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Research Article

Pellet quality and growth performance of whiteleg shrimp, *Litopenaeus vannamei,* fed with different dietary pellet binders

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Abstract

The current study investigated the effects of different pellet binders on the pellet quality, growth performance, hemolymph biochemistry, and intestinal morphology of whiteleg shrimp for 3 months. The shrimp were randomly assigned to four dietary groups (three replicates/ group) in a polyethylene circular tank containing seawater. Experimental treatments included, the control group fed with the basal diet (BD) (G1, no pellet binder), and G2, G3, and G4 received the BD containing different pellet binders, including calcium lignosulphonate (at 1 % of diet), starch + gum based-binder (at 0.4 % of diet), and polymethyl carbamide (PMC) (at 0.5 % of diet), respectively. Different binders improved the pellet quality parameters, including water stability, leaching rate ($p \geq 0.05$), and water activity ($p \leq 0.05$), with a particularly pronounced improvement effect in the PMC-bound pellets. No differences ($p \geq 0.05$) were found in shrimp growth and feed efficiency utilization, whole body composition of nutrients, and digestive enzyme activities in response to the different pellet binders. The diets processed with starch+ gum, as well as lignosulfonate binders, resulted in higher glucose levels in shrimp hemolymph. Feeding shrimp with pellet binders significantly reduced hemolymph concentrations of triglycerides, total cholesterol, high- and low-density lipoproteins, and very low-density lipoprotein, with the most pronounced reductions occurring in the PMC and starch + gum-based binder groups ($p \leq 0.05$). In conclusion, the pellet binders evaluated in this study were beneficial in enhancing pellet quality; however, they did not improve shrimp growth performance. Polymethyl carbamide (an inclusion level of 0.5% of diet), gave the best results in both pellet quality and performance. However, findings suggest that PMC is more suitable for short-term application during shrimp culture. Also, suggest that starch+ gum-based binder could be used as a practical alternative to PMC since it enhances pellet quality and nutritional characteristics, while supporting shrimp performance without adverse impacts.

Introduction

Crustaceans, including shrimp, crayfish, and crabs, are recognized globally as edible and nutritionally valuable aquatic organisms (FAO, 2020). The worldwide production of shrimp has increased rapidly with the development of aquaculture practices and the increasing demand for seafood products. The success and sustainability of the aquaculture sector depend on the continuous improvement of feed formulations and the efficiency of utilization to minimize feed operation costs, which typically account for 50–60% of total costs (Sinha *et al.,* 2011).

Pellet water stability and nutrient leaching are major concerns in crustacean feeding compared to fish. Crustaceans, including shrimp, are slow-feeding aquatic animals that extensively manipulate their food before ingesting it, which may cause pellet dispersion in water (Argüello-Guevara and Molina-Poveda 2013). Therefore, sinking and water-stable diets are necessary for these animals. Pellets should be bound firmly enough to remain intact in water for an extended period and to withstand disintegration and nutrient leaching during feeding (Goldblatt *et al.,* 1980; Farmanfarmaian *et al.,* 1982).

The water stability of aqua feeds is influenced by several factors, including diet composition, manufacturing process, and type of binders used (DeSilva and Anderson, 1995). Binders are crucial for maintaining nutrients in feed pellets, stabilizing their form during transport and storage, and preventing nutrient loss into the surrounding water when feed is delivered to fish (Yamamoto and Akiyama, 1995). Binders used in aqua feeds can be classified as, plant extracts (carrageenan, alginates, agar, starches, pectin's, molasses and a wide variety of gums), animal extracts (collagen), polymers (urea formaldehyde), wood-processing by-products (lignin sulfonate, hemicelluloses and carboxymethyl cellulose), and minerals (bentonite) (Cuzon *et al.,* 1994; Stephen Sampath Kumar and Judith Betsy, 2014). There are three ways in which binders impact the stability of pellets; they eliminate void spaces, resulting in more compact and durable pellets; they function as adhesives to hold particles together; and they perform chemical action on the components, altering the composition of the feed (DeSilva and Anderson 1995; Ruscoe *et al.,* 2005). Previous trials have evaluated some pellet binders, such as sodium alginate, wheat gluten, guar gum, agar, and carboxymethyl cellulose in shrimp, lobster, and crayfish diets, showing positive effects on growth performance (Dominy *et al.,* 2004; Palma *et al.,* 2008; Volpe *et al.,* 2008; Simon, 2009; Argüello-Guevara and Molina-Poveda, 2013). The effectiveness of a diet formulation's choice of binder and the level of its inclusion in the diet will influence the overall performance of pellets in terms of nutrient leaching and water stability. Therefore, research focusing on the selection of appropriate binders to ensure consistency in the experimental feed must consider their effects not only on feed stability but also on the productive performance of the species under study. In the present study, the binders examined (calcium lignosulphonate, starch+ gum-based binder, polymethyl carbamide) represent the different sources of binders mentioned previously. Diets containing

these binders were tested to assess their quality and were administered to shrimp, *Litopenaeus vannamei,* to examine their potential effects on growth performance, hemolymph biochemistry, and intestinal morphology.

Materials and methods

The current experiment was conducted at Ghalioun Farm, Kafrelsheikh, Egypt. The management procedures followed during this experiment were approved by the Animal Care and Ethics Committee of the Faculty of Veterinary Medicine, Alexandria University, Egypt (AU 013-2022/03/14-1-118).

Shrimp culture and study design

Two hundred and forty shrimp, *Litopenaeus vannamei* (2 months old), were used in this experiment. They were obtained from the Ghalioun hatchery, Kafr El-Sheikh, Egypt. Shrimp were acclimated for two weeks in experimental tanks (polyethylene circular tank with a capacity of 350 L) before the start of the study. They were individually weighed (body weight 9.3±0.25 g) and stocked at a density of 20 shrimp per replicate (three replicates per group) over a three-month rearing period. They were assigned to four treatments as follows: G1 (control) fed the basal diet (BD) without binder; G2 fed the BD containing calcium lignosulphonate (Lignobond which contains 93% calcium lignosulphonate, with a recommended addition level of 10 kg/ ton; Nutrivet Misr Co., Giza, Egypt; G3 fed the BD containing a starch + gum-based binder (Master cube, which contains guar gum (50 g/ kg), xanthan gum (50 g/ kg), starch (150 g/ kg) and calcium sulphate (750 g/ kg), with a recommended addition level of 4 kg/ ton, Al Buraq Co. El-mansoura, Dakhalia, Egypt; G4 which fed the BD containing PMC (HY-Bound, which contains 95% polymethyl carbamide, with a recommended addition level of 5 kg/ ton, produced by Hengyi Vet Co., Ltd, China, and distributed by Reda chemicals, Dammam, Saudi Arabia, as the sole agent in Egypt. These binders were added to the BD at the expense of wheat middlings.

The basal diet was formulated to meet the nutrient requirements of shrimp (NRC, 2011). These diets were prepared in a commercial feed plant at the Ghalioun feed factory (Ghalioun farm, Kafr El-Sheikh, Egypt), with a production capacity of 20 tons/ hour. The ground dry ingredients shown in Table 1 were mixed for 90 s in a feed mixer (first mixing), after which oil was added, followed by water (second mixing, 120 s). Water was added to the feed mixture to obtain a suitable consistency for pelleting. Diets were then pressure-pelleted using a pellet mill with a 2.5 mm die at a temperature of 80 ˚c and dried at 90-100˚C in a hot–air drier to achieve a moisture content of less than 10% (drying time about 15-20 min), followed by cooling of the pellets in a cooler.

Shrimp were fed their respective experimental diets at a feeding rate starting at 4% body weight (BW) at the beginning of the experiment and reduced to 3.8 % during the last month. The amount of feed was adjusted every two weeks according to the biomass of shrimp in each tank. The daily ration was divided into two feedings (8:00 am and 4:00 pm). Water quality was monitored after feeding, and care was taken

to ensure that no uneaten feed remained in the tanks. Approximately 30 % of the water in each tank was renewed with seawater two to three times per week.

Table 1: Ingredient composition of the used basal diet.

Ingredients (%)	
Wheat middlings	25.5
Fish meal (65%)	14.5
Soybean meal (46 %)	21.25
Corn gluten (60 %)	6.0
Poultry byproduct	13.0
Rice bran	9.065
Dried distiller's grains	7.0
Soya Oil	1.5
Shrimp vitamin premix[1]	0.15
Shrimp mineral premix[2]	0.15
Monocalcium phosphate[3]	1.0
Choline chloride	0.20
Vitamin C	0.025
Phospho-lipid soy lecithin	0.50
Mycotoxin binder	0.035
Immune stimulant[4]	0.125
Pellet binder	0.0
Chemical composition (%)	
Moisture	9.05
Crude protein	37.6
Ether Extract	7.77
Ash	10.13
Calcium	1.89
Phosphorus	1.18

[1]Each 1.5 kg contains: Vit A (13000000IU), Vit D (3500000IU), Vit E (150000 mg), Vit K_3 (50000mg), Vit B_1 (75000mg), Vit B_2 (50000mg), VitB_6 (75000mg), Vit B_{12} (30 mg), Nicotinic acid (30000mg), Pantothenic acid (150000mg), Folic acid (15000mg), Biotin (1500mg) and carrier limestone up to 1.5 kg. [2]Each 1.5 kg contains: Iron (100000mg), Copper (35000mg), Zinc (150000mg), Manganese (30000mg), Iodine (5000mg), Selenium (1000mg), Cobalt (50mg), and carrier limestone up to 1.5 kg**.** [3]Monocalcium phosphate contains 16.90% calcium and 22.73% phosphorus. [4]Diamond V XP yeast culture, diamond V mills, Cedar Rapids, Iowa, USA.

During the feeding trial, water temperature and dissolved oxygen (DO) were measured twice daily at the center of each aquarium using a multiparameter probe meter (HI9829-03042-HANNA® instruments, www.hannainst.com). Salinity was measured with a Handheld refractometer (ATAGO CO., LTD). Water PH, total ammonia nitrogen (TAN), and total dissolved solids (TDS) were measured once per week. TAN was determined using the hypo-bromate oxidimetric method, while TDS was measured with a turbidimeter (HACH2100N, China). Throughout the experiment, water quality parameters were maintained within the following ranges (mean±SE), dissolved oxygen, 5.09±0.03 mg/L; temperature, 26.54±0.21°C; TDS, 38.35±7.95 mg/L; pH, 7.8±0.05; salinity, 35.50±2.13 ppt; and TAN, 0.25±0.05 mg/L.

Sampling and measurements

Pellet quality

Feed samples from each group (n=3 /group) were collected and analyzed to assess quality parameters:

Water stability of feed: The samples were immersed in seawater for varying durations (30 min, 1 h, and 2 h). After immersion, pellets were recovered, oven-dried at 60°C for 24 h, and then reweighed (Ahamad-Ali *et al.,* 2005):

$$\text{Water stability (\%)} = \frac{Final\ pellet\ weight * dry\ matter}{Initial\ pellet\ weight * dry\ matter} \times 100$$

The percentage of dry matter leaching was recorded as an index of water stability:

$$\%\ Nutrient\ leaching = \frac{Initial\ pellet\ weight - Final\ pellet\ weight}{Initial\ pellet\ weight} \times 100$$

The water activity of the feed was measured using a water activity meter (Rotronic HP23-AW-A Water Activity Meter), while the moisture content was determined by oven-drying at 105ºC for 3 h (AOAC, 2005).

Shrimp growth and feed efficiency

Using a digital balance, shrimp were individually weighed at the beginning and end of the trial, counted, and weighed at two-week intervals throughout the whole experimental period. Feed intake (FI) was calculated as the difference between the feed offered and the residual feed. Shrimp length was measured using a measuring board. The following parameters were calculated:

$$Weight\ gain\ (WG.g) = \frac{Final\ body\ weight}{Initial\ body\ weight}$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{Feed\ intake\ (g)}{Weight\ gain\ (g)}$$

$$Protein\ efficency\ ratio\ (PER) = \frac{Weight\ gain\ (g)}{Protein\ intake\ (g)}$$

$$Efficency\ of\ energy\ utilization\ (EEU) = \frac{Energy\ intake\ (Kcal)}{Weight\ gain\ (g)}$$

$$Specific\ growth\ rate(SGR) = \frac{Log\ final\ weight - Log\ initial\ weight}{T\ (the\ rearing\ peroid\ in\ days)} \times 100$$

Hemolymph biochemical variables

Hemolymph was withdrawn (9 shrimp per treatment) from the ventral sinus into a 1-ml disposable syringe containing 1 mL of ice-cold anticoagulant solution (450 mM NaCl, 100 mM glucose, 26 mM citric acid, 30 mM sodium citrate, pH 4.6). Samples were centrifuged at 3000 g /10 min at 4◦C (Abdel-Rahim *et al.,* 2021). The obtained plasma was stored at -20°C until use. Triglyceride (TG), total cholesterol (TC), high- and low-density lipoprotein (HDL and LDL, respectively), glucose, kidney function-related parameters (uric acid and creatinine), liver function enzymes (glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT)), were measured using commercial kits obtained from Bio-diagnostic Co., Egypt.

Shrimp whole-body composition

At the end of the experiment, 9 shrimp per group were collected, weighed, oven-dried at 105°C for 3 h, ground, and analyzed for

crude protein (CP) and ether extract (EE) content according to AOAC methods (AOAC 2005). CP and EE retention were calculated as follows:

$$CP\ retention = \frac{Final\ body\ CP - Initial\ body\ CP}{CP\ intake} \times 100$$

$$EE\ retention = \frac{Final\ body\ EE - Initial\ body\ EE}{EE\ intake} \times 100$$

Shrimp intestinal morphology

By the end of the feeding trial, the whole intestine was randomly collected from three shrimps per aquarium, fixed in 10% neutral buffered formalin for 48h, sectioned, stained with hematoxylin and eosin (H&E), and examined under a light microscope (Bancroft *et al.,* 2013).

Determination of digestive enzymes

The digestive tract (n=9 shrimp per group) was excised, immediately frozen in liquid nitrogen, and stored at -80°C. Digestive tissue was homogenized in nine volumes of ice-cold 100 mM Tris-HCl buffer containing 0.1 mM EDTA and 0.1% (v/v) Triton X-100, pH 7.8 (Radhakrishnan *et al.,* 2015). Homogenates were centrifuged at 30,000 g for 30 min at 4°C, and the resulting supernatants were stored in aliquots at -80°C for subsequent enzyme assays. Amylase activity was determined by the starch-hydrolysis method of Bernfeld (1955). Lipase activity was measured according to Furné *et al.* (2005), and total protease activity was determined by the casein-hydrolysis method described by Furné *et al.* (2005).

Statistical Analysis

The obtained data were analyzed by one-way ANOVA to test the effects of different pellet binders in the shrimp diet. Duncan's post-hoc test was applied for multiple comparisons, and statistical significance was set at $p \leq 0.05$. Results are presented as means and standard error (SE).

Results

Pellet quality

Table 2 shows the effect of different pellet binders (calcium lignosulfonate, starch + gum-based binder, and PMC) on the pellet quality parameters. Pellet water stability measured at different immersion periods (30 min, 1 h, and 2 h) was non-significantly higher with PMC compared with other binders or the control pellets ($p \geq 0.05$). Additionally, dietary inclusion of different binders non-significantly reduced the pellet leaching rate over the same immersion periods compared with pellets manufactured without a binder ($p \geq 0.05$). Among the treatments, PMC- containing pellets recorded the lowest leaching rate. The water activity (aW) of pellets was significantly reduced with calcium lignosulfonate and PMC binders compared with the control or pellets manufactured with starch + gum-based binder ($p \leq 0.05$). Pellet moisture content showed a similar trend, with the lowest content recorded in pellets containing PMC and lignosulfonate ($p \leq 0.05$).

Table 2: Effect of different pellet binder sources on the pellet quality parameters.

Parameter	Control	Calcium lignosulphonate	Starch + gum-based binder	Polymethyl carbamide	*P-value*
Leaching rate 30 min	15.43±1.17	13.79±0.61	13.48±0.24	9.79±2.19	0.064
Leaching rate 1h	19.13±1.31	18.23±0.37	16.85±0.42	12.77±2.82	0.090
Leaching rate 2h	22.53±1.27	21.39±0.17	20.66±0.57	16.80±2.12	0.062
Water stability 30 min	84.57±1.17	86.20±0.61	86.52±0.28	90.21±2.19	0.073
Water stability 1h	80.87±1.31	81.77±0.37	83.15±0.42	87.22±2.82	0.086
Water stability 2h	77.64±1.27	78.60±0.17	79.33±0.57	83.19±2.12	0.059
Moisture content (%)	9.28±0.02 [a]	8.22±0.06 [b]	9.22±0.14 [a]	8.35±0.08 [b]	0.000
Water activity (aW)	0.60±0.00[b]	0.55±0.00[d]	0.62±0.00[a]	0.57±0.00[c]	<0.0001

Values are means ± SE. Mean values with different letters in the same row differ significantly at ($p \leq 0.05$).

Shrimp growth performance and feed efficiency

Table 3 illustrates shrimp growth performance in response to different pellet binders. Final BW and WG showed no significant difference among groups; however, both parameters tended to increase in shrimp fed PMC and starch + gum-based binders compared with the control ($p \geq 0.05$). Total FI was non-significantly higher in shrimp fed diets containing pellet binders, with the highest intake observed in the PMC and starch + gum-based binders ($p \geq 0.05$). Average FCR for the entire experiment followed the same trend, with the best values recorded in shrimp fed PMC binder and starch + gum-based binders, whereas the poorest FCR was observed in shrimp fed calcium lignosulfonate fed shrimp ($p \geq 0.05$). Similarly, average PER and EEU did not differ significantly among groups, though both parameters were numerically enhanced in shrimp fed PMC ($p \geq 0.05$).

Table 3: Growth performance and feed efficiency utilization of shrimp fed different pellet binders.

Growth performance	Control	Calcium lignosulphonate	Starch + gum-based binder	Polymethyl carbamide	*P-* value
Initial BW (g)[1]	9.32±0.41	9.15±0.35	9.53±0.09	9.15±0.17	0.757
Final BW (g)[2]	24.43±1.12	22.27±0.21	25.02±0.79	25.80±1.40	0.147
Weight gain (g)	14.98±1.16	13.02±0.43	15.36±0.70	16.55±1.27	0.149
Total FI (g)[3]	38.54±0.40	37.82±0.86	40.21±0.82	40.41±0.81	0.102
Average FCR[4]	2.63±0.24	2.92±0.05	2.65±0.07	2.48±0.19	0.323
Average SGR[5]	0.49±0.03	0.46±0.02	0.49±0.01	0.53±0.02	0.184
Average PER[6]	1.03±0.09	0.92±0.02	1.02±0.03	1.09±0.09	0.339
Average EEU [7]	9.34±0.84	10.36±0.18	9.40±0.26	8.78±0.67	0.323

Values are means ± SE. Mean values with different letters in the same row differ significantly at ($p \leq 0.05$). [1]Initial BW= initial body weight; [2]Final BW= final body weight; [3]FI= feed intake; [4]FCR= feed conversion ratio (FI/ weight gain); [5]SGR= specific growth rate; [6]PER= protein efficiency ratio (weight gain(g)/ protein intake); [7]EEU= efficiency of energy utilization.

Hemolymph biochemical parameters

As presented in Table 4, hemolymph concentrations of uric acid, creatinine, GPT, and GOT showed no significant difference among treatments; however, activities of liver function enzymes were numerically higher in shrimps fed PMC ($p \geq 0.05$). Additionally, glucose

concentration was significantly increased in shrimps fed diets containing different binders compared with the control group *(p≤0.05*). Shrimp fed starch+gum-based and lignosulfonate binders showed higher hemolymph glucose levels than those fed the control or PMC diets. Hemolymph lipid profile constituents (triglyceride, total cholesterol, LDL, HDL, VLDL, and TC/HDL ratio) varied significantly among binder groups ($p\leq$ 0.05) (Table 5). Triglyceride and VLDL concentrations were significantly reduced in shrimp fed diets containing different pellet binders, with the lowest values observed in those fed PMC and starch + gum-based binders ($p\leq$ 0.05) compared with the control. Hemolymph total cholesterol was also significantly reduced in shrimp fed the starch + gum-based binder compared with the other groups ($p\leq$ 0.05). A similar trend of reduction was observed for HDL, as dietary inclusion of different binders was associated with lower hemolymph HDL ($p\leq$0.05), with the lowest concentration recorded in shrimp fed PMC. In contrast, LDL concentrations were significantly higher in shrimp fed calcium lignosulfonate and PMC compared with other groups ($p\leq$0.05). Consequently, due to the combined effects on total cholesterol and HDL, the total cholesterol/HDL ratio was significantly increased in shrimp fed PMC ($p\leq$0.05) compared with other groups.

Table 4: Hemolymph biochemical changes in shrimp fed different pellet binders.

Parameter	Control	Calcium lignosulphonate	Starch + gum-based binder	Polymethyl carbamide	*P- value*
GOT (u/mL)[1]	15.55±1.02	16.11±0.48	14.64±0.74	17.24±0.49	0.105
GPT (u/mL)[2]	7.09±1.04	7.43±0.30	7.35±0.48	7.48±0.50	0.975
Glucose (mg/dl)	68.13±1.69[b]	100.2±4.47[a]	102.21±10.62[a]	75.96±2.11[b]	<0.0001
Uric acid (mg/dl)	5.4±0.42	4.78±0.32	4.91±0.25	5.42±0.22	0.386
Creatinine (mg/dl)	0.79±0.14	1.23±0.23	0.52±0.09	0.84±0.18	0.062

Values are means ± SE. Mean values with different letters in the same row differ significantly at ($p\leq$0.05).
[1] GOT=glutamic oxaloacetic transaminase;
[2] GPT=glutamic pyruvic transaminase.

Table 5: Hemolymph lipid profile in shrimp fed different sources of pellet binders.

Parameter	Control	Calcium lignosulphonate	Starch + gum-based binder	Polymethyl carbamide	*P-value*
TC (mg/dl)[1]	197.90± 10.36 [a]	198.06± 10.66 [a]	157.34 ±9.74 [b]	185.59±9.32[ab]	0.024
Triglyceride (mg/dl)	201.90±6.22[a]	177.68±10.38[ab]	151.52±10.99[b]	159.61±11.16[b]	0.004
HDL (mg/dl)[2]	57.73±1.80[a]	43.93±2.96[b]	44.06±3.09[b]	32.4±3.32[c]	0.000
LDL (mg/dl)[3]	٩٩,٩٠±6.57[b]	117.40± 3.44 [a]	84.76±11.87 [b]	120.14±4.4 [a]	0.001
VLDL(mg/dl)[4]	40.78±.56[a]	36.73±2.86[ab]	28.52±2.63[b]	33.05±2.01[ab]	0.007
TC/HDL	3.59±0.28 [b]	4.62±0.34 [b]	3.75±0.38 [b]	6.45±0.68 [a]	0.0000

Values are means ± standard error. Mean values with different letters in the same row differ significantly at ($P\leq$ 0.05).
[1] TC= total cholesterol;
[2] HDL= high-density lipoprotein;
[3] LDL= low-density lipoprotein;
[4] VLDL= very low-density lipoprotein.

Digestive enzymes

Dietary inclusion of different pellet binders had no significant effect on digestive enzyme concentrations (lipase, amylase, and protease) ($p \geq 0.05$), although enzyme levels tended to be higher in shrimps fed PMC binder (Table 6).

Table 6: Digestive enzymes of shrimp fed different sources of pellet binders.

Enzyme	Control	Calcium lignosulphonate	Starch + gum-based binder	Polymethyl carbamide	*P- value*
Protease	2.57±0.09	2.61±0.13	2.53±0.16	2.74±0.07	0.668
Lipase	436.76±5.1	446.63±6.76	447.8±3.39	451.36±5.96	0.336
Amylase	1175.83±26.66	1192.1±17.21	1201.56±14.12	1212.6±6.68	0.540

Values are means ± SE. Mean values with different letters in the same row differ significantly at ($p \leq 0.05$).

Shrimp whole body composition

Diets processed with different binders significantly affected carcass relative weight and head + shell relative weight of shrimp ($p \leq 0.05$) (Table 7). Shrimp fed the calcium lignosulfonate binder exhibited the lowest carcass relative weight and the highest head+ shell relative weight compared with other experimental groups ($p \leq 0.05$). In contrast, inclusion of pellet binders had no significant effect on body dry matter, moisture, or crude protein content ($p \geq 0.05$). However, shrimp fed diets containing starch+ gum-based binder showed increased ether extract percentage and improved ether extract retention.

Table 7: Carcass traits and whole-body composition in shrimp fed different sources of pellet binders.

	Control	Calcium lignosulphonate	Starch + gum-based binder	Polymethyl carbamide	*P- value*
Carcass traits					
Carcass relative wt.	53.25±0.88 [a]	48.19±1.75 [b]	51.63±0.71 [a]	53.51±.51 [a]	0.005
Head+ shell relative wt.	46.74±0.88[b]	51.80±1.75[a]	48.36±0.71 [b]	46.48±0.51 [b]	0.005
Whole-Body composition					
Dry matter (%)	16.75±4.06	17.53±4.32	18.84±5.19	18.94±4.95	0.983
Moisture (%)	83.25±4.08	82.47±4.30	81.16±5.19	81.04±4.93	0.983
Crude Protein (%)	68.38±0.45	67.81±0.68	68.12±0.91	68.68±0.72	0.849
Ether extract (%)	6.90±1.08	7.02±2.04	7.70±2.17	6.39±1.13	0.685
CP retention	12.24±4.77	11.64±5.06	14.83±6.45	15.28±5.73	0.953
EE retention	6.71±2.38	6.60±2.50	9.35±3.66	7.24±2.27	0.884

Values are means ± standard error. Mean values with different letters in the same row differ significantly at ($p \leq 0.05$).

Intestinal morphology

Figure 1 shows that shrimp fed diets containing pellet binders exhibited improved intestinal morphology compared with those fed the control diet without a binder. The observed improvement included better epithelial cell morphology and integrity of intestinal folds, enhanced lamina propria with increased immune cells, and greater intestinal wall thickness in the binder-fed groups, particularly in groups starch + gum-based and PMC, compared with the control group.

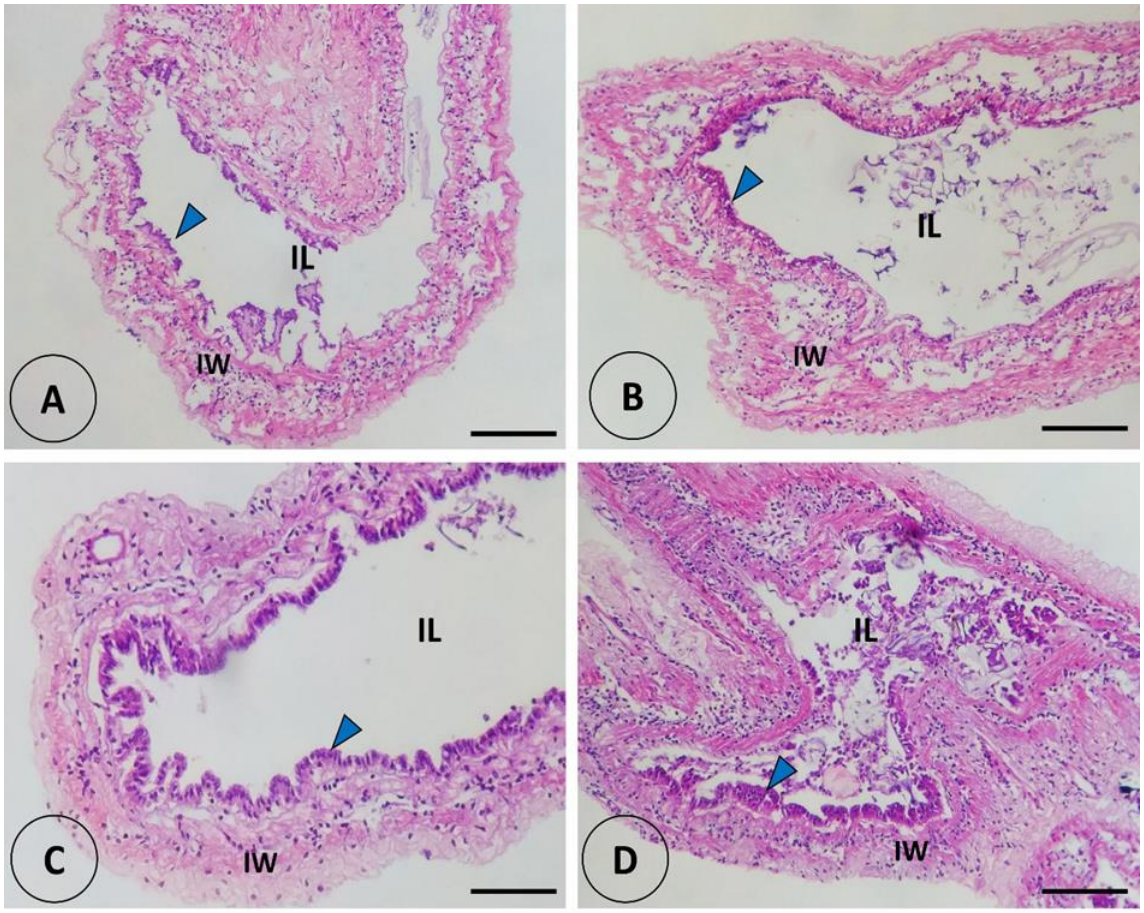

Figure 1: Histomorphology of mid gut of shrimp in the control group (A) as well as other pellet binder fed shrimps (B, C, and D) (calcium lignosulfonate, starch+ gum-based binder and polymethyl carbamide, respectively) showing the intestinal morphology including intestinal epithelial lining (blue arrowhead), intestinal wall (IW) and intestinal lumen (IL). There was a clear enhancement in both intestinal epithelium and intestinal wall, especially in groups C and D. (H&E Stain; scale bar=100 µm).

Discussion

Shrimp feeding habits highlight the importance of producing better, well-formulated diets that provide balanced nutrients throughout the feeding period while minimizing feed loss, nutrient leaching, and water pollution (Palma *et al.,* 2008). Results demonstrated that the tested binders (calcium lignosulfonate, starch + gum-based binder, and PMC) had no significant effect on shrimp growth but improved pellet quality parameters, including water stability, leaching rate, and water activity.

Pellet water stability is defined as the ability of the pellet to retain its integrity and nutrients while in water until consumed by the animal (Obaldo *et al.,* 2002). High water stability is essential to prevent nutrient loss through rapid disintegration in water and to extend the feed availability. This stability largely depends on the type of binder used, as effective binders minimize the void spaces, enhance pellet integrity, and produce more compact and durable pellets (Ruscoe *et al.,* 2005). In the present study, the inclusion of different pellet binders improved pellet water stability and reduced nutrient leaching. This effect was

most evident in pellets processed with PMC, which exhibited the highest water stability and lowest leaching rate, followed by starch + gum-based binder. These findings are consistent with Dominy *et al.* (2004), who reported that urea-formaldehyde containing binders (PMC) improved shrimp pellet structure and reduced nutrient leaching, particularly protein loss. The lower leaching rate in the PMC-binder could be attributed to its lower water absorption capacity, which limits water uptake of pellets containing this binder. In support, Ahamad-Ali *et al.* (2010) demonstrated that a low inclusion level of PMC (0.5%) produced the best water stability with minimal moisture absorption compared to wheat gluten or guar gum, also allowing greater flexibility for inclusion of other essential dietary ingredients, making it a cost-effective option. In comparison, starch+ gum-based binder produced moderate stability and leaching rate falling between PMC and lignosulfonate binders. This effect could be explained by the combined action of starch and gums, as the viscous properties of gums enhance starch granule swelling during gelatinization, thereby improving starch viscosity and overall pellet stability. Supporting this, Christianson *et al.* (1981) reported that wheat starch viscosity increases when a small amount of polysaccharide gums is added. These findings are consistent with Ahamad-Ali *et al.* (2010), who reported that guar gum produced shrimp pellets with moderate water stability. Similarly, Caltagirone *et al.* (1992) observed that guar gum, gelatin, and agar-agar enhanced pellet stability after 48 hours of water immersion compared to other binders such as cellulose, CMC, and sodium alginate. In contrast, lignosulfonate-bound pellets in the present study demonstrated lower stability and higher leaching rate relative to PMC and starch+ gum-based binders. Unlike our results, Acar *et al.* (1991) and Corey *et al.* (2014) reported that calcium lignosulfonate improved the pellet durability index in broiler diets. These discrepancies between trials may be attributed to the target species for whom pellets were manufactured and the variation in feed formulation and processing across studies.

Moreover, pellet moisture content was reduced in diets processed with calcium lignosulfonate and PMC binders. A similar trend was observed for water activity (aW), with the lowest activity recorded in lignosulfonate and PMC-bound pellets. Lower aW values are preferable, as they provide better protection against bacterial growth. The optimum aW level for formulated feeds has been standardized to 0.65, which represents the upper limit for safe storage of foods (Hemmingsen *et al.,* 2008). In decapod aquafeed such as shrimp pellets, the common aW is reported to be 0.5043 (Carter, 2016). Elevated moisture levels in formulated feeds promote microbial growth, as microbes proliferate more rapidly in a feed medium with higher aW, ultimately deteriorating feed quality. The higher water content observed in starch + gum-based binder could be attributed to the gelatinization process, during which starch becomes activated, absorbs large volumes of water, and, thus, increases pellet moisture. This, in turn, was reflected in the higher aW values of pellets manufactured with this binder. By contrast,

lignosulfonate-bound pellets demonstrated the lowest water activity. This may be due to the strong water affinity of lignosulfonate and its high solubility (Cecilia *et al.,* 2008), which enables it to bind with water molecules and lower water activity.

Regarding the shrimp growth performance and feed efficiency indices (FCR, PER, and EEU), no significant differences were observed among the experimental groups. However, slight improvements were noted in shrimp fed PMC and starch + gum-based binders. The enhanced growth performance in these groups appears to be associated with the improved pellet quality as reflected by greater pellet water stability and lower leaching rates. This likely allowed the pellets to maintain more of their nutritional value and, consequently, better growth. Also, these improvements could be linked to the enhanced intestinal morphology and the response of digestive enzymes in these groups. Unlike the obtained results, Dominy *et al.* (2004) reported that shrimp fed urea-formaldehyde (PMC) binders (Compact-PBX and Pell-Tuff) demonstrated significantly lower final weights (p<0.05) compared with other binders. The differences between trials may be explained by the inclusion level of PMC, as in their trial, binders were added at 0.75% and 1% of the diet, which may have negatively affected feed palatability or exerted toxic effects; while in our study, PMC was added at the level of 0.5 % of the diet. The same authors concluded that dietary inclusion of PMC above 0.5% reduced shrimp final weights and weekly growth. In the same regard, Castille and Lawrence (1995) observed reduced shrimp growth when dietary PMC levels exceeded 0.5%. The variation in effects observed across studies could be attributed to differences in the cultured species and the concentration of urea-formaldehyde included in the diet. Moreover, shrimp fed the starch + gum-based binder exhibited moderate growth performance and pellet quality falling between the PMC and lignosulfonate groups. In the same context, Indra Jasmine (2000) reported that pellets containing 4% guar gum were water stable, showed minimal weight loss, and supported good growth in *Penaeus indicus.* Similarly, Pearce *et al.* (2002) reported that a guar gum-based diet enhanced the growth of green sea urchin *Strongylocentrotus droebachiensis* compared to the control. The lack of difference in PER and EEU among shrimp groups in this study could be associated with the non-significant effects of different binders on digestive enzyme activities.

Shrimp fed calcium lignosulfonate exhibited lower WG and deteriorated feed efficiency compared to those fed other binders. This could be linked to the lower FI, reduced nutrient absorption from the diet, lower pellet water stability, and the associated increase in nutrient leaching, all of which likely resulted in impaired growth. Similarly, Yamamoto and Akiyama (1995) reported that feeding Japanese flounder fingerling CMC-containing diets reduced WG, feed efficiency, protein digestibility, and protein and energy retention compared to those fed starch or wheat gluten as binders. They suggested that CMC increases diet viscosity and decreases proteolytic enzymes' activities, thereby

contributing to poor growth performance. Moreover, shrimp total FI did not differ significantly among groups; however, it was increased in shrimp fed PMC and starch + gum-based binders. Similarly, Indra Jasmine (2000) reported that shrimp fed diets containing 2% potato starch had the highest FI, whereas the lowest feed consumed was observed in shrimp fed with 5% agar-agar.

On the other hand, a non-significant reduction in FI was observed in shrimp fed lignosulfonate, which may be related somehow to the lower pellet water stability. Pellets bound with lignosulfonate tend to absorb water and become soft, which results in difficulties for shrimp to manipulate before ingestion (Palma *et al.,* 2008). These results are partially in line with (Acar *et al.,* 1991; Corey *et al.,* 2014), who reported that broilers fed diets manufactured with calcium lignosulfonate exhibited higher FI and WG but poor FCR. Such discrepancies among trials may be explained by differences in species and experimental design, including binder inclusion levels. Overall, findings of the present study suggest that lignosulfonate use as a binder offers no clear advantages in terms of either pellet stability or shrimp growth.

Dietary inclusion of different binders significantly influenced hemolymph glucose concentration compared to shrimp fed the control diet without a binder. The present result could be linked to the role of binders in enhancing pellet stability, reducing nutrient leaching, and thereby preserving the nutrient density of feed and consequently improving its nutritive value. In particular, diets processed with starch+ gum-based and lignosulfonate binders resulted in higher hemolymph glucose levels, suggesting that shrimp may have partially utilized these binders as an additional energy source, resulting in a glycemic response. Carbohydrates are commonly incorporated in crustacean-formulated diets for their protein-sparing effect (Shiau and Peng, 1992; Rosas *et al.,* 2008); however, the type and source of the carbohydrates consumed must be carefully considered. In this context, Radford *et al.* (2005) reported that both agar and alginate (two algal polysaccharides) influenced blood sugar levels in lobster, *Jasus edwardsii,* suggesting that lobster may utilize these polysaccharides as both binders and energy sources. Also, Zhang *et al.* (2023) observed higher serum glucose levels in juvenile yellow catfish *Pelteobagrus fulvidraco* fed diets containing different cellulose levels (4, 6, 8 % of diet) compared to fish receiving the cellulose-free diet.

Furthermore, substantial differences were observed among binder groups in the shrimp hemolymph lipid profile. Triglyceride and VLDL concentrations were reduced in shrimp fed diets containing pellet binders, with the lowest concentrations observed in the PMC and starch + gum-based binder fed groups. Total cholesterol and HDL exhibited a similar pattern, showing the greatest reduction in shrimp fed starch + gum-based binder and PMC, respectively. In contrast, LDL levels were elevated in shrimp fed calcium lignosulfonate and PMC containing diets. Overall, starch + gum-based binder appeared to exert a hypolipidemic effect, as demonstrated by

lower lipid profile parameters. In support of these findings, Gao *et al.* (2019) reported lower plasma cholesterol and TG levels in gibel carp fed diets supplemented with guar gum. These results are in quite agreement with Yokoyama *et al.* (2020), who found that fish fed on an activated gluten + guar gum diet had significantly lower plasma TG levels compared to those fed the activated gluten diet alone, although total cholesterol levels didn't differ significantly among groups (activated gluten, activated gluten + guar gum, and CMC). Additionally, previous studies in other fish species have shown that dietary non-starch polysaccharides (NSPs) can reduce cholesterol levels (Kraugerud *et al.,* 2007; Kumar *et al.,* 2011). The cholesterol-lowering effect of NSPs might be due to impaired cholesterol absorption and/or bile acid reabsorption (Potter, 1995). In the present study, the TC/HDL ratio was considerably higher in the PMC-fed shrimp, reflecting the combined treatment effects on TC and HDL levels. The increased LDL concentration and TC/HDL ratio in PMC-fed shrimp may indicate potential adverse effects of PMC, possibly linked to the presence of free formaldehyde in urea formaldehyde. Unfortunately, residues of formalin inside the shrimp's whole body were not measured in the current study, so this hypothesis remains unconfirmed. Overall, the results suggest that the tested binders modified hemolymph lipid constituents in shrimp.

Crustaceans can adapt their digestive enzymatic profile and activities according to the diet composition (Johnston and Johnston, 2007). In the current study, we conducted a screening analysis for digestive enzyme activities in shrimp fed different experimental diets to determine whether the tested pellet binders could influence the enzymatic profile and, consequently, contribute to the observed variations in weight gain among the experimental groups. In the current study, pellets containing different binders had no significant effect on the activities of lipase, amylase, or protease enzymes. However, these enzymes were increased in shrimp fed PMC, followed by those fed starch+ gum-based binder. The elevated enzyme levels in these groups may help explain the comparatively higher weight gain observed. These results are in quite agreement with the findings of Volpe *et al.* (2012), who reported no significant differences in digestive enzyme activities in the digestive tract of juvenile *Cherax albidus,* except for amylase, which was significantly higher in shrimp fed pectin-containing pellets compared with those fed alginate or chitosan-based diets. In the same regard, Yamamoto and Akiyama (1995) observed lower levels of pepsin, lipase, and trypsin in flounder fish fed carboxymethyl cellulose (CMC) diets at 2 to 12 h after feeding compared to fish fed diets containing α-starch or wheat gluten; however, the differences in pepsin and lipase activities were not statistically significant. The lack of variation in the proximate composition of the experimental diets may have contributed to the non-significant difference in the activities of digestive enzymes, which in turn resulted in no major changes in nutrient digestion and may partially explain the observed growth in the experimental groups.

Analysis of the shrimp's whole body revealed no significant difference in dry matter, moisture, crude protein, or ether extract among the different groups. Similarly, retention of CP and EE inside the body showed no notable differences, although the percentage and retention of EE were slightly higher in shrimp fed the starch + gum-based binder compared to the other binders. Similarly, Yamamoto and Akiyama (1995) reported no differences in whole-body composition of nutrients among treatments, though fat content was higher in flounder fed diets containing α-starch or wheat gluten compared to those fed CMC-containing diets. The same authors also observed that protein retention increased significantly (p<0.05) in ascending order of CMC, α -starch and wheat gluten. Likewise, Yokoyama *et al.* (2020) stated no variation in whole body moisture, protein, or ash contents among amberjacks fed different binders (CMC, activated gluten, and guar gum), while whole body lipid content was significantly lower in those fed the CMC diet. The lack of difference in digestive enzyme activities among shrimp groups in the present study may explain the similar results in whole-body composition.

Since dietary components influence the intestinal morphology and its related physiological function, it is commonly used to evaluate dietary effects in fish (Huang *et al.,* 2022). In this study, dietary inclusion of different pellet binders enhanced shrimp intestinal morphology, by better epithelial cell morphology and intestinal folds integrity, more developed lamina propria with increased immune cells, and higher intestinal wall thickness. The response was particularly evident in shrimp fed diets containing starch+ gum-based binder and PMC. The results suggest that the enhanced growth performance in these groups may be partially linked to the improved intestinal morphology. Studies investigating the effects of these binders on intestinal morphology in shrimp and fish are limited. In this context, Yokoyama *et al.* (2020) reported that villus density in the anterior and middle regions of amberjacks' intestine showed no significant differences among fish fed different binders (CMC, activated gluten, and guar gum); however, villus density in the posterior segment of CMC-fed fish was significantly higher than those fed the other binders.

Conclusion

The dietary inclusion of calcium lignosulfonate; starch+ gum-based binder and polymethyl carbamide as pellet binders in shrimp was beneficial in improving pellet quality but didn’t markedly enhance shrimp growth performance. Among the tested binders, PMC at an inclusion level of 0.5% of the diet produced the best results in both pellet quality (greater water stability, lower leaching rate, and reduced water activity) and improved shrimp performance compared with calcium lignosulfonate at 1% of the diet. However, these findings suggest that PMC could be used for short periods during shrimp culture to minimize the potential long-term negative impacts. Also, starch+ gum-based binder could be used as a practical alternative to PMC, as it enhances pellet quality and nutritional characteristics while supporting shrimp performance without adverse impacts.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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