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Original Research Article

Effect of three polychaetes on growth and reproductive performance, biochemical indices and histology of different tissues in the female Pacific white shrimp, *Litopenaeus vannamei* broodstock

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ABSTRACT

A 30-day feeding trial was conducted to assess the effect of three fresh-live polychaetes as diets on growth and reproductive performance, biochemical indices and histology of different tissues in female Pacific white shrimp (*Litopenaeus vannamei*) broodstock. Two novel polychaete species, *Marphysa maxidenticulata* (MM group) and *Perinereis nuntia* (PN group), and a traditional species, *Perinereis aibuhitensis* (PA group), were used as a single diet and individual experimental groups, respectively. A total of 225 healthy female broodstock shrimp, initial weight of 59.70 ± 0.18 g, were randomly divided into three groups (three replicates of 25 shrimp each). The results showed that the MM group outperformed the PA and PN groups in growth indices, with the highest weight gain, specific growth rate, molting rate, protein efficiency ratio, gonadosomatic index, and lower feed conversion ratio compared to the PN group ($P < 0.05$). Regarding reproductive performance, the MM group had the highest daily number of sexually mature female broodstock shrimp, successful mating count, maturity rate, mating rate, spawning cycle, total number of fertilized eggs, individual fertilized egg yield, area of mature oocytes, total number of nauplii, naupliar yield/shrimp, hatching rate, and the lowest naupliar deformity rate than the other two groups ($P < 0.05$). Moreover, compared to the PA and PN groups, the MM group demonstrated superior activities of lipid metabolism-related enzymes and digestive enzymes, and antioxidant capacity in the hepatopancreas, intestine and serum, as well as reduced malondialdehyde levels. Meanwhile, the ovaries of the MM group showed a significant accumulation of triglycerides, estradiol, and vitellogenin compared to the other groups. Histology revealed more developed secretory cells in the hepatopancreas and larger mature oocytes in the MM group compared to the others. In conclusion, *M. maxidenticulata* can maximize growth, reproductive performance, the activities of lipid metabolism-related enzymes and digestive enzymes, antioxidant and immune ability of female broodstock shrimp. This study demonstrated that *M. maxidenticulata* could be used as a potential fresh-live diet for the female *L. vannamei* broodstock.

1. Introduction

Broodstock shrimp breeding is a crucial link in the shrimp industry chain. The condition of the broodstock during breeding directly affects the quality of the nauplii, as their initial nutrition and energy come from the fertilized eggs (Racotta, 2003). Therefore, enhancing broodstock nutrition is critical for improving both the reproductive performance and the quality of the broodstock, as

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well as the quality of their offspring (Benzie, 1997; Browdy, 1998; Peixoto et al., 2011).

Since the emergence of marine shrimp hatchery technology in the 1970s (Chamberlain, 2012), a variety of live marine organisms, including polychaetes, squid, oysters, fish, and shrimp, have been tested as feed for broodstock shrimp (Braga et al., 2010; Bray and Lawrence, 1992; Hoa et al., 2009). However, extensive practice has proven that polychaetes are the only indispensable diet during the broodstock shrimp breeding period (Chamberlain, 2012; Chimsung, 2014). Previous studies have shown that feeding polychaetes can significantly improve the growth and reproductive performance of broodstock shrimp (Leelatanawit et al., 2014; Meunpol et al., 2007; Nguyen et al., 2012). The gonadal development of broodstock shrimp is essentially a process of nutrient accumulation. Within the reproductive cycle, nutrients and energy from food are initially stored in the hepatopancreas, and then these reserves, including lipids and proteins, are mobilized and transferred via the hemolymph to support ovarian development (Bray and Lawrence, 1992; Peixoto et al., 2011; Teshima et al., 1988; Teshima and Kanazawa, 1983; Yan et al., 2017). The significant increase in the contents of arachidonic acid (ARA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) in the ovaries indicates that these essential fatty acids are transferred from the hepatopancreas to the ovaries, playing a critical role in the development and maturation of the ovaries (Mourente, 1996; Mourente and Rodrguez, 1991). Therefore, the richness in polyunsaturated fatty acids (PUFAs) such as ARA, DHA, and EPA in polychaetes may be the most fundamental reason for improved reproductive performance of broodstock shrimp (Jerónimo et al., 2021; Nguyen et al., 2012; Yang et al., 2022). In particular, ARA in polychaetes, serves as a lipid precursor for prostaglandins (Lee et al., 2020; Wang et al., 2021), and can promote the maturation and development of shrimp ovaries (Binh et al., 2008; Deenarn et al., 2020; Meunpol et al., 2010).

In recent years, most studies have focused on mixing polychaetes with other fresh diets (such as oysters, squid, fish meat, shrimp meat) as feed for broodstock shrimp, rather than using them as a single feed source (Braga et al., 2010; Jin et al., 2022; Omar et al., 2020). These studies have only demonstrated the positive effect of the fresh mixed diets, but not confirmed the exact species of diet or whether polychaetes are the only indispensable part of the broodstock shrimp feed. Previous studies have also not verified the effect of fresh-live polychaetes as a diet on the reproductive performance and the physiological and biochemical aspects of female broodstock shrimp. Commercial feeds are heat treated, do not carry viruses and are easy to store. Broodstock industry is lagging behind in developing a commercial feed due to higher research costs, lower economic value and being a small player in the shrimp farming industry. As a result, the development of commercial feed may not be the best solution to the nutritional problems of broodstock shrimp, and commercial feed may not be able to replace natural diets in the near future (Hoa et al., 2009; Leelatanawit et al., 2014; Nascimento et al., 1991).

In this study, we introduced the three polychaetes species that have currently achieved industrial-scale aquaculture in China, including two new species of polychaetes, *Marphysa maxidenticulata* (Liu et al., 2017) and *Perinereis nuntia*, and compared them with the widely used *Perinereis aibuhitensis*. The effect of several polychaetes species on growth, reproduction, and physiological and biochemical profiles, as well as the histological morphology of female *Litopenaeus vannamei* broodstock, were comprehensively investigated for the first time. This study initially fills the gap in the aforementioned research area regarding the use of polychaetes as a single feed for female broodstock reproductive performance. This study aims to explore the application effect of two new adult polychaetes species in broodstock shrimp, with the goal of screening the most effective polychaetes to provide a new direction and basis for developing future broodstock diets.

2. Materials and methods

2.1. Animal ethics statement

All animal experiments were conducted in compliance with the "Animal Research: Reporting of In Vivo Experiments" (ARRIVE) guidelines. Animal Ethics Committee of the Guangdong Ocean University accepted the protocols (ID: GDOU-IACUC-2022-A0502, dated May 2, 2022).

2.2. Fresh-live maturation diets

In this study, three adult polychaetes species: *P. aibuhitensis* (PA group), *M. maxidenticulata* (MM group), and *P. nuntia* (PN group), were selected as single fresh-live maturation diets of female *L. vannamei* broodstock, cultured from Dongfang Yixin Aquatic Products Co., Ltd., Hainan, China. Among them, *P. aibuhitensis* is currently the only polychaetes species that has achieved large-scale factory farming. However, *M. maxidenticulata* and *P. nuntia* are two emerging new polychaetes species whose cultivation techniques have been mastered but have not yet achieved a large-scale commercial cultivation model. The cultivation cycles for *P. aibuhitensis*, *M. maxidenticulata*, and *P. nuntia* are 6 to 8 months, 16 to 18 months, and 8 to 10 months, respectively. In the experiment, we adopted the cold seawater suspension culture method from Japan to process the three types of live polychaetes that were manually collected for 24 h (starved in a recirculating water device at 10–15 °C, with the entire process conducted under a temperature-controlled device). Cold water treatment causes the polychaetes' bodies to contract, resulting in the shedding of surface silt and mucus, thereby obtaining cleaner polychaetes. The treatment in this study was carried out in a cold storage room (10–15 °C). The morphological photographs and body indices of the three polychaetes species are shown in Fig. 1 and Table 1.

The standard methods (AOAC, 1995) were used to determine the proximate composition of three fresh-live maturation diets. The moisture content was determined using the oven dry matter method (at 105 °C) (method 934.01), the crude protein content was determined using the Kjeldahl method (nitrogen × 6.25) (method 976.05), the crude fat content was determined using the Soxhlet extraction method (method 920.29), and the ash content was determined using the incineration method heating the samples in a muffle furnace at 550 °C for 6 h (method 942.05). Furthermore, amino acid (AA) and fatty acid (FA) were measured by the Amino Acid Analyzer (L-8800, Hitachi, Ltd., Japan) and Gas Chromatograph (GC-2010, Shimadzu Corporation, Japan), respectively.

2.3. Feeding experiment

After purchasing domesticated F1 broodstock shrimp from Zhengda Aquatic Products Co., Ltd., they were continually cultured to the age of 8 months. A total of 225 male and 225 female *L. vannamei* broodstock were randomly separated into 18 rectangular concrete ponds (three replicates of 25 male or female broodstock shrimp in each pond), each with a base area of 5.00 m^2 and a water depth of 0.60 m. Before the experiment, the eyestalk ablation was performed on all the female broodstock shrimp.

At the start of a 30-day feeding trial, the initial body weight (IBW) of male broodstock shrimp was 50.40 ± 0.23 g and female broodstock shrimp was 59.70 ± 0.18 g. During the experiment, each group was fed 4 times/d (06:00, 12:00, 18:00 and 24:00). The daily

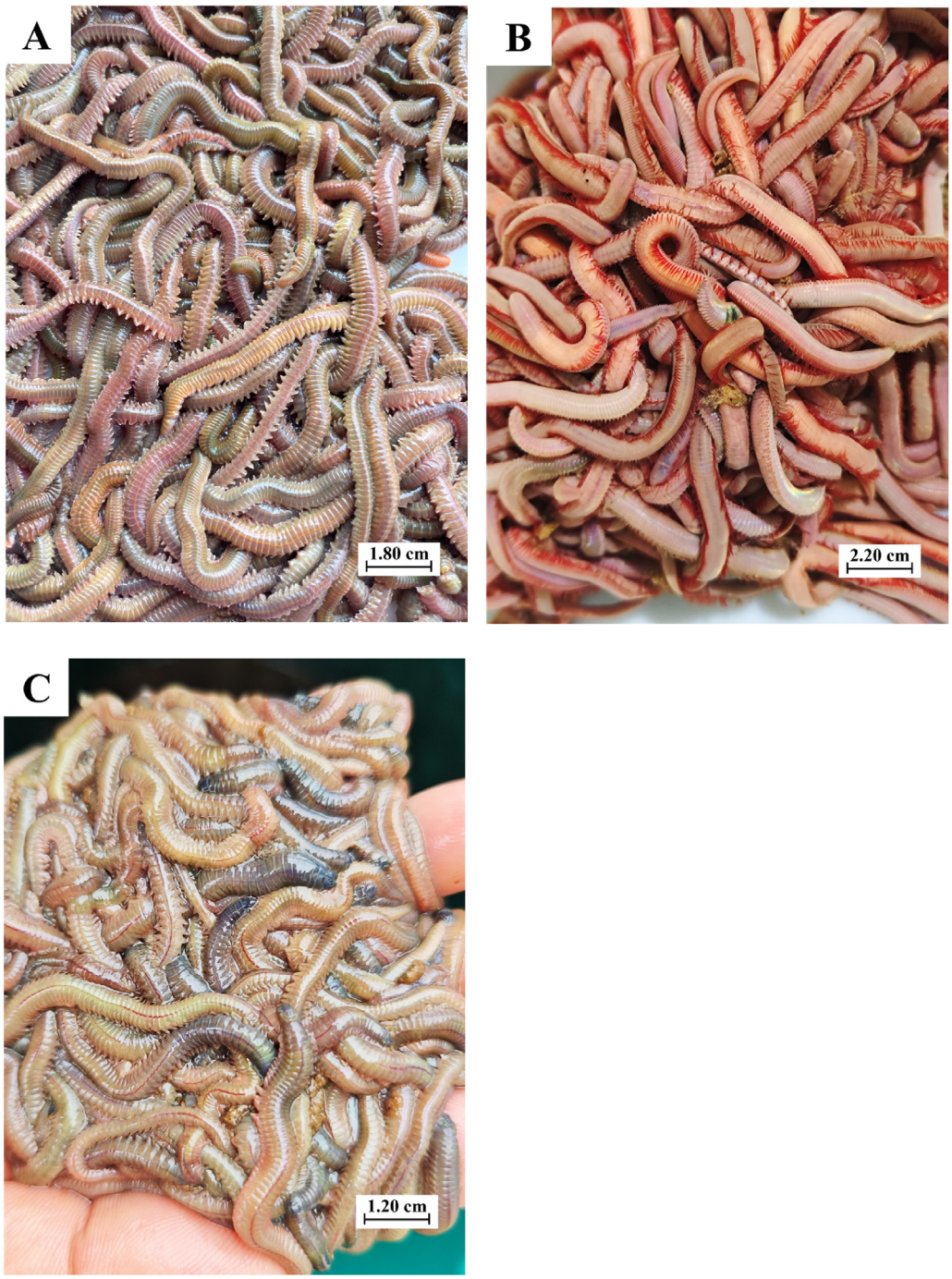

Fig. 1. Morphological photograph of three polychaetes species. (A) *Perinereis aibuhitensis*. (B) *Marphysa maxidenticulata*. (C) *Perinereis nuntia*.

Table 1
The average body weight and body length of three polychaetes species.

Item	*P. aibuhitensis*	*M. maxidenticulata*	*P. nuntia*	SEM	*P*-value
Body weight, g	2.43[b]	5.10[c]	0.97[a]	0.182	<0.001
Body length, cm	16.06[b]	21.13[c]	8.57[a]	0.557	<0.001

Within a row, means without a common superscript differ at $P < 0.05$.

feed intake of all experimental groups was the same, and it was weighed with an electronic scale (Meilen MTB500, Shenzhen Mei Foo Electronics Co., Ltd., China). The seawater temperature was determined using a probe-style electronic thermometer (AR212, SMART SENSOR, China). The salinity was determined using a water thermometer (AR8012, SMART SENSOR, China), the pH was

determined using a pH meter (pH 818, SMART SENSOR, China), and the total alkalinity was determined using the acid-base titration method. Similary, the dissolved oxygen was determined using a Dissolved Oxygen Meter (AR8010, SMART SENSOR, China), residual chlorine was determined using a residual chlorine meter (YL-2AZ, Shanghai Haizheng Electronic Technology Co., Ltd., China), ammonia nitrogen, nitrite, and nitrate concentrations were determined using reagent kits (Fujian Tianma Science and Technology group co., Ltd., China), 12 h light and 12 h dark. Detailed water quality parameters are provided in Table S1. During the feeding experiment, the seawater was changed 100% daily at 08:00 after residual polychaetes and excrement were removed.

The daily tasks and operational procedures during the experiment are shown in Fig. 2. Feed was provided starting at 06:00 every morning; the water was changed in all the ponds at 08:00; the nauplii was counted at 10:00; second feeding was done at 12:00. At 13:00, sexually mature female broodstock shrimps were retrieved to the male broodstock shrimp pond for mating; at 16:00, the successfully mated female broodstock shrimps were transferred to a cylindrical bucket with a volume of 1 cubic meter (each with a base area of 1.00 m^2 and a water depth of 0.60–0.80 m) for egg laying and incubation, and unsuccessful mated female shrimps were returned to the female broodstock shrimp pond; at 18:00, the egg-laying female shrimps were retrieved back to the female broodstock shrimp pond and fed for the third time, and at 19:00, the fertilized eggs were counted. In addition, from the end of egg laying at 18:00 pm until 00:00, the eggs were rolled once every 30 to 40 min; from 00:00 to 03:00, the eggs were rolled once every 60 to 90 min; from 03:00 to 06:00, the eggs were rolled once every 120 min. The interval of egg rolling was appropriately increased with time until the fertilized eggs metamorphosed into nauplii.

2.4. Sample collection

During a 30-day breeding period, the initial number of male broodstock shrimp (INM), initial number of female broodstock shrimp (INF); final number of male broodstock shrimp (FNM), and final number of female broodstock shrimp (FNF) were recorded to calculate male broodstock shrimp survive rate (MSR) and female broodstock shrimp survive rate (FSR). The number of molts (the number of carapace and tail was equal, and the number of shrimp shells equals the number of molts) was counted to calculate the molting rate (MR). In addition, daily statistics also included the average number of sexually mature female broodstock shrimp (SMFS), successful mating count (SMC) to calculate maturity rate (MATR), and mating rate (MTGR). Meanwhile, spawning cycle (SC), total number of fertilized eggs (TNFE), individual fertilized egg yield (IFEY), area of mature oocytes (AMO), total number of nauplii (TNN), naupliar yield per shrimp (NY) (each group took random samples three times using a 250-mL glass beaker, and counted while pouring under the illumination of a 20W flashlight with weak light) to calculate hatching rate (HR), naupliar deformity rate (NDR) (each group took 100 nauplii for observation under an optical microscope with 3 repetitions, using a magnification of 40×). Deformed shrimp nauplii mainly included abnormalities or damage in the development of setae, appendages, or telson spines.

At the end of the trial, first of all, the female *L. vannamei* broodstock was anesthetized in an ice bath for 10 to 15 min, and then their final body weight (FBW) was measured to calculate various growth performance, including weight gain rate (WGR), specific growth rate (SGR), daily feed intake (DFI), feed conversion ratio (FCR), protein efficiency ratio (PER), hepatosomatic index (HSI), and gonadosomatic index (GSI). The mature ovary of female broodstock shrimp was clearly visible (Fig. 3A). Secondly, 9 shrimps were randomly selected from each group, and hemolymph was collected using a 1-mL sterile syringe inserted from the first pair of pleopods to the third to fourth pair of pereiopods, and stored at 4 °C for 24 h. The hemolymph was pounded by a glass rod and centrifuged at 3000 × *g* at 4 °C for 20 min, and the supernatants were transferred to the centrifuge tube, and finally stored in a −80 °C freezer. Thirdly, during sampling, the soft tissue between the fifth pereiopods of the shrimp was first cut open using scissors that had been sterilized by incineration (Fig. 3B), and the posterior blind sac of the midgut was cut off. Then, the hepatopancreas was peeled off with forceps, and finally, the hepatopancreas and intestine (excluding the hindgut) were pulled out with forceps gripping the cardiac stomach. Fourthly, the abdomen was cut open between the pleopods with scissors and the ovaries were peeled off with forceps (Fig. 3C and D). In addition, the dissected hepatopancreas, intestine, and ovary were partly stored at −80 °C for subsequent biochemical analysis; the other part was preserved in 4% paraformaldehyde for subsequent histological analysis.

2.5. Enzymes activity and biochemical index analysis

Hepatopancreas sample were weighed and transferred into 1.5-mL centrifuge tubes. Then, nine times the volume of precooled 0.85% saline solution was added, followed by thorough grinding

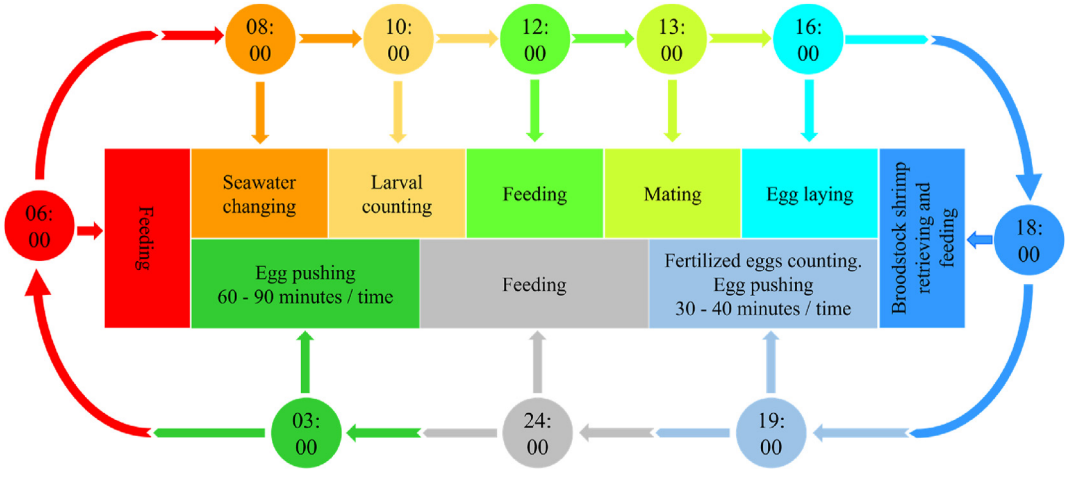

Fig. 2. The daily tasks and operational procedures during a 30-day feeding trial.

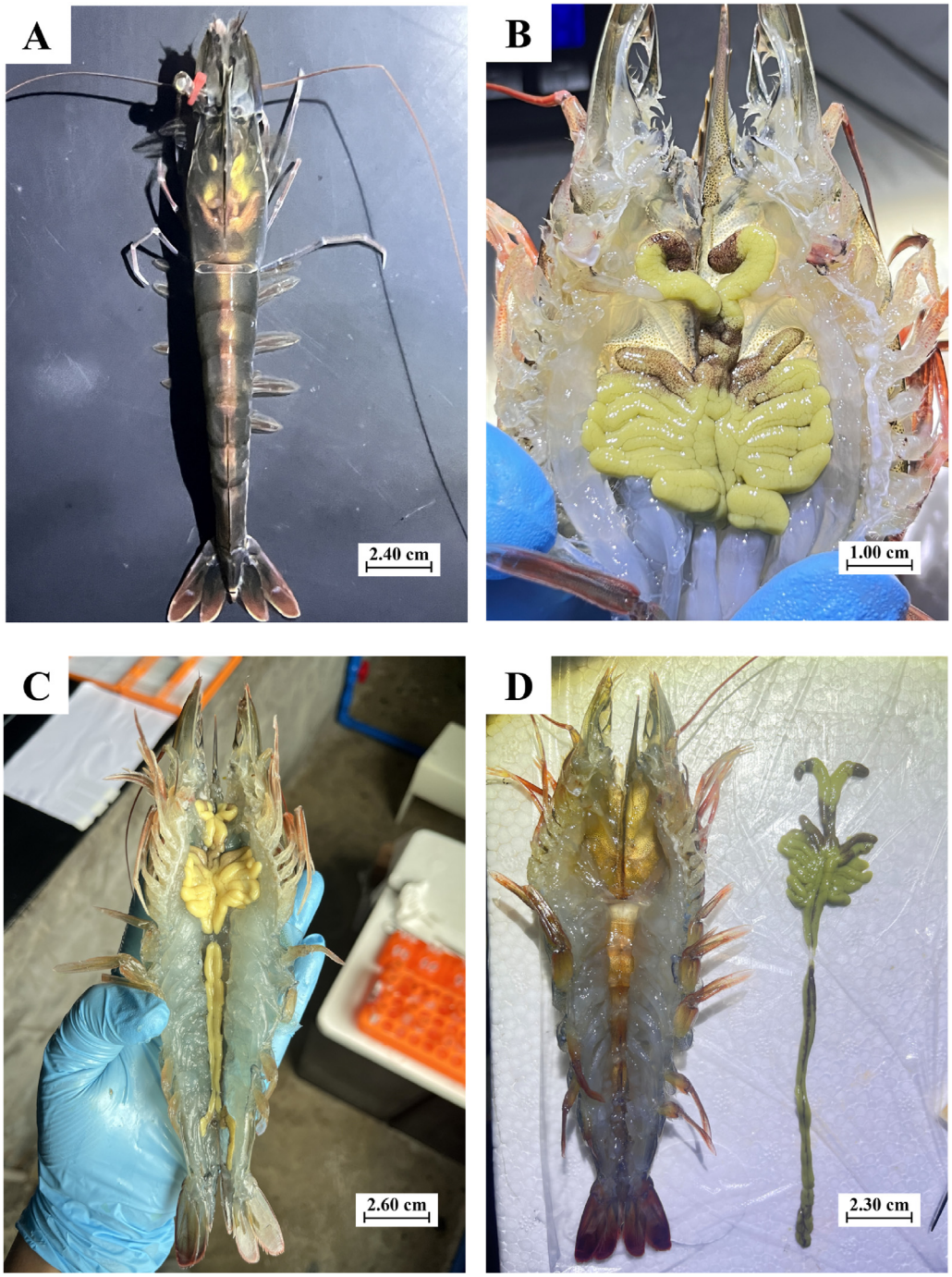

Fig. 3. The morphology of female *Litopenaeus vannamei* broodstock. (A) Top view of the broodstock shrimp. (B) A distribution map of the gonads inside the cephalothorax dissected from the ventral side of the broodstock shrimp. (C) A distribution map of the gonads within the broodstock shrimp's body (with gonads not dissected). (D) Carcass, and gonads that were peeled off from the broodstock shrimp.

with a tissue grinder and transfer of tissue into a centrifuge tube for 30 min of centrifugation (1500 × *g*, 4 °C). Finally, the supernatants were collected and the activities of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACCase), hepatic lipase (HL), lipoprotein lipase (LPL), pancreatic lipase (PL), total antioxidant capacity (T-AOC), amylase, lipase, trypsin, catalase (CAT), superoxide dismutase (SOD), lysozyme (LZM), glutathione peroxidase (GSH-Px), and the content of malondialdehyde (MDA), estradiol (E) and vitellogenin (VTG) were measured following the described methods by Liang et al. (2023). The methods for determining amylase, lipase and trypsin in the intestine was the same as that of hepatopancreas.

Stored hemolymph samples were centrifuged at 3000 × *g* at 4 °C for 10 min. The supernatants were collected and the activities of T-AOC, CAT, SOD, LZM, and GSH-Px, and the contents of MDA, E and VTG were measured according to Liang et al. (2023). These enzyme activities and biochemical indices in hepatopancreas, intestine, and hemolymph were determined using commercial kits (Nanjing Jiancheng Biological Engineering Institute, Nanjing, China). In addition, enzymatic-colorimetric analyses were

performed for the content of glucose (Glu), triglycerides (TG), and total protein (TP) in hepatopancreas, hemolymph and ovaries samples. Total protein content was determined using the bicinchoninic acid method; Glu and TG contents were measured using the micro methods. Their contents in hepatopancreas, hemolymph and ovaries were determined using the commercial kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). Detailed operation steps of all biochemical indices above were conducted according to the instructions provided by the manufacturer.

2.6. Histological analysis

The hepatopancreas and ovaries were fixed in a 4% paraformaldehyde solution, with the hepatopancreas being preserved for more than 48 h and the ovaries for more than 24 h. These samples were dehydrated in a series of ethanol with gradient concentrations, embedded in paraffin, sectioned and mounted. Staining was performed with hematoxylin and eosin (H&E). The histological sections of the hepatopancreas and ovaries were photographed by an Upright Fluorescence Microscope (Nikon Y-TV55,Guangzhou Xian Ni Instrument Co., Ltd., China; the magnification was 400 ×) for subsequent analysis.

2.7. Statistical analyses

The data were statistically analyzed using one-way ANOVA in SPSS (version 27, IBM SPSS Statistics, Armonk, NY, USA). The mathematical model for one-way ANOVA is represented as:

$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$, where Y_{ij} is the observation for the j-th trial in the i-th group; μ is the overall mean across all groups; α_i is the deviation of the mean of the i-th group from the grand mean; ϵ_{ij} is the random error term, which is assumed to be normally distributed with a mean of 0 and variance σ^2.

The Duncan method was used for multiple comparisons of the mean values among all treatment groups. The obtained outcomes are expressed as mean and between-group standard error of the mean (between-group SEM) in all tables are presented with one more decimal place than the means. A value of $P < 0.05$ suggests statistical significance, while $P < 0.01$ indicates a highly significant difference.

3. Results

3.1. Growth performance, hepatosomatic index and gonadosomatic index

The results on growth performance, HSI and GSI of female broodstock shrimp are shown in Table 2. During a 30-day breeding period, the FBW, WGR, and SGR in MM and PN groups were significantly higher than those in the PA group ($P < 0.05$). Meanwhile, the MR ($P = 0.056$) in the MM group was the highest among all experimental groups. The DFI ($P = 0.083$) in the PA group was substantially higher than that in other experimental groups. The FCR ($P = 0.009$) in the PA group was significantly higher than that in the MM and PN groups, while the PER ($P = 0.012$) in the MM and PN groups was significantly higher than that in the PA group. In addition, the HIS ($P = 0.009$) in the PA and MM groups was lower than that in the PN group, whereas the GSI ($P = 0.005$) in the MM group was significantly higher than that in the other two groups.

3.2. Reproductive performance

The reproductive performance of female broodstock shrimp during a 30-day period is presented in Table 3. By the end of the experiment, there were no significant differences in FNM, FNF, MSR, and FSR among the experimental groups ($P > 0.05$). However, the SMFS, SMC, MATR, MTGR, SC, TNFE, IFEY, AMO, TNN, NY and HR in the MM group were significantly higher than those in PA and PN groups ($P < 0.05$), but there were no significant differences in those between PA and PN groups. Additionally, there were significant differences in NDR among all the groups ($P < 0.001$). PA group had the highest NDR, followed by the PN group, and the MM group had the lowest NDR.

3.3. Lipid metabolism-related enzyme activities in hepatopancreas

The activities of lipid metabolism-related enzymes in hepatopancreas are presented in Table 4. The activities of FAS, ACC, HL, LPL, and PL in the MM group were the highest. Nevertheless, there were no significant differences in the activities of these fat metabolism-related enzymes among all the groups ($P > 0.05$).

3.4. Digestive enzyme activities in hepatopancreas and intestine

The activities of digestive enzymes in the hepatopancreas and intestine are listed in Table 5. The activities of the three digestive enzymes in the hepatopancreas and intestine of the MM group were the highest. The activities of lipase ($P = 0.011$) and trypsin ($P = 0.047$) in hepatopancreas and lipase ($P = 0.004$) in the intestine of the MM group were significantly higher than those in the PA group, meanwhile, the activities of amylase ($P = 0.041$) and lipase ($P = 0.011$) in hepatopancreas of MM group were significantly higher than those in the PN group. In addition, amylase ($P = 0.078$) in the intestine of MM group was the highest among all experimental groups. However, there were no significant differences in the trypsin activity ($P = 0.156$) of the intestine among all the groups.

3.5. Antioxidant and immune indicators in hepatopancreas and serum

The results of antioxidant and immune indicators in hepatopancreas and serum of female *L. vannamei* broodstock fed different diets are shown in Table 6. The T-AOC in hepatopancreas

Table 2
Growth performance of female *L. vannamei* broodstock fed three fresh-live maturation diets.

Item	PA group (control)	MM group	PN group	SEM	*P*-value
IBW, g	59.94	59.30	59.86	0.182	0.336
FBW, g	66.68[a]	69.12[b]	68.38[b]	0.403	0.008
WGR, %	11.25[a]	16.56[b]	15.14[b]	0.849	0.002
SGR, %/d	0.35[a]	0.51[b]	0.44[b]	0.256	0.005
MR, %	5.64	6.55	5.85	0.175	0.056
DFI, %	10.93	10.78	10.74	0.038	0.083
FCR	1.04[b]	0.71[a]	0.82[a]	0.055	0.009
PER	1.56[a]	2.10[b]	2.07[b]	0.100	0.012
HSI, %	3.06[a]	2.87[a]	3.47[b]	0.100	0.009
GSI, %	6.48[a]	7.59[b]	6.62[a]	0.192	0.005

PA = *P. aibuhitensis*; MM = *M. maxidenticulata*; PN = *P. nuntia*; IBW = initial body weight; FBW = final body weight; WGR = weight gain rate; SGR = specific growth rate; MR = molting rate; DFI = daily feed intake; FCR = feed conversion ratio; PER = protein efficiency ratio; HIS = hepatosomatic index; GSI = gonadosomatic index.
Within a row, means without a common superscript differ at $P < 0.05$.
WGR = (FBW − IBW)/IBW × 100.
SGR = (ln FBW − ln IBW)/days × 100.
MR = total number of molts/total number of shrimps × 100.
DFI = (total feed offered/total days)/[(IBW + FBW)/2] × 100.
FCR = total feed intake/total weight gain.
PER = shrimp weight gain/shrimp protein intake.
HSI = wet hepatopancreas weight/wet body weight × 100.
GSI = wet testicular weight/wet body weight × 100.

Table 3
The reproductive performance indexes of the female *L. vannamei* broodstock fed three fresh-live maturation diets.

Item	PA group (control)	MM group	PN group	SEM	*P*-value
INM/INF, tail/tail	25/25	25/25	25/25		
FNM, tail	23.67	24.33	24.00	0.289	0.702
FNF, tail	24.67	24.67	24.33	0.242	0.850
MSR, %	94.67	97.33	96.00	1.155	0.702
FSR, %	98.67	98.67	97.33	0.969	0.850
SMFS, tail	4.94^{a}	5.80^{b}	4.97^{a}	0.144	<0.001
SMC, tail	2.34^{a}	3.12^{c}	2.53^{b}	0.121	<0.001
MATR, %	19.83^{a}	23.31^{b}	20.01^{a}	0.583	<0.001
MTGR, %	9.41^{a}	12.56^{b}	10.20^{a}	0.490	<0.001
SC, d	5.64^{b}	4.82^{a}	5.76^{b}	0.160	0.003
TNFE, $\times 10^4$	2382.31^{a}	3404.84^{b}	2570.09^{a}	159.800	<0.001
IFEY, $\times 10^4$	33.74^{a}	36.17^{b}	33.77^{a}	0.417	<0.001
AMO, $\times 10^4$ μm^2	2.21^{a}	2.82^{b}	2.33^{ab}	0.106	0.039
TNN, $\times 10^4$	1835.33^{a}	2739.24^{b}	1958.18^{a}	143.357	<0.001
NY, $\times 10^4$	25.85^{a}	29.22^{b}	26.00^{a}	0.557	<0.001
HR, %	76.56^{a}	80.75^{b}	76.94^{a}	0.696	<0.001
NDR, %	4.47^{c}	3.45^{a}	4.21^{b}	0.157	<0.001

PA = *P. aibuhitensis*; MM = *M. maxidenticulata*; PN = *P. nuntia*; INM = initial number of males broodstock shrimp; INF = initial number of females broodstock shrimp; FNM = final number of males broodstock shrimp; FNF = final number of females broodstock shrimp; MSR = male broodstock shrimp survive rate; FSR = female broodstock shrimp survive rate; SMFS = the daily number of sexually mature female broodstock shrimp; SMC = daily successful mating count; MATR = maturity rate; MTGR = mating rate; SC = spawning cycle; TNFE = total number of fertilized eggs; IFEY = individual fertilized egg yield; AMO = area of mature oocytes; TNN = total number of nauplii; NY = naupliar yield per shrimp; HR = hatching rate; NDR = naupliar deformity rate.
Within a row, means without a common superscript differ at $P < 0.05$.
MSR = FNM/INM × 100.
FSR = FNF/INF × 100.
MATR = SMFS/daily number of females broodstock shrimp × 100.
MTGR = SMC/daily number of females broodstock shrimp × 100.
SC = daily number of females broodstock shrimp/SMFS.
HR = TNN/TNFE × 100.
NDR = the naupliar number of deformities/100 nauplii.

Table 4
Lipid metabolism-related enzymes activities in hepatopancreas of female *L. vannamei* broodstock fed three fresh-live maturation diets.

Item	PA group (control)	MM group	PN group	SEM	*P*-value
FAS, U/L	1803.56	1847.11	1688.40	60.475	0.607
ACCase, U/L	37.06	37.84	36.63	2.253	0.982
HL, U/mL	23.46	29.67	25.52	1.357	0.163
LPL, U/L	481.05	609.14	502.58	31.604	0.224
PL, U/L	97.70	105.60	94.38	7.397	0.856

PA = *P. aibuhitensis*; MM = *M. maxidenticulata*; PN = *P. nuntia*; FAS = fatty acid synthetase; ACCase = acetyl-CoA carboxylase; HL = hepatic lipase; LPL = lipoprotein lipase; PL = pancreatic lipase.
Within a row, means without a common superscript differ at $P < 0.05$.

($P = 0.003$) and serum ($P = 0.021$) of the MM group was significantly higher than that in the PA and PN groups. The activities of SOD ($P = 0.040$) in hepatopancreas and CAT ($P = 0.009$), SOD ($P = 0.015$), LZM ($P = 0.006$) in the serum of the MM group were significantly higher than those in the PA group, meanwhile, the activities of CAT ($P = 0.038$) in hepatopancreas and SOD ($P = 0.015$) in serum of the MM group were significantly higher than those in the PN group. In addition, the activities of CAT ($P = 0.009$) and LZM ($P = 0.006$) in the serum of the PN group were significantly higher than those in the PA group. However, there were no significant differences in the activities of LZM ($P = 0.115$) and GSH-Px ($P = 0.127$) in hepatopancreas, and the GSH-Px activity ($P = 0.639$) in the serum among all the groups. Furthermore, the MDA content in hepatopancreas ($P = 0.415$) and serum ($P = 0.390$) of the MM group was the lowest than that in the other two groups, but there was no significant difference among all the experimental groups.

Table 5
Digestive enzymes activities in hepatopancreas and intestine of female *L. vannamei* broodstock fed three fresh-live maturation diets.

Item	PA group (control)	MM group	PN group	SEM	*P*-value
Hepatopancreas					
Amylase, U/L	406.46^{b}	424.44^{b}	318.98^{a}	20.121	0.041
Lipase, U/L	673.38^{a}	793.43^{b}	632.67^{a}	27.382	0.011
Trypsin, U/mL	2690.73^{a}	3424.90^{b}	2906.29^{ab}	136.189	0.047
Intestine					
Amylase, U/L	287.16	313.07	263.89	9.377	0.078
Lipase, U/L	276.92^{a}	454.27^{b}	454.52^{b}	32.191	0.004
Trypsin, U/mL	2000.66	2373.06	2069.30	84.208	0.156

PA = *P. aibuhitensis*; MM = *M. maxidenticulata*; PN = *P. nuntia*.
Within a row, means without a common superscript differ at $P < 0.05$.

3.6. Biochemical composition in hepatopancreas, serum, and ovary

The biochemical composition in the hepatopancreas, serum and ovary are shown in Table 7. MM group had the highest Glu content in hepatopancreas ($P = 0.280$) and serum ($P = 0.922$), but lowest in the ovary ($P = 0.109$) and significantly lower than that in the PN group. The TG and E contents in all tissues of the MM group were the highest, and the TG content in both serum ($P = 0.051$) and ovary ($P = 0.013$) of the MM and PN groups were higher than that in the PA group. Additionally, the MM group had the lowest VTG content in serum ($P = 0.003$), but the MM group had the highest VTG content in the ovary ($P = 0.070$). However, there were no significant differences in contents of Glu, TG, TP, E and VTG in hepatopancreas, Glu, TP and E in serum, and TP and E in ovary among the three experimental groups ($P > 0.05$).

3.7. Histological analysis

The histological sections of hepatopancreas and ovary tissue are shown in Figs. 4 and 5, respectively. In hepatopancreas, the development degree of secretory cells in the MM group (Fig. 4B) was the highest, followed by the PN group (Fig. 4C), and PA group

Table 6
Antioxidant and immune ability in hepatopancreas and serum of female *L. vannamei* broodstock fed three fresh-live maturation diets.

Item	PA group (control)	MM group	PN group	SEM	*P*-value
Hepatopancreas					
T-AOC, mmol/g	1.74[a]	2.28[b]	1.96[a]	0.085	0.003
CAT, U/mL	43.13[ab]	54.29[b]	38.76[a]	2.840	0.038
SOD, U/mL	75.48[a]	117.98[b]	88.19[ab]	7.767	0.040
LZM, U/L	3.17	5.30	3.73	0.444	0.115
GSH-Px, U/L	81.27	113.82	97.18	6.667	0.127
MDA, nmol/mL	13.70	12.01	14.95	0.846	0.415
Serum					
T-AOC, μmol/mL	0.29[a]	0.40[b]	0.35[ab]	0.019	0.021
CAT, U/mL	47.36[a]	73.48[b]	65.14[b]	4.324	0.009
SOD, U/mL	89.68[a]	163.05[b]	109.94[a]	12.620	0.015
LZM, U/L	3.53[a]	7.19[b]	6.09[b]	0.600	0.006
GSH-Px, U/L	150.49	169.40	153.73	7.838	0.639
MDA, nmol/mL	9.97	8.19	8.73	0.508	0.390

PA = *P. aibuhitensis*; MM = *M. maxidenticulata*; PN = *P. nuntia*; T-AOC = total antioxidant capacity; CAT = catalase; SOD = superoxide dismutase; LZM = lysozyme; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.
Within a row, means without a common superscript differ at $P < 0.05$.

(Fig. 4A) was the lowest. In addition, there were more secretory cells (B-cells) in the PA group, whereas the B-cells in the PN group were more developed. The ovarian tissue sections are shown in Fig. 5. All experimental groups were at either the prematuration stage or the maturation stage, with cortical rods or those in which the cortical rods were gradually disappearing. Overall, the density of yolk granules in mature oocytes was highest in the MM group, followed by the PN group, while the PA group had the lowest density (Fig. 5A–C).

3.8. Nutritional composition of three polychaetes species

3.8.1. Proximate compositions of three polychaetes species

There were significant differences in moisture, crude protein, crude lipid, and ash contents among the three polychaetes species (Table 8). The moisture content in *P. aibuhitensis* was significantly higher than that in the other two polychaetes, and *P. nuntia* was significantly higher than *M. maxidenticulata* ($P < 0.001$). Crude protein content in *M. maxidenticulata* was significantly higher than that in the other two polychaetes, and *P. aibuhitensis* was significantly higher than that in *P. nuntia* ($P < 0.001$). Besides, the crude lipid content in *P. nuntia* was significantly higher than that in the other two polychaetes, and the crude lipid content in *M. maxidenticulata* was significantly higher than in *P. aibuhitensis* ($P < 0.001$). The ash content of *P. nuntia* was the highest, followed by *P. aibuhitensis*, with *M. maxidenticulata* being the lowest ($P < 0.001$).

Table 7
Biochemical composition in hepatopancreas, serum and ovary of the female *L. vannamei* broodstock fed three fresh-live maturation diets.

Item	PA group (control)	MM group	PN group	SEM	*P*-value
Hepatopancreas					
Glu, mmol/L	12.46	16.80	12.32	1.252	0.280
TG, mg/mL	0.37	0.47	0.40	0.030	0.444
TP, mg/mL	2.95	2.83	2.92	0.055	0.734
E, pmol/L	61.15	70.92	70.15	2.210	0.122
VTG, ng/mL	171.71	178.11	166.02	10.711	0.922
Serum					
Glu, mmol/L	5.03	5.58	4.44	1.007	0.922
TG, mg/mL	0.95	1.29	1.35	0.079	0.051
TP, mg/mL	15.40	24.38	17.09	1.918	0.113
E, pmol/L	59.65	64.49	64.24	4.421	0.908
VTG, ng/mL	235.65[a]	226.57[a]	420.15[b]	34.180	0.003
Ovary					
Glu, mmol/L	7.25	4.90	10.55	1.135	0.109
TG, mg/mL	0.22[a]	0.38[b]	0.32[b]	0.028	0.013
TP, mg/mL	2.70	2.53	2.37	0.093	0.404
E, pmol/L	74.36	86.85	81.53	4.878	0.642
VTG, ng/mL	444.74	634.72	607.98	38.714	0.070

PA = *P. aibuhitensis*; MM = *M. maxidenticulata*; PN = *P. nuntia*; Glu = glucose; TG = triglycerides; TP = total protein; E = estradiol; VTG = vitellogenin.
Within a row, means without a common superscript differ at $P < 0.05$.

3.8.2. Amino acid composition of three polychaetes species

The amino acid composition in three polychaetes species is shown in Table 9. The total contents of NEAA and EAA in *M. maxidenticulata* was the highest, followed by *P. aibuhitensis*, with *P. nuntia* being the lowest. Among the NEAA, *M. maxidenticulata* had the highest contents of Ser, Glu, Ala, Tyr and Pro, but the lowest content of Gly. *P. aibuhitensis* had the second-highest contents of Glu, Ala, Tyr and Pro, but the lowest level of Ser while *P. nuntia* had the highest content of Gly. In terms of EAA, *M. maxidenticulata* had the highest contents of His, Trp, Phe, Met, Lys, Leu, Ile and Val, *P. aibuhitensis* had the highest content of Ile, while *P. nuntia* had the highest content of Arg.

3.8.3. Fatty acid composition of three polychaetes species

The fatty acid composition in three polychaetes species is shown in Table 10. *P. nuntia* had the highest saturated fatty acids (SFA) content followed by *M. maxidenticulata* and *P. aibuhitensis*. Among SFA, C16:0, and C18:0 content in *M. maxidenticulata* and *P. nuntia* was higher than that in *P. aibuhitensis*, while C21:0 content in *P. nuntia* was higher than that in *P. aibuhitensis* and *M. maxidenticulata*. The difference in monounsaturated fatty acids content was mainly reflected in the C16:1, C18:1 n-9t and C18:1 n-9c among all the experimental groups. The contents of PUFAs in *M. maxidenticulata* and *P. aibuhitensis* were higher than in *P. nuntia*, while the content of high unsaturated fatty acids (HUFAs) in *M. maxidenticulata* was higher than that in the other two polychaetes, and the differences were mainly reflected in the contents of ARA, EPA and DHA.

4. Discussion

Due to the wide variety, comprehensive nutrition, and high freshness, fresh-live diets can meet the natural nutritional needs of broodstock shrimp, hence hatcheries generally mix multiple types of fresh-live diets for feeding broodstock shrimp (Alfaro-Montoya et al., 2019; Chimsung, 2014; Hoa et al., 2009). However, there are significant differences in nutrition among different types of diets, hence difficult to prove which species is the most effective for a complete

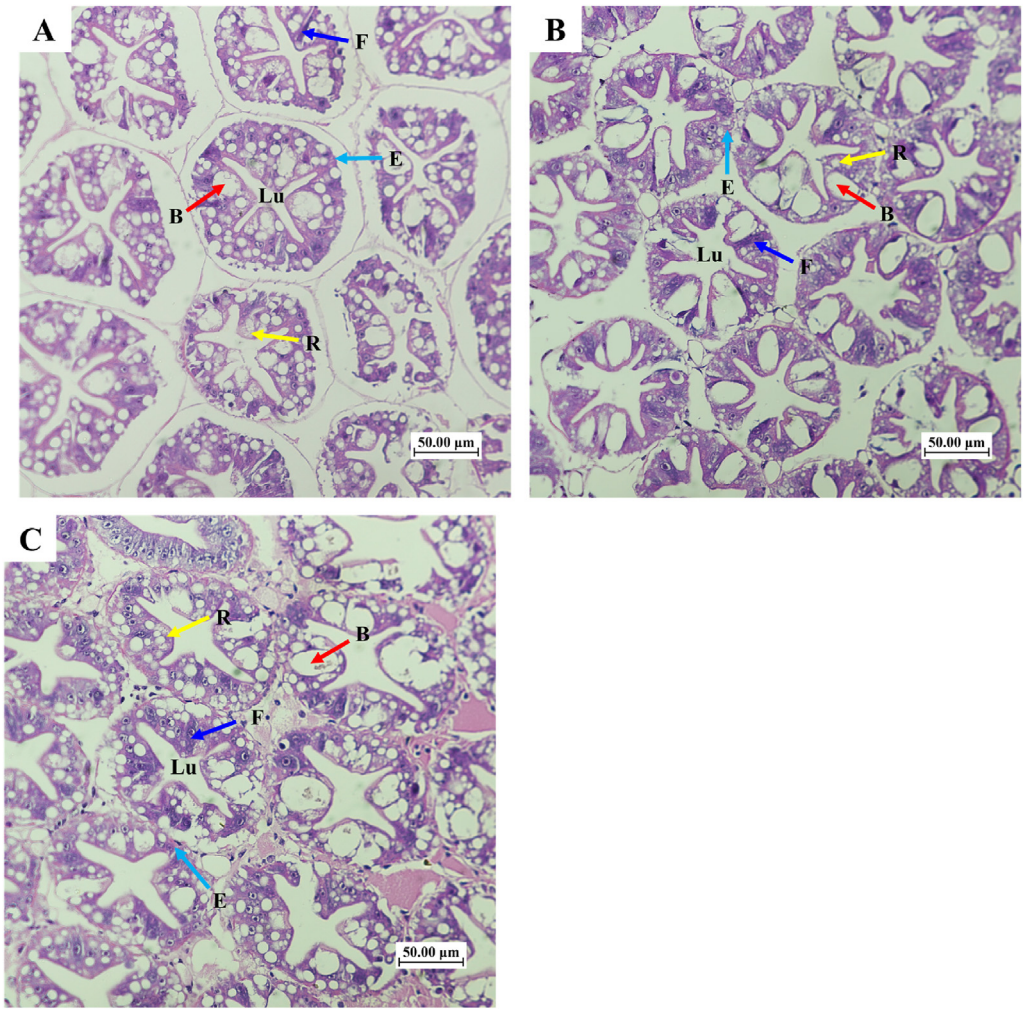

Fig. 4. Histology of hepatopancreas in female *L. vannamei*. broodstock fed three fresh-live maturation diets. (A) PA group (control). (B) MM group. (C) PN group. PA = *P. aibuhitensis*; MM = *M. maxidenticulata*; PN = *P. nuntia*; R = absorptive cells; B = secretory cells; F = fibrous cells; E = embryonic cells; Lu = lumen.

diet. The findings of this study indicated that the MM group had the highest total crude protein, amino acid contents, and PUFAs, especially those related to reproduction, such as ARA and EPA. From a nutritional perspective, the results indicate that *M. maxidenticulata* has a higher nutritional value. Studying the nutritional components of each type of fresh-live diet and its impact on the reproductive performance of broodstock shrimp individually may provide a more precise measure of the application value of each diet. This approach can also provide an accurate direction for the development of new diets and avoid the blind development of natural diets. Given that polychaetes are the most widely used in broodstock shrimp breeding, this study introduces two new species of polychaetes, using each as a single diet. For the first time, a comprehensive analyses of the growth, reproductive performance, biochemical indices, and histology were conducted to explore the feeding effect of three types of polychaetes on female broodstock shrimp. The aim was to verify the feasibility of polychaetes as a single diet and to select the best species of polychaetes among the three.

Growth performance is one of the most important indicators for evaluating the effect of diets. The type and content of protein in diets are the most critical factors affecting the growth and development of shrimps (Kureshy and Allen Davis, 2002; Lee and Lee, 2018; Xiao et al., 2023). In this study, the results on growth performance indicated that the MM group can significantly improve the WGR, SGR, and MR. These results were consistent with the highest crude protein content in *M. maxidenticulata* as MM group had the maximum PER. Additionally, the results of the proximate composition analysis showed that *P. nuntia* had lower crude protein and higher crude fat compared to *P. aibuhitensis*. However, the FBW, WGR and SGR in the PN group were significantly higher than those in the PA group. There may be two reasons for these results. First, the PN group had a higher PER. Second, when the protein content in the diet reaches a certain level, increasing the fat content can effectively conserve protein and still achieve the goal of improving the growth performance of aquatic animals, which might also be the main reason for the rapid development of high-fat feed in recent years (Wang et al., 2019).

During breeding stages, the HSI and GSI reflect the changes in nutrients in the hepatopancreas and the development of ovaries in shrimps (Bo et al., 2021; Flores et al., 2019). In this study, the MM group had the highest GSI and the lowest HSI, indicating that the MM group can maximize the transfer of substances from the hepatopancreas to the ovary during the process of ovarian development. Additionally, some studies have shown that highly unsaturated fatty acids (such as ARA, DHA, and EPA) in polychaetes can eventually accumulate in the ovaries and promote ovarian development (Deenarn et al., 2020; Meunpol et al., 2005). The findings of this study are consistent with this conclusion.

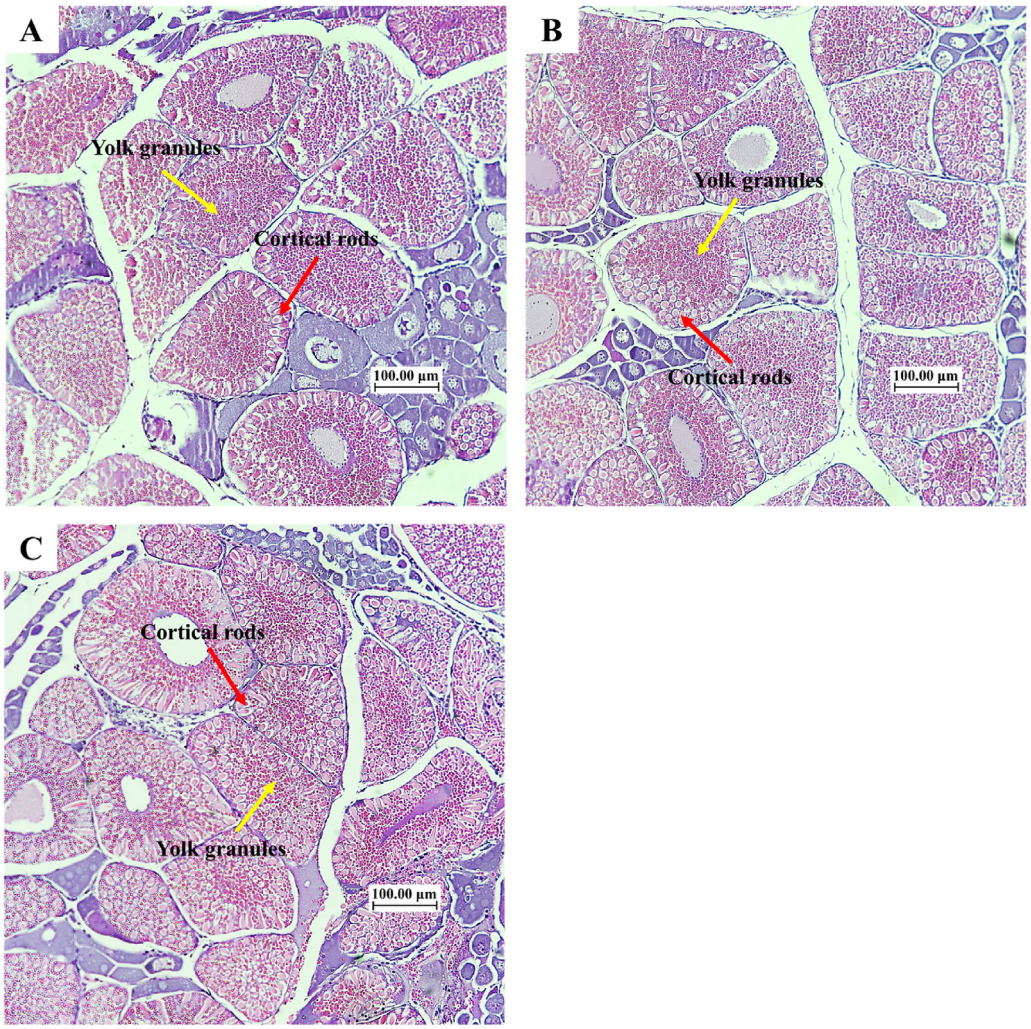

Fig. 5. Ovarian histology of female *L. vannamei*. broodstock fed three fresh-live maturation diets. (A) PA group (control). (B) MM group. (C) PN group. PA = *P. aibuhitensis*; MM = *M. maxidenticulata*; PN = *P. nuntia*. Red arrows point to cortical rods, yellow arrows point to yolk granules.

Table 8
Proximate composition of three polychaetes species (%, dry matter basis).

Item	*P. aibuhitensis*	*M. maxidenticulata*	*P. nuntia*	SEM	*P*-value
Moisture	80.19^{c}	72.74^{a}	77.30^{b}	0.834	<0.001
Crude protein	62.43^{b}	67.60^{c}	59.46^{a}	0.976	<0.001
Crude lipid	19.41^{a}	20.07^{b}	21.38^{c}	0.250	<0.001
Ash	10.75^{b}	8.43^{a}	12.01^{c}	0.415	<0.001

Within a row, means without a common superscript differ at $P < 0.05$.

Reproductive performance not only reflects the quality of broodstock shrimp and larvae but also serves as the most important and direct manifestation of the effect of diets (Racotta, 2003). Studies have shown that diets can significantly affect the reproductive performance of female broodstock shrimp (Hoa et al., 2009; Jin et al., 2022; Omar et al., 2020). In this study, MM group had the highest SMFS, SMC, MATR, MTGR, SC, TNFE, IFEY, AMO, TNN, INY and HR, indicating that feeding *M. maxidenticulata* can significantly enhance the reproductive performance of female broodstock shrimp. Yang et al. (2022) used four species of polychaetes, *M. maxidenticulata*, *Cheilonereis cyclurus*, *Nereis vexillosa*, and *Perinereis aibuhite* as diets for female *L. vannamei* broodstock, and the results showed that the broodstocks fed with *M. maxidenticulata* had higher GSI and higher gonadal development compared to those fed with *P. aibuhite*. This has also been confirmed in this study.

The hepatopancreas is the largest functional organ in shrimps, with functions of digestion and absorption as well as immune defense (Kulkarni et al., 2021; Vogt, 2019). During the maturation process of ovaries, the hepatopancreas absorb and digest plenty of nutrients to support the gonadal development, while lipid metabolism-related enzymes play an important role in this stage (Dyall et al., 2022; Teshima et al., 1988). The activities of lipid metabolism-related enzymes directly reflect the capacity for lipid digestion and absorption of the hepatopancreas. In this study, the highest activity of lipid metabolism-related enzymes in the MM group indicates that the broodstock shrimp fed with *M. maxidenticulata* can improve the digestion and absorption capacity of dietary lipids, implying that the hepatopancreas of the broodstock shrimp in the MM group has greater vitality. Additionally, the intestine is also one of the main organs for digestion and absorption, providing the main place for the colonization and proliferation of symbiotic microorganisms in aquatic animals (Ringø et al., 2016; Sun and Xu, 2021). The activities of amylase, lipase, and trypsin in the hepatopancreas and intestine of the MM group were the highest, followed by the PN group, with the PA group having the lowest. These results are consistent with the

Table 9
Amino acid composition in three polychaetes species (g/100 g, DM basis).

Item	*P. aibuhitensis*	*M. maxidenticulata*	*P. nuntia*
NEAA	28.80	31.21	28.31
Asp	5.19	5.35	5.04
Ser	2.03	2.34	2.07
Glu	8.22	8.28	7.30
Gly	4.43	3.07	6.12
Ala	3.69	4.80	3.29
Tyr	1.89	2.04	1.72
Pro	2.27	4.21	1.91
Cys	1.08	1.12	0.86
EAA	23.12	24.50	22.55
His	1.29	1.31	1.23
Arg	3.66	3.85	4.49
Trp	0.52	0.55	0.51
Phe	1.83	2.07	1.62
Met	1.23	1.27	0.93
Lys	3.70	4.32	4.20
Thr	2.49	2.58	2.00
Leu	3.61	3.68	3.44
Ile	2.30	2.29	1.96
Val	2.49	2.58	2.17

NEAA = non-essential amino acid; Asp = aspartic acid; Ser = serine; Glu = glutamic acid; Gly = glycine; Ala = alanine; Tyr = tyrosine; Pro = proline; Cys = cysteine; EAA = essential amino acid; His = histidine; Arg = arginine; Trp = tryptophan; Phe = phenylalanine; Met = methionine; Lys = lysine; Thr = threonine; Leu = leucine; Ile = isoleucine; Val = valine.

Table 10
Fatty acid composition in three polychaetes species (% of total FA).

Item	*P. aibuhitensis*	*M. maxidenticulata*	*P. nuntia*
C6:0	0.14	nd	0.19
C14:0	0.83	0.94	0.38
C15:0	0.49	0.45	0.72
C16:0	16.31	32.93	27.85
C17:0	1.10	1.61	2.03
C18;0	8.24	11.01	8.49
C20:0	0.24	0.20	0.20
C21:0	0.17	nd	11.63
C22:0	0.36	nd	0.07
C23:0	0.26	0.19	0.14
ΣSFAs	28.14	47.33	51.70
C15:1	0.53	0.33	0.66
C16:1	2.20	3.57	2.12
C17:1	0.91	0.63	0.52
C18:1 n-9t	2.30	9.00	6.86
C18:1 n-9c	21.73	14.77	14.40
C20:1 n-9	3.41	2.85	3.44
C22:1 n-9	0.20	0.24	0.23
C24:1 n-9	0.22	0.36	0.12
ΣMUFA	31.50	31.75	28.35
C20:2	7.46	4.60	0.27
C22:2	0.12	0.17	0.28
C18:2 n-6t	0.18	0.18	0.13
C18:2 n-6c	5.58	3.46	9.99
C18:3 n-3	1.12	0.44	1.22
C18:3 n-6	0.18	0.25	0.16
C20:3 n-3	0.26	0.15	0.49
C20:3 n-6	0.29	0.87	0.67
C20:4 n-6(ARA)	2.48	4.93	2.43
C20:5 n-3(EPA)	3.36	6.79	4.06
C22:6 n-3(DHA)	2.46	2.36	0.52
ΣPUFA	23.49	24.20	20.22
ΣHUFA	8.85	15.10	8.17

nd = not detected; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids (fatty acids with two or more double bonds); HUFA = highly unsaturated fatty acids (C20:3 n-3, C20:3 n-6, ARA, EPA, and DHA).

growth performance of each experimental group, including WGR, SGR, MR and PER. Previous research has indicated that, within a certain range, the activities of lipid metabolism-related enzymes and digestive enzymes in shrimp increase with the increase in lipid or protein contents in diets (Gong et al., 2012; Liou et al., 2023). In this study, the highest crude protein and unsaturated fatty acid contents in *M. maxidenticulata* may be the fundamental reason for the highest growth performance and the highest digestive enzyme activities in the hepatopancreas and intestine of the MM group.

Shrimp primarily rely on their innate immune system to defend against the invasion of foreign pathogens, while the hepatopancreas and serum are the most important components of the shrimp's immune system (Kulkarni et al., 2021; Vazquez et al., 2009; Vogt, 2019). Shrimp produce a variety of antioxidative enzymes (such as T-AOC, SOD, CAT, GSH-Px) and immune enzymes during their normal life activities to neutralize free radicals, protect cell membranes, and inhibit bacterial proliferation (Vazquez et al., 2009). Shrimp with higher T-AOC often exhibit greater reproductive capacity. Healthy gonads are vital for gamete production and maturation, and increased T-AOC helps preserve their integrity and function, enhancing reproductive performance. During critical developmental stages, embryos are particularly susceptible to oxidative damage, while higher T- AOC ensures a nurturing environment by neutralizing ROS, supporting normal embryonic development (Ramos-Rodríguez et al., 2024). SOD serves as the first enzymatic line of defense that converts superoxide radicals into less harmful molecules, and it is crucial for maintaining the redox balance necessary for gonadal health and normal gamete function (Bal et al., 2021; Ighodaro and Akinloye, 2018). Glutathione peroxidase can reduce hydrogen peroxide and lipid peroxides, playing a vital role in protecting gonadal cells from oxidative damage, thereby supporting reproductive health and the production of viable offspring (Covarrubias et al., 2008). In summary, a strong antioxidant system, including T-AOC, SOD, and GSH-Px, is crucial for optimal reproductive performance in broodstock shrimp. These antioxidants protect the gonads from oxidative stress, ensuring the production of healthy gametes and successful reproduction. Additionally, LZM in shrimp is one of the most important immune active enzymes in innate immunity. Lysozyme can break down bacterial cell walls, especially those of Gram-positive bacteria, finally killing or inhibiting bacterial growth (Peregrino-Uriarte et al., 2012). The findings indicated that the MM group had the highest activities of T-AOC, SOD, GSH-Px, and LZM suggesting that *M. maxidenticulata* significantly enhances the reproductive performance of female broodstock shrimp. These results were consistent with the reproductive performance outcomes observed in our study.

Additionally, the hepatopancreas, serum, and ovaries are the main metabolic sites in shrimp, so analyzing the biochemical composition of these tissues can help understand the nutritional requirements and metabolic processes of broodstock shrimp, especially during the critical ovarian maturation period (Kumar et al., 2018; Teshima et al., 1988; Teshima and Kanazawa, 1983). In this study, the Glu content in both the hepatopancreas and serum of the MM group was at the highest level, followed by the PA group, with the PN group having the lowest content. The result of this study was consistent with the finding that amylase activity was highest in the hepatopancreas of shrimp fed with *M. maxidenticulata*. Triglycerides, the most important intermediate products of fatty acid metabolic pathways, can indirectly reflect the level of lipid metabolism in various tissues during the ovaries maturation stage (Alves-Bezerra and Cohen, 2018; Teshima et al., 1988). The results of this study showed that the TG content in both serum and ovaries of the MM group was the highest, and was significantly higher than that in the PA group. The highest content of HUFAs (such as ARA, EPA, DHA) in the *M. maxidenticulata* may be the main reason (Deenarn et al., 2020; Meunpol et al., 2005). In addition, estradiol is an important sex hormone and plays a crucial

role in the development of shrimp ovaries. It not only directly participates in the maturation of the ovaries and the formation of yolk but may also indirectly affect ovarian development by influencing lipid metabolism and endocrine regulation (Liang et al., 2023). In this study, the contents of E and TVG in the hepatopancreas, serum, and ovaries of the MM group were the highest, and its TVG content was significantly higher than that in the PA group. This result was consistent with the highest reproductive indices in the MM group, such as TNFE, IFEY, TNN, INY and HR. These results indicate that the higher the content of TG and VTG in mature oocytes, the more energy substances accumulated, and the higher the quality of fertilized eggs and larvae. Overall, in this study, the hepatopancreas had the highest glucose content, serum had the highest TG and TP contents, and ovaries had the highest E and VTG contents. These results are consistent with previous research outcomes (Teshima et al., 1988).

The histological sections of hepatopancreas and ovaries enable the most direct observation of morphological and structural differences among all the experimental groups. The epithelial cells in the hepatic tubules are divided into absorptive cells (R-cells), B-cells, fibrous cells (F-cells) and embryonic cells (E-cells) according to their function (Al-Mohanna et al., 1985; Al-Mohanna and Nott, 1987; Vogt, 1993). The B-cells in the hepatic tubules mainly secrete various enzymes and immune active substances to degrade and absorb substances and defend against the invasion of foreign pathogens, while R-cells are responsible for the absorption and storage of nutrients (Vogt, 2019, 1994). The histological sections of hepatopancreas in this study showed that the MM group had the most developed B-cells. This finding was consistent with the highest levels of fat metabolism-related enzymes, digestive enzymes, antioxidant and immune capacity in the hepatopancreas of MM group. In addition, there were more B-cells in the PN group, whereas the B-cells in the PA group were more developed, which may also be the main reason why there was no significant difference in lipid metabolism-related enzymes and digestive enzyme activities between PA and PN groups. Furthermore, the histological results of the hepatopancreas were consistent with the results of growth and biochemical indices in each experimental group.

The development of shrimp gonads is primarily reflected in the morphological and structural changes of the ovaries and oocytes. During the process of ovarian development, the appearance of the cortical rods signifies that the oocyte has entered the maturation phase, and the size of the mature oocyte and the density of the yolk proteins within the oocyte directly reflect the quality of the oocytes (Craveiro et al., 2023; Farhana and Ohtomi, 2016). In this study, the histological section results showed that the structure of the cortical rods appeared in all the experimental groups, indicating that the oocytes had entered the maturation phase, which was consistent with the visual judgement that the broodstock shrimps were in the mature stage. Additionally, MM group had the highest density of yolk granules in mature oocytes, indicating better oocyte quality. This is reflected in the hatching rate of fertilized eggs and the deformity rate of nauplius larvae.

5. Conclusions

M. maxidenticulata can significantly improve growth, reproductive performance, the activities of lipid metabolism-related enzymes and digestive enzymes, antioxidant and immune ability of female *L. vannamei* broodstock. *M. maxidenticulata* had the highest crude protein and was rich in HPFAs compared with the other two polychaetes. The results of this study indicated that *M. maxidenticulata* is a potential high-quality diet for female broodstock shrimp, and higher crude protein and HUFAs contents in *M. maxidenticulata* can boost the development and maturation of ovaries in female broodstock shrimp. However, we do not support the in-depth development and large-scale factory farming of *P. nuntia*, because its effect on growth and reproductive performance of female broodstock shrimp did not outperform that of *P. aibuhitensis*. In the future, we will more precisely determine the fatty acid and amino acid composition of broodstock shrimp gonads at different stages and fertilized eggs to explore the effect of different polychaete species on the nutritional composition of broodstock gonads and fertilized eggs. Furthermore, by integrating multi-omics approaches, including transcriptomics, metabolomics, proteomics, and lipidomics, in conjunction with the nutrition of different polychaetes species, we will delve into the effect on broodstock gonadal development and the mechanisms underlying it.

Credit Author Statement

Shuaipeng Li: Writing – original draft, Investigation, Data curation. **Hao Liu:** Data curation. **Weibin Huang:** Writing – review & editing. **Shipei Yang:** Writing – review & editing. **Mingsheng Xie:** Software. **Menglong Zhou:** Software. **Baiquan Lu:** Conceptualization. **Biao Li:** Formal analysis. **Beiping Tan:** Supervision, Funding acquisition. **Yuanzhi Yang:** Data curation. **Xiaohui Dong:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.11.020.

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