17	6.61	6.56	6.92	6.91	7.21
Gly	2.04	2.30	2.39	2.58	2.76	2.93
Pro	2.43	2.58	2.54	2.59	2.62	2.59
EAA ^b						
Val	1.89	1.97	1.99	2.04	2.08	2.13
Met	0.99	1.01	1.01	1	1.02	1.02
Ile	1.73	1.79	1.75	1.78	1.80	1.81
Leu	2.69	2.86	2.89	3.02	3.09	3.18
Thr	1.42	1.54	1.58	1.65	1.70	1.69
Phe	1.61	1.70	1.67	1.73	1.75	1.75
His	0.68	0.74	0.78	0.87	0.90	0.95
Lys	2.47	2.61	2.58	2.68	2.74	2.8
Arg	2.12	2.27	2.26	2.37	2.42	2.42

^aNEAA:Non-essential amino acid; ^bEAA:Essential amino acid.

2.2 Experimental conditions

This study was conducted at Haimao Biotechnology Co., Ltd (Zhanjiang, China). Sexually immature female Pacific white shrimp broodstock (10 months old; initial weight: 27.95 ± 0.23 g) displaying robust health and uniform size were selected. Following a 7-day acclimatization period to stabilize feeding

activity and vitality, 180 shrimp were randomly distributed across six culture tanks (2.3 m × 1.9 m; water depth: 50 cm). Each tank was partitioned into three independent compartments using mesh screens, with 10 shrimp per compartment serving as triplicate experimental units. During the 70-day nutritional conditioning phase, shrimp were fed according to the following schedule: four daily feedings (06:00, 10:30, 15:00, 19:30) from Days 1-28, increasing to five feedings (additional 12:00 feeding) from Days 29-70. Water quality parameters were maintained at: temperature 26-28°C, salinity 25-27‰, pH 7.8-8.0, dissolved oxygen ≥5 mg/L, ammonia nitrogen ≤0.6 mg/L, and nitrite ≤0.1 mg/L. Daily husbandry included 80% water exchange with waste siphoning at 10:00, with concurrent removal of uneaten feed, feces, and exuviae.

2.3 Sample collection and index analysis methods

Upon completion of the nutritional enrichment phase, all female shrimp were weighed to calculate the weight gain rate (WGR) and specific growth rate (SGR). Concurrently, six sexually mature female shrimp per experimental group were randomly selected. Hemolymph was collected from the pericardial cavity via a 1 mL sterile syringe inserted posterior to the carapace. Samples were centrifuged at 4,000 rpm for 15 min. The supernatant (serum) was aspirated using a micropipette and stored at -80°C for subsequent biochemical and sex steroid hormone analyses. Eystalks were excised using sterilized scissors, immediately flash-frozen in liquid nitrogen, then stored at -80°C. Hepatopancreas and ovaries were dissected and weighed to calculate the hepatopancreas index (HSI), gonadosomatic index (GSI), and gonado-hepatosomatic ratio (GHR).

2.3.1 Calculation formulas for growth and reproductive performance indexes

Weight gain rate (WGR, %)

$$= 100 \times [(\text{final weight, g}) - (\text{initial weight, g})] / (\text{initial weight, g});$$

Specific growth rate (SGR, %/d)

$$= 100 \times [\ln(\text{final weight, g}) - \ln(\text{initial weight, g})] / (\text{days of experiment, d});$$

Hepatosomatic index (HSI, %)

$$= 100 \times (\text{hepatopancreas weight, g} / \text{body weight, g})$$

Gonadosomatic index (GSI, %)

$$= 100 \times (\text{ovary weight, g} / \text{body weight, g})$$

Gonado-hepatic ratio (GHR, %)

$$= 100 \times (\text{ovary weight, g} / \text{hepatopancreas weight, g})$$

2.3.2 Proximate composition analysis

Proximate composition of experimental diets was determined following AOAC standard methods (Latimer, 2023). Moisture content was measured by oven-drying at 105°C to constant weight. Crude protein was analyzed using a Skalar Dumas TN/TC/IC/TOC auto-analyzer (Skalar Analytical B.V., China). Crude lipid content was quantified by Soxhlet extraction with petroleum ether as the solvent.

2.3.3 Sample pretreatment, and biochemical and hormonal indicators analysis

Samples were homogenized (1:9 w/v) in pre-chilled 0.9% physiological saline using an FJ-200cL high-speed homogenizer (15,000 rpm, 3 min). Homogenates were centrifuged at 4,000 rpm for 10 min (4°C), and supernatants were aliquoted into 1.5-mL centrifuge tubes for storage at -80°C pending biochemical and hormonal assays.

Triglyceride (TG) and total cholesterol (TC) contents in hemolymph, hepatopancreas, and ovaries were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, China) strictly following manufacturer's protocols. Serum progesterone (PROG), estradiol (E₂), and vitellogenin (VTG) concentrations were quantified alongside eyestalk gonad-inhibiting hormone (GIH) and molting-inhibiting hormone (MIH) levels. Hepatopancreatic and ovarian PROG, E₂, and VTG were determined using ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., China) according to standardized protocols.

2.3.4 RNA extraction and cDNA synthesis

The fully matured ovaries from 6 shrimp per group (2 shrimp in each replicate) were randomly selected and immersed in 1.5 mL centrifuge tubes containing RNAlater, and then placed in a refrigerator at 4°C for 12 h before being transferred to -80°C for long-term storage. Total ovary RNA was extracted by the TRIzol method, and then reverse transcribed into cDNA using the Prime Script™ RT Master Mix reagent kit (Takara, Japan), and the primers were designed using PrimerQuest, and the sequence list is shown in Table 3. The amplification was carried out in a quantitative thermal cycler (LightCycler 480, Roche Diagnostics, Switzerland) using the following reaction program: 2 min at 95°C for one cycle, 15 s at 95°C, 15 s at 60°C, 20 s at 72°C for 40 cycles. The cDNA data were normalized to β-actin as the internal reference gene, and the relative gene expression was calculated using the 2^{-ΔΔCt} method.

Table 3. Primers and sequences used for real-time PCR amplification

Primer names	Forward and reverse primers sequence (5–3')	Genbank accession no.
<i>β-actin</i>	F: AACATGATCTCTAAGGGTAC R: CCTCTCTGACCACTTTAG	XM_027364954.1
<i>vtgr</i>	F: TCTCCTCGTCTTGGCTCT R: GCAAACCTGCGGCGGCTGG	MN807241.1
<i>vtg</i>	F: GAACCCTAAGGCTATCATCACTG R: AGGTCGCTCTTCCATCTTTACT	XM_070117824.1
<i>vasa</i>	F: GGT TTTGGCTCTGGTTCTGG R: CTTCAATTCACATCCGCTGGG	DQ095772
<i>e75</i>	F: GCGTCCGAATCTCCTCTG R: CCTCGCTCTTCTCGTCAAT	XM_070124398.1
<i>rxr</i>	F: CCCTCTCCTCTCTCCCTC R: GGTTGCTGCTCCATTAC	XM_070117987.1
<i>star</i>	F: TGTGAATGTGCGACACTGGA R: CTGTGTCCAGCAACCACTGA	XM_027369781.1
<i>3β-hsd</i>	F: CGCCCGTAACCTCTCCTATT R: CAGAGTAGTAGGCCGTCGAG	XM_027367407.2
<i>17β-hsd</i>	F: CGAAATCAGCGTGCAGAGAA R: CTCCAACAGCACTGAGAGGA	XM_027359161.2

vtgr: vitellogenin receptor; *vtg*: vitellogenin; *vasa*: probable ATP-dependent RNA helicase vasa-like;

e75: ecdysone-induced protein 75B; *rxr*: retinoid X receptor; *star*: steroidogenic acute regulatory protein; *3β-hsd*: 3 beta-hydroxysteroid dehydrogenase; *17β-hsd*: 17 beta-hydroxysteroid dehydrogenase.

2.4 Statistical Analysis

All data were analyzed using one-way analysis of variance (one-way ANOVA) in SPSS 24.0 (SPSS Inc., USA). Each compartment was considered as one replicate (n = 3). When significant differences were detected (P < 0.05), Tukey's multiple comparison test was applied. Results are presented as mean ± standard error (SEM).

3. Results

3.1 Growth performance

As shown in Table 4, the M6 group resulted in the lowest WGR and SGR, which were significantly lower than those of the other groups ($P < 0.05$). WGR and SGR showed a quadratic response to increasing AKM replacement, peaking at intermediate levels and declining at the highest replacement level. The M4 group achieved the highest values for these parameters. The second-order polynomial curve based on WGR (Fig. 1) indicated the estimated optimal AKM replacement level of 27.22%.

Table 4. Effects of AKM replacement levels on the growth performance of *Litopenaeus vannamei* broodstock

Groups	IBW (g)	FBW (g)	WGR (%)	SGR (%)
M1	27.91±0.72	61.47±1.30 ^b	120.52±7.45 ^b	1.13±0.05 ^b
M2	27.96±0.96	62.41±0.61 ^b	123.74±7.94 ^b	1.15±0.05 ^b
M3	28.09±0.49	59.41±2.32 ^{ab}	111.40±5.52 ^{ab}	1.07±0.04 ^{ab}
M4	27.90±0.38	63.41±1.43 ^b	127.38±5.97 ^b	1.17±0.04 ^b
M5	27.93±0.64	57.47±1.27 ^{ab}	105.84±3.23 ^{ab}	1.03±0.02 ^{ab}
M6	27.91±0.62	52.78±2.02 ^a	88.94±3.37 ^a	0.91±0.03 ^a

Data are expressed as mean ± standard error (n=3). Different superscript letters within the same column indicate significant differences ($P < 0.05$). IBW: initial body weight, FBW: final body weight, WGR: weight gain rate, SGR: specific growth rate. M1: whole fish meal diet, M2: 20% AKM replacement diet, M3: 40% AKM replacement diet, M4: 60% AKM replacement diet, M5: 80% AKM replacement diet, M6: 100% AKM replacement diet.

3.2 Reproductive performance

As shown in Table 5, HSI decreased at lower AKM replacement levels and then increased with further increases in replacement level. The M2 group showed a significantly lower HSI than the control group ($P < 0.05$), whereas the M6 group showed a significantly higher HSI than the control group ($P < 0.05$). GSI increased markedly at moderate replacement levels and then declined at higher replacement levels, with the highest values recorded in M2 group and M3 group, both significantly higher than the control ($P < 0.05$). Similarly, GHR peaked in the M2 group and remained significantly higher in M2 and M3 groups than in M1 group ($P < 0.05$), while no significant differences were detected between M4–M6 group and the control group ($P > 0.05$). Second-order polynomial curve based on GSI (Fig. 2) indicated the estimated optimal AKM replacement level of 41.78%.

Table 5. Effects of AKM replacement levels on the reproductive performance of *Litopenaeus vannamei* broodstock

Groups	HSI (%)	GSI (%)	GHR (%)
M1	3.69±0.05 ^b	4.20±0.21 ^{bc}	1.14±0.04 ^a
M2	3.10±0.07 ^a	6.01±0.30 ^a	2.48±0.05 ^c
M3	3.36±0.20 ^{ab}	5.62±0.19 ^a	1.76±0.15 ^b
M4	3.85±0.13 ^{bc}	5.34±0.03 ^{ab}	1.39±0.05 ^{ab}
M5	4.05±0.07 ^{bc}	4.32±0.47 ^{bc}	1.28±0.10 ^a
M6	4.31±0.07 ^c	3.83±0.03 ^c	1.19±0.06 ^a

Data are expressed as mean ± standard error (n=3). Different superscript letters within the same column indicate significant differences ($P < 0.05$). HSI: hepatic somatic index, GSI: gonadosomatic index, GHR: gonado-hepatic ratio. M1: whole fish meal diet, M2:20% AKM replacement diet, M3:40% AKM replacement diet, M4:60% AKM replacement diet, M5:80% AKM replacement diet, M6:100% AKM replacement diet.

3.3 Eyestalk hormonal parameters

As shown in Table 6, GIH decreased first and then increased with increasing AKM replacement. GIH was significantly lower in M2–M4 than in the control ($P < 0.05$), with the lowest level observed in M2. Similarly, MIH levels demonstrated a quadratic trend, showing the lowest value in the M4 group that was significantly lower than the control group ($P < 0.05$). Conversely, M5 and M6 groups exhibited significantly elevated MIH levels relative to the control group ($P < 0.05$).

Table 6. Effects of AKM replacement levels on hormone levels in the eyestalk of *Litopenaeus vannamei* broodstock

Groups	GIH (ng/mL)	MIH (pg/mL)
M1	5.15±0.10 ^c	557.41±11.78 ^b
M2	3.11±0.07 ^a	520.46±9.19 ^b
M3	3.17±0.09 ^a	545.46±7.63 ^b
M4	3.83±0.45 ^{ab}	473.19±2.49 ^a
M5	4.72±0.17 ^{bc}	612.75±5.46 ^c
M6	5.00±0.14 ^c	670.32±12.80 ^d

Data are expressed as mean ± standard error (n=3). Different superscript letters within the same column indicate significant differences ($P < 0.05$). GIH:gonad-inhibiting hormone, MIH:molting-inhibiting hormone. M1:whole fish meal diet, M2:20% AKM replacement diet, M3:40% AKM replacement diet, M4:60% AKM replacement diet, M5:80% AKM replacement diet, M6:100% AKM replacement diet.

3.4 Hemolymph biochemical and hormonal parameters

As shown in Table 7, the experimental groups exhibited significantly higher hemolymph TG content than the control group ($P < 0.05$). TC peaked in M2 and then declined as AKM replacement increased further (Table 7). VTG rose at moderate replacement levels and peaked in M3, and all AKM groups remained significantly higher than the control ($P < 0.05$). Both E_2 and PROG levels initially increased and subsequently decreased: E_2 reached maximum levels in the M3 group, with M2-M4 groups being significantly higher than the control group ($P < 0.05$); similarly, PROG peaked in the M3 group, with M2 and M3 groups demonstrating significantly increased concentrations relative to the control group ($P < 0.05$).

Table 7. Effects of AKM replacement levels on hemolymph biochemical indices and hormone levels of *Litopenaeus vannamei* broodstock

Groups	TG (mg/mL)	TC (mmol/L)	VTG (ng/mL)	E ₂ (pmol/L)	PROG (ng/mL)
M1	0.79±0.07 ^a	4.97±0.13 ^a	275.73±4.67 ^a	42.70±1.40 ^a	10.62±0.09 ^a
M2	1.25±0.03 ^{bc}	5.85±0.14 ^b	355.14±15.42 ^b	52.36±1.03 ^b	13.29±0.27 ^{bc}
M3	1.08±0.04 ^b	5.44±0.15 ^{ab}	410.17±5.00 ^b	58.42±1.56 ^c	15.03±0.28 ^c
M4	1.36±0.07 ^{cd}	5.17±0.21 ^{ab}	384.54±20.10 ^b	49.32±0.68 ^b	12.54±0.64 ^{ab}
M5	1.49±0.03 ^d	5.06±0.17 ^a	389.98±6.35 ^b	43.17±0.59 ^a	11.49±0.61 ^{ab}
M6	1.74±0.02 ^e	5.04±0.16 ^a	394.58±20.69 ^b	43.70±0.68 ^a	11.03±0.32 ^a

Data are expressed as mean ± standard error (n=3). Different superscript letters within the same column indicate significant differences ($P < 0.05$). TG: triglyceride, TC: total cholesterol, VTG: vitellogenin, E₂: estradiol, PROG: progesterone. M1: whole fish meal diet, M2:20% AKM replacement diet, M3:40% AKM replacement diet, M4:60% AKM replacement diet, M5:80% AKM replacement diet, M6:100% AKM replacement diet.

3.5 Hepatopancreatic biochemical and hormonal parameters

As shown in Table 8, hepatopancreatic TG increased with higher AKM replacement, and values in M3–M6 were significantly higher than those in the control ($P < 0.05$). TC peaked in M5, which was significantly higher than the control ($P < 0.05$). VTG levels initially decreased and then increased, with the highest concentration observed in the control group, which was significantly higher than those in the M2–M5 groups ($P < 0.05$), with minimum values observed in M2. With increasing dietary fishmeal replacement levels by AKM, E₂ initially increased and subsequently decreased, peaking in the M2 group at levels significantly exceeding the control group ($P < 0.05$).

Table 8. Effects of AKM replacement levels on hepatopancreatic biochemical indices and hormone levels of *Litopenaeus vannamei* broodstock

Groups	TG (mmol/gprot)	TC (mmol/gprot)	VTG (ng/mL)	E ₂ (pmol/L)
M1	0.26±0.01 ^a	1.05±0.04 ^{ab}	167.95±6.10 ^c	48.58±1.49 ^a
M2	0.28±0.014 ^a	1.09±0.02 ^{ab}	115.63±1.93 ^a	56.63±0.96 ^b
M3	0.40±0.013 ^b	1.23±0.04 ^{bc}	144.47±1.84 ^b	50.90±3.54 ^{ab}
M4	0.48±0.01 ^c	1.36±0.06 ^{cd}	143.59±4.69 ^b	52.36±0.33 ^{ab}
M5	0.52±0.010 ^c	1.52±0.04 ^d	143.70±2.31 ^b	51.98±1.29 ^{ab}
M6	0.48±0.013 ^c	0.95±0.02 ^a	162.25±1.20 ^c	49.35±0.97 ^{ab}

Data are expressed as mean ± standard error (n=3). Different superscript letters within the same column indicate significant differences ($P < 0.05$). TG: triglyceride, TC: total cholesterol, VTG: vitellogenin, E₂: estradiol. M1: whole fish meal diet, M2: 20% AKM replacement diet, M3: 40% AKM replacement diet, M4: 60% AKM replacement diet, M5: 80% AKM replacement diet, M6: 100% AKM replacement diet.

3.6 Ovarian biochemical and hormonal parameters

As shown in Table 9, ovarian TG levels were significantly elevated in M2-M5 groups compared to the control group ($P < 0.05$), peaking in the M2 group. TC level initially increased and subsequently decreased, reaching maximum values in M2 group with all groups showing significantly higher concentrations than the control group ($P < 0.05$). VTG showed a progressive increase with increasing krill meal replacement, with the lowest concentration observed in the control group, which was significantly lower than those in the M2, M5, and M6 groups. E₂ levels surpassed control values across all experimental groups, with M2 and M5 groups exhibiting statistically significant elevations ($P < 0.05$).

Table 9. Effects of AKM replacement levels on ovarian biochemical indices and hormone levels of *Litopenaeus vannamei* broodstock

Groups	TG (mmol/gprot)	TC (mmol/gprot)	VTG (ng/mL)	E ₂ (pmol/L)
M1	0.21±0.01 ^a	0.63±0.01 ^a	218.02±14.32 ^a	60.74±2.27 ^a
M2	0.29±0.01 ^b	1.07±0.03 ^d	276.59±4.54 ^{bc}	67.21±0.99 ^b
M3	0.27±0.01 ^b	0.98±0.04 ^d	241.43±7.69 ^{ab}	64.36±1.05 ^{ab}
M4	0.26±0.02 ^b	0.76±0.01 ^{bc}	271.33±5.77 ^{bc}	64.71±0.70 ^{ab}
M5	0.27±0.01 ^b	0.74±0.01 ^b	309.84±17.05 ^c	70.27±1.23 ^b
M6	0.25±0.01 ^{ab}	0.85±0.02 ^c	310.58±6.34 ^c	65.73±0.67 ^{ab}

Data are expressed as mean $\pm$ standard error (n=3). Different superscript letters within the same column indicate significant differences ($P < 0.05$). TG: triglyceride, TC: total cholesterol, VTG: vitellogenin, E₂: estradiol. M1: whole fish meal diet, M2: 20% AKM replacement diet, M3: 40% AKM replacement diet, M4: 60% AKM replacement diet, M5: 80% AKM replacement diet, M6: 100% AKM replacement diet.

3.7 Ovarian development-related genes expression

As shown in Fig. 3, the relative expression of *e75*, *vtgr*, *vtg*, *vasa*, and *rxr* initially increased and subsequently decreased with increasing dietary AKM levels, with maximum values observed in the M2 group. Significantly elevated expression was detected for all five genes in M2 and M3 groups compared to the control group ($P < 0.05$).

Fig. 3. Effects of AKM replacement levels on the expression of genes related to ovarian development of *Litopenaeus vannamei* broodstock

Data are expressed as mean $\pm$ standard error (n=3). Different letters above bars indicate significant differences ($P < 0.05$). *e75*: ecdysone-induced protein 75B, *vtgr*: vitellogenin receptor, *vtg*: vitellogenin, *vasa*: probable ATP-dependent RNA helicase vasa-like, *rxr*: retinoid X receptor. M1: whole fish meal diet, M2: 20% AKM replacement diet, M3: 40% AKM replacement diet, M4: 60% AKM replacement diet, M5: 80% AKM replacement diet, M6: 100% AKM replacement diet.

3.8 Ovarian hormone biosynthesis pathway-related genes expression

As shown in Fig. 4, the relative expression of *star* and *3 β -hsd* initially increased and subsequently decreased with increasing AKM replacement. The relative expression of both genes in M2, M3, M4 and M5 groups was significantly higher than that of the control group ($P < 0.05$), with *star* reaching its peak value in M4 group and *3 β -hsd* in M2 group. For *17 β -hsd*, the relative expression in all AKM treatment groups was significantly higher than that of the control group ($P < 0.05$), peaking in M4 group. However, no significant difference in *17 β -hsd* expression was observed between the M4 group and the M2 or M3 groups ($P > 0.05$).

Data are expressed as mean $\pm$ standard error (n=3). Different letters above bars indicate significant differences ($P < 0.05$). *star*: steroidogenic acute regulatory protein, *17 β -hsd*: 17 beta-hydroxysteroid dehydrogenase, *3 β -hsd*: 3 beta-hydroxysteroid dehydrogenase. M1: whole fish meal diet, M2: 20% AKM replacement diet, M3: 40% AKM replacement diet, M4: 60% AKM replacement diet, M5: 80% AKM replacement diet, M6: 100% AKM replacement diet.

4. Discussion

AKM, as a premium protein source, has been extensively studied in aquaculture for species including silver pomfret (*Pampus argenteus*) (Yang, 2020), largemouth bass (*Micropterus salmoides*) (Zhu et al., 2019), large yellow croaker (*Larimichthys crocea*) (Wei et al., 2019b), rainbow trout (*Oncorhynchus mykiss*) (Wei et al., 2019a), and Chinese mitten crab (*Eriocheir sinensis*) (Wang, 2020), demonstrating its efficacy in enhancing growth performance, metabolic activity, immune competence, body coloration, and muscular nutritional quality. In the present study, nutritional conditioning of *Litopenaeus vannamei* broodstock with graded AKM levels significantly improved energy metabolism efficiency and promoted targeted nutrient accumulation through optimized dietary formulation. Enhanced digestive function in conditioned broodstock may have facilitated synergistic increases in body length and weight (Wang et al., 2022), establishing critical nutritional reserves for gonadal development, fertilization, and embryonic/naupliar metamorphosis. Our results indicated that 20% and 60% AKM replacement maintained or marginally increased WGR and SGR compared to the control, while 100% significantly reduced them ($P < 0.05$). This aligns with observations in Asian swamp eel (*Monopterus albus*) where 60%-100% AKM replacement suppressed WGR/SGR (Fu et al., 2023), and in spotted halibut (*Verasper variegatus*) where 50% replacement decreased survival rate (Yan et al., 2016), suggesting growth inhibition at excessive replacement levels. This may stem from fluoride accumulation in non-muscular tissues (e.g., exoskeleton), inducing chronic toxicity despite low muscular fluoride retention (Peng et al., 2024; Zhang et al., 2018). Second-order polynomial regression of WGR determined the optimal AKM replacement level at 27.22%.

The GSI reflects gonadal development status, whereas the GHR indicates nutrient translocation to the ovaries. As the primary nutrient reservoir, the hepatopancreas stores macromolecules during non-reproductive phases, mobilizing proteins, lipids, and glycogen to support ovarian maturation under hormonal regulation (gonadotropins, ecdysteroids, sex steroids) (Jiang et al., 2022; Song et al., 2019; Wen et al., 2001). This nutrient shift reduces HSI while elevating GSI. In this study, the optimal AKM replacement levels significantly increased GSI while decreasing HSI, indicating enhanced lipid mobilization from hepatopancreas to ovaries—consistent with findings in kuruma shrimp (*Marsupenaeus japonicus*) (Alava et al., 1993) and Chinese mitten crab (*Eriocheir sinensis*) (Sui et al., 2008; Wu et al., 2007). Regression analysis of GSI determined the optimal AKM replacement level at 41.78%.

GIH, a neuropeptide synthesized in the X-organ-sinus gland (XO-SG) complex of eyestalks, suppresses ovarian development by inhibiting vitellogenin synthesis and oocyte maturation in crustaceans including giant river prawn (*Macrobrachium rosenbergii*) (Zeng and Chen, 2023), and scalloped spiny lobster (*Panulirus homarus*) (Luo et al., 2021). Declining GIH levels during ovarian maturation enables reproductive progression (Luo et al., 2021). Similarly, MIH regulates ecdysis by suppressing ecdysteroid synthesis in Y-organs. Elevated MIH during intermolt phases maintains growth stability, while its decline initiates premolt ecdysteroid surges (Cheng and Chang, 1991; Mykles, 2021). MIH coordinates resource allocation between molting and reproduction under environmental cues. In the life cycle of crustaceans, molting (ecdysis) and reproduction represent two critical physiological processes, both requiring precise

timing (Webster et al., 2012). MIH suppresses molting, enabling crustaceans to allocate greater energy and resources toward gonadal development during appropriate periods. This mechanism facilitates a balance between somatic growth and reproduction (Zmora et al., 2009). Here, 20%-60% AKM replacement levels significantly reduced GIH in eyestalk, while 20%-40% did not affect MIH. Higher AKM replacement levels elevated MIH, indicating AKM-dependent modulation of neuroendocrine axes governing ovarian development and growth.

During crustacean ovarian maturation, lipid metabolism homeostasis exerts dual regulatory roles over germ cell development. At the energy metabolism level, TG stored in the hepatopancreas are transported via hemolymph to the ovary. Subsequently, TG are catabolized into acetyl-CoA through mitochondrial β -oxidation, entering the tricarboxylic acid (TCA) cycle to provide ATP and NADH for oocyte meiotic recombination and cytoplasmic maturation. Concurrently, generated precursors such as ketone bodies participate in zona pellucida biosynthesis (Dunning et al., 2014). At the signaling regulation level, lipid derivatives, including prostaglandins metabolized from arachidonic acid (ARA), activate the PPAR γ pathway to promote follicular granulosa cell proliferation. They also synergistically enhance the activity of steroidogenic enzymes (e.g., cholesterol side-chain cleavage enzyme, P450 scc), thereby achieving spatiotemporal coordination between reproductive development and energy supply (Kumar et al., 2018). As the central organ for lipid metabolism, the hepatopancreas transports TG, phospholipids, and TC to the ovary via secreted apolipoproteins. TC exhibits dual functions: (1) serving as a structural component of cell membranes and a lipoprotein carrier for hemolymph lipid transport (De Geest and Mishra, 2023; Zhu et al., 2022); and (2) acting as a precursor for sex steroids (e.g., pregnenolone), influencing vitellogenin (Vtg) gene expression through modulation of 17 β -estradiol (E_2) levels (Pan, 2020). Studies indicate that TC deficiency impedes ovarian steroidogenesis, while TG insufficiency triggers defective energy supply via embryonic β -oxidation, ultimately leading to reduced offspring survival and abnormal organ differentiation (Aslam et al., 2025). This lipid metabolic imbalance compromises reproductive success through an "energy-hormone" coupling mechanism. In this experiment, appropriate dietary AKM replacement levels significantly increased TG and TC content in hemolymph, hepatopancreas, and ovary, with the most pronounced elevation observed in the ovary. Similar results have been reported in *Macrobrachium nipponense* (Yan et al., 2024).

VTG, the precursor molecule of yolk protein (Vn), is synthesized primarily in the hepatopancreas (with ovarian contribution in some species) and transported via hemolymph to the ovary. It provides essential lipids, amino acids, and other nutrients to oocytes, acting as a core regulatory factor in yolk accumulation and ovarian maturation (Feng et al., 2022). Within the ovary, oocytes recognize and bind VTG via specific receptors (VgR), internalizing it through endocytosis. VTG is then processed into Vn within lysosomes and stored in yolk platelets, directly supporting germ cell developmental potential (Li et al., 2022; Luo et al., 2024). Concurrently, E_2 is widely distributed in organs including the ovary, hepatopancreas, and thoracic ganglia, exhibiting dynamic changes during ovarian development. E_2 peaks in the hepatopancreas during stage III and in the ovary during stage IV, suggesting multi-organ synergistic regulation of ovarian development (Liu et al., 2017). Similarly, PROG is distributed across

various crustacean tissues and associated with the initiation of vitellogenesis. During peak vitellogenic stages, PROG is converted into other steroid hormones, such as 17 α -hydroxyprogesterone, testosterone, and deoxycorticosterone (Teshima and Kanazawa, 1971; Zhao et al., 1999). In this experiment, appropriate dietary AKM replacement levels elevated hemolymph VTG, E₂, and PROG, increased hepatopancreatic E₂, and raised ovarian VTG and E₂ levels. These findings align with studies on silver pomfret (Yang et al., 2019), ricefield eel (Fu et al., 2023), and tongue sole (Xu et al., 2017), indicating that appropriate krill meal replacement promotes ovarian development by influencing sex hormone secretion. Compared to the control, krill meal supplementation reduced hepatopancreatic VTG. This reduction may result from an accelerated transport of VTG to the rapidly developing ovary.

VASA protein, an ATP-dependent RNA helicase, plays a crucial role in germ cell migration and differentiation. This gene exhibits specific expression patterns in gonadal tissues and indirectly regulates ovarian development by participating in VTG assembly and transport. Studies confirm a significant positive correlation between VASA transcript levels and VTG expression (Li et al., 2024). VTG synthesis in crustaceans displays tissue specificity, with the ovary and hepatopancreas being the primary sites. VTG expression increases progressively with advancing gonadal maturity (Manfrin et al., 2021; J. Wang et al., 2024). Exogenous VTG synthesized by the hepatopancreas undergoes transmembrane transport via vitellogenin receptor (VTGR)-mediated endocytosis, a critical step for oocyte nutrient accumulation (Chen et al., 2018; Li et al., 2001). Consequently, VTG and VTGR expression levels serve as vital indicators for assessing oocyte maturity. The *e75* gene, encoding a key regulator of the ecdysone signaling pathway, functions as a primary target for the ecdysone receptor (EcR). This gene plays a significant regulatory role in crustacean molting, metamorphosis, and reproduction (Girish et al., 2015; Gouveia et al., 2018), with expression patterns highly coordinated with EcR dynamics. The active form, 20-hydroxyecdysone (20E), forms a heterodimer with EcR and the retinoid X receptor (RXR), binding to target gene promoter regions to regulate VTG synthesis (Girish et al., 2015; Xie et al., 2024). In this experiment, specific dietary krill meal levels significantly upregulated the relative expression of *vtg*, *vtgr*, *vasa*, *e75*, and *rxr* in the ovary, while levels exceeding this threshold resulted in downregulation. These results suggest that AKM replacement may promote vitellogenin synthesis and thereby ovarian development by upregulating the ecdysteroid signaling pathway. However, the mechanism by which nutrients mediate ecdysteroid pathway expression requires further elucidation.

Sex steroid hormone biosynthesis involves a multi-step enzymatic cascade: Cholesterol is first transported to the mitochondrial inner membrane mediated by the steroidogenic acute regulatory protein (StAR). It is then catalyzed by cholesterol side-chain cleavage enzyme (P450_{scc}) to form the key precursor pregnenolone. Pregnenolone undergoes sequential modifications by key enzymes, including 3 β -hydroxysteroid dehydrogenase (3 β -HSD) and 17 β -hydroxysteroid dehydrogenase (17 β -HSD), ultimately yielding PROG and E₂ (Bhargava et al., 2021; Chakraborty et al., 2021). In this study, the relative expression levels of *star*, *17 β -hsd*, and *3 β -hsd* in the ovary exhibited an initial increase followed by a decrease. These results suggest that appropriate AKM replacement levels promote sex steroid hormone synthesis. The observed changes in sex steroid hormone levels aligned with the expression trends of

star, *17 β -hsd*, and *3 β -hsd*. Collectively, these findings demonstrate that appropriate AKM replacement promotes the synthesis and secretion of sex steroid hormones by enhancing the steroidogenic pathway, thereby facilitating vitellogenesis and ovarian development.

5. Conclusion

Under the present experimental conditions, replacing 20%-40% of dietary fishmeal with AKM had no significant effect on growth performance. However, these levels significantly promoted ovarian development, suppressed the secretion of gonad-inhibiting hormones, enhanced the secretion of sex steroid hormones, and upregulated the expression of key genes associated with the ovarian ecdysteroid signaling pathway and steroidogenesis pathway.

Based on polynomial regression analyses of WGR and GSI, the optimal replacement level of dietary fishmeal by Antarctic krill meal for *Litopenaeus vannamei* broodstock was estimated to be 27.22%-41.78% under the present experimental conditions.

Declarations

Conflicts of interest statement

The authors have no relevant financial or non-financial interests to disclose.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding Declaration

This study was supported by the National Key R&D Program of China (2023YFD2402000) and the Modern Seed Industry Park for Whiteleg Shrimp of Guangdong Province.

Acknowledgments

This study was supported financially by the National Key R&D Program of China (2023YFD2402000), the Modern Seed Industry Park for Whiteleg Shrimp of Guangdong Province.

CRedit authorship contribution statement

Beiping Tan: Funding acquisition. **Xiaohui Dong**: Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Songjing He**: Software. **Wenshan Cai**: Formal analysis, Data curation. **Guoyong Huang and Hengyang Song**: Investigation, Conceptualization. **Weibin Huang**: Supervision, Software. **Baiquan Lu**: Methodology, Investigation. **Yubin Liu**: Writing – review & editing, Writing – original draft, Conceptualization.

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Figures

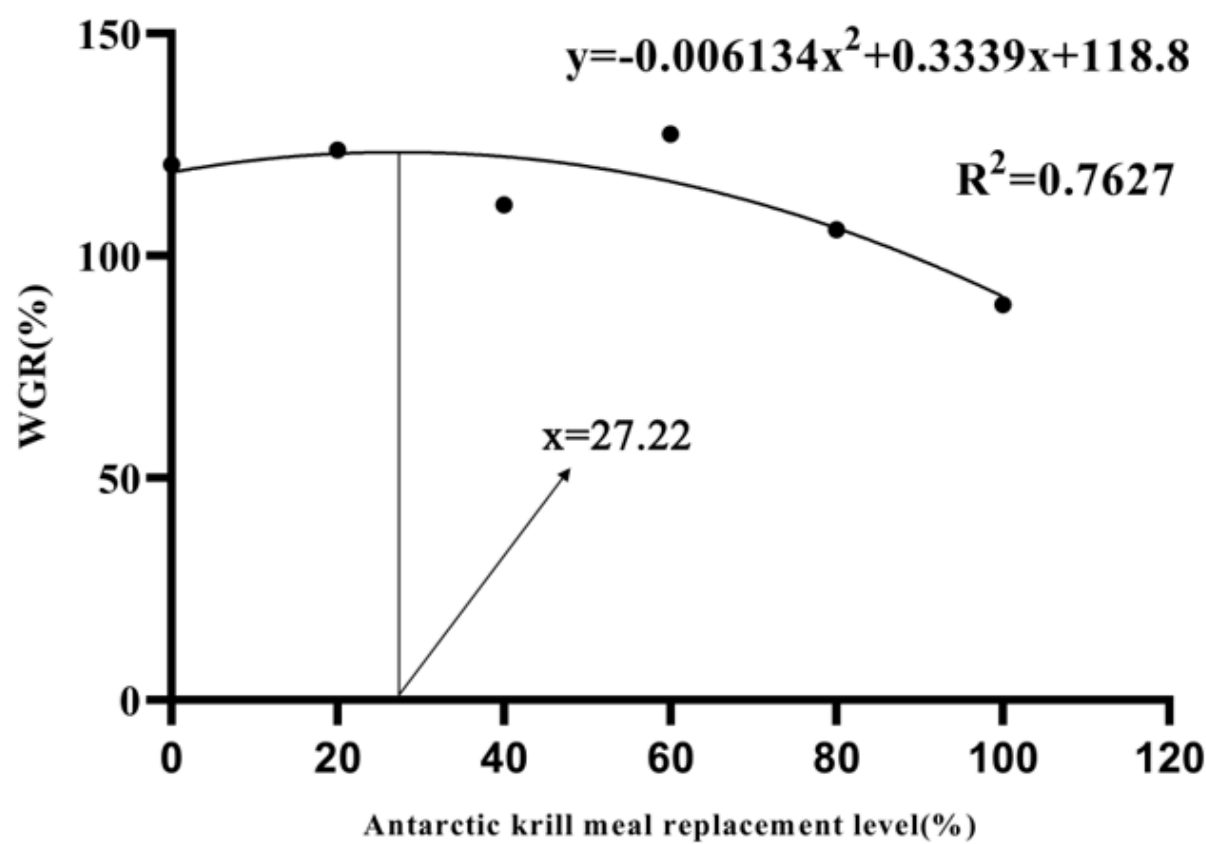


Figure 1

Association between AKM replacement levels and WGR of *Litopenaeus vannamei* broodstock

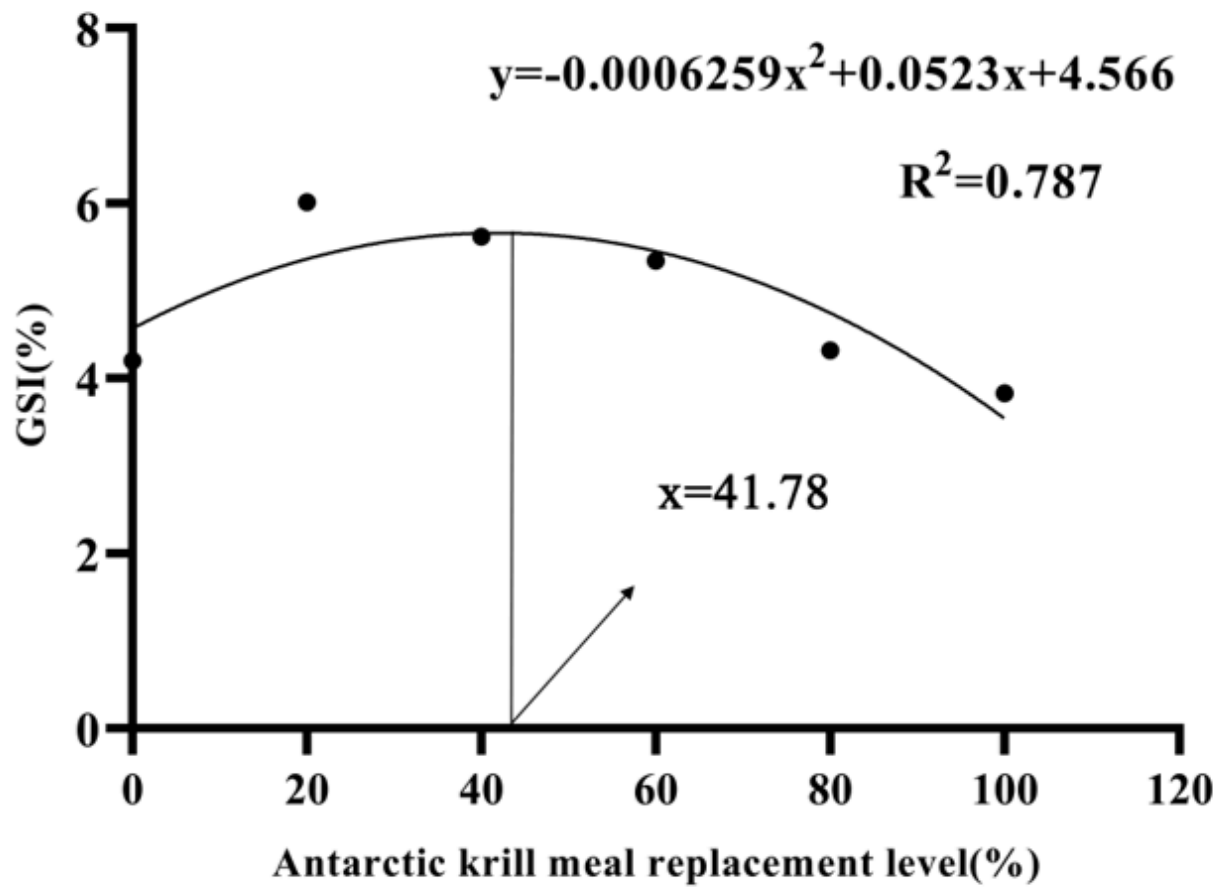


Figure 2

Association between AKM replacement levels and GSI of *Litopenaeus vannamei* broodstock

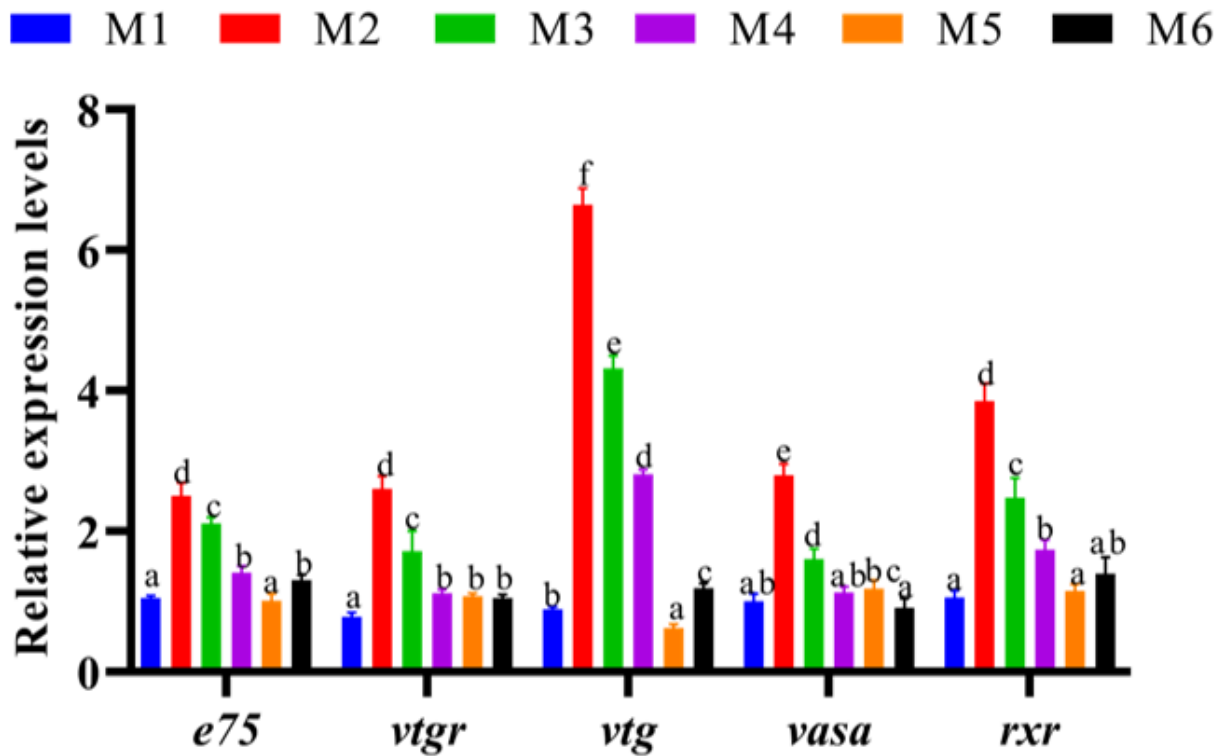


Figure 3

Effects of AKM replacement levels on the expression of genes related to ovarian development of *Litopenaeus vannamei* broodstock

Data are expressed as mean $\pm$ standard error ($n=3$). Different letters above bars indicate significant differences ($P < 0.05$). *e75*:ecdysone-induced protein 75B, *vtgr*:vitellogenin receptor, *vtg*:vitellogenin, *vasa*:probable ATP-dependent RNA helicase vasa-like, *rxr*:retinoid X receptor. M1: whole fish meal diet, M2:20% AKM replacement diet, M3:40% AKM replacement diet, M4:60% AKM replacement diet, M5:80% AKM replacement diet, M6:100% AKM replacement diet.

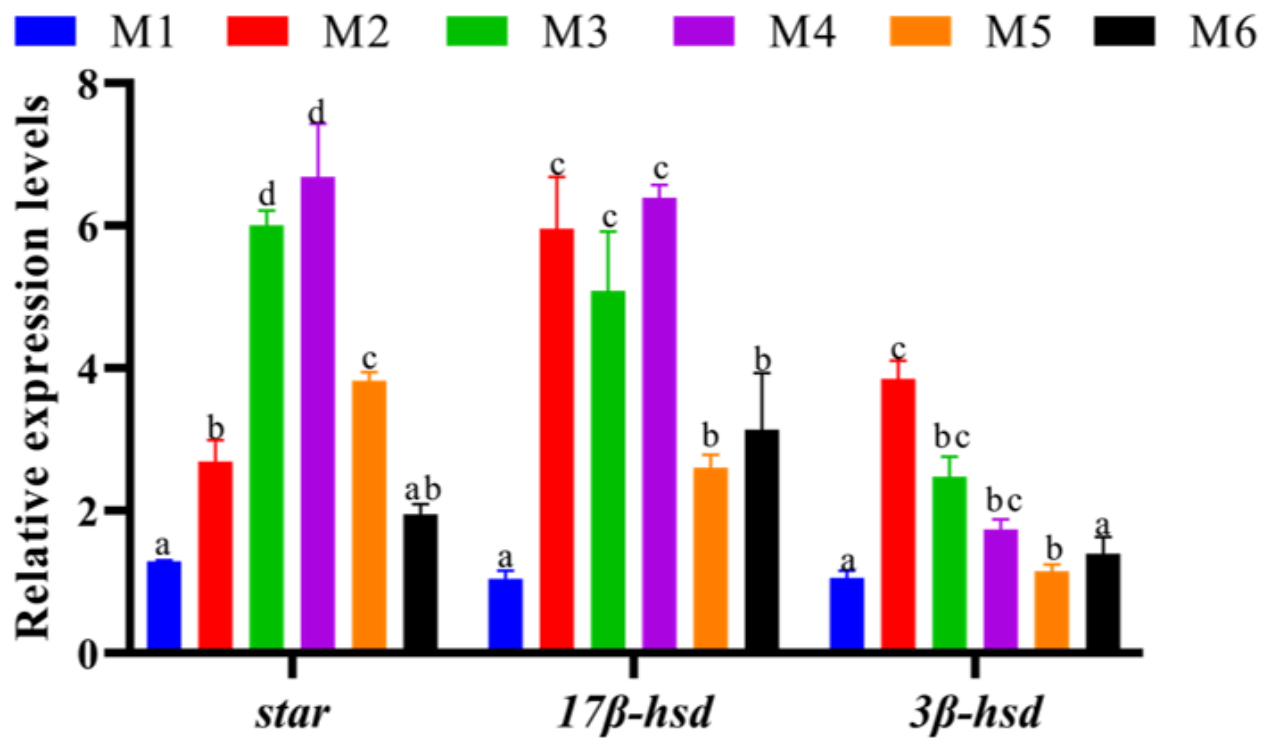


Figure 4

Effects of AKM replacement levels on the expression of genes related to the ovarian hormone synthesis pathway of *Litopenaeus vannamei* broodstock

Data are expressed as mean $\pm$ standard error (n=3). Different letters above bars indicate significant differences ($P < 0.05$). *star*:steroidogenic acute regulatory protein, *17β-hsd*:17 beta-hydroxysteroid dehydrogenase, *3β-hsd*:3 beta-hydroxysteroid dehydrogenase. M1:whole fish meal diet, M2:20% AKM replacement diet, M3:40% AKM replacement diet, M4:60% AKM replacement diet, M5:80% AKM replacement diet, M6:100% AKM replacement diet.