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Dietary Sago Caterpillar Meal Enhances Innate Immunity and Resistance to Vibriosis in Nile Tilapia (*Oreochromis niloticus*)

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Abstract

Vibriosis, caused by *Vibrio* spp., is a major bacterial infection leading to high mortality rates in fish. Enhancing the innate immune response through dietary immunostimulants is a promising preventive approach. This study investigates the effects of dietary inclusion of sago caterpillar meal (*Rhynchophorus ferrugineus*) on the growth performance and innate immune response of Nile tilapia (*Oreochromis niloticus*) following infection with *Vibrio harveyi*. A completely randomized design was employed with four dietary treatments (0%, 10%, 20% and 30% sago caterpillar meal) and three replicates per treatment. Tilapia were fed these diets for 30 days, after which a bacterial challenge test was conducted. Immune response was assessed by measuring total leukocyte count and bacterial load in fish gills, while growth performance was evaluated based on daily weight and length gain, specific growth rate (SGR), feed conversion ratio (FCR) and survival rate. Results showed that dietary sago caterpillar meal significantly influenced total leukocyte count, bacterial concentration in the gills, daily weight gain, SGR and survival rate. However, no significant effects were observed on daily length gain or FCR. The optimal concentration for enhancing immune response and growth performance was found to be 20% sago caterpillar meal. These findings suggest that sago caterpillar meal is a potential immunostimulant and growth enhancer in Nile tilapia culture.

Keywords: *Oreochromis niloticus*; Sago caterpillar meal; Immune response; Vibriosis; Growth performance

Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most important cultured fish species globally, widely farmed in freshwater and brackish environments (Fouz *et al*. 2002). It is commercially produced in several Asian countries (Dong *et al*. 2015) and ranks as the third most cultivated aquatic species worldwide, with production reaching 4.2 to 4.5 million tons between 2016 and 2018 (Dietz and Liebert 2018; Alves *et al*. 2020). The increasing demand for tilapia is driven by its rapid growth rate, adaptability to diverse aquatic conditions, high resistance to pathogens and suitability for various aquaculture systems, including ponds, rivers and reservoirs (Jabir *et al*. 2012; Pakingking *et al*. 2015). Despite its relative resistance to disease, tilapia remains vulnerable to infections caused by opportunistic pathogens, leading to significant economic losses in aquaculture (Al-Harbi and Uddin 2005).

Among the major bacterial pathogens affecting tilapia is *Vibrio* spp., which causes vibriosis—a serious disease commonly found in marine and estuarine aquaculture systems. Vibriosis has been linked to high mortality rates and substantial economic losses in fish farming (Pakingking *et al*. 2015; Ceballos-Francisco *et al*. 2020). *Vibrio* spp. naturally colonizes the skin, gills and gastrointestinal tract of tilapia, particularly in brackish water environments. The risk of infection increases under stressful conditions, such as high stocking densities and poor water quality (Fouz *et al*. 2002). In addition to its impact on fish health, *Vibrio* spp. is also present in the gut microbiota of tilapia, posing potential zoonotic risks to humans (Pakingking *et al*. 2015). Enhancing the innate immune response of tilapia is therefore crucial for disease prevention and improved

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aquaculture sustainability.

The use of immunostimulants is a promising strategy to enhance fish immunity and resistance to bacterial infections. Immunostimulants activate phagocytes and enhance the production of immune-related molecules, thereby strengthening the host's defense mechanisms (Immanuel *et al*. 2009; Rozik and Rochman 2021). These compounds can be derived from various sources, including bacterial components, carbohydrates, plant extracts and animal-derived materials (Immanuel *et al*. 2009). One emerging source of immunostimulants in aquaculture is insect meal, which has gained attention due to its high protein content and bioactive compounds with immune-boosting properties.

Insects are increasingly recognized as a sustainable alternative protein source for aquaculture feeds, offering high levels of protein (60–70%), essential amino acids and beneficial lipids (Dietz and Liebert 2018; Fontes *et al*. 2019; Tippayadara *et al*. 2021). Diptera insects have the potential to serve as an alternative protein source for use in aquaculture (Barroso *et al.* 2014). In addition to their nutritional value, insects contain bioactive compounds such as antimicrobial peptides, lauric acid, chitin, carotenoids and tocopherols, which exhibit antioxidant, anti-inflammatory and immunostimulatory effects (Dicke 2018; Sogari *et al.* 2019; Alves *et al*. 2020).

One promising insect-derived feed ingredient is sago caterpillar meal (*Rhynchophorus ferrugineus*), obtained from the larvae of the red palm weevil, a common pest of sago, coconut and oil palm trees (Leatemia *et al.* 2021). Sago caterpillars are rich in protein (33.68%), fat (18.09%) and essential amino acids such as methionine, leucine, valine and lysine (Ariani *et al*. 2018). They also contain high antioxidant levels (78.6% per 100 g), which help neutralize free radicals and modulate immune function (Leatemia *et al*. 2021). Additionally, chitin, carotenoids and tocopherols present in sago caterpillars contribute to fish health by enhancing immune responses and protecting cell membranes from oxidative damage (Chinarak *et al*. 2020). Chitin and its derivatives have been shown to stimulate both innate and adaptive immunity in vertebrates (Dicke 2018).

Given its nutritional and bioactive properties, sago caterpillar meal has the potential to improve fish growth and immune function. This study evaluates the effects of dietary sago caterpillar meal on the growth performance and innate immune response of Nile tilapia (*O. niloticus*) following infection with *Vibrio harveyi.*

Materials and Methods

Experimental protocol

Juvenile Nile tilapia (*Oreochromis niloticus*), measuring 4–5 cm in body length, were obtained from BPBAP Takalar, South Sulawesi, Indonesia. The fish were transferred to 12 glass aquaria (42 L capacity) filled with 30 L of freshwater, with each aquarium housing 20 fish. Prior to the experiment, tilapia was acclimatized for seven days in a fiber tank equipped with an aeration system. During this period, fish were fed a commercial diet twice daily (08:00 and 17:00) to satiation.

Sago caterpillars (*Rhynchophorus ferrugineus*) were collected from Luwu District, South Sulawesi, Indonesia. The caterpillars were washed, oven-dried at 60°C for 5–7 hours and ground into a fine meal. The experimental diets were formulated by incorporating sago caterpillar meal at final concentrations of 0% (control), 10%, 20% and 30%, as shown in Table 1. Dry ingredients were mixed until homogeneous, combined with water, pelletized (2 mm diameter), air-dried and stored until use.

Tilapia were fed their respective experimental diets for 30 days, with a total water change every 10 days. Daily maintenance included siphoning uneaten feed and feces and a 10–20% water exchange to maintain optimal water quality parameters:

- Temperature: 26–30°C
- pH: 6.8–7.3
- Dissolved oxygen (DO): 5.5–6.4 mg. kg^{-1}
- Ammonia (NH_3): 0–1 mg. kg^{-1}

A completely randomized design (CRD) was employed, consisting of four dietary treatments with three replicates each. The treatments tested were the percentage use of sago caterpillar meal at a concentration of 0%, 10%, 20% and 30%.

Cellular innate immune response

The bacterial challenge test was conducted using *Vibrio harveyi*, obtained from the Laboratory of Fish Parasites and Diseases, Faculty of Marine and Fisheries Sciences, Hasanuddin University. Bacterial isolates were cultured in Trypticase Soy Broth (TSB) for 24 h before use.

Following the 30-day feeding trial, fish were exposed to a *V. harveyi* suspension at 10^5 CFU/mL via immersion for seven days. Immune response and microbiological assessments were conducted post-challenge: Leukocyte Count: Total leukocyte levels were determined by blood sampling. Bacterial Load: A 1 g sample of fish gill tissue was collected from each aquarium, serially diluted (10^{-1} to 10^{-3}) and plated on Thiosulfate Citrate Bile Sucrose Agar (TCBSA). Bacterial colonies were counted using the plate count method and *V. harveyi* concentrations were compared among treatment groups.

Growth performance analysis: Fish growth performance was evaluated based on daily weight gain, daily length gain, specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR).

- *Average* Daily Weight Gain (ADG) (Hasan *et al*. 2021):

Table 1: Experimental feed composition

No.	Feed Raw Materials	Treatment			
		A (%)	B (%)	C (%)	D (%)
1	Sago caterpillar meal	0	10	20	30
2	Fish meal	56	46	36	26
3	Cornstarch	20	20	20	20
4	Tapioca flour	10	10	10	10
5	Fine bran	10	10	10	10
6	Vitamin mix.[a]	2	2	2	2
7	Mineral mix.[b]	2	2	2	2
	Total	100	100	100	100

[a]According to vitamin mixture (mg kg^{-1} dry diet) of Halver (1995): thiamin HCl; riboflavin 20; pyridoxine HCl 5; choline chloride 500; nicotinic acid 75; ca-pantothenate 50; inositol 200; biotin 0.5; folic acid 1.5; ascorbic acid 100; vitamin B1 10; menadione (K) 4; α-tocopherol-acetate (E) 40

[b]According to mineral mixture (mg kg^{-1} dry diet) of Halver (1995): Salt mix #2 USP XI11 100 g; $AlCl_3$ 15; $ZnSo_4$ 300; CuCl 10; $MnSo_4$ 80; KI 15; $CoCl_2$ 80; Na_2SeO_3 10

$$\text{ADG} = \frac{\text{Wt}-\text{Wo}}{\text{H}}$$

Where:
Wt: Final weight (g)
W0: Initial weight (g)
H: Days.

- *Average* Daily Length Gain (ADL) (Hasan *et al.* 2021):

$$\text{ADL} = \frac{\text{Lt}-\text{Lo}}{\text{H}}$$

Where:
Lt: Final length (cm)
Lo: Initial length (cm)
H: Days.

- The specific growth rate (α) of fish was calculated using the formula proposed by Tilami *et al.* (2020):

$$\alpha = 100 \text{ x } \frac{\text{Ln(Wt)}-\text{Ln(Wo)}}{\text{t}}$$

Where:
α: Specific growth rate (% days-1)
Wt: Final weight (g)
Wo: Initial weight (g)
t: Days.

Feed conversion rate (FCR): According to Tilami *et al.* (2020), the FCR formula is as follows:

$$\text{FCR} = \frac{\text{F}}{\text{(Wt)-W0}}$$

Where:
F: Total amount of the feed consumed (g)
Wt: Final weight (g)
D: Weight of dead fish (g)
W0: Initial weight (g).

Survival rate (SR): The survival rate (SR) was calculated 2 times, namely 30 days after feeding treatments and 7 days after the bacteria challenge test. According to Tilami *et al.* (2020), the formula is stated as follows:

$$\text{SR} = \frac{\text{Nt}}{\text{No}} \, x \, 100\%$$

Where:
SR : Survival Rate (%)
Nt : Final number of fish
N0 : Initial number of fish.

Statistical analysis

Bacterial counts, growth performance and survival rate data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post hoc test. Statistical significance was determined at $P < 0.05$. All statistical analyses were performed using SPSS version 22.0. Immune response data were descriptively analyzed based on tilapia viability post-challenge.

Results

Sago caterpillar meal as an immunostimulant

The innate immune system, particularly leukocytes (white blood cells), plays a crucial role in defending fish against pathogenic microorganisms and viral infections (Ali *et al.* 2019; Alves *et al.* 2020; Rozik and Rochman 2021). Leukocyte counts in fish are influenced by factors such as species, age, diet and environmental conditions (Alves *et al.* 2020).

In this study, fish fed 20% sago caterpillar meal exhibited leukocyte levels within the normal physiological range, indicating enhanced immune function and reduced stress levels compared to other treatment groups. The antibacterial effects of sago caterpillar meal are likely due to its high content of bioactive polysaccharides, chitin and antioxidants, which stimulate phagocytic activity and enhance host defense mechanisms (Dicke 2018; Sogari *et al.* 2019).

Chitin and its derivatives (chitosan, Chito oligosaccharides) enhance immunity by stimulating the production of beneficial gut bacteria while inhibiting pathogenic microbes (Huis 2013). Additionally, chitin increases lysozyme activity, which helps degrade bacterial cell walls, thereby enhancing resistance to infections (Alves *et al.* 2020; Tippayadara *et al.* 2021).

To further evaluate the efficacy of sago caterpillar meal in mitigating bacterial infection, the total *Vibrio harveyi* concentration in the gills was assessed post-challenge. The bacterial load was significantly lower ($P < 0.05$) in fish fed sago caterpillar meal-containing diets compared to the control. A dose-dependent decrease in *V. harveyi* concentration was observed, indicating that dietary inclusion of sago caterpillar meal enhanced disease resistance in tilapia.

Growth performance

The specific growth rate (SGR) of tilapia fed with sago caterpillar meal is presented in Fig. 2. The results showed that dietary inclusion had a significant effect ($P < 0.05$) on SGR.

Daily weight gain and specific growth rate (SGR): Statistical analysis (Fig. 1) showed that dietary inclusion of sago caterpillar meal had a significant effect ($P < 0.05$) on daily weight gain, whereas it had no significant impact ($P > 0.05$) on daily length gain. Similarly, SGR was significantly higher ($p < 0.05$) in fish fed sago caterpillar meal diets compared to the control (Fig. 2). These results suggest that sago caterpillar meal positively influences tilapia growth, particularly at a 20% inclusion level.

Feed conversion ratio (FCR): The feed conversion ratio (FCR), which measures feed efficiency, did not differ significantly ($P > 0.05$) among treatment groups (Fig. 3). However, the lowest FCR was observed in fish fed 20% sago caterpillar meal (1.27 ± 0.17), suggesting improved feed utilization compared to other groups.

The FCR values obtained in this study fall within the optimal range for tilapia (0.8–1.6) (Ihsanudin *et al.* 2014) and are lower than those reported in previous studies using Black Soldier Fly (*Hermetia illucens*) (2.14–2.23) (Tippayadara *et al*. 2021) and Housefly (*Musca domestica*) (1.32–1.86) (Wang *et al*. 2017). The higher digestibility and palatability of sago caterpillar meal likely contributed to the improved FCR observed in this study.

Survival rate (SR): Survival rate was assessed both after the 30-day feeding trial and after the 7-day bacterial challenge test. The highest survival rates were recorded in fish fed 20% and 30% sago caterpillar meal, indicating that sago caterpillar meal enhances disease resistance and overall fish health (Fig. 4).

These findings align with Rahman *et al*. (2022), who reported that survival rates of cultured tilapia typically exceed 100% when optimal feed and environmental conditions are maintained (Table 2-3).

Discussion

Sago caterpillar meal as an immunostimulant

The antibacterial effects of sago caterpillar meal are likely due to its high content of bioactive polysaccharides, chitin and antioxidants, which stimulate phagocytic activity and enhance host defense mechanisms (Dicke 2018; Sogari *et al*. 2019). Chitin and its derivatives (chitosan, chitooligosaccharides) enhance immunity by stimulating the production of beneficial gut bacteria while inhibiting pathogenic microbes (Huis 2013).

Additionally, chitin increases lysozyme activity, which helps degrade bacterial cell walls, thereby enhancing resistance to infections (Alves *et al.* 2020; Tippayadara *et al.* 2021). The dose-dependent reduction in bacterial load further confirms the immunostimulatory effect of sago caterpillar meal.

Growth performance and feed utilization

Despite concerns that chitin-rich diets may negatively impact protein and lipid digestibility (Sánchez-Muros *et al*. 2015; Fontes *et al*. 2019), this study demonstrated that sago caterpillar meal positively affects tilapia growth performance.

The improved FCR observed in this study suggests that sago caterpillar meal provides an efficient protein source, supporting optimal feed utilization and fish growth.

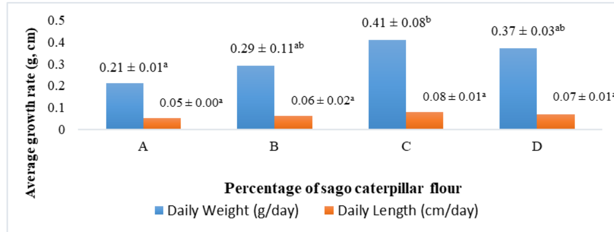

Fig. 1: Dietary inclusion of sago caterpillar meal increases the growth rate of tilapia (*O. niloticus*). Tilapia (n = 20/treatment) were fed with feed containing sago caterpillar meal at a concentration of 0% (A), 10% (B), 20% (C), or 30% (D) for 30 days. Fish body weight and fork length were measured daily. Sago caterpillar meal at a concentration of 20% (C) significantly increased daily body weight but not fork length of tilapia. Data are presented as means of three duplications (n = 3) and the different letters above show a significant difference ($P < 0.05$)

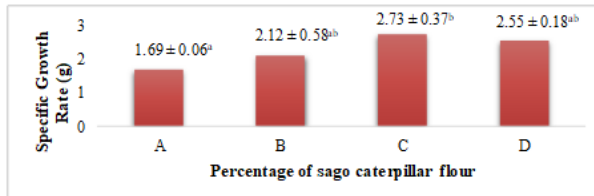

Fig. 2: Dietary inclusion of sago caterpillar meal improves the specific growth rate (SGR) of tilapia (*O. niloticus*). Tilapia (n = 20/treatment) were fed feed containing sago caterpillar meal at concentrations of 0% (A), 10% (B), 20% (C), or 30% (D) for 30 days. Sago caterpillar meal at a concentration of 20% (C) significantly increased the specific growth rate of tilapia. Data are presented as means of three duplications (n = 3) and the different letters above show a significant difference ($P < 0.05$)

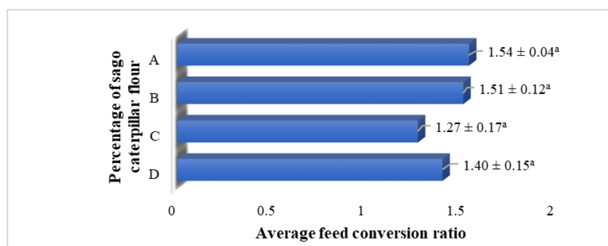

Fig. 3: Dietary inclusion of sago caterpillar meal does not affect the feed conversion ratio (FCR) of tilapia (*O. niloticus*). Tilapia (n = 20/treatment) were fed feed containing sago caterpillar meal at concentrations of 0% (A), 10% (B), 20% (C), or 30% (D) for 30 days. No significant differences were observed between fish fed with control and sago caterpillar meal-containing diets. Data are presented as means of three duplications (n = 3)

Table 2: Tilapia Leukocyte Examination Results

No.	Treatment (*n* = 3)	Leukocyte Total (cell/mm^3)
1	Early (before challenge)	1.96×10^5
2	A[a]	2.32×10^5
3	B[b]	2.36×10^5
4	C[c]	1.04×10^5
5	D[d]	3.40×10^5

Nb: Different letters showed significant differences between treatments with a level of 5% (*P < 0.05*). n = number of samples; [a] 0% sago caterpillar flour; [b] 10% sago caterpillar flour; [c] 20% sago caterpillar flour; [d] 30% sago caterpillar flour

Table 3: The mean of total bacteria in tilapia after rearing and challenge test of *V. harveyi*

Treatment (*n* = 3)	Total of *V. harveyi* bactery (cfu/mL)
A[a]	$8.85 \times 10^{6(a)}$
B[b]	$5.30 \times 10^{5(b)}$
C[c]	$4.97 \times 10^{5(b)}$
D[d]	$3.60 \times 10^{5(b)}$

Nb: Different letters showed significant differences between treatments with a level of 5% (*P < 0.05*). n= number of samples; [a] 0% sago caterpillar flour; [b] 10% sago caterpillar flour; [c] 20% sago caterpillar flour; [d]30% sago caterpillar flour

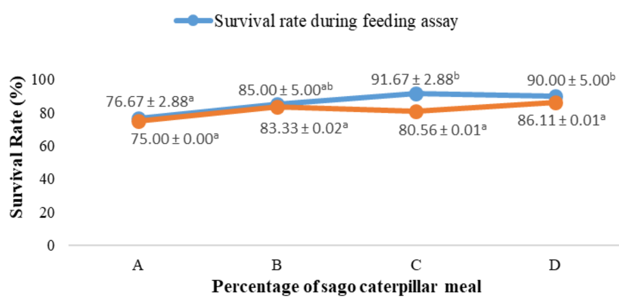

Fig. 4: Dietary inclusion of sago caterpillar meal enhances the survival rate of tilapia (*O. niloticus*). Tilapia (n = 20/treatment) were fed with feed containing sago caterpillar meal at concentrations of 0% (A), 10% (B), 20% (C), or 30% (D) for 30 days. The fish survival rate was calculated twice during the feeding treatment period (30 days) and after the challenge test period (7 days). Sago caterpillar meal at all concentrations significantly increased the survival rate of tilapia compared to the control. Data are presented as means of three duplications (n = 3) and the different letters above show a significant difference (*P < 0.05*)

The protein content of sago caterpillar meal (33.68% per 100 g) is comparable to fish meal, with high essential amino acid composition, including glycine, leucine and methionine (Ariani *et al*. 2018; Chinarak *et al*. 2020).

Fatty acid composition and survival rate

Sago caterpillar meal is also rich in omega-6 fatty acids, including palmitic, oleic and linoleic acids (Chinarak *et al*. 2020; Leatemia *et al*. 2021). These fatty acids play a crucial role in immune function and membrane integrity, further contributing to the high survival rates observed in tilapia fed sago caterpillar meal diets (Barakat *et al*. 2017; Qiang *et al*. 2019).

Other bioactive compounds present in sago caterpillar meal, including carotenoids, tocopherols, zinc and iron, contribute to antioxidant defense mechanisms, protecting fish from oxidative stress and disease (Dobermann *et al*. 2017; Dicke 2018; Huis 2020).

Conclusion

The inclusion of sago caterpillar meal at a 20% dietary concentration significantly enhances the immune response of tilapia (*Oreochromis niloticus*) against bacterial infections. Additionally, it promotes improved growth performance, as evidenced by increased daily weight gain, specific growth rate and overall survival. These findings suggest that sago caterpillar meal is a promising alternative protein source for optimizing the health and growth of tilapia in aquaculture.

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Author Contributions

The authors contributed to the study's conception and design. Grant acquisitions were contributed by GL, AAH and MA. Fish and Sago Caterpillar preparation was performed by ATS and AMFRF. Data collection and analysis were performed by DDA and DMI. Visualization and data validation were performed by MFZA. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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