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Article

Feeding and Social Behavior as Indicators of Dietary Acceptance and Growth Performance in *Oreochromis Niloticus* Fed Watermelon Rind Powder

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ABSTRACT
Integrating fish ethology into nutritional studies can provide valuable insights into dietary acceptance and help explain variations in feed intake and growth performance in aquaculture systems. This study evaluated the effects of dietary inclusion of watermelon rind powder (WRP, Citrullus lanatus), on feeding and social behavior, feed leftovers, and growth performance of Nile tilapia (Oreochromis niloticus). Fingerlings (4.20 ± 0.22 g) were randomly assigned to six dietary treatments containing increasing levels of WRP (0–20%) as a dietary ingredient, with three replicates per treatment. Behavioral responses, including foraging, feed approach, feed apprehension, feed fragmentation, shoaling behavior, and agonistic interactions, were systematically evaluated to characterize feeding activity and social responses associated with dietary acceptance. Before each feeding, all experimental units were cleaned by siphoning to remove residual waste accumulated overnight, ensuring that only leftovers from the experimental meal were collected. After feed delivery, fish were allowed to feed for 10 min, after which the remaining feed was carefully recovered by siphoning for subsequent quantification. Intermediate WRP inclusion levels were associated with more active feeding responses, greater feeding vigor, increased feed apprehension, and better growth performance, whereas higher inclusion levels (16% and 20%) were associated with reduced feeding activity, feed apprehension, and feeding vigor, increased feed leftovers, and lower weight gain. These behavioral responses provide a plausible explanation for the lower apparent feed intake observed at higher WRP inclusion levels. The observed responses may be associated with changes in dietary characteristics, particularly the higher fiber content of diets containing greater levels of WRP, although digestibility was not evaluated in the present study. Overall, the findings indicate that feeding and social behavior can provide complementary information for interpreting dietary acceptance, feed leftovers, and growth performance, supporting ethological assessment as a useful approach in the evaluation of functional aquafeeds.
Keywords: dietary acceptance; feeding behavior; social behavior; feed intake; Nile tilapia.

RESUMO
A integração da etologia de peixes aos estudos nutricionais pode fornecer informações valiosas sobre a aceitação da dieta e ajudar a explicar variações no consumo de ração e no desempenho de crescimento em sistemas de aquicultura. Este estudo avaliou os efeitos da inclusão dietética do pó do pericarpo da melancia (PCM, Citrullus lanatus), sobre o comportamento alimentar e social, as sobras de ração e o desempenho de crescimento da tilápia do Nilo (Oreochromis niloticus). Alevinos (4,20 ± 0,22 g) foram distribuídos aleatoriamente em seis tratamentos dietéticos contendo níveis crescentes de PCM (0–20%) como ingrediente da dieta, com três repetições por tratamento. As respostas comportamentais, incluindo forrageamento, aproximação à ração, apreensão da ração, fragmentação da ração, comportamento de cardume e interações agonísticas, foram avaliadas sistematicamente para caracterizar a atividade alimentar e as respostas sociais associadas à aceitação da dieta. Antes de cada alimentação, todas as unidades experimentais foram submetidas à limpeza por sifonamento para remover os resíduos acumulados durante a noite, garantindo que somente as sobras provenientes da refeição experimental fossem coletadas. Após o fornecimento da ração, os peixes foram deixados para se alimentar por 10 min, e, posteriormente, a ração remanescente foi cuidadosamente recuperada por sifonamento para posterior quantificação. Níveis intermediários de inclusão de PCM foram associados a respostas alimentares mais ativas, maior vigor alimentar, maior apreensão da ração e melhor desempenho de crescimento, enquanto níveis mais elevados (16% e 20%) foram associados à redução da atividade alimentar, da apreensão da ração e do vigor alimentar, ao aumento das sobras de ração e à redução do ganho de peso. Essas

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respostas comportamentais fornecem uma explicação plausível para o menor consumo aparente de ração observado nos níveis mais elevados de inclusão de PCM. As respostas observadas podem estar associadas a alterações nas características das dietas, particularmente ao maior teor de fibra nas dietas contendo níveis mais elevados de PCM, embora a digestibilidade não tenha sido avaliada no presente estudo. De modo geral, os resultados indicam que o comportamento alimentar e social pode fornecer informações complementares para a interpretação do desempenho de crescimento, reforçando a avaliação etológica como uma abordagem útil na avaliação de rações funcionais para a aquicultura.
Palavras-chave: composição da dieta; comportamento alimentar; comportamento social; consumo de ração; tilápia do Nilo.

Introduction

Feeding is one of the main factors influencing productive performance in aquaculture, directly affecting growth, feed conversion, and fish welfare. Beyond nutritional aspects, feeding and social behavior play a crucial role in consumption efficiency and competition among individuals, with applied ethology serving as a valuable tool to understand how different dietary ingredients influence feeding dynamics (Huntingford & Karlsen, 2003). Animals rely on dietary resources to meet their energy and nutritional requirements, which are essential for vital physiological processes such as growth, locomotion, and reproduction (Wang et al. 2006; Secor & Carey, 2016). Adequate nutrition promotes higher weight gain and body development, being fundamental for improving economic production indices, particularly in tilapia farming. However, rising feed costs and competition for conventional ingredients, such as fishmeal, soybean meal, and corn, underscore the need for sustainable and innovative feed alternatives (Dawood & Koshio, 2019; Jannathulla et al. 2019).

Fish health is another critical factor for sustainable production. Bacterial infections can cause significant economic losses, necessitating effective strategies to improve health and welfare. In this context, supplementation with functional additives, such as fruit by-products, has shown considerable potential (Arumugam et al. 2023; Sampathkumar et al. 2023). Agro-industrial residues, often discarded, contain bioactive compounds capable of enhancing immunity, reducing infections, and promoting animal growth (Richa et al. 2023; Veja et al. 2024).

Watermelon rind, in particular, is rich in antioxidants (lycopene, L-citrulline, β-carotene), vitamins (A, C, folic acid), minerals (potassium, magnesium), and fibers (mainly pectin), which can benefit fish health and support sustainable feed production (Zamuz et al. 2021; Burton-Freeman et al. 2020; Manivannan et al. 2020; Perdana et al. 2023; Milios, 2018; Mendes et al., 2026). However, its fiber content may interfere with feed intake and feeding behavior, affecting both food apprehension and social interactions among individuals (Bai et al. 2022). Therefore, evaluating feeding and social behavior is essential to understand how diet influences individual dynamics, performance, and welfare (Volpato et al. 2007).

In this study, watermelon rind powder (WRP) was tested as a partial replacement for corn meal, rather than protein-rich ingredients, due to its high energy content. During diet formulation, a natural reduction in corn meal and an increase in WRP inclusion (~17% crude protein) were observed, maintaining an adequate nutritional profile. Previous studies have evaluated WRP as a replacement for fishmeal or soybean meal, reporting benefits at inclusion levels up to 50%, but negative effects at higher levels due to fiber content (Vijayan et al. 2024; Samuel et al. 2022; Van Doan et al. 2020; Oladipupo & Salame, 2020). This study differs by prioritizing the replacement of an energy ingredient and, for the first time, integrating the analysis of feeding and social behavior with zootechnical performance parameters, such as feed intake, weight gain, and production efficiency.

Therefore, this study aimed to evaluate the effects of dietary inclusion of watermelon rind powder (Crimson Select cultivar) as a partial replacement for corn meal on the growth performance and behavioral responses of Nile tilapia (*Oreochromis niloticus*). Particular emphasis was placed on feeding and social behaviors as indirect indicators of feed intake. Additionally, the study sought to identify the maximum inclusion level that maintains fish welfare and production efficiency, proposing an integrative approach in which behavioral responses are used to explain zootechnical performance.

Methodology

Local

The experiment was conducted between October and December 2024, at the facilities of the Federal University of Vale do São Francisco (UNIVASF), in Petrolina, Pernambuco, Brazil. The specimens used here were of sexually reversed Nile tilapia (*Oreochromis niloticus*) with an average initial weight of 4.20 ± 0.22 g, donated

by the São Francisco and Parnaíba Valley Development Company (CODEVASF). The fish were randomly distributed at a population density of 20 fingerlings/m³.

Ethics

All the experimental stages were conducted in accordance with Normative Resolution No. 52 of Brazil's National Council for the Control of Animal Experimentation (CONCEA), dated May 19, 2021, and previously approved by the Ethics Committee on Animal Use of UNIVASF under Protocol No. 20240817.

Experimental conditions

The experimental units consisted of blue polyethylene tanks with white lids, with a useful volume of 1000 liters. The experimental design adopted here was completely randomized, with the tanks operating in a closed water recirculation system, equipped with continuous aeration and partial water renewal of 10 to 15 L min^{-1}. Water quality was monitored daily by measuring dissolved oxygen, temperature and pH, using a digital oximeter (YSI, Inc.) and a duly calibrated pH meter. In addition, total ammonia, nitrite, nitrate, alkalinity and water hardness levels were analyzed weekly, using specific Labcon® test kits to ensure adequate pisciculture conditions throughout the experiment.

The average water quality parameters recorded during the experimental period were as follows: pH 6.45, total ammonia 0.001 ppm, nitrate 2 ppm, nitrite 0.25 ppm, suspended solids (SSG) 0.01%, oxidation-reduction potential (ORP) 75 mV, water temperature 26°C, dissolved oxygen 7.59 mg/L, salinity 114 ppm, electrical conductivity 171 μS/cm, moderate water hardness, and alkalinity of 80 mg/L.

Development

The watermelon rind powder (WRP) additive was produced from the rind of Crimson Select watermelons, planted on December 5, 2023, and harvested on February 7, 2024. During cultivation, the crop was managed according to conventional agricultural practices using the pesticides Vertimec®, Polytrin®, Cefanol®, and Provado®. Experimental diets containing 32% crude protein were formulated and used in both the zootechnical performance trial and the feeding and social behavior assessments. The diets were formulated to be isoproteic and isocaloric, meeting the nutritional requirements of juvenile Nile tilapia (*Oreochromis niloticus*). To maintain equivalent nutritional profiles among treatments, adjustments were made to the proportions of soybean meal, fish meal, wheat bran, soybean oil, mineral supplements, and amino acids (L-lysine and DL-methionine) as WRP progressively replaced ground corn in the formulations.

Six experimental diets were prepared with increasing dietary inclusion levels of WRP (0, 4, 8, 12, 16, and 20%), corresponding to the replacement of 0, 20, 40, 60, 80, and 100% of the ground corn, respectively. Therefore, dietary WRP inclusion level was established as the sole experimental variable. Adjustments to the remaining ingredients were performed solely to maintain comparable nutrient profiles among diets with respect to crude protein, dietary energy, and essential amino acid contents, and should not be interpreted as independent experimental factors. The diets were pelleted into 1.0- and 2.0-mm diameter pellets and supplied *ad libitum* three times daily at 08:00, 12:00, and 16:00 h. The experiment consisted of six dietary treatments with three replicates each, totaling 18 experimental units. The ingredient composition and the calculated nutritional composition of the experimental diets (Tables 1 and 2).

Table 1: Ingredient composition of the experimental diets (% as-fed basis).

Nutrients	***Composition***					
Crude protein (%)	***32.00***	***32.00***	***32.00***	***32.00***	***32.00***	***32.00***
Gross energy (Kcal/Kg)	***3.490***	***3.488***	***3.466***	***3.444***	***3.422***	***3.400***
Raw fiber (%)	***5.13***	***5.53***	***5.93***	***6.32***	***6.72***	***7.12***
Ether Extract	***0.82***	***2.00***	***3.17***	***4.35***	***5.52***	***6.70***
Mineral matter	***11.74***	***12.56***	***12.32***	***12.28***	***11.82***	***11.25***
Nitrogen-Free Extract (%)	***50.31***	***47.91***	***46.58***	***45.05***	***43.94***	***42.93***
Lysine (%)	***2.10***	***2.10***	***2.10***	***2.10***	***2.10***	***2.10***
Methionine	***0.80***	***0.80***	***0.80***	***0.80***	***0.80***	***0.80***

Source: Authors (2026).

Vitamin and mineral mix (Premix fish Agromix®; Jaboticabal, Brazil) (guaranteed levels per kg of premix) — folic acid: 1200 mg; nicotinic acid: 20 g; pantothenic acid: 10 mg; **BHT: 5 mg; vit A: 2400 UI; vit D3: 600 UI; vit E: 30 UI mg; vit K3: 3 mg; vit C: 60 g; pantothenic acid:10 mg; biotin: B6: 3 mg; Co: 80 mg; Fe: 20 g;

Se: 100 mg; Cu: 3500 mg; Mn: 10 mg; Zn: 24 mg; I: 160 mg. 200 mg; choline: 100 g; Inositol: 25 g; Vit B1: 4 mg; Vit B2: 4 mg; Vit B12: 8 mg; Vit B2: 4 mg; Vit

Table 2: Calculated nutritional composition of the experimental diets.

Ingredients (%)	Watermelon Rind Powder content					
	0%	4 %	8 %	12%	16 %	20%
Ground corn 7.88%	20.00	16.00	12.00	8.00	4.00	0.00
Soy bran 45%	52.18	46.89	41.61	36.32	31.03	25.75
Wheat bran	5.24	7.19	9.14	11.09	13.05	15.00
Fish meal 54%	7.19	12.27	17.35	22.43	27.51	32.59
Broken rice	1.00	1.20	1.40	1.60	1.81	2.01
WRP	0.00	4.00	8.00	12.00	16.00	20.00
Vitamin C	0.06	0.06	0.06	0.06	0.06	0.06
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Dicalcium phosphate	3.50	2.80	2.10	1.40	0.70	0.00
Fish oil	0.50	1.07	1.64	2.21	2.78	3.35
L- Lysine	0.53	0.47	0.41	0.35	0.28	0.22
DL- Methionine	0.35	0.30	0.25	0.21	0.16	0.12
Vitamin mineral premix*	0.50	0.50	0.50	0.50	0.50	0.50
BHT**	0.01	0.01	0.01	0.01	0.01	0.01
Total	100 kg	100 kg	100 kg	100 kg	100 kg	100 kg

Source: Authors (2026).

Zootechnical performance and apparent feed intake

Zootechnical performance was evaluated during a 45-day feeding trial. A total of 360 Nile tilapia (*Oreochromis niloticus*) fingerlings, with an initial body weight of 4.20 ± 0.22 g, were randomly selected from a stock population of approximately 1,000 fish and distributed into 18 experimental units at a stocking density of 20 fish per tank. Fish were individually weighed on experimental days 0, 15, 30, and 45 to monitor growth performance. Following each biometric assessment, the daily feed allowance was adjusted according to the average body weight of the fish and the recommended feeding rate for the species, ensuring that feed allowance remained appropriate throughout the experimental period. The zootechnical parameters evaluated included weight gain (WG), apparent feed intake (AFI), and apparent feed conversion ratio (AFCR), calculated as follows:

$$WG\ (g) = \text{Final body weight} - \text{Initial body weight}$$
$$AFI\ (g\ fish^{-1}) = \text{Feed offered} - \text{Uneaten feed recovered}$$
$$AFCR = AFI / WG$$

Because feed intake was estimated indirectly through the recovery of uneaten feed, the values obtained are referred to throughout this study as apparent feed intake (AFI). Accordingly, feed conversion was calculated using AFI values and is therefore expressed throughout this study as the apparent feed conversion ratio (AFCR). Uneaten feed was quantified on experimental days 0, 10, 20, and 30 following a standardized recovery protocol. Before the first daily feeding (08:00 h), after a 12-h fasting period, all experimental tanks were cleaned by siphoning to remove feces and residual feed accumulated overnight, ensuring that only uneaten feed originating from the experimental meal was recovered for quantification.

The daily feed allowance for each experimental unit was weighed in advance according to the respective feeding rate. After feed delivery, fish were allowed to feed for 10 min, which was considered sufficient to allow voluntary feed intake before uneaten feed recovery. Subsequently, uneaten pellets that had settled on the bottom of the tanks were recovered by siphoning using a custom-made device consisting of a flexible hose connected to a PVC tube. Floating pellets remaining after the cessation of feeding activity were collected separately.

The recovered material was filtered through a cotton cloth to remove excess water, individually identified, and dried in a forced-air convection oven at 55 °C for 24 h. After drying, the remaining feed was weighed using an analytical balance, and the recovered mass was used to estimate apparent feed intake and evaluate diet acceptance based on the amount of uneaten feed recovered from each experimental unit.

Uneaten feed recovery was performed until experimental day 30. This procedure was not conducted on day 45 because fish underwent final biometric measurements and blood sampling as part of concurrent

physiological assessments. To minimize cumulative handling stress immediately before these terminal procedures, feed recovery by siphoning was discontinued after day 30, whereas zootechnical performance continued to be monitored until the end of the feeding trial.

Feeding and social behavior

Behavioral evaluations were performed using a structured ethogram developed to characterize the feeding and social responses of Nile tilapia throughout the experimental period (Figure 1). The ethogram was adapted from Huntingford et al. (2006) and comprised seven behavioral categories: foraging, feed approach, feed apprehension, feed fragmentation (laceration), schooling, feeding vigor, and agonistic behavior. Each behavior was defined using objective operational criteria to ensure consistency and repeatability throughout the behavioral assessments.

Behavior Evaluation

BEHAVIOR	DESCRIPTION	EVALUATION
Foraging	Movement in the tank and search for food (floating or sedimented).	(1) Absence (2) Discreet (0) Discreet
Approach	Olfactory recognition of food.	(1) No Recognition (2) Recognition
Apprehension	Slow or voracious capture of food.	(1) Slow (2) Voracious
Laceration	Food crushed or swallowed whole.	(1) Absence (2) Swallowing Whole
Schooling	Collective swimming behavior.	(1) Absence (2) Discreet (3) Effective
Vigor	Activity level during feeding.	(1) Absence (2) Discreet (3) Effective
Agonistic Behavior	Aggressive or dominant interactions.	(1) No Agonist (2) Presence of Agonist

Figure 1: Food and behavioral classification and description, protocol adapted from Huntingford et al. (2006). Source: Authors (2026).

Behavioral assessments were conducted by direct visual observation performed by previously trained observers who remained blinded to the dietary treatments throughout the experimental period, thereby minimizing observer bias. To ensure methodological consistency, all behavioral evaluations were conducted by the same trained observers. All fish within each experimental unit (20 fish per tank) were considered during every behavioral assessment. Behavioral observations were conducted on experimental days 0, 10, 20, and 30, immediately after feed delivery, when feeding activity was most intense. Each observation session lasted 10 min, during which the occurrence of each behavioral category was recorded according to the criteria established in the ethogram (Figure 1). Immediately after the observation period, uneaten feed was recovered by siphoning following the standardized protocol described in the Apparent Feed Intake section.

Behavioral responses were recorded using predefined numerical scores for each behavioral category and subsequently converted into percentages for graphical presentation. Because the behavioral assessments generated qualitative categorical data rather than continuous quantitative variables, the dataset was analyzed descriptively. Therefore, no inferential statistical analyses were performed, and the behavioral findings should be interpreted as exploratory indicators that complement the interpretation of growth performance, apparent feed intake, apparent feed conversion ratio, and dietary acceptance.

Growth performance data, including initial body weight, final body weight, weight gain, apparent feed intake, apparent feed conversion ratio, and uneaten feed recovered (Uneaten feed), were tested for normality and homogeneity of variances. Subsequently, the data were subjected to one-way analysis of variance

(ANOVA), followed by Tukey's honestly significant difference (HSD) test for multiple comparisons at a significance level of $P < 0.05$, using Statistica® software in UEMASUL.

Results

The inclusion of WRP significantly affected the zootechnical performance of Nile tilapia (Table 3). After 45 days of feeding, fish fed diets containing intermediate WRP inclusion levels (8–12%) exhibited the highest final weight and weight gain, whereas fish fed the control diet and the highest WRP inclusion level (20%) showed lower values. A significant quadratic response was observed for final weight and weight gain, indicating a non-linear effect of WRP inclusion on growth performance. Feed conversion ratio was significantly improved by WRP inclusion, with lower values observed in fish fed WRP-containing diets compared with the control. Apparent feed intake also varied among treatments, with the highest value observed at 16% WRP and the lowest at 20% WRP. Feed leftovers increased with increasing WRP inclusion, reaching the highest value at 20% WRP (T6).

Table 3: Effects of the inclusion of WRP to the diet on the zootechnical performance of Nile tilapia (*Oreochromis niloticus*).

Variables	***Treatments***						***CV***	***EPM***	***P value***	***Regression***	
	T1	***T2***	***T3***	***T4***	***T5***	***T6***				***L***	***Q***
Initial weight (g)	4.30^{A}	4.17^{A}	4.21^{A}	4.20^{A}	4.26^{A}	4.24^{A}	***2.58***	***0.02***	***0.7204***	***0.9261***	***0.2807***
Final weight (g)	36.5^{D}	49.50 BC	59.53 A	63.27 A	57.25 AB	45.81 CD	6,58	***2.3***	<0.0001	***0.0008***	<0.0001
Weight gain (g)	32.2^{D}	45.33 BC	55.32 A	59.07 A	52.99 AB	41.57 CD	***7.27***	***2.31***	<0.0001	***0.0009***	<0.0001
Apparent feed intake (g)	52.20 AB	51.01 AB	54.08 AB	54.79 A	55.82 A	49.57 B	***3.48***	***0.64***	***0.0100***	***0.8230***	***0.0050***
Feed Conversion Ratio	1.61^{A}	1.13^{B}	0,98^{B}	1.01^{B}	1.04^{B}	***1.20 ^{B}***	***8.49***	***0.06***	<0.0001	0,0004	<0.0001
Feed leftovers	0.62^{B}	0.66^{B}	0.75^{B}	0.80^{B}	1.04^{A} B	1.49^{A}	***19.59***	***0.10***	***0.0015***	***0.001***	***0.0186***

Source: Authors (2026).

* EPM: Standard Error of the Mean.

Means followed by the same capital letter on the line do not differ from each other by Tukey's test at 5% probability.

Treatment T1 (0% inclusion of WRP – control), T2 (4% inclusion of WRP), T3 (8% inclusion of WRP), T4 (12% inclusion of WRP), T5 (16% inclusion of WRP), and T6 (20% inclusion of WRP).

Feeding behavior can be divided into five consecutive stages: foraging, approach, grasping, tearing, and ingestion of food (Gray, 1990; Price et al., 2008). Figure 2 presents the feeding and social behavior observed during the adaptation period. During the initial adaptation phase, differences in feeding behavior were observed among treatments (Figure 2). Fish fed intermediate WRP inclusion levels showed predominantly active feeding responses, characterized by regular approach and ingestion behavior. In contrast, fish exposed to higher inclusion levels exhibited reduced feeding activity and a higher occurrence of discreet foraging behavior, indicating changes in feeding dynamics during dietary adaptation. Social behavior followed a similar pattern, with reduced group activity and feeding vigor observed at higher inclusion levels.

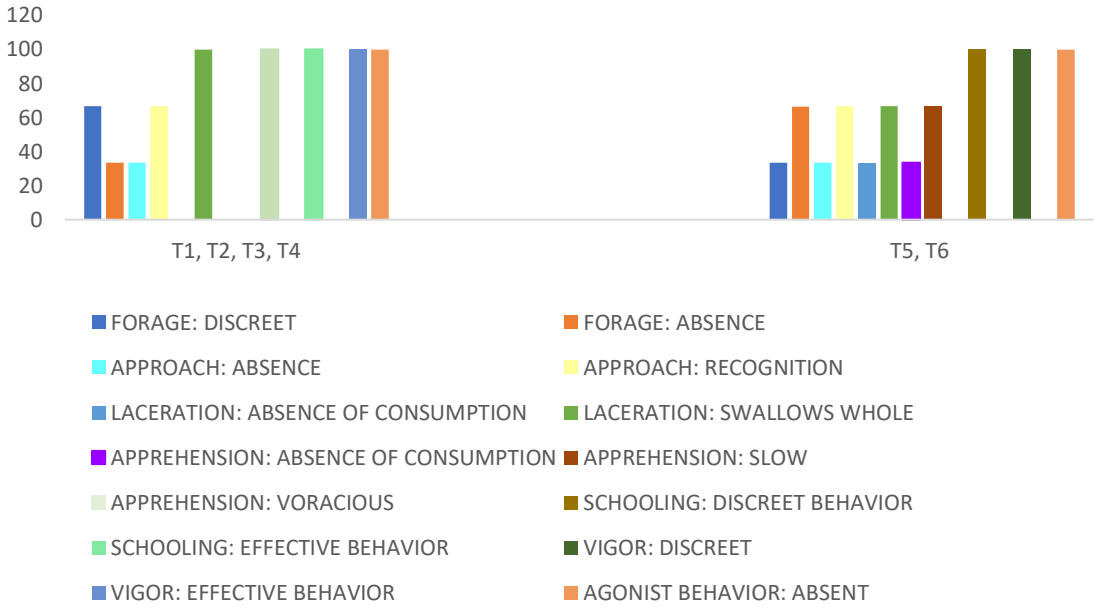


Figure 2: Scale of feeding and social behavior of tilapia fingerlings during the adaptation period to experimental diets, per treatment. Source: Authors (2026).

At day 10 of the experimental period (Figure 3), most fish across treatments exhibited active feeding behavior and normal social interactions, indicating successful initial adaptation to the diets. By days 20–30 (Figure 4), behavioral differences became more evident among treatments. Fish fed intermediate WRP inclusion levels maintained active feeding behavior, whereas those fed higher inclusion levels showed a higher proportion of discreet feeding responses and reduced feeding vigor. A comparative overview of feeding behavior and feed leftovers is presented in Figure 5. Overall, behavioral responses were consistent with the zootechnical results, with more active feeding responses at intermediate WRP inclusion levels and reduced feeding activity accompanied by greater feed leftovers at the highest inclusion level. These behavioral patterns provide a plausible explanation for the lower apparent feed intake and reduced weight gain observed at 20% WRP.



Figure 3: Assessment of the scale of feeding and social behavior of tilapia fingerlings at baseline (day 1) and after 10 days of exposure to experimental diets. Source: Authors (2026).

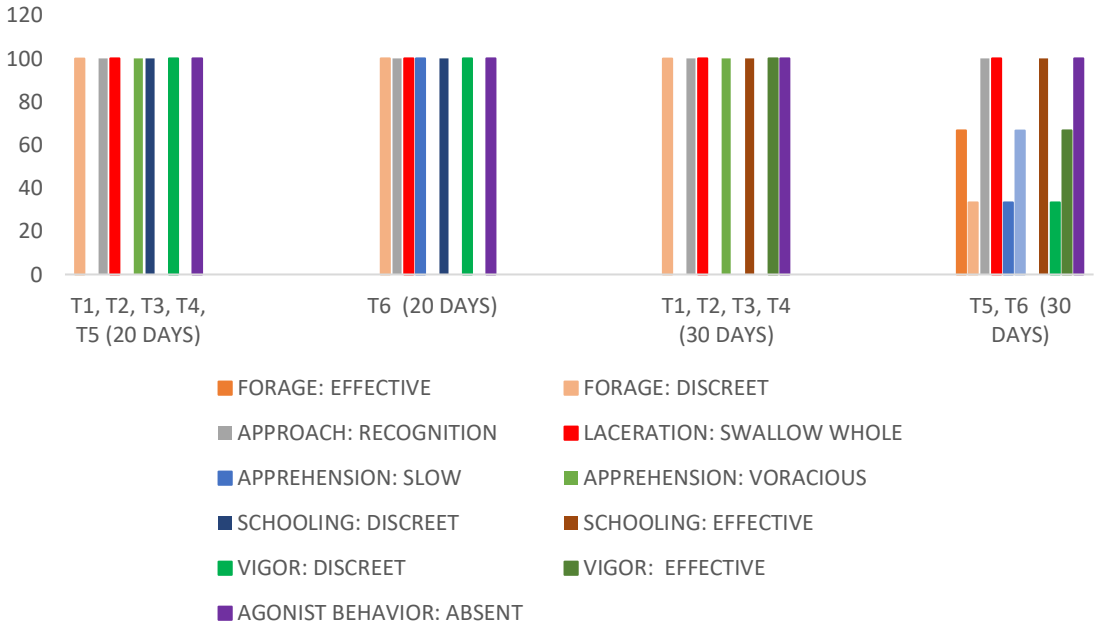


Figure 4: Assessment, per treatment, of the scale of feeding and social behavior of tilapia fingerlings on days 20 and 30 of the experiment, during adaptation to the experimental diets. Source: Authors (2026).

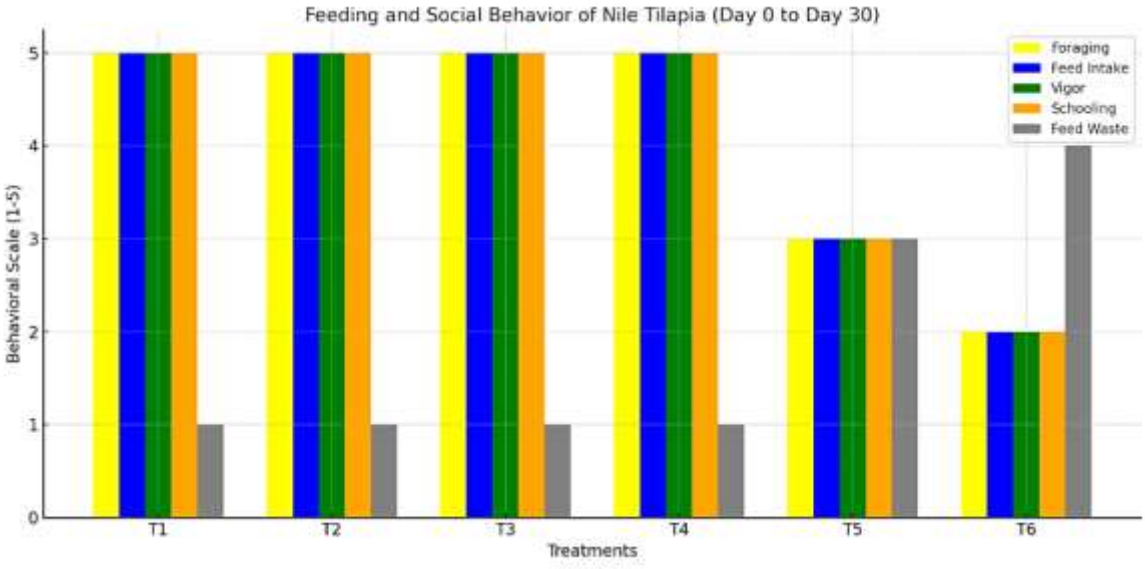


Figure 5: Comparative summary of the main feeding behaviors of tilapia in each treatment, and amount of leftover feed. Source: Authors (2026).

Discussion

The present study demonstrates that the effects of watermelon rind powder (WRP) on Nile tilapia performance cannot be adequately described by growth parameters alone. The integration of zootechnical measurements with feeding and social behavior revealed a consistent behavioral–performance relationship, in which intermediate WRP inclusion levels (8–12%) were associated with more active feeding responses, greater feeding vigor, increased feed apprehension, and better growth performance, whereas the highest inclusion level (20%) was characterized by reduced feeding activity, greater feed leftovers, lower apparent feed intake, and lower weight gain. Thus, behavioral assessment provided complementary information that helped interpret differences in dietary acceptance and performance that would not be fully captured by conventional zootechnical measurements alone. Feeding behavior has been recognized as a useful complementary approach in aquaculture nutrition studies because it integrates responses related to feed recognition, dietary acceptance, and the physiological condition of fish (Lugert et al., 2019; Brosset et al., 2021).

The growth response followed a significant quadratic pattern, with the highest final weights and weight gains observed at 8–12% WRP inclusion. This response indicates that WRP may provide beneficial effects within an intermediate inclusion range, whereas excessive inclusion may exceed the level at which these potential benefits can be maintained. Similar non-linear responses have been reported in aquaculture studies using plant-derived by-products and functional ingredients, in which moderate inclusion levels may improve performance, whereas higher concentrations may result in reduced responses (Rahman et al., 2019; Van Doan et al., 2020; Sukri et al., 2022; Vijayan et al., 2024). Similarly, Wannavijit et al. (2026) observed improved growth and feed efficiency at intermediate inclusion levels of a fruit-derived by-product, followed by reduced performance at higher inclusion levels. These dose-dependent responses highlight the importance of identifying an optimal inclusion range rather than assuming that increasing the proportion of an alternative ingredient will result in proportional benefits. The presence of bioactive compounds in watermelon-derived materials may also contribute to their potential functional effects at appropriate inclusion levels (Aras et al., 2021).

An important finding of the present study was the improvement in feed conversion ratio following WRP inclusion. Feed conversion ratio decreased from 1.61 in the control treatment to values ranging from 0.98 to 1.20 in the WRP-containing diets, with the most favorable values observed at intermediate inclusion levels. Therefore, WRP inclusion did not impair feed utilization efficiency compared with the control. Instead, the main limitation observed at the highest inclusion level was associated with feeding activity, apparent feed intake, feed leftovers, and weight gain. This distinction is important because the lower growth observed at 20% WRP should not be interpreted as a consequence of poorer feed conversion, since feed conversion actually improved.

The feeding behavior results provide an important explanation for this pattern. Fish receiving intermediate WRP inclusion levels maintained active feeding responses, characterized by regular approach to the feed, greater feeding vigor, and increased feed apprehension. These behavioral responses were consistent with the higher weight gain observed at 8–12% WRP inclusion. In contrast, fish receiving 16 and 20% WRP showed reduced feed apprehension activity and feeding vigor, together with a greater occurrence of discreet foraging and feeding behaviors. Feeding behavior may reflect changes in dietary acceptance and sensory perception, including responses associated with feed detection and olfactory stimuli (Rodrigues et al., 2021; Lugert et al., 2019). The behavioral reduction was particularly relevant at 20% WRP, at which apparent feed intake was significantly lower and feed leftovers reached their highest value. Thus, the behavioral observations provide a plausible explanation for the lower apparent feed intake and lower weight gain observed at the highest inclusion level.

The relationship between feeding behavior and feed leftovers is particularly relevant for interpreting dietary acceptance. Feed leftovers increased from 0.62 g in the control treatment to 1.49 g at 20% WRP, whereas apparent feed intake decreased at this highest inclusion level. This combination suggests that the reduction in feeding activity was accompanied by a greater proportion of uneaten feed. As feed leftovers were specifically quantified after the experimental feeding period and the tanks were cleaned before each feeding event, these differences are unlikely to reflect residual feed accumulated from previous meals. Rather, they are consistent with the behavioral differences observed among treatments and indicate that feeding behavior can provide useful information for interpreting dietary acceptance. In this context, behavioral indicators can complement conventional measures of feed intake and growth by providing information on how fish respond to the diet during the feeding process itself (Brosset et al., 2021).

The mechanisms underlying the reduced feeding activity at high WRP inclusion levels remain uncertain. The higher fiber content of WRP-containing diets represents one possible explanation, as increased dietary fiber may alter the physical and nutritional characteristics of diets, including bulk, energy density, digesta characteristics, and gastrointestinal transit (Oladipupo & Salami, 2020; Wannavijit et al., 2026). Dietary fiber may influence gastrointestinal processes and nutrient utilization in fish, particularly when included at high levels (Montagne et al., 2003; Tacon & Metian, 2008). Thus, the structural fraction, which is rich in cellulose, hemicellulose, and lignin, may impose nutritional limitations at high inclusion levels (Najafi et al., 2023; Brandão et al., 2025). In addition, pectin has a high water-retention capacity, which may increase digesta viscosity in the gastrointestinal tract (Busti et al., 2020; Freitas Júnior et al., 2025). Furthermore, excessive levels of insoluble fiber may accelerate gastrointestinal transit, reducing the time available for nutrient hydrolysis and absorption (El-Sayed et al., 2025). Another factor is the reduction in dietary energy density associated with increasing fiber levels, which may also contribute to changes in feed intake and growth responses (Tacon & Metian, 2008). However, because digestibility and post-ingestive physiological responses were not evaluated in the present study, the contribution of dietary fiber cannot be established as a direct causal mechanism. Therefore, this association should be interpreted as a plausible nutritional explanation rather than as demonstrated evidence of impaired digestion or nutrient absorption.

Dietary sensory characteristics may also have contributed to the behavioral responses observed at the highest WRP inclusion levels. Fish rely strongly on chemosensory stimuli to detect and recognize feed, and changes in dietary composition may affect feeding responses through sensory mechanisms (Jobling, 1994; Rodrigues et al., 2021). Watermelon rind contains a complex mixture of phytochemicals, including phenolic compounds and other secondary metabolites, whose effects may vary according to inclusion level. At moderate concentrations, these compounds may contribute to the functional potential of WRP, whereas higher concentrations may modify the sensory characteristics of the diet. Plant-derived compounds may influence feed acceptance when present at sufficiently high concentrations, potentially affecting the willingness of fish to approach and ingest the diet (Portz et al., 2006; Osinubi et al., 2020; Brosset et al., 2021). However, the present study did not directly evaluate palatability, olfactory preference, or the sensory properties of the diets. Consequently, sensory rejection should be considered a possible mechanism rather than a confirmed explanation.

The temporal pattern of behavioral responses further strengthens this interpretation. During the initial adaptation period, differences in feeding behavior were already evident among treatments, with more active feeding responses at intermediate WRP inclusion levels and reduced activity at higher inclusion levels. By day 10, most fish exhibited active feeding and normal social interactions, indicating successful general adaptation to the experimental conditions. However, between days 20 and 30, differences among dietary treatments became more evident, particularly in feeding vigor and discreet feeding responses. This temporal progression suggests that the behavioral responses were not restricted to an immediate novelty effect associated with the introduction of the diets but were maintained throughout the experimental period. The use of behavioral observations at different stages of the feeding process may be particularly valuable for detecting changes in feeding dynamics that may not be immediately evident in cumulative performance variables (Lugert et al., 2019; Brosset et al., 2021).

The social behavior results provide an additional dimension to this interpretation. The maintenance of schooling behavior and the absence of marked agonistic interactions among treatments suggest that differences in growth performance were not primarily determined by increased social aggression or alterations in group cohesion. Instead, the main behavioral differences were associated with feeding activity and feeding vigor. Social stability is relevant because changes in agonistic interactions and social organization may influence access to feed and energy allocation in cultured fish (Falcon, 2008). In the present study, the relative stability of social interactions strengthens the interpretation that dietary factors were more closely associated with the observed differences in feeding behavior and growth.

In addition to immediate feeding responses, post-ingestive mechanisms may also contribute to the behavioral changes observed at higher WRP inclusion levels. Increased dietary fiber may influence satiety, digesta characteristics, intestinal transit, and the duration of gastrointestinal stimulation, potentially modifying subsequent feeding responses (Perar et al., 2017; Xavier et al., 2019; Araujo et al., 2020; Freitas Júnior et al., 2025). These mechanisms provide a plausible physiological framework for understanding why changes in dietary composition may be expressed not only through growth performance but also through observable feeding behavior. However, because these physiological processes were not directly measured in the present study, they should be considered hypotheses requiring experimental confirmation.

Furthermore, the results obtained are consistent with reports in other fish species in which excessive inclusion of watermelon rind or other plant-derived by-products was associated with reduced growth performance, particularly when the fibrous fraction became more pronounced in the diet (Tacon & Metian, 2008; Vijayan et al., 2024). These findings reinforce the concept that alternative plant ingredients should be evaluated within species-specific inclusion ranges rather than considered universally beneficial or detrimental. The present study extends this interpretation by demonstrating that behavioral responses can help identify the point at which increasing inclusion begins to affect dietary acceptance.

Taken together, the zootechnical and behavioral results reveal a coherent biological pattern. Intermediate WRP inclusion levels promoted active feeding behavior and were associated with the highest growth performance and improved feed conversion, whereas the highest inclusion level was characterized by reduced feeding activity, increased feed leftovers, lower apparent feed intake, and lower weight gain. Importantly, this pattern demonstrates that feeding behavior can function as a complementary indicator of dietary acceptance, helping to identify changes in feeding responses that may occur before or simultaneously with changes in conventional performance variables. The value of behavioral assessment, therefore, lies not only in describing fish activity but also in relating observable feeding responses to practical outcomes such as feed waste, apparent feed intake, and growth (Lugert et al., 2019; Brosset et al., 2021).

From an aquaculture nutrition perspective, these findings support the use of WRP as a potential functional feed ingredient within an appropriate inclusion range. The results also emphasize that the evaluation of alternative ingredients should not rely exclusively on final weight, weight gain, or feed conversion ratio. Behavioral indicators such as approach to feed, feed apprehension, feeding vigor, foraging patterns, and schooling behavior can provide complementary information on how fish respond to dietary changes. In this sense, ethological assessment may serve as an additional tool for refining dietary inclusion levels and identifying potential limitations before they are expressed exclusively as reductions in production performance (Lugert et al., 2019; Brosset et al., 2021).

The absence of direct digestibility measurements represents an important limitation of the present study. Although the higher fiber content of WRP-containing diets provides a plausible explanation for some of the responses observed at higher inclusion levels, the data obtained do not allow direct conclusions regarding nutrient digestibility, intestinal transit, satiety mechanisms, or nutrient absorption. Future studies should therefore integrate behavioral assessment with digestibility trials, characterization of diet physical properties, sensory or preference tests, and evaluations of digestive and metabolic responses. Such an integrated approach may clarify the mechanisms underlying the behavioral changes observed at high WRP inclusion levels and contribute to optimizing the use of watermelon rind as a functional ingredient in Nile tilapia diets.

Conclusions

This study demonstrates that the inclusion of watermelon rind powder (WRP) in diets for Nile tilapia (***Oreochromis niloticus***) follows a non-linear dose–response pattern, with the best zootechnical performance observed at inclusion levels of 8–12%. At these levels, final body weight and weight gain were improved, while feed conversion ratio was also more favorable than in the control treatment, indicating that WRP may function as a potential functional ingredient when included at moderate levels. These positive responses may be associated with the bioactive compounds present in watermelon rind, although the specific contribution of these compounds was not directly evaluated in the present study.

At higher inclusion levels, particularly 16–20%, a decline in growth performance was observed, accompanied by reduced feeding activity and vigor, lower apparent feed intake, and increased feed leftovers. These responses suggest that excessive WRP inclusion may negatively affect dietary acceptance and feeding dynamics. The higher dietary fiber content and other characteristics of WRP-containing diets may have contributed to these responses; however, because digestibility and sensory properties were not directly evaluated, these mechanisms should be interpreted as plausible explanations rather than confirmed causal effects.

The integration of zootechnical and behavioral data highlights feeding behavior as a valuable complementary indicator for interpreting dietary acceptance, feed leftovers, and growth performance. The maintenance of social stability and the absence of marked agonistic interactions among treatments further indicate that the observed differences in productive performance were primarily associated with dietary responses rather than pronounced social disturbances.

From a practical perspective, WRP represents a promising agro-industrial by-product for use in Nile tilapia nutrition, with potential applications within a circular economy framework. Based on the present results, inclusion levels of 8–12% are recommended when the objective is to maximize growth performance and feed efficiency while maintaining active feeding behavior and minimizing feed leftovers. Future studies should investigate apparent digestibility, sensory acceptance, digestive and metabolic responses, and nutritional strategies such as enzyme supplementation to clarify the mechanisms underlying the reduced performance at higher WRP inclusion levels and to optimize its utilization in tilapia diets.

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