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EVALUATION OF PHYSICAL QUALITY OF EXTRUDED FEED SUPPLEMENTED WITH BASIL (Ocimum basilicum)

QUALIDADE FÍSICA DA RAÇÃO COMERCIAL EXTRUSADA APÓS INCORPORAÇÃO DE MANJERICÃO (*Ocimum basilicum*)

CALIDAD FÍSICA DEL PIENSO EXTRUSIONADO COMERCIAL TRAS LA INCORPORACIÓN DE ALBAHACA (Ocimum basilicum)

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ABSTRACT

This study evaluated the effects of basil (Ocimum basilicum) incorporation in two forms (essential oil and aqueous extract) into commercial extruded fish feed. The physical quality of the feed was assessed under four treatments: control (without additives), 0.5% basil essential oil, 0.5% basil aqueous extract, and 0.5% soybean oil. Experimental units consisted of 20 L aquariums, with three replicates per treatment. Additive retention rate was evaluated. Additionally, floating capacity and dry matter leaching tests were conducted in a factorial arrangement involving four treatments and four floating times (5, 10, 20, and 30 minutes), with three replicates. The results showed that basil essential oil and soybean oil presented higher ($p<0.05$) retention rates (97.51% and 97.14%, respectively) than the basil aqueous extract (93.96%). The floating test over time showed a significant effect ($p<0.05$) among additives at 10 and 30 min, in which soybean oil exhibited lower floating capacity. No differences were observed in the percentage of dry matter leaching of the feeds as a function of floating time. It was concluded that the incorporation of oil-based additives is more efficient than powder-based additives, as they are better retained and do not impair the physical quality of the feed regarding water stability.

KEYWORDS: *Additives. Extract. Essential oil. Fish farming.*

RESUMO

Este estudo avaliou os efeitos da incorporação de manjericão (*Ocimum basilicum*) em duas formas, óleo essencial e extrato aquoso, em ração comercial extrusada para peixes. A incorporação dos aditivos foi avaliada por testes de retenção com quatro tratamentos: controle (sem aditivos); 0,5% de óleo essencial de manjericão; 0,5% de extrato aquoso de manjericão e 0,5% de óleo de soja, com três repetições. Os testes de flutuabilidade e lixiviação da matéria seca foram realizados em arranjo fatorial com quatro tratamentos: controle (sem aditivos); 0,5% de óleo essencial; 0,5% de extrato aquoso de manjericão, e 0,5% de óleo de soja, e quatro tempos de flutuação (5, 10, 20 e 30 min) com três repetições. Os resultados demonstraram que o óleo essencial de manjericão e o óleo de soja obtiveram maiores taxas de retenção (97,51% e 97,14%, respectivamente) que o extrato aquoso de manjericão (93,96%). O teste de flutuabilidade em função de tempo demonstrou efeito significativo ($p<0,05$) entre os aditivos nos tempos de 10 e 30 minutos, em que o óleo de soja apresentou menor taxa de flutuabilidade.

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Não foram observadas diferenças na porcentagem de lixiviação da matéria seca das rações em função do tempo em que os grãos ficaram sob flutuação. Concluímos que a incorporação de aditivos em forma de óleos é mais eficiente do que os aditivos em pó, pois são mais bem incorporados e não afetam a qualidade física da ração durante a estabilidade na água.

PALAVRAS-CHAVE: Aditivos. Extratos. Óleo essencial. Piscicultura.

RESUMEN

Este estudio evaluó los efectos de la incorporación de albahaca (Ocimum basilicum) en dos formas (aceite esencial y extracto acuoso) en alimento extruido comercial para peces. La incorporación de los aditivos se evaluó mediante pruebas de retención con cuatro tratamientos: control (sin aditivos); 0,5 % de aceite esencial de albahaca; 0,5 % de extracto acuoso de albahaca; y 0,5 % de aceite de soja, con tres réplicas. Las pruebas de flotabilidad y lixiviación de materia seca se realizaron en un diseño factorial con cuatro tratamientos: control (sin aditivos); 0,5 % de aceite esencial; 0,5 % de extracto acuoso de albahaca; y 0,5 % de aceite de soja, y cuatro tiempos de flotación (5, 10, 20 y 30 min) con tres réplicas. Los resultados mostraron que el aceite esencial de albahaca y el aceite de soja tuvieron tasas de retención más altas (97,51 % y 97,14 %, respectivamente) que el extracto acuoso de albahaca (93,96 %). La prueba de flotabilidad en función del tiempo mostró un efecto significativo (p<0,05) entre los aditivos a los 10 y 30 minutos, donde el aceite de soja presentó una menor tasa de flotabilidad. No se observaron diferencias en el porcentaje de lixiviación de materia seca del alimento en función del tiempo que los granos permanecieron flotando. Concluimos que la incorporación de aditivos en forma de aceite es más eficiente que la de aditivos en polvo, ya que se incorporan mejor y no afectan la calidad física del alimento durante la estabilidad en agua.

***PALABRAS CLAVE**: Aditivos. Extractos. Aceite esencial. Piscicultura.*

INTRODUCTION

The growth in fish production has driven an increase in the supply of sector inputs, particularly feeds. Feed production in Brazil grew by 2.4% in 2024, with the aquaculture sector showing the highest growth (8.6%) in feed production (PANORAMA DA AQUICULTURA, 2025). To keep pace with this expansion, it is crucial to improve production technologies and implement rigorous quality control in the manufacturing and storage of feeds, given that feed accounts for approximately 70% of production costs and is one of the most important factors in fish farming (NASS *et al.*, 2020). Therefore, the use of high-quality feeds capable of supplying the nutrients required for good zootechnical performance is essential in farmed fish production.

The inclusion of a new product in an already-prepared feed results in lower costs for the producer, since conventional manufacturing requires the use of artificial binding additives with high market value (KARIM *et al.*, 2024). As an alternative, the manual incorporation of these additives is both viable and more economical, while also improving the appearance of extruded pellets and reducing the number of fines (DAIRIKI *et al.*, 2013; MOREIRA *et al.*, 2015). Among

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the additives considered safest, the use of plant extracts and oils has shown satisfactory results as growth and health-promoting agents in fish production (SUTILI *et al.*, 2018; DE SOUZA *et al.*, 2019; ACAR *et al.*, 2015). Among these, basil stands out, used both as an essential oil (LIMMA-NETTO; OLIVEIRA; COPATTI, 2017; DE SOUZA *et al.*, 2019; VENTURA *et al.*, 2021) and as an extract (AMIRKHANI; FIROUZBAKHSH, 2015; SOUSA *et al.*, 2024). Basil (*Ocimum basilicum*) is a plant belonging to the Lamiaceae family, containing numerous chemical compounds such as methyl chavicol, linalool, eucalyptol, eugenol, and cyclohexane (OLIVEIRA; MOREIRA; OLIVEIRA, 2013), and in folk medicine it is used for its potential antifungal, antioxidant, anti-inflammatory, antimicrobial, calming, sedative, and anesthetic properties (VENTURA *et al.*, 2020).

In fish farming, the use of natural plant extracts has proven highly promising in the context of increased production and intensified farming systems, aiming to improve feed efficiency, control diseases, and promote growth, while also being easy to handle during application (HAI, 2015). For use in aquaculture, these substances are available as extracts and essential oils containing various active compounds such as phenols, polyphenols, alkaloids, quinones, terpenoids, lectins, and polypeptides (HARIKRISHNAN; BALASUNDARAM; HEO, 2011), which may contribute to fish health, promote growth performance, exert antioxidant activity (LEE *et al.*, 2005) and antimicrobial action, modulate innate and adaptive immune responses (MOGHADDAM *et al.*, 2011; CAMPAGNOLO *et al.*, 2013), and act as anesthetic agents, increasing stress resistance (VENTURA *et al.*, 2020).

Although studies have demonstrated beneficial effects on the growth of common carp (*Cyprinus carpio*; AMIRKHANI; FIROUZBAKHSH, 2015), Nile tilapia (*Oreochromis niloticus*; DE SOUZA *et al.*, 2019), and arapaima (*Arapaima gigas*; CHUNG *et al.*, 2020) following supplementation with basil-based additives, studies evaluating the physical quality of commercial extruded fish feeds after the inclusion of natural basil-derived additives are still lacking. In this context, the objective of this study was to evaluate the physical quality of commercial extruded fish feed following the incorporation of natural basil (*Ocimum basilicum*) additives in two forms: essential oil and aqueous extract.

MATERIAL AND METHODS

Location and feed

The experiment was carried out at the Experimental Fish Farming Station of the Faculty of Veterinary Medicine and Animal Science – FAMEZ – at the Federal University of Mato Grosso do Sul (UFMS), in Campo Grande, MS, Brazil (20°25’57’’S and 55°17’11’’W). Commercial extruded feed (36% crude protein) for omnivorous fish, with a particle size of 2 to 3 mm, purchased

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from a local supplier, was used in the experiment. Basil essential oil was obtained commercially (Phytoterápica®), and the aqueous extract was prepared from basil leaves (*O. basilicum*) harvested at the Medicinal Plant Garden of the Federal University of Grande Dourados – UFGD, following the methodology described by SOUSA *et al.* (2024).

Experimental design

For the retention test, a completely randomized design with four treatments was used: positive control (no additive); negative control (0.5% soybean oil); 0.5% basil essential oil; and 0.5% basil aqueous extract. For the floating test, a completely randomized design in a 4×4 factorial arrangement was employed. The factors consisted of four treatments: (i) control (no additive); (ii) 0.5% basil essential oil; (iii) 0.5% basil aqueous extract; and (iv) 0.5% soybean oil, combined with four floating times: 5, 10, 20, and 30 minutes. The leaching test was conducted immediately after the floating test, using the same experimental design. Three replicates were used for all variables.

Sample preparation

For the incorporation of basil essential oil, basil aqueous extract, and soybean oil, 1,000 g of commercial extruded feed (36% crude protein) was used. Initially, a dilution solution was prepared for both basil essential oil and soybean oil at a 1:10 ratio (1 mL of oil to 10 mL of 92.8% ethyl alcohol). Subsequently, 100 mL of grain alcohol (96%) was used per 1,000 g of feed (DAIRIKI *et al.*, 2013).

For the powdered extract, incorporation was initially performed by dissolving it in 50 mL of ethyl alcohol, followed by the addition of 100 mL of grain alcohol per 1,000 g of commercial feed. In the control treatment, the same quantities of alcohol (ethyl and grain) were applied to the feed to ensure it underwent the same procedure.

The feed from each treatment was placed in an inflated and sealed plastic bag and manually shaken for 10 minutes. After shaking, the feed was spread over a paper towel-lined polyethylene trough (100 L) and left to dry at room temperature for 24 hours (DAIRIKI *et al.*, 2013; MENEZES, 2019). The procedures adopted aimed to maximize additive incorporation into the feed pellets.

Proximate composition

Proximate composition was determined using Near-Infrared Spectroscopy (NIRS) to quantify nutrients present in feed samples (DARDENNE; SINNAEVE; BAETEN, 2000). Moisture,

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protein, ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed (Table 1).

Table 1. Ingredients and proximate composition of diets

		Additives 0.5% per kg		
		Incorporation of *Ocimum basilicum*		
Ingredients	**[1]Control**	**Aqueous extract**	**Essential oil**	**Soybean oil**
	0	5 g	5 mL	5 mL
		[2]Calculated composition		
Protein	36.73±0.19	36.70±0.34	35.91±0.12	35.29±0.29
Moisture	7.08±0.16	8.28±0.12	8.2±0.13	8.28±0.14
Ash	7.33±0.04	7.60±0.19	7.63±0.10	7.68±0.24
[3]NDF	11.78±0.17	10.85±0.57	11.14±0.15	11.12±0.30
[4]ADF	3.44±0.07	3.47±0.09	3.95±0.23	3.84±0.23

[1]Commercial feed basic ingredients (g kg^{-1}): soybean meal, full-fat rice bran, wheat bran, meat and bone meal, fish meal, blood meal, ground whole corn, degummed soybean oil, sodium chloride (common salt), dicalcium phosphate, ferrous sulfate, manganese monoxide, zinc oxide, copper sulfate, cobalt, sodium selenite, vitamins A, D_3, E, K_3, B_1, B_2, B_{12}, C, niacin, pantothenic acid, threonine, lysine, methionine, and propionic acid. Data are expressed as mean ± standard deviation. [2]Analyses were performed at the Multiuser Laboratory of the Faculty of Veterinary Medicine and Animal Science (FAMEZ), Federal University of Mato Grosso do Sul (UFMS), using the NIRS method. [3]Neutral detergent fiber. [4]Acid detergent fiber. Number of samples: n = 3.

Retention rate

To evaluate the additive retention rate, a transparent plastic bag (80×40 cm) was first weighed, and then 1,000 g of feed was added. The corresponding solution (essential oil + alcohol; aqueous extract + alcohol; soybean oil + alcohol; or feed + alcohol) was slowly poured over the feed pellets. The bag was then sealed and manually shaken for 10 minutes. The retention rate (RR) was calculated using the following formula: RR (%) = (final weight − initial weight) / 100. The weight of the plastic bag was subtracted prior to additive incorporation; after 10 minutes of shaking, the feed was reweighed, and the initial feed weight (before inclusion of oils and extract) was subtracted (MENEZES, 2019).

Particle size distribution and uniformity

Particle size distribution and uniformity were assessed using a commercially purchased 25 kg bag of feed with 36% crude protein and a declared particle size of 2 to 3 mm. Following the

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methodology of FREITAS *et al.* (2016), the entire bag was sieved, and the collected fines were weighed. A sample of 100 pellets was individually measured using a digital caliper, and the recorded values were compared against the manufacturer's label specifications. As described by the authors, pellet size should correspond to the mode of the measurements, i.e., the most frequently occurring size in the sample. The total fines obtained from the 25 kg bag amounted to 78.42 g, corresponding to 0.3%, a value considered within acceptable standards. The uniformity among the evaluated pellets was 2.45 mm, within the range indicated by the manufacturer (2–3 mm).

Floating capacity

To evaluate floating, 100 feed pellets (after additive incorporation) were randomly sampled and placed in an aquarium (40×25×20 cm) containing 4 L of water, and assessed at 5, 10, 20, and 30 minutes of floating time. After each interval, the number of sunken pellets was counted and recorded (FREITAS *et al.*, 2016). Floating capacity was calculated using the following formula (CRUZ *et al.*, 2015): Floating (%) = (number of remaining floating pellets / total number of pellets evaluated) × 100.

Dry matter leaching

Dry matter leaching was assessed immediately after the floating test, following the methodology adapted from HASHIM and SAAT (1992). After each floating interval, the pellets were placed in disposable cups and left to air-dry for 24 hours, then transferred to an oven at 45°C for an additional 24 hours. Leaching was calculated from the difference in dry matter weight before and after immersion (reflecting pellet structural loss during the floating period) using the following formula (adapted from CARVALHO; NUNES, 2006): Leaching (%) = [1 − (dry weight after immersion (g) / dry weight before immersion (g))] × 100.

Statistical analyses

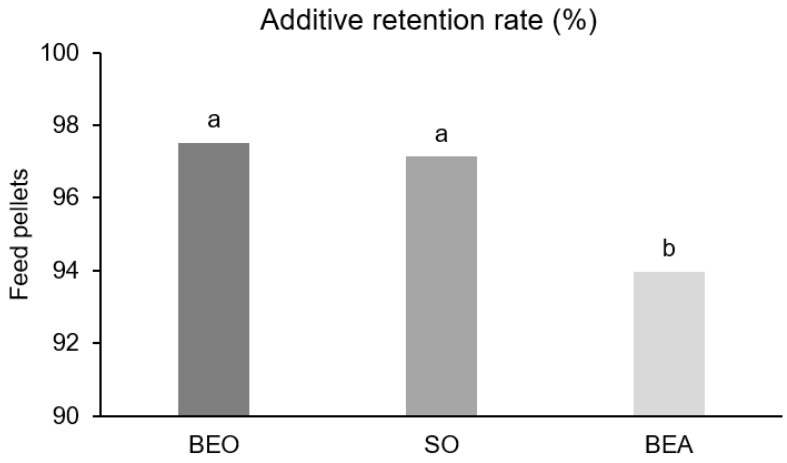
Data from the incorporation and leaching tests were subjected to normality and homogeneity tests. When significant differences were detected, Tukey's test was applied at a 5% significance level. Floating data underwent angular transformation and were submitted to the Shapiro-Wilk normality test and Levene's test for homogeneity of variances. Analysis of variance was performed with the two independent variables and their interaction as sources of variation, and Student's t-test was used for multiple comparisons. The interaction between feed type and floating time was significant ($p < 0.05$). All analyses were performed using SAS software.

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RESULTS AND DISCUSSION

A significant difference was observed in the retention rate of basil aqueous extract, which yielded the lowest value (93.96%). No significant difference ($p > 0.05$) was detected between basil essential oil (97.51%) and soybean oil (97.14%) (Figure 1). Research on additive incorporation in fish feeds has focused primarily on the effects on fish growth performance. Studies evaluating the influence of these products on the physical quality of feed after additive incorporation are scarce, given that feeds are required to be durable and must not interfere with fish feeding behavior. Furthermore, several factors may affect the physical quality of feed, including the processing method (HAKIM *et al.*, 2021).

Figure 1. Retention rate of basil essential oil (BEO), soybean oil (SO) and basil aqueous extract (BEA) after manual incorporation into commercial feed (36% crude protein)



The incorporation of additives into extruded feeds must be carried out correctly to ensure adequate adhesion of the additive to the feed pellets, thereby guaranteeing efficiency in the diet preparation process. However, the high costs associated with extruded feed manufacturing led producers to adopt alternative, manual methods that can still ensure process efficiency (DAIRIKI *et al.*, 2013). One such method is the manual incorporation of additives, which can be performed by mixing, when the additive is in powder form, or by spraying, when additives such as essential oils are used. Consistent with this being an effective management practice, our study

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demonstrated that oil-based additives are more efficient than aqueous extract, as they yielded higher retention rates.

Floating is a key parameter for assessing feed quality in fish farming, as feed that sinks too rapidly cannot be efficiently consumed by fish, reducing nutritional efficiency and contributing to water quality deterioration (MULIA; WULANDARI; MARYANTO, 2017). Results regarding feed floating after additive incorporation showed that the additives did not affect overall floating as a function of immersion time. However, when comparing floating time effects among treatments ($p < 0.05$), significant differences were found at 10 and 30 minutes, with soybean oil presenting the lowest floating rate (Table 2). This may have occurred due to insufficient additive coating on some pellets in the soybean oil treatment.

Table 2. Mean floating capacity (%) of commercial feed (36% CP) with different additives and evaluation times

	Floating capacity (%)			
Time (min)	**Additives 0.5% per kg**			
	[1]Control	**[2]Essential oil (5 mL)**	**[2]Aqueous extract (5 g)**	**Soybean oil (5 mL)**
5	99.36 Aa	98.39 Aa	99.56 Aa	97.78 Aa
10	98.24 Aab	99.11 Aa	99.01 Aab	92.92 Ab
20	99.11 Aa	98.09 Aa	99.56 Aa	95.70 Aa
30	99.66 Aa	98.72 Aa	100.00 Aa	90.21 Ab

[1]Commercial feed without additive inclusion; [2]Basil essential oil and basil aqueous extract (*Ocimum basilicum*). Mean values followed by different lowercase letters differ within rows, and different uppercase letters differ within columns ($p<0.05$) according to Student's t-test. The p-value for the Additive × Time interaction was 0.0445, and the coefficient of variation (CV) was 3.92%. Values are expressed as percentages and were back-transformed from the angular (arcsine) transformation.

Water stability and nutrient leaching are considered the main challenges in feed formulation (IGHWELA; AHMAD; ABOL-MUNAFI, 2013). Leaching occurs progressively as feed remains in the water, causing pellets to disintegrate and release nutrients into the environment; the longer the pellets remain in the water column, the greater the degree of disintegration. In the present study, no significant differences were observed in dry matter leaching as a function of floating time. Given that most fish tend to consume feed within 10 to 15 minutes (MASSER; WURTS, 1992), it can be concluded that additive incorporation did not influence dry matter

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leaching, and no significant changes were detected up to 30 minutes of immersion (Table 3).

Table 3. Evaluation of dry matter leaching from commercial feed (36% CP) after additive incorporation subjected to different floating times

Time (min)	Dry matter leaching (%)			
	Additives 0.5% per kg			
	[1]Control	[2]Essential oil	[2]Aqueous extract	Soybean oil
5	1.47	6.19	3.24	2.85
10	2.24	4.06	3.24	3.42
20	4.48	1.80	6.43	5.65
30	4.48	3.93	6.43	8.85

[1]Commercial feed without additive inclusion; [2]Basil essential oil and basil powder extract (*Ocimum basilicum*). Mean values did not differ within rows or columns ($p > 0.05$) according to Tukey's test. The *p-value* for the Additive × Time interaction was 0.1100, and the coefficient of variation (CV%) was 28.01%.

Feed supplied to fish must remain stable in the water for sufficient time to allow fish to locate and consume it (CYRINO *et al.*, 2006). Extruded feeds perform better than pelleted and mash feeds in terms of feed efficiency and reduced water quality deterioration (POTRICH *et al.*, 2011). Thus, additive incorporation techniques, whether aimed at improving feed quality or at oral drug administration via feed, indicate that the use of a carrier such as an essential oil and/or vegetable oil is more effective than the incorporation of powdered additives.

CONCLUSION

The incorporation of oil-based additives, such as basil essential oil and soybean oil, into commercial extruded feeds proved to be efficient, yielding high retention rates without compromising the physical quality of the feed. These additives represent a viable and effective alternative for enhancing fish feeds, with potential benefits for aquaculture production.

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