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Porcine Mucosal Protein Hydrolyzate as a food stimulant for Nile tilapia juveniles

Hidrolisado Proteico de Mucosa Suína como estimulante alimentar para juvenis de tilápia do Nilo

Hidrolizado de proteína de mucosa porcina como alimento estimulante para juveniles de tilapia del Nilo

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Abstract
This study aimed to evaluate the inclusion of porcine mucosa hydrolysate (PMH) as a flavor stimulant in diets for juvenile tilapia. For this, 12 juvenile tilapia were used, distributed in 12 aquariums, where video recordings were performed to obtain data on attractiveness and palatability: (a) time of capture of the first pellet; (b) rejection of the pellets by the tilapia; (c) approach without ingestion; (d) percentage of ingestion; (e) time taken for the tilapia to consume all the pellets. Six treatments were evaluated in comparison with the control, namely without inclusion of PMH and from one to five percent of inclusion of PMH. The treatment with 1% inclusion presented the highest intake of pellets and, for the other variables, no differences were observed between the evaluated treatments, however, compared to the control, there was a greater decrease in the consumption of the treatment with 3% inclusion. Thus, the inclusion of 1% of PMH is suggested to improve the palatability of the ration offered.
Keywords: Food behavior; Taste stimulant; Low molecular Weight peptides.

Resumo
Este trabalho teve como objetivo avaliar a inclusão do hidrolisado de mucosa suína (HMS) como estimulante de consumo em dietas para juvenis de tilápias. Para isso, foram utilizados 12 juvenis de tilápias, distribuídos em 12 aquários, onde foram realizadas gravações em vídeo para obter dados de atratividade e palatabilidade: (a) tempo de captura do primeiro pélete; (b) rejeição dos péletes pela tilápia; (c) aproximação sem ingestão; (d) percentual de ingestão; (e) tempo que a tilápia levou para consumir todos os péletes. Foram avaliados seis tratamentos em relação ao controle, sendo eles sem inclusão de HMS e de um a cinco por cento de inclusão do HMS. O tratamento com 1% de inclusão apresentou a maior ingestão dos péletes, e as demais variáveis não diferenças entre os tratamentos avaliados, porém em comparação com o controle, houve uma redução maior no consumo do tratamento com 3% de inclusão. Deste modo, sugere-se a inclusão de 1% do HMS para melhorar a palatabilidade da ração ofertada.
Palavras-chave: Comportamento alimentar; Estimulante de sabor; Peptídeos de baixo peso molecular.

Resumen
Este estudio tuvo como objetivo evaluar la inclusión de hidrolizado de mucosa porcina (HMP) como estimulante del sabor en dietas para juveniles de tilapia. Para ello se utilizaron 12 juveniles de tilapia, distribuidos en 12 acuarios, donde se realizaron videograbaciones para obtener datos de atractivo y palatabilidad: (a) tiempo de captura de la primera bolita; (b) rechazo de los gránulos por parte de la tilapia; (c) acercamiento sin ingestión; (d) porcentaje de ingestión; (e) tiempo que tarda la tilapia en consumir todos los gránulos. Se evaluaron seis tratamientos, el testigo, sin inclusión de HMP y de uno a cinco por ciento de inclusión de HMP. El tratamiento con 1% de inclusión presentó el

mayor consumo de pellets y, para las demás variables, no se observaron diferencias entre los tratamientos evaluados, sin embargo, en comparación con el control, hubo una mayor disminución en el consumo del tratamiento con 3% de inclusión. Así, se sugiere la inclusión de 1% de HMP para mejorar la palatabilidad de la ración ofrecida.
Palabras clave: Comportamiento alimentario; Estimulante del gusto; Péptidos de bajo peso molecular.

1. Introduction

Protein hydrolysates are products of protein degradation and can be obtained from chemical, microbial and enzymatic methods (Hou et al., 2017), among others. An example is enzymatic hydrolysis, which is characterized by improving the functional properties of the protein from the release of amino acids and peptides of different sizes, especially small peptides (smaller than 5000 Da), promoting an efficient breakdown of these proteins (Yatisha et al., 2022) as well as helping to remove anti-nutritional factors (Kowalczewski et al., 2021).

Low molecular weight peptides have bioactivities that improve that can act as palatabilizers and attractants for the ingestion of offered diets (Saadoui et al., 2019; Yang et al., 2021). Porcine mucosa hydrolyzate (PMH) is an enzymatic hydrolyzate, being a by-product generated from the production process of heparin sodium, an anticoagulant drug (Mateos et al., 2014). This hydrolyzate has a profile of essential and non-essential amino acids, in addition to more than 80% of low molecular weight peptides (Yang et al., 2022).

Nile tilapia (*Oreochromis niloticus*) is one of the most produced species around the world. It is the third most cultivated fish species worldwide, representing 8.3% of world production (FAO, 2020). In the latest FAO report (2020), it is possible to observe that there has been an increase of more than 170% since 2010 in its production, resulting in an increase in *per capita* consumption rates. Despite being one of the most produced species in world aquaculture, there are relatively few behavioral studies on this species.

It is known that fish have the ability to differ the taste of chemical substances (Kasumyan & Mouromtsev, 2020), therefore it is important to observe the taste preferences of these animals so that, increasingly, studies are carried out to include foods/ingredients that can act as growth producers and improve the health of the animal, since more attractive and palatable foods can increase the consumption of the offered diets (Al-Souti et al., 2019; Jingting et al., 2020; Yang et al., 2020). According to Al-Souti et al. (2019), when the food offered is more palatable, it will consequently be consumed more quickly, requiring lower proportions of more expensive ingredients, such as proteins.

Protein hydrolysates have the ability to improve the health of animals due to their bioactive properties, acting as antioxidant, antihypertensive, and antidiabetic (Zou et al., 2021), so their inclusion in the diet of juveniles can improve health parameters of the fish. Since the feeding behavior of fish is stimulated by low molecular weight elements, including amino acids and peptides (Lokkeborg et al., 2014) that are present in protein hydrolysates, this study aimed to evaluate the palatability of different levels of porcine mucosa hydrolysate (PMH) inclusion for Nile tilapia.

2. Methodology

To evaluate the attractiveness and palatability of the porcine mucosa hydrolysate (PMH), 12 Nile tilapia were used, distributed in 12 10-liter aquariums, all equipped with constant temperature and aeration control, opaque side walls to prevent visual contact between fish, and an opening in the lid for the introduction of pellets. These aquariums were covered by a physical barrier of styrofoam on the front with a front opening for the camera to fit in so that the impacts caused by the daily routine of the laboratory were minimized.

Six isoprotein (40% crude protein) and isoenergetic (3,250 Kcal) diets were formulated: Control (without PMH addition) and treatments with increasing levels of PMH, from 1% to 5% inclusion — T1%, T2%, T3%, T4% and T5% (Table 1). For the preparation of the diets, the ingredients were weighed separately. The macro nutrients were ground for better homogenization and then the micronutrients were added and mixed until ideal homogenization. following the methodology proposed by the NRC (2011) The rations were processed in an extruder machine and dried in a forced circulation oven at 55°C for 24 hours. The composition of the ingredients used and their respective amounts are shown in Table 1. The amino acid composition of PMH is shown in Table 2.

In total, the experiment was carried out over 16 days, 10 for the adaptation period and six days for the tests themselves.

The experiment was carried out in a Latin square design with two blocks, six treatments and two replications over time (Table 3). The diets were offered twice a day (at 10 am and at 2 pm), totaling 144 test videos (6 treatments × 2 blocks × 2 meals × 6 days).

During the experiment, the quantity of pellets to be supplied was evaluated (in this period only the control diet was used) and the offer of 25 pellets per trial for each aquarium was defined.

The pellets were counted before filming and placed on the lid of each aquarium to be used at the time of filming. A time of 180 seconds was set for the tests. Counting started as soon as the pellets touched the water. After the tests, the pellets that had not been consumed were removed and at the end of the last test the aquariums were cleaned, with a renewal of approximately 80% of the water.

Table 1. Percentage and centesimal composition of experimental diets containing different levels of hydrolyzed porcine mucosa hydrolysate (PMH) protein inclusion to assess the attractiveness and palatability for juvenile Nile tilapia *Oreochromis niloticus*.

[1]Warranty levels per kilogram of product: vit. A — 500,000 IU; vit. D3 — 200,000 IU; vit. E — 5000 mg; vit. K3 — 1000 mg; vit. B1 — 1500 mg; vit. B2 — 1500 mg; vit. B6 — 1500 mg; vit. B12 — 4000 mg; folic acid — 500 mg; calcium pantothenate — 4,000 mg; vit. C — 15,000 mg; biotin — 50 mg; inositol — 10,000; nicotinamide — 7,000; choline — 40,000 mg; cobalt — 10 mg; copper — 500 mg; iron — 5,000 mg; iodine — 50 mg; manganese — 1,500 mg; selenium — 10 mg; zinc — 5,000 mg.

Ingredients	Diets containing PMH (%)					
	0	1	2	3	4	5
Feather meal	12.00	12.00	12.00	12.00	12.00	12.00
Blood meal	5.00	5.00	5.00	5.00	5.00	5.00
Poultry offal meal	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal 48%	16.33	15.99	15.65	14.31	14.96	14.62
Tilapia flour	5.00	4.00	3.00	2.00	1.00	0.00
Porcine mucosa hydrolysate	**0.00**	**1.00**	**2.00**	**3.00**	**4.00**	**5.00**
Corn meal	34.13	34.16	34.16	34.20	34.23	34.27
Rice grits	5.00	5.00	5.00	5.00	5.00	5.00
Soy oil	0.59	0.65	0.71	0.78	0.84	0.90
Premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Common salt	0.30	0.30	0.30	0.30	0.30	0.30
Limestone	0.11	0.21	0.30	0.40	0.50	0.60
Bicalcium phosphate	0.35	0.49	0.62	0.76	0.89	1.02
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20

Antifungal	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.02	0.02	0.02	0.02	0.02	0.02
Vitamin C	0.14	0.14	0.14	0.14	0.14	0.14
L-lysine HCL	0.08	0.09	0.11	0.12	0.13	0.14
L-tryptophan	0.01	0.02	0.02	0.03	0.04	0.05
DL-methionine	0.15	0.15	0.15	0.15	0.15	0.15
Nutrients (%)						
Linoleic acid	1.82	1.84	1.86	1.88	1.90	1.92
Starch	25.60	25.61	25.62	25.63	25.64	25.65
Tilapia digestible energy (Kcal)	3250	3250	3250	3250	3250	3250
Crude protein	40.00	40.00	40.00	40.00	40.00	40.00
Raw fiber	1.30	1.29	1.27	1.26	1.25	1.23
Total phosphorus	1	1	1	1	1	1
Total fat	6.01	6.09	6.17	6.25	6.33	6.41
Calcium	1.50	1.50	1.50	1.50	1.50	1.50
Mineral matter	6.20	6.06	5.92	5.77	5.63	5.48
	490.0	490.0	490.0	490.0	490.0	490.0
Vitamin C	0	0	0	0	0	0
Choline	1200	1200	1200	1200	1200	1200
Total arginine	2.60	2.57	2.53	2.50	2.46	2.43
Phenylalanine + total tyrosine	3.24	3.23	3.22	3.21	3.20	3.19
Total histidine	0.96	0.96	0.96	0.96	0.96	0.96
Total isoleucine	1.55	1.55	1.54	1.54	1.54	1.54
Total leucine	3.07	3.07	3.07	3.07	3.07	3.07
Total lysine	2.13	2.13	2.13	2.13	2.13	2.13
Total methionine + total cysteine	1.57	1.57	1.57	1.57	1.57	1.57
Total methionine	0.73	0.73	0.73	0.73	0.73	0.73
Threonine	1.69	1.69	1.68	1.67	1.66	1.65
Tryptophan	0.40	0.40	0.40	0.40	0.40	0.40
Valine	2.31	2.31	2.31	2.31	2.31	2.31

Source: Data from the present research (2022).

Table 2. Percentage composition of amino acids (total and free) of porcine mucosa hydrolysate (PMH).

Porcine mucosa hydrolysate		
Amino acid	**Total amino acid**	**Free amino acid**
Aspartic acid	1.73%	0.33%
Glutamic acid	1.40%	0.37%
Serine	1.07%	0.12%
Glycine	0.92%	0.28%
Histidine	0.47%	0.14%
Taurine	0.17%	0.02%
Arginine	1.04%	0.30%
Threonine	1.05%	0.24%
Alanine	1.71%	0.38%
Proline	1.02%	0.26%
Tyrosine	1.22%	0.22%
Valine	1.64%	0.35%
Methionine	0.77%	0.12%
Cystine	0.29%	0.13%
Isoleucine	1.30%	0.28%
Leucine	2.49%	0.49%
Phenylalanine	1.19%	0.25%
Lysine	1.98%	0.31%
Asparagine	0.11%	0.03%
Sum (%)	**21.57%**	**4.62%**

Source: Data from the company BRF Ingredients SA (2022).

Table 3: Distribution of treatments used for each block along the experimental days.

	AQUARIUM					
DAY	**1**	**2**	**3**	**4**	**5**	**6**
1	CON	T1%	T2%	T3%	T4%	T5%
2	T5%	CON	T1%	T2%	T3%	T4%
3	T4%	T5%	CON	T1%	T2%	T3%
4	T3%	T4%	T5%	CON	T1%	T2%
5	T2%	T3%	T4%	T5%	CON	T1%
6	T1%	T2%	T3%	T4%	T5%	CON

Source: Data from the present research (2022).

After filming, the videos were analyzed to obtain the following data:

a) The capture time of the first pellet from the moment it touched the water, given by the capturing of the first pellet (CAP1), in seconds;

b) How many times the tilapia grab the pellet and regurgitate it, taken as rejection (REJ);

c) How many times the tilapia approached the pellet, but did not catch or swallow it, taken as approach without ingestion (APP);

d) The percentage of pellets ingested by the tilapia up to the end of the 180 seconds, taken as ingestion (ING%);

e) The time it took the tilapia to consume all the pellets (up to 180 seconds limit), taken as time to consume all (TTCA).

After obtaining all these data, the palatability index suggested by Kasumyan and Doving (2003) was performed, in which:

$$PI = [(F - C) / (F + C)] \times 100$$

Where:

PI = palatability index;

F = test feed intake;

C = control feed intake.

In addition, it was also calculated whether there was an increase or decrease in feed consumption with the inclusion of PMH in relation to the control, given by:

$$IC = [(CP_{Treat} \times 100) / CP_{Con}] - 100$$

Where:

IC = increment;

CP_{Treat} = consumption of treatment pellets;

CP_{Con} = control pellet consumption.

Data were analyzed in Statistic 7.0 (Statsoft, 2004). A main effects ANOVA, in which the experimental days, fish and treatments were considered as sources of variation, was performed for each response variable. As the data did not comply with the assumptions of normality and homoscedasticity, it underwent transformation into Rank. Tukey's *PostHoc* analysis were performed to verify the response variables that presented significant effects from treatments.

3. Results

The attractiveness data, determined by the variables capture of the first pellet (CAP1) and approach without ingestion (APP), showed no significant differences (Table 4). However, differences were observed for palatability variables: ingestion (ING%) and palatability index (PI) pointed to better results in the treatment with 1% inclusion of PMH (Figures 1 and 2, respectively). For the other variables, no differences were observed between the treatments tested (Table 4).

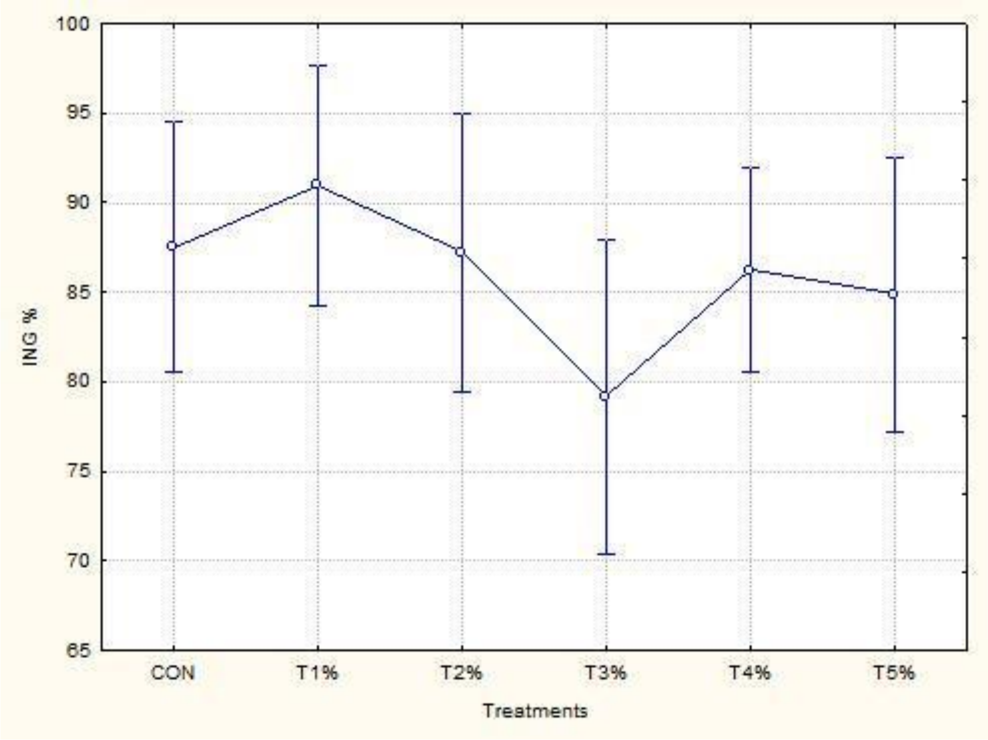
To assess whether there was an increase or a decrease in feed intake with the inclusion of PMH, the percentage of intake based on the control treatment was calculated. With this, it was possible to observe a preference for the treatment with 1% and a decrease in the treatment with 3% of inclusion (Figure 3).

Table 4: Mean values and values transformed into rank ± standard error of attractiveness and palatability variables for the inclusion of different levels of porcine mucosa hydrolysate inclusion. CP1 = time of capture of the first pellet (in seconds); REJ = rejection — number of times the tilapia regurgitated the pellet after ingesting it; APP = approach without ingestion — number of times that the tilapia approached the pellet, but did not ingest it; TTCA = time to consume all — time it took the tilapia to consume all the pellets offered (in seconds).
*Non-interpretable data.

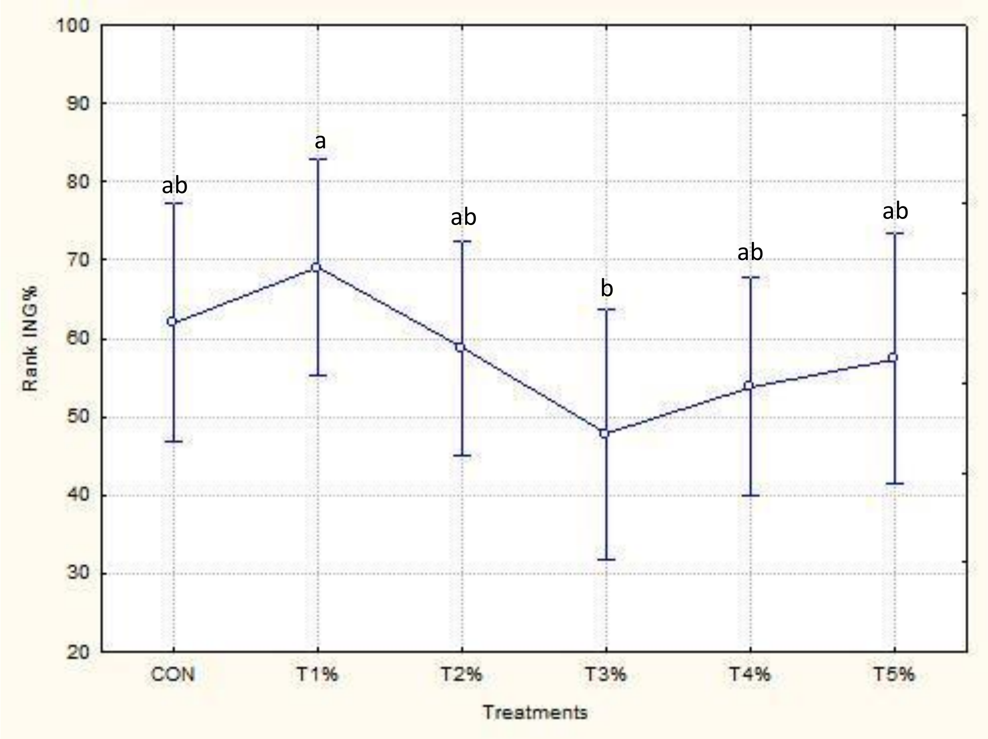
		TREATMENTS						
		CON	**T1%**	**T2%**	**T3%**	**T4%**	**T5%**	***p***
MEAN	CP1	4.91 ± 2.0	3.62 ± 1.7	12.87 ± 7.5	11.70 ± 5.7	3.91 ± 1.0	7.87 ± 2.8	*
	REJ	0.41 ± 0.1	0.41 ± 0.2	0.29 ± 0.1	0.79 ± 0.4	0.33 ± 0.1	0.41 ± 0.3	*
	APP	5.16 ± 1.8	6.95 ± 1.3	4.20 ± 1.0	4.16 ± 0.8	4.20 ± 0.8	6.12 ± 1.3	*
	TTCA	144.12 ± 1.85	132.54 ± 12.14	151.66 ±10.42	155.16 ± 9.29	151.62 ± 11.01	147.87 ± 9.94	*
RANK	CP1	67.27 ± 8.18	61.35 ± 8.07	73.89 ± 8.68	78.37 ± 8.34	75.45 ± 8.65	78.64 ±8.20	>0.05
	REJ	76.54 ± 6.36	71.12 ± 5.68	70.62 ±5.44	77.25 ± 6.63	71.04 ±5.64	68.41 ± 5.22	>0.05
	APP	67.91 ± 7.84	84.45 ± 8.83	64.77 ± 8.86	69.93 ± 7.69	71.33 ± 7.88	76.58 ± 9.68	>0.05
	TTCA	68.60 ± 7.65	63.47 ±8.04	76.85 ±7.02	77.70 ± 6.72	76.27 ± 7.31	72.08 ± 7.13	>0.05

Source: Data from the present research (2022).

Figure 1: Graphs depicting percent intake data for all treatments tested. A: means of the data without transformation. B: means of data transformed into Rank. Distinct letters indicate statistical differences at 5% significance.



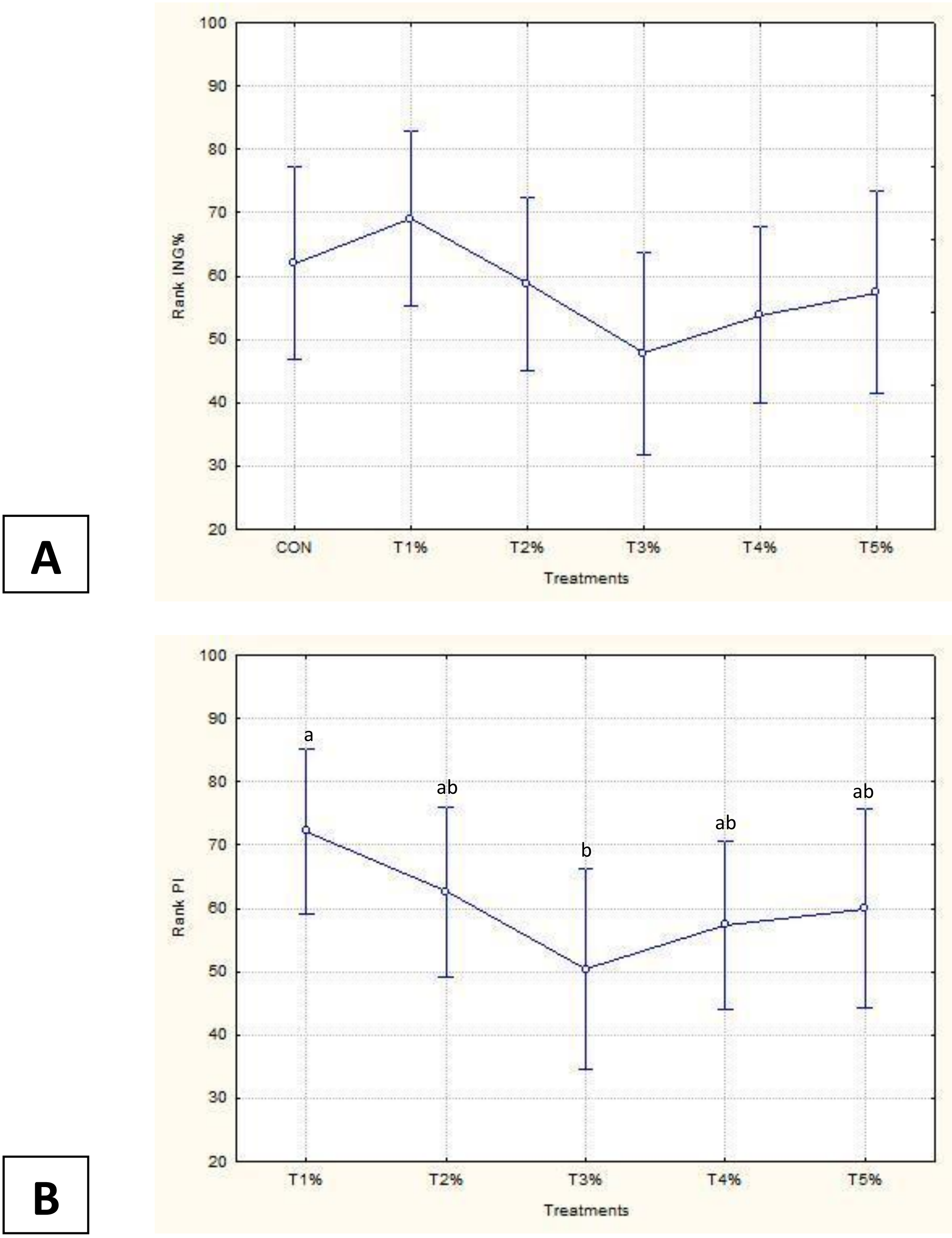
A



B

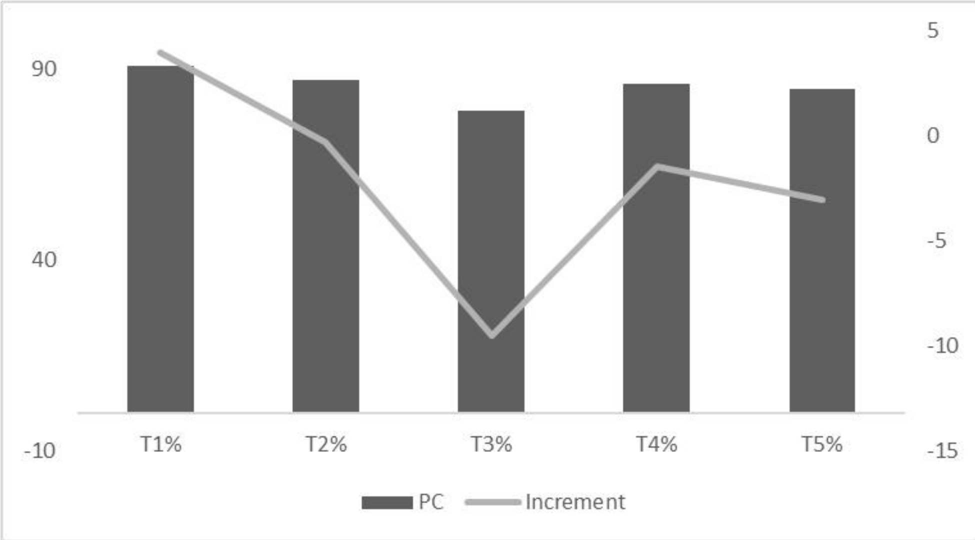
Source: Data from the present research (2022).

Figure 2: Graphs representing the palatability index (PI) data for all treatments tested. A: means of the data without transformation. B: means of data transformed into Rank. Distinct letters indicate statistical differences at 5% significance.



Source: Data from the present research (2022).

Figure 3: Graph of the increase in feed consumption with the inclusion of porcine mucosa hydrolysate compared to the control treatment. PC = pellet consumption. Left vertical axis corresponds to PC, right vertical axis corresponds to increment.



Source: Data from the present research (2022).

4. Discussion

The attractiveness in the present work was measured through the data of first capture and of approach without ingestion, since these are attributes that concern the olfactory sense of the analyzed animals. It is from this sense that the search for food begins (Lokkeborg et al., 2014), which causes agitation, especially through swimming behavior in search of food and attraction to the odor source (Ólsen & Lundh, 2016). Although there were no significant differences in attractiveness attributes, the treatment with 1% inlcusion had the lowest average time for capturing the first pellet (3.62 seconds), proving to be as attractive as the control. In general, the fish reacted soon after the pellets touched the water. Studies show that foods considered attractive to tilapia are consumed, on average, 1.6 to 5 seconds after the pellet touches the water (Kasumyan &Vinogradskaya, 2019; Kasumyan & Mouromtsev, 2020). The capture time of the first pellet is counted from the moment it touches the water, thus also starting the test count. The short time span in which the feed is available in the water also prevents food leaching, so it may also be able to decrease the accumulation of organic matter in the environment. It was possible to observe, during the recordings, that the tilapia were readily attracted to the food, whatever the treatment.

For the approach without ingestion, no differences were observed between treatments either. This parameter was evaluated from the observation of how many times the tilapia approached the pellet, but did not ingest it. The fish usually swam away but often came back and grabbed it. There is probably some substance that causes a slight repulsion to the animals (this occurred for all treatments) but not enough for them to completely refuse food, a behavior which is typical for several species of fish (Kasumyan & Mouromtsey, 2020). Protein hydrolysates are products rich in amino acids and low molecular weight peptides (Kasumyan & Doving, 2003; Chotikachinda et al., 2013; Dieterich et al., 2014) which incites the olfactory sensitivity of the animal; a sensitivity so great that it can be detect something even smaller than the dimension/size of free amino acids (Kasumyan & Vinogradskayaa, 2019).

The rejection was given by the regurgitation of the pellets by the tilapia and, despite having occurred a few times, this behavior was observed for all fish and all treatments. When this occurred, the tilapia would grab then spit out the pellets, a few times grabbing and spitting the pellet a second time. This action was observed by other authors, being a typical behavior of several fish species, especially tilapia (Kasumyan & Vinodraskayaa, 2019), repeated in more than 50% of the tests, according to several authors (Kasumyan et al., 2021; Kasumyan & Mouromtsev, 2020). In the present study, this occurred infrequently. According to Levina et al. (2021), when compared to other fish species, this behavior is lower in tilapia. The same authors found that such behavior reflects the social life of the species, which live in a complex social hierarchy, especially the juveniles, objects of this study, who report agonistic behaviors, leading them to consume the pellet more quickly so that the other does not consume. Another fact observed by the authors is related to the pellet retention time in the mouth, which is always longer the first time it is gripped than the second time. In this way, there is a longer time of orosensory evaluation, which leads to a better judgment in the decision making between swallowing or rejecting the pellet. The same occurred in the present study, where, of the few times that there was a second rejection of the same pellet, the first time took longer.

A time of 180 seconds was delimited for the evaluation of the tests. After that time, when the rations were not fully consumed, the tilapia seemed to lose interest in them. In most of the trials, the pellets were all consumed, with the average time varying from 132.54 to 155.16 seconds, respectively for treatments with 1% and 3% PMH inclusion. This suggests that the treatments offered were both attractive and palatable for the tilapia, since no differences were observed between them.

Porcine mucosal protein hydrolysate (PMH) is obtained from the mucosa of the small intestine of pigs for the production of sodium heparin, an anticoagulant drug (Mateos et al., 2014), and is characterized by a high content of free amino acids, generated from enzymatic hydrolysis (Mora et al., 2014) and low molecular weight peptides (Yang et al., 2021), which makes this ingredient more attractive to animals (Soares et al., 2020; Vázquez et al., 2022), increasing its consumption, as observed in this work, where there was a preference for the ration with the addition of 1% of PMH. This fact may reflect on the growth performance of the animal, since ingestion is essential to ensuring the consumption of the required nutrients (Jingting et al., 2020). In addition, a more palatable feed will be ingested more quickly, and will allow for lower proportions of costly components, such as proteins (Al-Souti et al., 2019).

Yang et al. (2021) and Yang et al. (2022), when characterizing PMH, observed that more than 88% of molecular peptides have a size smaller than 5000 Da, and of these, most (40.02%) have sizes smaller than 180 Da, among which is a large amount of amino acids, including alanine and glycine, that evoke food-seeking behavior for *Acipenser persicus*, according to Shamushaki et al. (2011), as well as alanine and serine, that act as food stimulants for tilapia (Al-Souti et al., 2019) and are also present in the composition of PMH (Table 2). This may explain the preference of tilapia for the feed with the inclusion of PMH. However, a decrease in consumption was also observed, in relation to the control, especially for the treatment with 3% inclusion. Kasumyan and Mouromtsev (2020) observed that some chemicals had different flavor qualities, despite being structurally similar. This fact seems to have occurred in an analogous way in the present study, however, instead of differences in the structures of the substances (which were not verified in this study), the greater amount of inclusion of PMH seems to have caused a more aversive effect, when compared to the control treatment, but not enough for the tilapia not to ingest the rations offered.

The inclusion of PMH does not cause refusal by the tilapia, as it improves palatability, and, as no significant differences were observed between treatments for attractiveness, it can be inferred that PMH is as attractive as fishmeal for these animals. Therefore, it becomes evident that the 1% of inclusion level is the most indicated, as it was more palatable than the other treatments offered.

5. Final Considerations

This work contributes to the evaluation of the inclusion of porcine mucosa hydrolysate (PMH) as a consumption stimulant in diets for tilapia, one of the most cultivated species in the world and an animal whose behavior is scarcely researched. From this study, other reports can be carried out on the functioning of this substance in the animal's tract, since it is well accepted and quickly consumed by them. Therefore, based on the data obtained, we suggest the inclusion of 1% of PMH to improve the palatability of the ration offered.

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References

Al-Souti, A., Gallardo, W., Claereboudt, M., & Mahgoub, O. (2019). Attractability and palatability of formulated diets incorporated with chicken feather and algal meals for juvenile gilthead seabream, *Sparus aurata*. *Aquaculture Reports*, 14, 100199. https://doi.org/10.1016/j.aqrep.2019.100199.

Chotikachinda, R., Tantikitti, C., Benjakul, S., Rustad, T., & Kumarnsit, E. (2013). Production of protein hydrolysates from skipjack tuna (*Katsuwonus pelamis*) viscera as feeding attractants for Asian seabass (*Lates calcarifer*). *Aquaculture Nutrition*, 19(5), 773-784. https://doi.org/10.1111/anu.12024.

Dieterich, F., Boscolo, W. R., Pacheco, M. T. B., Silva, V.; S. N., Gonçalves, G. S., & Vidotti, R. M. (2014). Development and characterization of protein hydrolysates originated from animal agro industrial byproducts. *Journal of Dairy, Veterinary & Animal Research*, 1(2), 1-7. https://doi.org/10.15406/jdvar.2014.01.00012.

FAO. 2020. The State of World Fisheries and Aquaculture (2020). Sustainability in action. Rome. https://doi.org/10.4060/ca9229en.

Hou, Y., Wu, Z., Dai, Z., Wang, G., & Wu, G. (2017). Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. *Journal of Animal Science and Biotechnology*, 8(1), 1-13. https://doi.org/10.1186/s40104-017-0153-9.

Jingting, Y., Danting, G., Chun, K., Min, J., & Xueming, H. (2020). Effect of soybean antigenic protein on feed palatability of fishmeal replaced diets for obscure puffer (*Takifugu fasciatus*) and the alternation of diet preference by domestication. *Aquaculture Reports*, 17, 100332. https://doi.org/10.1016/j.aqrep.2020.100332.

Kasumyan, A. O., & DÖving, K. B. (2003). Taste preferences in fishes. *Fish and fisheries*, 4(4), 289-347. https://doi.org/10.1046/j.1467-2979.2003.00121.x.

Kasumyan, A. O., & Mouromtsev, G. E. (2020). The teleost fish, blue gourami *Trichopodus trichopterus*, distinguishes the taste of chemically similar substances. *Scientific reports*, 10(1), 1-10. https://doi.org/10.1038/s41598-020-64556-6.

Kasumyan, A. O., & Vinogradskaya, M. I. (2019). Palatability of bile substances for fish. *Journal of Ichthyology*, 59(4), 610-618. https://doi.org/10.1134/S0032945219040088.

Kasumyan, A., Isaeva, O., & Zvonareva, S. 2021. Coloration type of two allied cowries (Ovulidae: Gastropoda) tested through palatability evaluation in feeding experiments with fish. *Journal of Experimental Marine Biology and Ecology*, 538, 151529. https://doi.org/10.1016/j.jembe.2021.151529.

Kowalczewski, P. Ł., Olejnik, A., Rybicka, I., Zielińska-Dawidziak, M., Białas, W., & Lewandowicz, G. (2021). Membrane filtration-assisted enzymatic hydrolysis affects the biological activity of potato juice. *Molecules*, 26(4), 852. https://doi.org/10.3390/molecules26040852.

Levina, A. D., Mikhailova, E. S., & Kasumyan, A. O. (2021). Taste preferences and feeding behaviour in the facultative herbivorous fish, Nile tilapia *Oreochromis niloticus*. *Journal of Fish Biology*, 98(5), 1385-1400. https://doi.org/10.1111/jfb.14675.

Løkkeborg, S., Siikavuopio, S. I., Humborstad, O. B, A. C., Utne-Palm., & Ferter, K. (2014). Towards more efficient longline fisheries: fish feeding behaviour, bait characteristics and development of alternative baits. *Rev Fish Biol Fisheries* 24, 985–1003. 10.1007/s11160-014-9360-z.

Mateos, G. G., Mohiti-Aslia, M., Bordab. E., Mirzaiea, S., & Frikhaa, M. (2014). Effect of inclusion of porcine mucosa hydrolysate in diets varying in lysine content on growth performance and ileal histomorphology of broilers. *Animal Feed Science and Technology*.187, 53– 60. http://dx.doi.org/10.1016/j.anifeedsci.2013.09.013

Mora, L., Reig, M., & Toldrá, F. (2014). Bioactive peptides generated from meat industry by-products. *Food Research International*, 65, 344-349. http://dx.doi.org/10.1016/j.foodres.2014.09.014.

National Research Council (NRC). (2011). Nutrient requirements of fish and shrimp. The national academies press, Washington, 379p.

Olséna, K. H., & Lundh, T. (2016). Feeding stimulants in an omnivorous species, crucian carp *Carassius carassius* (Linnaeus 1758). *Aquaculture Reports* 4, 66–73. http://dx.doi.org/10.1016/j.aqrep.2016.06.005.

Saadaoui, H., Espejo-Carpio, F. J., Guadix, E. M., Amar, R. B., & Pérez-Gálvez, R. (2019). Bi-objective optimization of tuna protein hydrolysis to produce aquaculture feed ingredients. *Food and Bioproducts Processing*, 115, 26-35. https://doi.org/10.1016/j.fbp.2019.03.001.

Shamushaki, V. A. J., Abtahi, B., & Kasumyan, A. O. (2011). Olfactory and taste attractiveness of free amino acids for Persian sturgeon juveniles, *Acipenser persicus*: a comparison with other acipenserids. *Journal of Applied Ichthyology*, 27(2), 241-245. https://doi.org/10.1111/j.1439-0426.2011.01687.x.

Soares, M., Rezende, P. C., Correa, N. M., Rocha, J. S., Martins, M. A., Andrade, T. C., Fracalossi, D. M., & do NascimentoVieira, F. (2020). Protein hydrolysates from poultry by-product and swine liver as an alternative dietary protein source for the Pacific white shrimp. Aquaculture Reports, 17, 100344. https://doi.org/10.1016/jaqrep.2020.100344.

Statsoft, Statistica (Data Analysis Software System). 2004. Inc. Version 7.

Vázquez, J. A., Pedreira, A., Durán, S., Cabanelas, D., Souto-Montero, P., Martínez, P., & Valcarcel, J. (2022). Biorefinery for tuna head wastes: Production of protein hydrolysates, high-quality oils, minerals and bacterial peptones. Journal of Cleaner Production, 357, 131909. https://doi.org/10.1016/j.jclepro.2022.131909.

Yang, X., Wang, G., Zhao, X., Dong, X., Chi, S., & Tan, B. (2021). Addition of hydrolysed porcine mucosa to low-fishmeal feed improves intestinal morphology and the expressions of intestinal amino acids and small peptide transporters in hybrid groupers (*Epinephelus fuscoguttatus*♀× *E. lanceolatus*♂). *Aquaculture*, 535, 736389. https://doi.org/10.1016/j.aquaculture.2021.736389.

Yang, X., Zhi, X., Song, Z., Wang, G., Zhao, X., Chi, S., & Tan, B. (2022). Flesh quality of hybrid grouper (Epinephelus fuscoguttatus♀× Epinephelus lanceolatus♂) fed with hydrolyzed porcine mucosa-supplemented low fishmeal diet. Animal Nutrition, 8(1), 114-124. https://doi.org/10.1016/j.aninu.2021.05.011.

Yang, X., Dong, X., Yang, Q., Wang, G., Zhao, X., Lui, H., Zhang, S., Chi, S., & Tan, B. (2020). Addition of enzyme-digested hydrolysed porcine mucosa to low-fishmeal feed improves growth, intestinal microbiota, and intestinal peptide and amino acid transporter expressions in hybrid groupers (Epinephelus fuscoguttatus ♀ × E. lanceolatus ♂). 00, 1 – 20. https://doi.org/10.1111/anu.13190.

Yathisha, U. G., Vaidya, S., & Sheshappa, M. B. (2022). Functional Properties of Protein Hydrolyzate from Ribbon Fish (Lepturacanthus Savala) as Prepared by Enzymatic hydrolysis. International Journal of Food Properties, 25(1), 187-203. https://doi.org/10.1080/10942912.2022.2027964.

Zou, Y., Shahidi, F., Shi, H., Wang, J., Huang, Y., Xu, W., & Wang, D. (2021). Values-added utilization of protein and hydrolysates from animal processing by-product livers: A review. Trends in Food Science & Technology, 110, 432-442. https://doi.org/10.1016/j.tifs.2021.02.033.