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Growth performance and digestibility of juvenile Nile tilapia fed diets containing acid silage viscera of surubim catfish

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ABSTRACT. This study aimed to evaluate increasing levels (0, 4, 8 and 12%) of acid silage viscera of surubim catfish as an alternative protein source on growth performance and apparent digestibility coefficients (ADC) for Nile tilapia. Weight gain (WG), feed intake (FI), feed conversion ratio (FCR), and protein efficiency ratio (PER) were evaluated. The digestibility for dry matter (DM), crude protein (CP), ether extract (EE), gross energy (GE) and availability of phosphorus (P) were also assessed. The experimental design was completely randomized with four treatments and four repetitions. The results indicated no difference ($p > 0.05$) for growth performance parameters. The ADC of CP of treatment containing 8% of silage was lower ($p < 0.05$) when compared to the others. On the other hand, for the GE, the same level provided a higher ADC ($p < 0.05$). In conclusion, acid silage viscera of surubim catfish can be included in diets for Nile tilapia up to 12%, with no negative effects on growth performance and digestibility as well as to reduce feed cost.

Keywords: aquaculture, by-product, protein source.

Desempenho e digestibilidade de rações contendo silagem ácida de vísceras de surubim para juvenis de tilápia-do-nilo

RESUMO. Níveis crescentes (0%, 4%, 8% e 12%) de inclusão de silagem ácida de vísceras de surubim na alimentação de tilápia-do-nilo foram avaliados sobre o desempenho e coeficiente de digestibilidade aparente (CDA). Os parâmetros de desempenho analisados foram: ganho de peso (GP), consumo de ração (CR), conversão alimentar (CA) e taxa de eficiência proteica (TEP). Avaliou-se o CDA da matéria seca (MS), proteína bruta (PB), extrato etéreo (EE), energia bruta (EB) e disponibilidade de fósforo (P). O delineamento utilizado foi o inteiramente casualizado, com quatro tratamentos e quatro repetições. Os resultados obtidos não demonstraram diferença ($p > 0,05$) para os parâmetros de desempenho avaliados. O CDA da PB para o tratamento contendo 8% de silagem foi menor ($p < 0,05$), quando comparado aos demais. Por outro lado, para a EB, este mesmo nível de inclusão proporcionou melhor CDA ($p < 0,05$). Conclui-se que a silagem ácida de vísceras de surubim pode ser incluída em rações para tilápia-do-nilo em até 12%, sem que haja interferência negativa no desempenho e na digestibilidade, além de reduzir o custo da ração.

Palavras-chave: aquicultura, subproduto, fonte proteica.

Introduction

Fish meal is the most used animal protein source in fish diets, due to its adequate amino acid balance, high biological values, and positive characteristics of attraction and palatability (FURUYA et al., 2001). Nevertheless, given its growing scarcity due to increasing demand, the partial or total replacement by by-products and co-products is essential for economic and environmental development of aquaculture.

According to Nunes (1999), the waste produced by fish processing plants generates several problems of environmental impact, when incorrectly handled, with the need to improve systems of application and management of residues. A sustainable and viable alternative is to convert this material into silage for animal feeding, which may be economically advantageous for fish processing industry and allow the use of this waste of difficult disposal.

Moreover, the silage has high potential for application in diets for livestock due to the similarity with the feedstock, providing protein of high quality, high digestibility and low cost (FAGBENRO; JAUNCEY, 1995). However, its chemical composition differs according to the type of silage (acidic, biological, and enzymatic), and proportion and type of residue.

In order to preserve the fish acid silage it is necessary to reduce the pH by adding inorganic or organic acids. Although low-cost, the use of inorganic acids result in material with greater acidity, being

recurrent its neutralization before providing to the animals (ARRUDA et al., 2007). Among the most used organic acids stand out formic and propionic acids that ensure a stable product free of pathogenic microorganisms (MORALES-ULLOA; OETTERER, 1995), but the propionic acid is expensive. The citric acid is an alternative to reduce the cost but it is advisable the addition combined with formic acid, which has a more effective antimicrobial activity, besides providing a better stability to the material (FAGBENRO; FASAKIN, 1996; GAO et al., 1992).

Gao et al. (1992) employed different proportions of formic and citric acid in silage of salmon farm mortalities and concluded that the mixture of these acids in different proportions resulted in a good quality silage. They also reported that the increase in the proportion of citric acid has reduced the cost of the product, as well as its preservation period. Besides that, organic acids are commercially used in poultry, swine and fish diets, aiming to reduce the stomach pH, improve the digestibility of some nutrients and the enzyme activity, and reduce the number of pathogenic bacteria (IZAD et al., 1990; VIELMA; LALL, 2006), which could increase the benefits of using silage of fish prepared with organic acids.

This study aimed to evaluate the performance and digestibility of diets with acid silage of surubim viscera for Nile tilapia juvenile.

Material and methods

The experiment was conducted at the Fish Culture Laboratory of *Embrapa Agropecuária Oeste*. Four isoproteic (32% DP) and isoenergetic (3,200 kcal DE kg^{-1}) diets were prepared with different levels of silage of surubim *Pseudoplatystoma* spp. viscera (0, 4, 8 and 12%). The formulations were based on Nutrient Requirements of Fish - National Research Council (NRC, 1993). To produce the silage it was used viscera of surubim from a fish processing plant under federal inspection. The residue was grinded to obtain a homogeneous mass and a mixture formic and citric acids (1.0: 0.75), and 0.02% BHT was added. Periodically the mixture was homogenized until completing 30 days. The acidity was kept around pH 4.0, and was measured using digital pHmeter.

The acid silage was mixed with the ingredients previously ground (0.5 mm) to prepare the experimental diets. Diets were processed into granules (1.5 mm), and dried in forced-air oven at 60ºC for 24 hours and stored under refrigeration (5°C). By the end of the preparation and processing of diets, samples were taken from each diet to determine the dry matter (DM), crude protein (CP), ether extract (EE), calcium (Ca) and phosphorus (P), according to the methodology described by Association of Official Analytical Chemists (AOAC, 2000). The pH of the diets was assessed by the methodology of the Instituto Adolfo Lutz (IAL, 1985). The cost for silage production considered the price of the acids, labor, electric energy, and depreciation of equipments.

For performance assays, 96 fingerlings of Nile tilapia (*Oreochromis niloticus*) GIFT lineage were used, with initial mean weight of 4.26 ± 0.16 g. Fish were previously anesthetized with clove oil (40 mg L^{-1}). It was used four fiberglass tank (1000 L) with four net cages (60 L) inside each (six fish/cage). The experimental design was completely randomized, with four treatments and four repetitions.

Fish were fed four times a day, at 7.30, 11.00 a.m., 1.30 and 4.00 p.m., until apparent satiety. In order to control the amount of diet consumed, it was performed observations during the feeding, to prevent losses. Tanks were siphoned on alternate days to avoid the accumulation of organic matter.

Parameters of water quality, such as dissolved oxygen (mg L^{-1}) and temperature (°C), were measured twice a day (at 8.00 a.m. and 1.30 p.m.) using digital oximeter and thermometer. The content of ammonia, nitrate and nitrite were determined through a colorimetric kit, and pH was measured using a pHmeter, both every seven days. After the experimental period of 30 days, weight gain (WG), feed intake (FI), feed conversion rate (FCR) and protein efficiency ratio (PER) were evaluated.

The apparent digestibility coefficient (ADC) of the experimental diets were assessed using the diets of the performance test, added of 0.1% chromium oxide III (Cr_2O_3), as inert external marker (Table 1).

Table 1. Chemical composition and cost of experimental diets.

Ingredient (%)	Silage inclusion level (%)			
	0	4	8	12
Soybean meal	66.20	64.00	61.40	59.10
Silage	0.00	4.00	8.00	12.00
Corn	13.08	13.48	14.38	14.38
Wheat middlings	9.50	9.50	9.50	9.50
DL – methionine	0.40	0.30	0.20	0.10
Soybean oil	5.80	3.70	1.60	0.0
Dicalcium phosphate	3.90	3.90	3.80	3.80
Salt	0.10	0.10	0.10	0.10
Vit min^{-1} premix[1]	1.00	1.00	1.00	1.00
BHT (g)[2]	0.02	0.02	0.02	0.02
TOTAL	100.00	100.00	100.00	100.00
Cost (R$)	1.21	1.13	1.09	1.03
	Composition (%)			
Dry matter (%)[3]	97.07	97.21	97.12	96.18
Digestible protein (%)[4]	33.24	32.70	31.74	32.10
Digestible energy (kcal kg^{-1})[4]	3,144	3,154	3,254	3,158
Ether extract (%)[4]	7.16	7.09	7.20	7.78
Calcium (%)[3]	1.37	1.37	1.36	1.39
Available phosphorus (%)[4]	0.52	0.52	0.50	0.57

[1]Guarantee level (ingredient kg^{-1}): Vit. A. 500.000UI; Vit. D3. 250.000 UI; Vit. E. 5.000 mg; Vit. K3. 500 mg; Vit. B1. 1.000 mg; Vit. B2. 1.000 mg; Vit. B6. 1.000 mg; Vit. B12. 2.000 mg; Folic acid. 500 mg; Vit. C. 10.000 mg; Biotin. 10 mg; Inositol. 1.000; Choline. 100.000 mg; Calcium pantothenate. 4.000 mg; Co. 50 mg; Cu. 1.000 mg; Fe. 5.000 mg; I. 200 mg; Mn. 5000 mg; Se. 30 mg; Zn. 9.000 mg. [2]Butil hidroxi toluene (antioxidant). [3]Analyzed values. [4]Determined values according to apparent digestibility coefficients of experimental diets.

Two fiberglass tanks (1000 L each) were used for feeding, with four cages of 60 L inside each, with 15 fish (35.0 ± 5.0 g), where fish received the experimental diets. Tanks were continuously supplied with water from an artesian well. The period for acclimation to experimental diets and collection management lasted for five days.

Fish remained in these tanks and fed to apparent satiety (eight times a day), with higher frequency by the afternoon. Cages were used aiming to ease the transference of fish to aquaria for feces collection, minimizing the stress and contamination of feces by feed. In the late of each afternoon, cages with fish were taken to four aquaria for feces collection, with conical bottom and capacity of 250 L. At the bottom of each aquarium, plastic vials were coupled to ease the feces collection. Fish remained in aquaria under constant aeration until the next morning. Water temperature and oxygen were monitored daily with a digital oximeter and thermometer.

The feces collected were dried in oven at 55ºC and manually ground in a mortar. Scales, whenever present, were removed using tweezers and placed on plastic bags, kept under refrigeration at -20ºC, for further chemical analysis.

Analyses of DM, CP, EE, Ca and P were accomplished in the Laboratory of Soil of the *Embrapa Agropecuária Oeste*, while the analyses of gross energy (GE) of the diet and feces were performed by the Laboratory of Animal Nutrition of the *Universidade Estadual de Santa Cruz* - UESC. The ADC of nutrients was determined following the methodology described by Cho et al. (1985).

$$ADC_{(n)} = 100 - \left[100 \left(\frac{\%Cr_2O_{3d}}{\%Cr_2O_{3f}}\right) \times \left(\frac{\%N_f}{\%N_d}\right)\right]$$

where:

$ADC_{(n)}$ = apparent digestibility coefficient;

Cr_2O_{3d} = % chromium oxide in the diet;

Cr_2O_{3f} = % chromium oxide in the feces;

N_d = Nutrients in the diet;

N_f = Nutrients in the feces.

Eight feces collection were carried out, grouped every two days, amounting four repetitions. For the values of chemical composition and ADC it was considered the mean value of these repetitions.

Data of performance were subjected to an analysis of variance at 5% probability and polynomial regression analysis, and data of nutrient digestibility and phosphorus availability were subjected to the Scott Knott test, both using the software SISVAR 5.3 (FERREIRA, 2008).

Results and discussion

Physical and chemical parameters analyzed were within acceptable limits for tilapia rearing (POPMA; GREEN, 1990). The average temperature during the experiment was 25.00 ± 0.61°C; pH 7.56 ± 0.09; dissolved oxygen 6.33 ± 0.59 mg L^{-1} and total ammonia 0.09 ± 0.01. The experimental silage presented 21.68% DM, 25.70% CP, 42.40% EE, 0.06% Ca and 0.27% P.

The cost of the formulation decreased with increased level of inclusion of silage (Table 1). The production cost of acid silage viscera of catfish surubim was R$ 0.38 kg^{-1}. The diet with the greatest inclusion of silage has costed 15% less that the diet with no test ingredient. This reduction in the cost with increasing inclusion of silage was also observed by Costa et al. (2009), with acid silage of shrimp waste, and by Abimorad et al. (2009), using fish waste silage, both for feeding tilapia.

In Table 2 are listed the mean results of performance of Nile tilapia fingerlings fed diets with increasing levels of acid silage viscera of surubim as well as pH of the diets. No significant difference ($p > 0.05$) was detected between the levels of replacement of silage on the variables WG, FI, FCR and PER.

Table 2. Average values of weight gain (WG), feed intake (FI), feed conversion ratio (FCR) and protein efficiency ratio (PER) of Nile tilapia juveniles fed diets of increase levels of silage and pH of experimental diets.

Parameter	Silage inclusion level (%)				CV[1]
	0	4	8	12	(%)
WG(g animal)$^{-1}$	6.47 ± 1.08	8.20 ± 1.52	7.72 ± 1.06	7.56 ± 1.77	19.55
FI (g animal^{-1})	9.22 ± 0.59	10.81 ± 2.10	12.43 ± 1.49	9.82 ± 1.70	14.67
FCR	1.43 ± 0.31	1.32 ± 0.10	1.61 ± 0.27	1.30 ± 0.14	16.10
PER	2.01 ± 0.48	2.17 ± 0.16	1.77 ± 0.26	2.17 ± 0.22	15.36
pH	6.05 ± 0.01^a	5.85 ± 0.03^b	5.64 ± 0.02^c	5.47 ± 0.01^d	0.23

[1]Coefficient of variation (%). Means followed by the same letter do not differ by Scott Knott test ($p < 0.05$).

Considering the WG, the results of Carvalho et al. (2006) with Nile tilapia fed with acid silage of discarded filleting of the same species, and Cavalheiro et al. (2007) with Nile tilapia fed with acid silage of shrimp head corroborate with the present study. Both researches verified that the inclusion of silage did not lead to significant difference in WG. On the other hand, Enke et al. (2009) evaluated the inclusion of up to 50% of fish silage into five diets (0, 12.5, 25, 37.5 and 50%) for feeding silver catfish *Rhamdia quelen*, and concluded that values above 37.5% of inclusion have negatively interfered with the WG of animals, possibly by influencing the palatability, attractiveness and digestibility of the diets.

As well as the FI of tilapia that received increasing levels of silage of surubim viscera, Oliveira et al. (2006) analyzed the performance of tilapia fingerlings fed diets with inclusion of up to 40% of acid silage of tilapia filleting residue and found no significant difference for the FI. The same was observed for the FCR, i.e. the viscera silage did not cause any difference ($p > 0.05$) between the treatments, agreeing with Carvalho et al. (2006) who have used 30% of acid silage of tilapia filleting residue for fingerlings of the same species, and with Vidotti et al. (2002), which studied four different acid and fermented silages (whole fish unfit for human consumption, disposal of marine and freshwater fish, and tilapia filleting residue) in diets for pacu *Piaractus mesopotamicus*.

On the other hand, Pimenta et al. (2008), working with Nile tilapia, and Llanes - Llanes-Iglesias et al. (2009), with African catfish *Clarias gariepinus*, registered the negative effect on the FCR by the increased level of inclusion of acid silage of tilapia filleting residue and filleting residue of big head carp *Aristichthys nobilis*, respectively.

The values obtained for the PER were similar to those recorded by Carvalho et al. (2006), with Nile tilapia fed with increasing levels of acid silage of fish filleting, by Fagbenro and Fasakin (1996), analyzing the acid silage of poultry viscera for feeding fingerlings of African catfish and by Llanes-Iglesias et al. (2009), studying the African catfish fed with increasing sources of acid silage of big head carp viscera, which also did not present any significant difference for the PER.

Regarding the addition of organic acids (formic and citric) to prepare the acid silage of catfish surubim viscera, the pH of the experimental diets was significantly different ($p < 0.05$) between the treatments. The increased level of silage inclusion led to a lower pH (Table 2).

Organic acids have been used in animal production, especially in pig farming and aviculture, due to their antimicrobial activity and beneficial effects on the performance of the animals (METZLER; MOSENTHIN, 2009). For fish these same effects were also observed (RINGØ, 1991; ZHOU et al. 2009). Despite the incorporation of formic and citric acids into the silage and into the diets for juvenile tilapia, the results did not evidence ($p > 0.05$) any improvement in the performance parameters evaluated.

When included different levels of acid silage viscera of catfish surubim the results of growth performance reinforced the considerations of Borghesi et al. (2007), which highlighted the high biological value of fish waste silage. In this study, this characteristic was observed, since the experimental diets were isoproteic and isoenergetic, and thus any difference in the performance could be a consequence of the tested ingredient.

In the Table 3 are found the ADC of DM, CP, GE and EE and availability of P in experimental diets with different levels of supplementation of acid silage viscera of catfish surubim.

Table 3. Apparent coefficient of digestibility (ADC) of dry matter (DM), crude protein (CP), gross energy (GE), ether extract (EE) and availability of phosphorus (P) in diets with increased levels of acid silage viscera of surubim catfish to Nile tilapia juveniles.

ADC	Silage inclusion level (%)				CV[1] (%)
	0%	4%	8%	12%	
DM (%)	64.52	60.94	65.20	63.42	3.43
CP (%)	89.40[b]	88.66[b]	87.00[a]	88.57[b]	0.47
GE (%)	60.09[a]	60.42[a]	65.18[b]	58.70[a]	3.19
EE (%)	81.00	82.05	79.54	81.60	1.48
P (%)	33.39	32.61	32.15	35.78	6.70

[1]Coefficient of variation (%). Means fallowed by the same letter do not differ by Scott Knott test ($p < 0.05$).

A significant difference ($p < 0.05$) was registered for the ADC of CP and GE. For the CP, the treatment containing 8% of silage had the lowest ADC. On the other hand, this same level of inclusion provided the highest ADC for the GE.

Mach et al. (2010) investigated the digestibility of diets with acid silage of residue of lizard fish *Saurida undosquamis* for cobia juveniles *Rachycentron canadum* and observed values of ADC for the CP of 69% and for GE of 82.8%. Comparing with the present study, the ADC of CP of diets with silage of surubim catfish viscera, on average, was higher (88.4%). For the ADC of GE, the mean result was lower (61.1%) than the above mentioned study

There was a pH reduction of the experimental diets with increased level of silage inclusion, and consequent incorporation of formic and citric acids in the experimental diets. According to Mroz et al. (2000), by including 1.4% of formic acid, 1.8% of fumaric acid or 2.7% of n-butyric acid in diets for piglets, have verified an increase ($p < 0.05$) above 6% in the ileal digestibility of protein and several essential and non-essential amino acids

Metzler and Mosenthin (2009) argued that the supplementation with organic acids for weaned piglets leads to a decrease in stomach pH, with optimal values between 3 and 4, easing the activation of pepsinogen to pepsin, and as a result the protein digestibility can be improved, as well as the gastric retention. Moreover, the addition of organic acids in the diet influences the mucosa morphology, and these acids work as substrate in intermediate metabolism, causing a better digestion, absorption and retention of nutrient of the diet (PARTANEN; MROZ, 1999). In the present study, the ADC of CP

had no difference between no addition of silage and the treatments with 4 and 12%.

The availability of P, although not significantly different (Table 3) between the treatments, has indicated a trend for improvement in the use of P with increased addition of silage and reduction in pH of the diet. Viana et al. (1996) suggested that the formic acid has a significant effect on the pH and on the intestinal absorption by an indirect mechanism, and that further studies are required to determine the amount of acids (organic and inorganic) necessary to keep an optimal pH for the normal growth and health of fish, in addition to improve the efficiency of absorption and retention of minerals, especially the phosphorus.

In agreement with Vielma and Lall (2006) when evaluated the acidification of diets for rainbow trout with formic acid, have verified that its use improved the availability of phosphorus from 69.5 to 75%, when compared with the basal diet with that containing 10 mg kg^{-1} of formic acid. The values of availability of magnesium and Ca also significantly increased with diet acidification.

Fish viscera represented a great environmental problem in fish industry of Norway, with 50% of total disposed in landfills and in the sea (STRØM; EGGUM, 1981). Even considering the advance of appropriate technologies for processing viscera, this material is still considered a by-product of low utilization.

The bioconversion and preservation of fish viscera in the form of silage is a low cost alternative, which can be used as an alternative protein source in aquaculture diets. In Nordic countries, the preservation of a great volume of fish waste as silage is still a reality. In Brazil there is no large scale production of silage. In this way, Abimorad et al. (2009) stressed that the fish silage can be a low cost alternative for tilapia, and can be especially used in diets.

On the other hand, the production can be increased, considering the preservation of viscera as acid silage, which can be later processed and dried, as also ease the logistic for production of a greater volume and application in commercial diets. Besides that, the use of silage of surubim viscera in aquaculture diets contributes to reduce the environmental impact caused by fish processing plant, and can bring important advantages to reduce the total cost of feeding.

Conclusion

The acid silage of surubim viscera can be included in diets for juvenile Nile tilapia up to 12% with no negative interference on growth performance and digestibility, besides reducing the cost of the diets.

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