GABRIEL SOBRAL MICHEREFF

EFEITO DA SUPLEMENTAÇÃO DIETÉTICA COM MANANOLIGOSSACARÍDEO (MOS) E β-GLUCANO SOBRE O DESEMPENHO ZOOTÉCNICO, RESPOSTA IMUNE E VARIÁVEIS HISTOLÓGICAS DE *Penaeus vannamei* MANTIDOS EM SISTEMA SIMBIÓTICO

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PROGRAMA DE PÓS-GRADUAÇÃO EM RECURSOS PESQUEIROS E AQUICULTURA

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Gabriel Sobral Michereff

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Resumo

A eficácia de suplementos contendo mananoligossacarídeos (MOS) e β-glucanos em *Penaeus vannamei* pode variar conforme sua pureza e composição, principalmente em sistemas intensivos de cultivo, onde o aumento da densidade compromete a qualidade da água e a saúde dos animais. Neste contexto, o presente estudo avaliou o efeito da pureza de mananoligossacarídeos (MOS) e β-glucanos em dietas de *P. vannamei* mantidos em sistema simbiótico sobre o crescimento e variáveis histológicas. Para tal, o desenho experimental foi composto por quatro tratamentos com diferentes graus de pureza e composição e um grupo controle: CON(Controle); MOS1(72%); MOS2(76,5%); MOS3(80,2%) e; MOS4(83,3%). O experimento durou 60 dias e foram avaliadas: variáveis de qualidade de água e de desempenho zootécnico; contagens presuntivas totais de *Vibrio sp.*, *Bacillus sp.*, e fungos e; Índice de Alterações Histológicas (HAI) do intestino. As variáveis de qualidade de água estiveram dentro dos padrões recomendados. Em relação às variáveis de desempenho zootécnico, foram obtidos melhores valores para CON e MOS3. Quanto à análise microbiológica, observou-se inibição parcial de *Vibrio spp.* nos tratamentos MOS1, MOS3 e MOS4 com 60 dias de oferta, além de maior desenvolvimento de colônias de *Bacillus spp*. e preservação da integridade intestinal no tratamento MOS3. A análise histológica revelou que apenas o tratamento MOS3 apresentou baixos valores de HAI (<10), enquanto os demais tratamentos apresentaram lesões graves e irreversíveis (HAI >100), incluindo vacuolização citoplasmática, descolamento epitelial e melanização da mucosa. Conclui-se que a composição e pureza do MOS são fatores determinantes na manutenção da integridade da parede intestinal de *P. vannamei* mantidos em sistemas simbióticos.

Palavras-chave: *Penaeus vannamei*, Pureza de Mananoligossacarídeos (MOS) e β-glucano, Desempenho zootécnico, Histologia intestinal.

Abstract

The efficacy of supplements containing mannan oligosaccharides (MOS) and β-glucans in *Penaeus vannamei* can vary according to their purity and composition, especially in intensive farming systems, were increased stocking density compromises water quality and animal health. In this context, the present study evaluated the effect of the purity of mannan oligosaccharides (MOS) and β-glucans in diets for *P. vannamei* maintained in a symbiotic system on growth and histological variables. To this end, the experimental design consisted of four treatments with different levels of purity and composition, and one control group: CON (Control); MOS1(72%); MOS2(76,5%), MOS3(80,2%) and; MOS4(83,3%). The experiment lasted 60 days, and the following were evaluated: water quality and zootechnical performance variables; total presumptive counts of *Vibrio sp.*, *Bacillus sp.*, and fungi; and the Histological Alteration Index (HAI) of the intestine. The water quality variables remained within the recommended standards. Regarding zootechnical performance variables, better values were obtained for CON and MOS3. As for microbiological analysis, partial inhibition of *Vibrio spp.* was observed in the MOS1, MOS3, and MOS4 treatments after 60 days of supplementation, as well as greater development of *Bacillus spp.* colonies and preservation of intestinal integrity in the MOS3 treatment. Histological analysis revealed that only the MOS3 treatment showed low HAI values (<10), while the other treatments exhibited severe and irreversible lesions (HAI >100), including cytoplasmic vacuolization, epithelial detachment, and mucosal melanization. It is concluded that the composition and purity of MOS are determining factors for maintaining the integrity of the intestinal wall of *P. vannamei* maintained in symbiotic systems.

Keywords: *Penaeus vannamei*, Mannan oligosaccharides (MOS) and β-glucan purity, Zootechnical performance, Intestinal Histology

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1 Introdução

O camarão peneídeo *Penaeus vannamei* compõe 53% da produção mundial de camarão (FAO, 2024), sendo cultivado ao redor do mundo em sistemas extensivos, semi-intensivos e intensivos (Li et al. 2017). O alto interesse pela espécie se dá pela sua adaptabilidade a diferentes condições de salinidade e temperatura, além da alta taxa de crescimento (El-Saadony et al. 2022). A espécie também possui baixos requisitos proteicos em comparação a outras como o *P. monodon* e, um alto rendimento de carne, cerca de 66 a 68%, tornando-a ideal para processamento e consumo (Dai et al. 2023).

Devido ao bom desempenho da espécie, seu mercado tem se desenvolvido cada vez mais o que vem demandando densidades de estocagem cada vez maiores, visando o aumento da produtividade (Mugwanya et al. 2022). No entanto, a intensificação das densidades, associada ao uso de rações com alto teor proteico tem criado cenário propício à deterioração ambiental de sistemas de cultivo, aumentando o estresse e comprometendo o equilíbrio fisiológico dos animais, bem como seu sistema imunológico e reduzindo sua capacidade de resposta a infecções (Li et al. 2017).

Frente ao comprometimento da função imunológica, o *P. vannamei* se torna susceptível a infecções bacterianas, com destaque ao gênero *Vibrio spp.* (*V. harveyi, V. alginolyticus, V. parahaemolyticus, V. vulnificus, e V. cholerae*) (Li et al. 2018). Infecções por *Vibrio spp* representam uma ameaça recorrente à cadeia produtiva por sua alta severidade e possibilidade de coinfecção, especialmente, ao afetar estágios larvais de produção (Kumar et al. 2017).

Comumente utilizados para o controle de infecções bacterianas, os antibióticos possuem uma ação rápida e eficaz, porém, seu uso indiscriminado em sistemas intensivos pode levar ao desenvolvimento de resistência bacteriana (Valente et al. 2021). Cepas de *Vibrio spp.*, por exemplo, já demonstraram resistência a antibióticos frequentemente utilizados, tais como ampicilina, cloranfenicol, tetraciclina, estreptomicina, canamicina, trimetoprim e carbapenem (Lee et al. 2019), com possibilidade de transferência horizontal de genes de resistência ao longo de novas gerações bacterianas (Osunla & Okoh 2017). A banalização no uso dos antibióticos não só limita as opções terapêuticas disponíveis, como também resulta na liberação desses compostos no ambiente aquático, contribuindo para a contaminação ambiental e a disseminação de genes de resistência bacteriana na microbiota aquática (Amoah et al. 2020).

Neste sentido, visando o controle ou eliminação do uso de antibióticos na indústria aquícola, novos métodos e sistemas vêm sendo desenvolvidos e implementados. Dentre estes, a utilização de sistemas simbióticos, que integram o uso de microrganismos probióticos associados a prebióticos, têm ganhado o interesse entre produtores e pesquisadores por sua relação sinérgica, resultando em melhorias significativas na eficácia da dieta e no desempenho do crescimento de *P. vannamei* (Amenyogbe et al. 2020)

Bindels et al. (2015) definem prebióticos como "ingredientes alimentares não digeríveis que são decompostos em substâncias mais simples, aumentando o crescimento e a atividade dos microrganismos benéficos encontrados no trato gastrointestinal e beneficiando a saúde do hospedeiro". Embora prebióticos possam ser obtidos de diversas fontes, destaca-se o uso da parede celular de leveduras, como *Saccharomyces cerevisiae* como principal fonte (Amenyogbe et al. 2020). Essa preferência se deve à presença de componentes bioativos de grande importância nutricional como frutooligossacarídeos de cadeia curta (FOS), mananoligossacarídeos (MOS), β-glucano, xilooligossacarídeos e isomaltooligossacarídeos (Abdel-Latif et al. 2022).

Os prebióticos à base de leveduras vêm sendo cada vez mais utilizados na produção de camarões *P. vannamei*, devido a sua capacidade de melhorar o desenvolvimento de bactérias benéficas no sistema gastrointestinal dos animais (Guo et al. 2022), servindo de fonte de alimento e de substrato para as bactérias probióticas, estimulando a multiplicação e a atividade desses microrganismos, promovendo benefícios sobre a saúde intestinal, modulando o sistema imune e contribuindo para o desempenho zootécnico dos animais (Bozkurt et al. 2014).

A extração de componentes da parede celular de leveduras usadas como prebióticos (*S. cerevisiae*) gera uma série de compostos como: mananoligossacarídeo (MOS), β-glucano e nucleotídeos. O MOS é um oligossacarídeo composto por unidades de manose extraídas da parede celular das leveduras (Merrifield et al. 2010). Além de possuir propriedade prebiótica, atua como agente antagônico a patógenos ao ligar-se a receptores de sítio ativo da mucosa intestinal, impedindo a fixação destes organismos e facilitando sua eliminação pelo trato intestinal (Gainza & Romero 2020). Do ponto de vista imunológico, o MOS auxilia no aumento da atividade de enzimas antioxidantes e imunológicas como as fenoloxidases (PO), superóxido dismutase (SOD) e aumento da contagem total de hemócitos (Apines-Amar et al. 2014), desempenhando papel de suma importância na defesa antioxidante e imunológica em sistemas de cultivo.

O β-glucano, que também pode ser extraído da parede celular de leveduras, é um polissacarídeo não digerível que pode desempenhar um papel importante na modulação do sistema imune de *P. vannamei*. Sua ação ocorre principalmente por meio da ativação de receptores de reconhecimento de padrões como os receptores de β-glucano tipo 1 (Dectin-1) e β-glucano tipo 2 (CR3). A interação com esses receptores desencadeia vias de sinalização intracelular que ativam diferentes células do sistema imunológico inato, como os granulócitos e hialinócitos, fundamentais na coagulação e encapsulamento de patógenos (Li et al. 2023). A suplementação conjunta de mananoligossacarídeo (MOS) e β-glucanos apresenta potencial para promover a saúde e o desempenho zootécnico de *P. vannamei*, contribuindo em melhores taxas de crescimento e conversão alimentar (Mameloco & Trafalgar 2020).

Entretanto, dentre os principais desafios na aplicação dos compostos de parede celular de *S. cerevisiae* está a falta de consenso na indústria com relação à pureza do suplemento, em especial, quando utilizado de forma continuada, tendo em vista o alto grau de processamento químico envolvido na desproteinização celular e processamento em MOS e β-glucanos (Huang et al. 2010), dificultando a padronização de protocolos alimentares, limitando a utilização deste tipo de suplemento em cultivos comerciais. Dessa forma, o presente estudo avaliou o efeito da pureza de mananoligossacarídeos (MOS) e β-glucanos nas dietas de *P. vannamei* em sistema simbiótico sobre o crescimento e saúde intestinal.

1.1 Objetivo geral

Avaliar o efeito da pureza de mananoligossacarídeos (MOS) e β-glucanos nas dietas de *P. vannamei* mantidos em sistema simbiótico sobre o crescimento e variáveis histológicas.

1.2 Objetivos específicos

- Determinar o ganho de biomassa, taxa de crescimento específico, fator de conversão alimentar e sobrevivência dos camarões alimentados com dietas contendo diferentes graus de pureza de MOS e β-glucano;
- Determinar o efeito da pureza de MOS e β-glucanos sobre a histologia do intestino e a contagem presuntiva total de bactérias do gênero *Vibrio* spp., *Bacillus spp.* e leveduras neste tecido.

2 Artigo científico

Os resultados obtidos durante o trabalho experimental desta dissertação estão apresentados no artigo intitulado: **"Efeito da pureza de mananoligossacarídeos (MOS) e β-glucanos em dietas para *Penaeus vannamei* em sistema simbiótico"**

2.1. Artigo – Effect of mannan oligosaccharide (MOS) and β-glucan purity and composition in diets for *Penaeus vannamei* reared in a symbiotic system

Artigo científico a ser encaminhado a Revista – Aquaculture International (ISSN: 0967-6120). Todas as normas de redação e citação, deste capítulo, atendem as estabelecidas pela referida revista (https://link.springer.com/journal/10499).

Effect of mannan oligosaccharide (MOS) and β-glucan purity and composition in diets for *Penaeus vannamei* reared in a synbiotic system

Abstract

The effectiveness of supplements containing mannan-oligosaccharides (MOS) and β-glucans in *P. vannamei* may vary depending on their purity and composition, especially in intensive farming systems, where increasing density compromises water quality and animal health. In this context, the present study evaluated the effect of four supplements containing MOS and β-glucans with four different degrees of purity and nutritional compositions (72%–83.3%) in *P. vannamei* diets on zootechnical performance, microbial counts, and Histological Alteration Index (HAI) for 60 days. The MOS3 treatment (80.2%), by presenting higher quality in the extraction and purification process, maintained the integrity of the mucosa (HAI <10), increased *Bacillus spp.* colonization and partially dropped the Vibrio spp. counts without significant improvement in zootechnical performance. In contrast, the MOS1 (72%), MOS2(76.5%), and MOS4(83.3%) treatments resulted in severe histological lesions on the intestinal tissue (HAI > 100), which include vacuolization, epithelial detachment and mucosal melanization, associated with high mortality rates (>50%). The results indicate that the mere use of supplements does not guarantee physiological safety, but the nutritional quality of these supplements is a key factor in gut preservation. The combined analysis of zootechnical performance, microbial counts, and Histological Alteration Index (HAI) reinforces the need for additional studies in the direction of developing intermittent protocols, combined with minimum criteria for purity and nutritional quality of supplements, to guarantee intestinal integrity without compromising animal health in synbiotic cultures of *P. vannamei*.

Keywords: *Penaeus vannamei*, Mannan-oligosaccharide, β-glucan, HAI, Synbiotic system

Introduction

The increase in stocking densities of *Penaeus vannamei* aggravates the deterioration of water quality due to excess feed and excreta, resulting in stress, immunosuppression, and higher occurrence of bacterial outbreaks (Li et al., 2017). Such circumstances lead to extensive use of antibiotics in intensive farming systems, resulting in bacterial resistance and environmental impacts (Amoah et al., 2020). These challenges reinforce the relevance of sustainable alternatives, such as synbiotic systems that integrate probiotics and prebiotics and provide beneficial effects on animal performance and health (Amenyogbe et al., 2020).

Mannan-oligosaccharides (MOS) and β-glucans are prebiotics obtained from the cell wall of *Saccharomyces cerevisiae* that stand out as alternatives to the use of antibiotics, since they stimulate the immune response, favor microbiota, and maintain the integrity of the intestinal mucosa, enhancing nutrient absorption (Gainza & Romero, 2020). However, prolonged exposure to these feed additives can lead to adverse effects such as immune fatigue, chronic inflammation, and histological lesions in the mucosa (Jiang et al., 2019).

Another aspect associated with the application of these compounds is the variation in purity and composition of supplements caused by chemical processes involved in cell deproteinization and compound formation (Huang et al., 2010). Such variations can change the proportions of soluble mannan, insoluble mannan, and β-glucan, as well as different levels of impurities such as trace proteins and minerals (Bzducha-Wróbel et al., 2014). In this context, histological analysis proves to be a sensitive tool to identify changes in the intestinal mucosa of *P. vannamei*, enabling the compositional profile of supplements to be related to tissue responses (Huang et al., 2010). Therefore, this study evaluated the effect of purity and composition of mannan-oligosaccharides (MOS) and β-glucans on the intestinal histology of *P. vannamei* cultivated in a synbiotic system.

Material and methods

Stocking and experimental design

Shrimp post-larvae were acquired from commercial hatcheries 10 days after metamorphosis (PL_{10}). Post-larvae were stocked at density of 1,000 PL/m³ in 800 L units until reaching an average weight of 1.5 ± 0.3 g. During this period, commercial feed with

45% crude protein was offered four times a day. Once the shrimp reached the target weight, the juveniles were transferred to new 800 L units at an initial density of 100 shrimp/m³ to receive experimental diets, also provided four times a day.

Throughout the experiment, the pH was maintained between 7.5 and 8.5, with alkalinity above 100 mg $CaCO_3$/L, adjusted with calcium hydroxide at 25 g/m³ every 5 days. Constant aeration was provided by compressor and diffused through porous hoses to maintain oxygen above 5 mg/L without water exchange, except for the inclusion of fresh water to compensate for evaporation losses. All parameters remained in the proper range for species, with no differences observed between treatments (Table S1).

Experimental diets

The experimental diets were prepared at the Advanced Fish Center – Fisheries Institute (São José do Rio Preto, São Paulo, Brazil) with an average of 35% crude protein and 8% lipids (Table S1). An identical basal diet was used for all treatments (isolipidic, isoenergetic and isoproteic), differing only in the inclusion of supplements provided by the manufacturer (Table 1). Four grams of MOS supplement were incorporated per kilogram of feed, varying in proportions of soluble mannans, insoluble mannans and β-glucans among treatments (MOS1–4). The combination of these compounds resulted in the supplement's total purity value, which was the differentiating factor between treatments, as specified by the manufacturer (Table 1). The feed was pelletized to 2 mm and stored under refrigeration until use.

Zootechnical performance

Every ten days, biometry was performed by weighting and counting the total number of animals per treatment to obtain the following variables: Biomass gain (g) = final biomass (g) – initial biomass (g); Final average weight (g) = final biomass/number of individuals at the end of cultivation; Feed conversion ratio (FCR) = total feed offered (g)/biomass gain (g); Survival (%) = (number of individuals at the end of cultivation/number of individuals initially stocked) × 100; and Productivity (kg/m³) = (final biomass (g)/volume of the experimental unit (m³))/1,000.

Histological analysis

After 60 days of experimental feeding, five shrimp per treatment were collected for intestinal histology analysis. The samples were immersed in Davidson AFA for fixation, followed by transfer to 70% ethanol. After fixation, the samples were dehydrated through a series of increasing ethanol, cleared in xylene and embedded in paraffin blocks. The blocks were trimmed and cut into 5 µm-thick sections with microtome and stained with hematoxylin and eosin (HE), following the protocol of Lightner (1996). The histological sections were analyzed and photographed using an optical microscope with 10×, 20×, 40× and 100× objectives to detect damage as well as cellular and structural alterations.

Qualitative analysis of the overall integrity of the intestinal walls and quantitative analysis of height, density, and length of the villi were conducted according to Zhang et al. (2020). To quantify the level of tissue damage, the Histological Alteration Index (HAI) was used, initially proposed by Poleksić & Mitrović-Tutundžić (1994) for fish and later adapted for penaeid shrimp (Fregoso-López et al., 2017, 2018; Jamshidizadeh & Biuki, 2023). This method classifies injuries into three progressive stages of tissue damage, according to their intensity and potential for reversibility: stage I (minor to reversible injuries), stage II (moderate injuries), and stage III (severe and irreversible injuries). Each stage is given a different weight reflecting its severity (1 for I, 10 for II, and 100 for III), with HAI calculated using the following formula:

$$HAI = [1 \times \sum(stageI)] + [10 \times \sum(stageII)] + [100 \times \sum(stageIII)]$$

Bacteriological analysis

On days 0, 30 and 60, intestinal samples were submitted to total presumptive counts of *Vibrio* spp. in TCBS medium, *Bacillus* sp. in MYP medium, and fungi (yeasts) in Saboraud medium to evaluate the response of intestinal microbiota to supplementation. A detailed description of these procedures can be found in the supplementary material (Supplementary methods 1 and 2).

Statistical analysis

Data normality was checked using the Shapiro-Wilk test ($p < 0.05$), and homogeneity of variances using Levene’s test ($p < 0.05$). For parametric data (zootechnical, water quality variables and histological data), mean comparisons were performed using Tukey’s test ($p < 0.05$). For nonparametric microbial count data (CFU/g), the Kruskal-Wallis test with Bonferroni correction was applied. All statistical analyses were conducted using IBM SPSS Statistics software version 25.0.

Results

Zootechnical performance

No significant differences were observed between the parameters evaluated up to 30 days of the trial. At the end of 60 days, the average weight (g) did not differ between treatments ($p > 0.05$), but feed conversion ratio, survival rate, and productivity were significantly higher ($p < 0.05$) in MOS3 and Control treatments (Table 2)

Histological analysis

The intestines remained intact in the Control and MOS3 (80.2%) groups, with occasional detachment of basal membranes. Villi height and width remained inside expected limits, and HAI was under 10 in both treatments (Table 3). In contrast, MOS4 (83.3%) and MOS1 (72%) showed detachment of enterocytes from epithelial base, cytoplasmic vacuolization, chromatin fragmentation, and nuclear displacement (Figure 1), as well as reduction in villi height and width. In these groups, HAI reached 128.8 and 129.8, respectively (Table 3). In treatment MOS2 (76.5%), in addition to the lesions previously described, melanization of the epithelial base was observed, associated with complete detachment of intestinal walls, with minimum values for mucosal height and width (Figure 1) and an average HAI of 221.0 (Table 3).

Supplementary results

Microbiological analyses revealed significant differences between treatments with higher *Vibrio* spp. loads in Control and MOS2 (76.5%) groups, while MOS4 (83.3%) promoted a greater reduction in this pathogen load. Regarding probiotic bacteria, MOS3 (80.2%) showed the highest counts of *Bacillus* spp. in the intestinal tract (Table S2).

Discussion

Despite the similarity in zootechnical performance between treatments at 30 days (Table 2), significant differences were observed in the zootechnical performance and intestinal histology at the end of 60 days. Treatments MOS1 (72%), MOS2 (76.5%), and MOS4 (83.3%) presented severe and irreversible lesions (Figure 1), while MOS3 (80.2%) preserved mucosa, with HAI < 10 without evident alterations. This contrast highlights that deleterious effects associated with supplement formulation may not be detectable by short-term zootechnical parameters, becoming evident in histological and zootechnical analyses after prolonged exposure.

The increase in mortality rates in treatments MOS1 (72%), MOS2 (76.5%), and MOS4 (83.3%) with high concentration administration of immunostimulants suggests a condition of immune fatigue as described by Jiang et al. (2019). In that study, the authors demonstrated that prolonged exposure to β-glucans can lead to prolonged activation of LGBP and βGBP receptors, responsible for activating the ProPo system, promoting degranulation and release of reactive oxygen species (ROS), triggering a chronic inflammatory response. The oxidative stress caused by continuous activation of the immune system can lead to energy redirection in the body, compromising the intestinal mucosa due to its high energy demand associated with nutrient absorption and epithelial maintenance (Garibay-Valdez et al., 2021; Tran et al., 2022; Wang et al., 2024). This scenario directly compromises intestinal health, favoring the appearance of lesions and contributing to the high mortality rates observed.

The histological analysis of intestinal lesions was conducted through qualitative and semi-quantitative methods described by Lightner (1996), allowing the classification of lesions according to progressive levels of severity and functional impairment. To quantify the damage, the Histological Alteration Index (HAI) was applied, adapted from Mitrović-Tutundžić (1994) for penaeid shrimp (Fregoso-López et al., 2017; Jamshidizadeh & Biuki, 2023). This adaptation enabled standardized qualification of histological alterations, revealing the progressive deterioration of intestinal tissue. A similar approach was applied to different fish species by Dane et al. (2015) to quantify contaminant-induced mucosal damage, reinforcing the feasibility of adapting the index for intestinal tissue evaluation.

Based on the quantification of lesions, it was possible to associate the severity of damage with the compositional quality of the supplements. Treatment MOS3 (80.2%) exhibited the lowest ash content (3.4%) and a low protein content (21.9%), indicating a lower presence of mineral and protein impurities, often associated with poor yeast purification process quality (Huang et al., 2010), which suggests higher quality in the extraction and purification process of the supplement. Although no zootechnical gains were observed compared to the control group, this treatment stood out by not compromising intestinal integrity (HAI < 10), suggesting a potential therapeutic or preventive role under environmental stress conditions. Previous studies report that MOS supplementation can promote mucosal integrity and modulate intestinal microbiota (Wang et al., 2021), a tendency reinforced by the high level of *Bacillus* spp. in the MOS3 treatment (80.2%) (Table S2). These bacteria are recognized for their probiotic action and improvement in nutrient digestibility (Yaqub et al. 2022).

Studies in crustaceans have demonstrated that prolonged exposure to stressors and low energy availability trigger cellular damage to the digestive epithelium. Similarly, treatments MOS1 (72%), MOS2 (76.5%), and MOS4 (83.3%) exhibited cytoplasmic vacuolization, chromatin fragmentation, epithelial detachment, and villous atrophy. In the MOS2 (76.5%) treatment, complete epithelial detachment, greater villous atrophy (Table 3), and melanization at the epithelial base (Figure 1) were also observed, indicating advanced necrosis and chronic inflammation caused by activation of the ProPo system (Kumar et al., 2024). The lesions were associated with mean HAI values of 128.8 in MOS4 (83.3%), 129.8 in MOS1 (72%), and 221.0 in MOS2 (76.5%), consistent with irreversible damage. According to Schwaiger et al. (1997), HAI > 100 indicates severe functional impairment and high risk of mortality, a pattern reinforced by Lestari et al. (2025) in freshwater fish exposed to environmental contaminants, where HAI values above 100 were associated with irreparable lesions in the gills, such as necrosis and lamellar fusion, as well as mortality.

Thus, this study highlights that the use of MOS and β-glucans in *P. vannamei* should consider not only the presence of functional compounds but also the quality of the extraction process and the proportion between components. While MOS3 (80.2%) demonstrated physiological safety, continuous exposure to supplements with lower purity and quality resulted in irreversible histological changes and compromised zootechnical performance.

Conclusion

The results of this study demonstrate that the efficacy of supplements containing MOS and β-glucans are directly related to their purity and composition at the tested concentration of 4 g additive/kg feed. Although the MOS3 (80.2%) treatment did not significantly improve zootechnical performance compared to the control, it preserved intestinal integrity, exhibiting a low level of histological alterations (HAI < 10). In contrast, treatments with supplements of lower nutritional quality resulted in severe lesions in the intestinal mucosa (HAI > 100) and high mortality (> 50). These findings reinforce the need for further studies focused on developing intermittent supplementation protocols, associated with minimum standards of purity and nutritional quality, to ensure intestinal integrity without compromising animal health in synbiotic culture systems of *Penaeus vannamei*.

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3 Tables and figures

Table 1 Proximate composition values of MOS supplements provided by the manufacturer (%).

Proximate composition of MOS supplements (%)				
Component	**MOS1 (72)**	**MOS2 (76.5)**	**MOS3 (80.2)**	**MOS4 (83.3)**
Protein	22.4	18.83	21.87	23.16
Moisture	3.34	3.61	4.08	4.35
Ash	5.53	5	3.4	4.1

β-glucans	29.6	31.2	29.5	29.2
Insoluble mannans	22.4	25.1	26.4	27.7
Soluble mannans	20	20.2	24.3	26.4
Purity	**72.0**	**76.5**	**80.2**	**83.3**

Table 2 Zootechnical performance (mean ± SD) for *Penaeus vannamei* after 30 and 60 days of feeding experimental diets containing MOS at different degrees of purity.

Zootechnical performance (30 days)					
Variable	**Treatment**				
	Control	**MOS1 (72%)**	**MOS2 (76.5%)**	**MOS3 (80.2%)**	**MOS4 (83.3%)**
Final mean weight (g)	4.10 ± 0.28^{a}	3.82 ± 0.27^{a}	3.90 ± 0.21^{a}	4.02 ± 0.05^{a}	3.87 ± 0.20^{a}
Feed conversion ratio (FCR)	0.7 ± 0.09^{a}	0.89 ± 0.05^{a}	0.81 ± 0.04^{a}	0.79 ± 0.05^{a}	0.93 ± 0.14^{a}
Survival rate (%)	96.72 ± 6.5^{a}	89.5 ± 9.0^{a}	94.7 ± 6.0^{a}	97.0 ± 3.5^{a}	89.0 ± 7.4^{a}
Productivity (kg/m³)	0.51 ± 0.07^{a}	0.43 ± 0.05^{a}	0.46 ± 0.02^{a}	0.43 ± 0.05^{a}	0.43 ± 0.05^{a}
Zootechnical performance (60 days)					
Variable	**Treatment**				
	Control	**MOS1 (72%)**	**MOS2 (76.5%)**	**MOS3 (80.2%)**	**MOS4 (83.3%)**
Final mean weight (g)	5.83 ± 0.28^{a}	5.34 ± 0.43^{a}	5.62 ± 0.21^{a}	5.89 ± 0.20^{a}	5.59 ± 0.50^{a}
Feed conversion ratio (FCR)	1.4 ± 0.12^{a}	2.1 ± 0.16^{c}	2.6 ± 0.36^{c}	1.6 ± 0.05^{ab}	2.4 ± 0.44^{bc}
Survival rate (%)	80.25 ± 11.3^{a}	46 ± 6.2^{b}	43 ± 8.4^{b}	67 ± 6.0^{a}	41 ± 6.0^{b}
Productivity (kg/m³)	0.58 ± 0.07^{a}	0.34 ± 0.04^{b}	0.26 ± 0.05^{b}	0.49 ± 0.04^{a}	0.28 ± 0.03^{b}

Values with different letters are significantly different from each other ($p < 0.05$).

Table 3 Histological Alteration Index (mean ± SD) along with mean villus height and width for *P. vannamei* after experimental diets.

Treatment	**HAI**	**Villus height (µm)**	**Villus width (µm)**
Control	2.1 ± 1.5^{a}	29 ± 4^{ab}	16 ± 6^{a}
MOS1 (72%)	129.8 ± 29.80^{b}	26 ± 5^{b}	11 ± 6^{b}
MOS2 (76.5%)	221.0 ± 66.40^{c}	7 ± 1^{c}	4 ± 1^{c}
MOS3 (80.2%)	1.3 ± 1.25^{a}	37 ± 7^{a}	12 ± 6^{ab}
MOS4 (83.3%)	128.8 ± 33.79^{b}	18 ± 3^{c}	10 ± 2^{ab}

Values with different letters are significantly different from each other ($p < 0.05$).

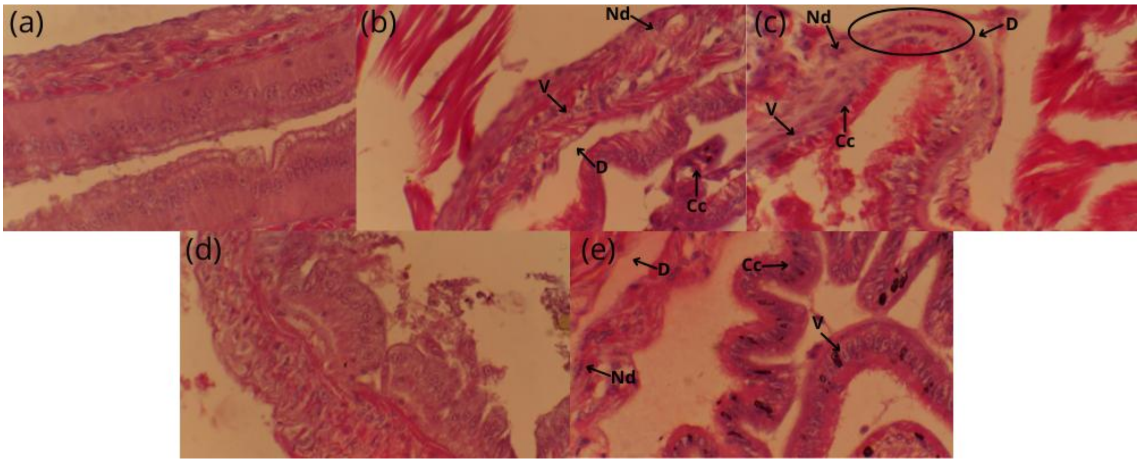

Figure 1. Histopathology of shrimp feed with MOS at different purity levels (400×). **(a)** Control group and **(d)** MOS3 (80.2%): conserved morphology with no apparent lesions in most of the intestinal tract; **(e)** MOS4 (83.3%) and **(b)** MOS1 (72%): damaged morphology with reduced villi width and height (stage III lesions); **(c)** MOS2 (76.5%): severe damage to most of the intestine with melanization at the epithelial base and severe atrophy of intestinal walls (stage III lesions). The main damage observed is indicated by the following abbreviations: **D** – Epithelial detachment; **V** – Cytoplasmic vacuolization; **Nd** – Nuclear displacement; **Cc** – Condensed chromatin; **Circle** – Region of epithelial melanization.

4 Considerações finais

- A pureza e composição dos suplementos MOS/β-glucanos influenciaram diretamente a integridade intestinal de *Penaeus vannamei*.
- O suplemento MOS3(80,2%) preservou a mucosa intestinal (HAI <10) e promoveu maior colonização por *Bacillus* spp. mesmo sem ganhos zootécnicos significativos após 60 dias de cultivo.
- Suplementos com menor qualidade nutricional MOS4(83,3%), MOS2(76%) e MOS1(72%) causaram lesões severas (HAI>100), inflamação crônica e mortalidade elevada (>50%) após 60 dias de experimento.
- É necessário o desenvolvimento de protocolos de suplementação intermitentes aliados a critérios mínimos de pureza e qualidade composicional de forma a garantir a integridade intestinal sem comprometimento na saúde dos animais em cultivos simbióticos intensivos.

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