

Full Length Research Paper

## Crambe meal in diets supplemented with enzyme complex solid state fermentation (SSF) for Nile tilapia

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Nile tilapia performance fed with diets containing crambe meal supplied with enzyme complex SSF was evaluated. With initial average weight ranging between  $1.133\text{g} \pm 0.105$ , 280 Nile tilapias were randomized into seven treatments, with four replicates and 10 fish per tank, totaling 28 experimental units. The temperature-controlled recirculation system had 30 L per tank, with individual water supply and aeration. The physico-chemical parameters of the water (temperature, dissolved oxygen, ammonia and pH) were monitored periodically. The fish went through an adaptation period of one week prior to starting the trial. The treatments consisted of a control diet and others diets containing three levels of inclusion of crambe meal, being replaced in their proper proportion, the protein soya by the protein ingredient evaluated (5, 10 and 20%) with 500 ppm of enzyme complex SSF or not. The isoproteic diets ( $360\text{ g.kg}^{-1}$ ) contained the same amount of the ingredients, changing only the levels of inclusion of soya meal, crambe meal and inert. Fish were fed *ad libitum* four times per day. At 56 days, the tilapia performance was evaluated. The physic and chemistry parameters of water were within of the levels recommended for the species during all experimental time There was statistical difference for final weight, weight gain and feed conversion. It is concluded that the inclusion of up to 10% crambe meal supplemented with 500 ppm of enzyme complex SSF provides better performance and higher nitrogen retention for Nile tilapia.

**Key words:** Additives, *Crambe abyssinica*, fish nutrition, fish performance, *Oreochromis niloticus*.

### INTRODUCTION

In Brazil, the main raw material for production of oil is soy, responsible for over 70% of the biodiesel produced in the country. In this sense, there is a search for new oilseeds non-edible to biodiesel production within the international quality standards. Cultures little known in Brazil, such as crambe (*Crambe abyssinica*) and *Jatropha*

*curcas*), appear as interesting alternatives for biodiesel production (Roscoe et al., 2007).

Recently, the interest in commercial cultivation of crambe is growing in several countries, including United States, Canada, Germany and Netherland. The planting of crambe in the Brazil reached over 10000 ha in 2009,

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being this oilseed used for culture rotation in grain production systems (Ferreira and Silva, 2011). Liu et al. (1993) reported that the main attractive for crambe production are: (a) Domestic source of erucic acid that may be used in additives of rubber, plastic, coatings and lubricants; (b) promise alternative for biodiesel production; (c) Byproduct with higher nutritional value that may be used in animal nutrition; (d) alternative culture as a income source for farmers.

For animal feed, the economic viability of crambe depends of possibilities and limitations of this byproduct, because it has also in its composition some antinutritional factors. This product contains up to 10% of glucosinolate and hydrolases enzymes (TGSase), which limit the ingestion and affect directly the animal performance (Daxenbichler et al., 1968). With this, the crambe needs to pass by treatment for reduction of TGSase and glucosinolate levels. Some methods are used, as: Heat (Pereira et al., 1981), processing with chemistry additives, irradiation (Lessman and McCaslin, 1987) and water extraction after TGSase inactivation (Mustakas et al., 1976).

The presence of glucosinolates in diets for non-ruminants may cause tissue alterations, health problems and reduce intake (Kloss et al., 1994). Lesions in certain tissues (Van Etten et al., 1969) and high mortality (Tookey et al., 1980) were observed in rats that consumed diets containing high concentrations of glucosinolates. Broilers can be fed diets containing up to 50 g crambe meal.kg<sup>-1</sup> diet with no adverse effects on gain and health (Ledoux et al., 1999).

Reducing the antinutritional factors, the crambe may be an alternative ingredient with high nutritional value for formulation of fish extruded diets, since the high temperature involved in this process reduces the levels of glucosinolate and inactivate the enzymes that release toxic products. In rapeseed meal, Huang et al. (1995) observed that the glucosinolate levels decreased in the extrusion process. However, how is not possible to remove all antinutritional factors present in crambe, probably its participation will be limited in diet formulation for fish.

Therefore, the replacement of soya meal by crambe meal in extruded diets supplied with enzyme complex SSF on performance and body composition of Nile tilapia was evaluated.

## MATERIALS AND METHODS

The trial was conducted during the period of 56 days at the Laboratory of Aquatic Ecology and Fish Nutrition, Department of Animal Science, Federal University of Jequitinhonha and Mucuri (UFVJM). This lab is equipped with recirculating water system, which is endowed with biofilter, individual aeration and thermostat for temperature control. The temperature was controlled with a thermostat and measured, daily, at 8 am and 5 pm with thermometer of mercury bulb. The photoperiod used was of 12 h of light controlled by electronic timer and total ammonia measured

weekly. For dissolved oxygen and pH were used oximeter (YSI 55) and pH meter (Quimis Q400H), respectively, being measured directly every seven tanks, one repetition of each treatment. In these same tanks were collected samples of 20 ml of water each for total ammonia analysis according Koroleff (1976). The tanks were cleaned by siphoning each two days, being removed the faeces and others possible decanted materials. After all analyzes of water quality, averages and standard deviations for each treatment were obtained.

It was used 280 Nile tilapia (*Oreochromis niloticus*) fingerlings with average initial weight of  $1.133 \pm 0.105$  g distributed in a completely randomized design with seven treatments, four replicates and 10 fish by experimental unit.

The fingerlings were fed *ad libitum*, divided into four meal daily (8 am, 11 am, 2 pm and 5 pm), during every experimental time. For maximum intake without leftover, the ration were given in little amount until satiety. The extruded diets (Table 1) were processed in Animal Science Department of Federal University of Viçosa (UFV) using an extruder machine model Inbramaq MX40.

The enzyme complex SSF (Allzyme SSF, Alltech Inc.) was incorporated by jelly top coat. In the Lab, 42 g jelly was dissolved in 600 ml of boiling water. After the jelly to cool, but in liquid state, enzyme complex SSF previously weighed was added and mixed. In the bucket, eight kilogram of the diet was mixed with the enzymes jelly solution. The diet was spread on trays and dried overnight in a cool area with the help of fans.

The following performance parameters were evaluated: initial weight, final weight, weight gain (WG), feed conversion ratio (FCR), survival (SOB) and chemical body composition (dry matter, crude protein, ether extract, calcium and phosphorus).

All fish in experimental unit were weighed at the beginning and end of the trial to determination of weight gain. For body composition analysis, every tilapias of each treatment were desensitization with eugenol, slaughtered and frozen in freezer (-18°C) at end of the trial.

The diets and fish samples were sent at the Animal Nutrition Lab of Animal Science Department (LNA/DZO/UFVJM) for analysis, being used procedures described by Silva and Queiroz (2002).

For evaluation of treatments, the averages were compared by Duncan's Test at 0.05. Through the F Test at 0.05 were measured the enzyme effects between the treatments with the same level of replacement of protein from soya meal by protein of crambe meal (50, 100 and 200g.kg<sup>-1</sup>). The statistics analysis was done using the SAS (2002).

## RESULTS AND DISCUSSION

The recirculation system maintained the water quality into of accepted levels during all experimental time. It was observed average values of  $28.35 \pm 0.43$ °C to temperature;  $6.64 \pm 0.41$  to pH;  $5.59 \pm 0.39$  ppm to dissolved oxygen and  $0.05 \pm 0.006$  mg.L<sup>-1</sup> to total ammonia. Significant difference ( $p < 0.05$ ) was observed for final weight, weight gain and feed conversion (Table 2). In general, it was verified that the gradual addition of crambe meal worsened the performance of tilapia. However, by including the enzyme complex SSF, there was an improvement in performance parameters, mainly in feed conversion.

Significant difference ( $p < 0.05$ ) was observed only for phosphorus retention in relation to body composition analysis (Table 3). There was improvement in the body phosphorus retention by adding the enzyme complex in

**Table 1.** Composition of experimental diets.

Ingredients (g.kg <sup>-1</sup> )	Replacement levels with or not SSF (g.kg <sup>-1</sup> )						
	0	50	100	200	50SSF	100SSF	200SSF
Soya meal	612.1	562.1	512.1	412.1	562.1	512.1	412.1
Crambe meal	0	67.4	134.8	269.5	67.4	134.8	269.5
Corn	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Rice meal	124.6	124.6	124.6	124.6	124.6	124.6	124.6
Gluten 60	101.3	101.3	101.3	101.3	101.3	101.3	101.3
Fish meal 60%	15.1	15.1	15.1	15.1	15.1	15.1	15.1
Dicalcium phosphate	27.7	27.7	27.7	27.7	27.7	27.7	27.7
Limestone	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Mix mineral vitaminic <sup>1</sup>	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin C	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Salt	5.0	5.0	5.0	5.0	5.0	5.0	5.0
SSF <sup>2</sup>	0	0	0	0	0.5	0.5	0.5
Inert (Caulin)	70.0	52.6	34.8	0.5	52.1	34.3	0
BHT	0.2	0.2	0.2	0.02	0.2	0.2	0.2
<b>Calculated<sup>(3)</sup> and analysed<sup>(4)</sup> composition</b>							
Dry matter (g/kg) <sup>(3)</sup>	897.1	898.2	899.3	901.6	898.2	899.3	901.6
Dry matter (g/kg) <sup>(4)</sup>	881.2	890.9	874.4	884.7	881.4	875.9	888.3
Crude protein (g/kg) <sup>(3)</sup>	360.0	360.0	360.0	360.0	360.0	360.0	360.0
Crude protein (g/kg) <sup>(4)</sup>	359.0	354.3	353.1	359.7	354.3	351.6	352.9
Digestible energy (kcal/kg) <sup>(3)</sup>	3200	3239	3279	3356	3239	3279	3356
Crude fiber (g/kg) <sup>(3)</sup>	38.3	54.2	70.1	101.8	54.2	70.1	101.8
Etereo extract (g/kg) <sup>(3)</sup>	33.2	33.5	33.7	34.1	33.5	33.7	34.1
Total calcium (g/kg) <sup>(3)</sup>	10.0	10.7	11.4	12.7	10.7	11.4	12.7
Total calcium (g/kg) <sup>(4)</sup>	9.4	9.6	9.8	1.1	1.0	1.1	1.2
Total fósforo (g/kg) <sup>(3)</sup>	10.0	10.3	10.6	11.2	10.3	10.6	11.2
Total fósforo (g/kg) <sup>(4)</sup>	10.6	10.8	10.9	10.9	10.7	10.8	10.8
Total lysine (g/kg) <sup>(3)</sup>	19.0	17.6	16.2	13.4	17.6	16.2	13.4
Starch (g/kg) <sup>(3)</sup>	200.0	203.3	206.8	213.4	203.3	206.8	213.4
Linoleic acid (g/kg) <sup>(3)</sup>	16.5	16.2	15.8	15.1	16.2	15.8	15.1

<sup>(1)</sup> Composition per kg of product: 1,200,000 IU of Vitamin A; 200,000 IU of Vitamin D<sub>3</sub>; 1,200 mg of Vitamin E; 2,400 mg of Vitamin K<sub>3</sub>; 4,800 mg of Vitamin B<sub>1</sub>; 4,800 mg of Vitamin B<sub>2</sub>; 4,800 mg of Vitamin B<sub>6</sub>; 4,800 mg of Vitamin B<sub>12</sub>; 48 g of Vitamin C; 1200 mg of folic acid; 12,000 mg of pantothenic acid; 48 mg of biotin; 108 g of choline chloride; 24,000 mg of niacin; 50,000 mg of Fe; 3,000 mg of Cu; 20,000 mg of Mn; 30,000 mg of Zn; 100 mg of I; 10 mg of Co; 100 mg of Se. <sup>(2)</sup> Allzyme SSF, Alltech Inc. - Minimum levels of guaranteed enzyme activity: α-amylase, 30 AU/g; β-glucanase, 200 IU/g; cellulase, 40 IU/g; fungal protease, 700 IU/g; pectinase, 4000 IU/g; phytase, 300 IU/g; xylanase, 100 IU/g.

<sup>(3)</sup> Values based on the coefficient of digestibility of the ingredients according to Furuya et al. (1996), Furuya et al. (2000), Bomfim et al. (2008), Botaro et al. (2007); Gonçalves et al. (2009); Lanna et al.(2004); Furuya et al. (2004), Furuya et al. (2006), Takishita et al. (2009) and Bomfim et al. (2010); Rostagno et al. (2011).

**Table 2.** Nile tilapia performance fed with diets contained crambe meal and enzyme complex SSF.

Parameters	Replacement levels with or not SSF (g.kg <sup>-1</sup> )							CV (%)
	0	50	100	200	50+SSF	100+SSF	200+SSF	
Initial weight (g)	1.124	1.139	1.124	1.149	1.137	1.134	1.125	10.485
Final weight (g)	40.15 <sup>a</sup>	40.20 <sup>a</sup>	38.89 <sup>ab</sup>	34.74 <sup>b</sup>	42.31 <sup>a</sup>	42.17 <sup>a</sup>	38.01 <sup>ab</sup>	7.083
Weight gain (g) <sup>1</sup>	39.02 <sup>a</sup>	39.06 <sup>a</sup>	37.77 <sup>ab</sup>	33.59 <sup>b</sup>	41.18 <sup>a</sup>	41.04 <sup>a</sup>	36.89 <sup>ab</sup>	7.380
FCR (g.g <sup>-1</sup> ) <sup>1,2,3</sup>	1.112 <sup>b</sup>	1.115 <sup>bb</sup>	1.122 <sup>bb</sup>	1.365 <sup>db</sup>	1.020 <sup>aa</sup>	1.035 <sup>aa</sup>	1.180 <sup>ca</sup>	2.310
Survival (%)	92.5	92.5	85.0	92.5	95.0	92.5	95.0	8.793

<sup>1</sup> Average following per same lowercase are similar by Duncan's Test (p<0.05); <sup>2</sup> Average following per same letters are similar by F test (p<0.05); <sup>3</sup> Feed conversion.

**Table 3.** Body analysis of Nile tilapia performance fed with diets contained crambe meal and enzyme complex SSF.

Parameters (g.kg <sup>-1</sup> )	Replacement levels with or not SSF (g.kg <sup>-1</sup> )						CV (%)	
	0	50	100	200	50+SSF	100+SSF		200+SSF
Dry matter	188.3	193.2	188.9	190.4	193.4	192.9	190.1	9.685
Crude protein	712.9	718.4	711.2	701.9	720.6	728.9	711.7	7.684
Ether extract	203.1	224.5	213.0	206.3	222.9	226.6	219.3	9.326
Calcium	22.35	22.55	22.54	21.89	22.87	23.89	22.76	7.215
Phosphorus	22.18 <sup>a</sup>	22.60 <sup>aA</sup>	23.10 <sup>aA</sup>	22.17 <sup>aA</sup>	23.76 <sup>abB</sup>	25.89 <sup>bB</sup>	24.02 <sup>bB</sup>	8.179

<sup>1</sup>Average following per same lowercase are similar by Duncan's Test ( $p < 0.05$ ); <sup>2</sup> Average following per same letters are similar by F test ( $p < 0.05$ ).

the diet, become more evident when comparing within the same level of substitution.

For Nile tilapia, Kubitza (2000) recommend that the range of thermal comfort of the species should be of 26 to 30°C for temperature, 6 to 8.5 for pH, dissolved oxygen above of 4 mg.L<sup>-1</sup> and total ammonia under of 0.2 mg.L<sup>-1</sup>, which are according with the results of this study.

The final weight and weight gain were influenced by the inclusion of crambe meal and enzyme supplementation.

It is observed that the replacement of 20% from soybean meal protein to meal crambe protein had the worst weight gain. Although the replacement has been made regarding the crude protein, the levels of the other nutrients were also altered, modifying qualitatively the diet. The inclusion of crambe meal in the diet increased the amount of fiber, which may have promoted low retention time of feed in the digestive tract. Moreover, although the protein level being the same for all treatments, there was a gradual decrease in the levels of lysine, limiting amino acid directly related to protein deposition. Unlike the present study, Pretto et al. (2014) observed no difference in the performance of silver catfish (*Rhamdia quelen*) fed with diets containing up to 20% of replacement of crude protein from soya meal by crude protein crambe meal in nature or chemically treated. However, the authors corrected the synthetic amino acid levels in the formulation of diets, which may have "masked" the effect of crambe meal on the silver catfish performance.

Other authors studied alternatives of vegetables origin contained glucosinolate for feeding of fish. Working with Nile tilapia (*Oreochromis niloticus*), Santos et al. (2009) concluded that there was no loss in performance and chemical composition of the fillet to replace 25% of soy protein by protein turnip (*Brassica rapa*). With the same fish species, Soares et al. (2001) verified that canola meal can be included to 35.40% (replacing 48.17% protein from soybean meal) in the diet without loss in performance. In pacus (*Piaractus mesopotamicus*), the addition of up to 19% of canola meal in the diet did not affect performance. Already in piavuçu (*Leporinus macrocephalus*), the maximum level of substitution was

11.19% for the same ingredient, without affecting performance (Gonçalves et al., 2002).

The plants belonging to the family of cruciferous vegetables such as radish, crambe, canola and rapeseed have in their composition the glucosinolates, which when intact, are not toxic to fish. However, the products of its hydrolysis by the action of the enzyme myrosinase or thioglucosidase may be detrimental to performance and health of this group of animals (Bell, 1993). More than 90% of the glucosinolates can be converted into epigoitrin (epi -PG) during the metabolism. In the seed, epi - PG is biologically separate of the enzyme thioglucosidase (TGSase). A reaction between the enzyme and epigoitrin can occur if the seed is crushed, if germinated, or when plant tissues are softened (Tookey et al., 1980). However, Oginsky et al. (1965) and Tani et al. (1974) reported that some intestinal bacteria (e.g., *Enterobacter cloacae*) are capable of displaying TGSase activity. The intake epi-PG and its subsequent hydrolysis can lead to the formation of a toxic product called aglucon in digestive tract of animals. Thus, the nutritional value of crambe depends on the relative toxicity of epi - PG intact and product levels aglucon present. These products are toxic and have a bitter taste which makes it unpalatable meal.

The feed conversion also was influenced by treatments. It was observed that, in general way, there is a tendency of worsening in feed conversion with the inclusion of crambe meal in diet. However, the addition of enzyme complex SSF in diet improved the feed conversion up to 10% of replacement of soya meal protein by crambe meal protein. The fish fed with diets contained 10% of replacement and enzyme complex improved the feed conversion in 7.43 and 8.41%, when compared at control and the same level of replacement, respectively.

Enzyme action arising from the complex SSF probably provided greater amount of nutrients and reduced the effects of anti-nutritional factors. In a study testing inclusion levels of the same enzyme complex in diets for Nile tilapia, Moura et al. (2012) observed that there was increases in levels of sucrose, glucose and fructose in

the chyme of this species, indicating that occurred an greater bioavailability of nutrients, positively influencing the performance. This is clearly evident when comparing the same inclusion levels of crambe meal with enzyme supplementation. Thus, it was observed an improvement in feed conversion of 9.31, 8.41 and 15.68% for the replacement levels of 50, 100 and 200, respectively.

For body analysis, the inclusion of enzyme complex SSF improved only the phosphorus retention. The phytase from enzyme complex acted on the phytate present in vegetable ingredients of the diet, releasing phosphorus that was unavailable. The diet with 10% of replacement plus SSF increased the body phosphorus level in 12% when compared the treatment with 10% of replacement. Similar results were verified by Bock et al. (2007) that found higher amount of phosphorus and calcium in the body composition when tilapias were fed with diets contained phytase. These same authors also mentioned that the use of phytase in diets for Nile tilapia in growth phase can reduce levels of inclusion of inorganic phosphorus in feed and minimize environmental impacts. Using phytase in diets, Furuya et al. (2005) observed that tilapias improved the deposition of phosphorus on bone and weight gain in 13.15 and 39.9%, respectively.

Therefore, it is concluded that the inclusion of enzyme complex SSF (solid state fermentation) improves the feed conversion and phosphorus retention in Nile tilapias fed with diets containing up to 10% of replacement of crude protein from soya meal to crude protein of crambe meal.

## Conflict of Interest

The authors have not declared any conflict of interest.

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