



Effects of enzyme complex SSF (solid state fermentation) in pellet diets for Nile tilapia

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ABSTRACT - The effects of enzyme complex SSF (solid state fermentation) on growth performance and the availability of sucrose and monosaccharides in the chyme of Nile were involved. The study included 360 fish (70g±4.43) in a completely randomized design with six dietary treatments (0, 50, 100, 150, 200 and 250 ppm of SSF) arranged in six replicates, with 10 fish per replicate. Every 15 days, one tilapia of each experimental unit was sacrificed for analyses of carbohydrate in the chyme. On day 60 of the experiment, the performance parameters were measured. There was a linear effect according to treatment for final weight and weight gain. For the other performance parameters, there were no differences. There was quadratic effect for sucrose and glucose in function of the treatment, whereas the fructose levels increased linearly. The addition of 150 ppm of the enzyme complex SSF in the feed improves the performance of Nile tilapia and increases the availability of sucrose and monosaccharides in the chyme.

Key Words: aquaculture, enzymes, fish nutrition, HPLC, *Oreochromis niloticus*

Introduction

The inclusion of enzymes in fish diets can be an alternative to improve the utilization of nutrients coming from plant ingredients. In addition to reducing production costs by increasing digestibility, it reduces the load of pollutants from waste, enabling better sustainability. Several scientific papers stress the benefits of using enzymes in diets for aquatic animals (Furuya et al., 2005; Nunes et al., 2006; Tesser et al., 2006; Filer & Shafer, 2011). However, in addition to performance, information about the exact mechanism of the enzymatic effect are important.

Trials with supply *ad libitum* can mask the enzymatic effect due to excess available nutrients from the diet. So, it is difficult to separate the weight gain arising from the nutrient already available in the diet from those that became available by enzyme action.

Among several options of cocktails and enzyme complexes, an alternative mechanism, SSF (solid state fermentation), enables enzyme production by a culture of microorganisms in a solid matrix (Del Bianchi et al., 2001). The greatest benefit of this additive is its combination of natural enzymes (protease, α -amylase, cellulase, xylanase, α -galactosidase, pectinase, phytase, endoglucanase, sucrase,

and others), which can improve the digestibility of plant feedstuffs in the diet.

Considering this, the effects of enzyme complex SSF on the growth performance and on the availability of sucrose, glucose and fructose generated by the carbohydrases of SSF found in the chyme of Nile tilapias were evaluated.

Material and Methods

Performance was assessed at the Laboratório de Nutrição de Peixes (Labnut) of the Departamento de Zootecnia (DZO) of Universidade Federal de Viçosa (UFV) from August to October 2009.

In order to meet the objective of this study, 360 Nile tilapias (Thai line) with average weight of 70±4.43 g were randomized into six treatments, with six replicates and 10 fish per tank, which was considered the experimental unit. The recirculation system contained thermostat so as to maintain the temperature at 28 °C. The 130 L tanks had individual water supply and aeration. Water physical-chemical parameters (temperature, dissolved oxygen, ammonia and pH) were monitored weekly. Before the start of the trials, the fish went through a period of adaptation of one week.

In addition to the 36 experimental units, another tank was installed at the laboratory under the same conditions in order to control growth. The purpose of this tank was to simulate the fish growth and adjust the feeding rate of the treatments assessed, in order to prevent problems of sampling and stress in the control treatment. The fish in this tank were fed a control diet at a sub-optimal rate of 15 g/kg of the total biomass supplied in four daily meals (08:00, 11:00, 14:00 and 17:00). At every 15 days, all fish were removed and individually weighed, in order to calculate the average weight. The feeding rate was adjusted again according to the growth control tank, and the 15-day weight gain was multiplied by 90% to maintain the sub-optimal feeding level. The amount fed to the fish was obtained with the adjustment of the current weight multiplied by the feeding rate.

At the beginning of the trial, fish were fed at a sub-optimal feeding rate of 15 g/kg of general average biomass, divided into four meals. The treatments consisted of a

control diet and other diets containing five inclusion levels of enzyme complex SSF (50, 100, 150, 200 and 250 ppm). The experimental diets had the same amount of nitrogen (320 g CP/kg diet) and calories (3000 kcal DE/kg diet). The ingredients were ground, weighed and mixed in plastic bag.

Thereafter, the enzyme complex SSF was incorporated into the mixtures according to the respective treatment. These mixtures were wetted with water at 55 °C and pelletized in a steel CAF-22STB processor with 2 mm matrix. Subsequently, the pellets formed were dried in a forced-ventilation oven for 14 hours at 55 °C.

All diets were sent to Laboratório de Análise de Alimentos of the Departamento de Zootecnia (UFV) for evaluation of the nutritional composition (Table 1).

At every 15 days in the experiment, a tilapia from each experimental unit was removed 50 minutes after the third meal of the day (2 pm) and immediately taken to an ice bath (60% of ice and 40% of water) for stunning and slaughtering, totaling six samples per treatment.

Table 1 - Composition of the experimental diets (natural matter)

Ingredient (g/kg)	Levels of enzyme complex SSF (ppm)					
	0	50	100	150	200	250
Soybean meal, 45%	458.0	458.0	458.0	458.0	458.0	458.0
Corn grain	350.9	350.9	350.9	350.9	350.9	350.9
Gluten meal, 60%	100.0	100.0	100.0	100.0	100.0	100.0
Wheat meal	20.0	20.0	20.0	20.0	20.0	20.0
Dicalcium phosphate	27.4	27.4	27.4	27.4	27.4	27.4
Calcitic lime	2.35	2.35	2.35	2.35	2.35	2.35
Soybean oil	29.4	29.4	29.4	29.4	29.4	29.4
Vitamin supplement ¹	4.0	4.0	4.0	4.0	4.0	4.0
Mineral supplement ²	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin C	0.5	0.5	0.5	0.5	0.5	0.5
Salt	6.0	6.0	6.0	6.0	6.0	6.0
Enzyme complex SSF ³	0	0.05	0.10	0.15	0.20	0.25
Inert (caulin)	0.25	0.20	0.15	0.10	0.05	0
Antioxidant (BHT)	0.2	0.2	0.2	0.2	0.2	0.2
Analyzed ⁴ and calculated ⁵ composition						
Dry matter (g/kg) ⁴	943.9	942.6	941.0	941.2	941.8	941.1
Crude protein (g/kg) ⁴	320.5	320.8	320.1	320.0	320.0	300.5
Gross energy (kcal/kg) ⁴	4539	4563	4540	4545	4540	4554
Digestible energy (kcal/kg) ^{5,6}	3000	3000	3000	3000	3000	3000
Crude fiber (g/kg) ^{5,7}	33.7	33.7	33.7	33.7	33.7	33.7
Ether extract (g/kg) ⁴	53.6	52.6	52.6	53.9	53.3	54.6
Total calcium (g/kg) ⁴	9.4	9.1	9.2	9.2	9.3	9.1
Total phosphorus (g/kg) ⁴	9.3	9.1	9.0	9.2	9.2	9.1
Ash (g/kg) ⁴	62.6	63.4	62.3	63.0	62.5	63.2
Total lysine (g/kg) ^{5,8}	14.7	14.7	14.7	14.7	14.7	14.7
Linoleic acid (g/kg) ^{5,9}	27.4	27.4	27.4	27.4	27.4	27.4

SSF - solid state fermentation; BHT - butylated hydroxytoluene.

¹ Composition per kg of product: vit. A - 1,200,000 IU; vit. D3 - 200,000 IU; vit. E - 1,200 mg; vit. K3 - 2,400 mg; vit. B1 - 4,800 mg; vit. B2 - 4,800 mg; vit. B6 - 4,800 mg; vit. B12 - 4,800 mg; vit. C - 48 g; folic acid - 1,200 mg; calcium pantothenate - 12,000 mg; biotin - 48 mg; choline chloride - 108 g; niacin - 24,000 mg.

² Composition per kg of product: iron - 50,000 mg; copper - 3,000 mg; manganese - 20,000 mg; zinc - 30,000 mg; iodine - 100 mg; cobalt - 10 mg; selenium - 100 mg.

³ Allzyme® SSF, Alltech Inc.

⁴ Analysed in Laboratory of Food Analysis of DZO (UFV).

⁵ Values calculated based on the coefficients of digestibility of the ingredients according to:

⁶ Furuya et al. (1996) and Furuya et al. (2000).

⁷ Lanna et al. (2004).

⁸ Furuya et al. (2004) and Furuya et al. (2006).

⁹ Rostagno et al. (2011).

Fish were subjected to longitudinal ventral incision, followed by subsequent isolation of the cranial portion of the esophagus and the caudal portion of the rectum, through double ligatures to prevent chyme leakage. After isolation, the medium and posterior intestine were removed and stored in polyethylene flasks. This material was freeze-dried, ground, and frozen at $-80\text{ }^{\circ}\text{C}$, to be subsequently analyzed for glucose, fructose and sucrose.

At 60 days of trial, the following parameters were assessed: feed intake (g), initial weight (g), final weight (g), weight gain (g), feed conversion rate (g/g) and survival (%).

The freeze-dried samples were taken to Laboratório de Análises Bioquímicas of Bioagro for assessment of sucrose, glucose and fructose levels; the experiment started in November 2008 and ended in February 2009. The Soxhlet Method was used to extract fat from each sample. After fat extraction, individual soluble sugars were quantified by HPLC (Shimadzu series 10A chromatography; column module CTO-10A; RI Detector RID-6A; Windows® software LC-10 version 2.2). A supelcosil™ LC-NH₂ was used to separate sugars according to molecule size at a flow rate of 0.7 mL/minute; the mobile phase was formed by a mixture of acetonitrile-water (80:20 v/v) at 35 °C (Guimarães et al., 2001).

Statistical analyses of the variables for performance and availability of carbohydrates were carried out by analysis of variance and linear regression with SAEG (Sistema para Análises Estatísticas e Genéticas, version 9.1). Linear or quadratic models of each parameter in function of the enzyme complex SSF were tested and the choice of the best equation was given by the greatest coefficient of determination (r^2), for the significance of the coefficients of regression by the t test at 5% probability and for its biological adequacy.

Results and Discussion

The recirculation system maintained water quality within acceptable levels during the experimental period.

Values of $27.96\pm 0.38\text{ }^{\circ}\text{C}$ for water temperature, 6.40 ± 0.34 for pH, 5.59 ± 0.31 ppm for dissolved oxygen and $6.00\pm 0.001 \times 10^{-3}\text{ mg.L}^{-1}$ for toxic ammonia were measured. These values are within the range recommended by Kubitzka (2000) for Nile tilapia, with the temperature between 26 and 30 °C, pH between 6 and 8.5, dissolved oxygen above 4 mg.L⁻¹ and ammonia concentration lower than 0.2 mg.L⁻¹.

The feed intake by fish was the same ($P>0.05$) between treatments, with no leftovers observed. According to the methodology, the use of a feeding rate at sub-optimal level with periodic adjustments based solely on the growth control tank was efficient to observe only the effects from the enzymatic action. As the same amount of diet was used, regardless of treatment, it was observed that the tilapias obtained positive response from enzymatic treatment (Table 2), even being restricted to growth in its entirety. Thus, the improvement in performance was due to the higher nutrient availability promoted by the enzymatic action of complex SSF. If the amounts of diet were adjusted periodically, considering the fish weight gain of each treatment or if the feed intake were *ad libitum*, it would not be possible to determine if the weight gain was in function of the amount of nutrients from feeding or of the amount of nutrients released by enzyme action.

The levels of enzyme complex SSF influenced the fish weight gain, which increased ($P<0.05$) linearly ($Y=66.5409 + 1.58023X$). Although the weight gain varied linearly, it was found that from the 150 ppm level of the enzyme complex SSF, there was no increase in the absolute value of this parameter, indicating that this level corresponds to the maximum efficiency of these enzymes. The improvement in the growth rate of the tilapias would be related to possible increases in the bioavailability of nutrients promoted by the enzymatic action of the enzyme complex SSF. Positive effect of inclusion of amylase and lipase and swine pancreatic enzyme on the weight gain of tambaqui (*Colossoma macropomum*) and pacu (*Piaractus mesopotamicus*) were observed, respectively, by Nunes et al. (2006) and Tesser et al. (2006).

Table 2 - Performance of Nile tilapia subjected to diets containing the enzyme complex SSF

Parameters	Levels of enzyme complex SSF (ppm)						CV (%)	P value		
	0	50	100	150	200	250		L	Q	LF
Feed intake (g)	83.76	83.76	83.76	83.76	83.76	83.76	-	-	-	-
Initial weight (g)	70.42	70.85	69.87	69.52	71.62	71.50	6.668	-	-	-
Final weight (g) ¹	138.72	139.75	140.08	145.81	144.70	147.15	4.790	0.012	0.957	0.775
Weight gain (g) ²	68.30	68.90	70.21	76.30	73.06	75.65	10.066	0.033	0.762	0.643
Feed conversion (g/g)	1.23	1.22	1.20	1.11	1.16	1.14	10.053	-	-	-
Survival (%)	98.3	100	100	96.7	96.7	100	4.342	-	-	-

SSF - solid state fermentation; L and Q - linear and quadratic order effects of the inclusion of enzyme complex SSF in the diet; LF - lack of fit.

¹ Linear effect ($P<0.05$): $\hat{Y} = 136.43 + 1.79213X$; $r^2 = 0.87$.

² Linear effect ($P<0.05$): $\hat{Y} = 66.5409 + 1.58023X$; $r^2 = 0.74$.

No effect ($P>0.05$) of levels of the enzyme complex SSF was observed on feed conversion. In contrast to what was observed in this study, Soares et al. (2008) verified that the inclusion of exogenous protease improved feed conversion, weight gain and specific growth rate for tucunare paca juveniles (*Cichla temensis*).

In pirarucu (*Arapaima gigas*), Cavero (2004) observed that the addition of protease and lipase in the diet improved the performance, unlike amylase, which showed no significant difference compared with the control treatment.

There was no variation ($P>0.05$) in the survival of tilapias between the different levels of inclusion of enzyme complex SSF.

Quadratic effect ($P<0.05$) was observed for sucrose in function of the treatments. This indicates that the α -galactosidase acted on the oligosaccharides (raffinose, stachyose and verbascose) present in the diet, since there was considerable increase in the chyme of its product, sucrose (Table 3). This fact is beneficial because the oligosaccharides are anti-nutritional factors that may compromise animal performance. According to Coon et al. (1990), the oligosaccharides of the α -galactoside family reduces the energy of the diet, fiber digestion, and the time of gastrointestinal transit. Furthermore, the fermentation of oligosaccharides in the intestine leads to an increase in gas production, causing discomfort to the animal.

In a study with rainbow trout (*Oncorhynchus mykiss*), Farhangi & Carter (2007) reported that fish fed diets based on shelled lupines (*Lupinus angustifolius*) supplemented with α -galactosidase significantly improved weight gain, protein efficiency and apparent digestibility of dry matter and crude protein.

No quadratic effect ($P<0.05$) was observed for glucose according to the levels of enzyme complex SSF in the diet. The inclusion of 150 ppm of this additive increased the available glucose level in the chyme in 9.57%, when

compared with the control treatment, which can be attributed to the combined action of carbohydrases.

However, part of the sucrose found in the feed and of the product resulting from the α -galactosidase of SSF in oligosaccharides underwent hydrolysis by the sucrase activity. As the fructose levels increased linearly ($P<0.05$) and concomitantly with glucose levels, this finding is a strong evidence of the action of sucrase.

Simultaneously to the levels of glucose, fructose increased linearly ($P<0.05$), which is a strong indication of the action of sucrase. Thus, the sucrose levels may be higher than those measured, because part of this carbohydrate present in the ingredients and of the product of the action of α -galactosidase on the oligosaccharides may have been hydrolyzed by this enzyme.

Furthermore, it must also be emphasized that the blood glucose levels may still be underestimated, because usually, when this substrate increases in the intestinal lumen, there is increase in the rate of absorption. This was reported by Stokes & Fromm (1964) in a study with rainbow trout. They found increase in the absorption and transport of glucose at higher temperatures and concentrations.

Another question to be considered is that with the more efficient use of glucose and fructose as energy sources, the protein could be spared for the structural function, in others words, tissue development. This can be confirmed by these experiments, since the fish with higher levels of these monosaccharides in the chyme were also those with better performance. This better energy with the inclusion of enzyme complex SSF in the diet, besides improving the animal performance, can also be an alternative to decrease the excretion by greater feed efficiency or still reduce the oil in the diet formulation. One can also conclude that the energy levels estimated in diet formulation can be considered apparent, because with the gradual addition of enzymes, the levels of digestible energy probably increased.

Table 3 - Levels of sucrose, glucose and fructose per gram of dry chyme of Nile tilapia fed diet containing enzyme complex SSF

Carbohydrate (mg.g ⁻¹)	Levels of enzyme complex SSF (ppm)				CV (%)	P value		
	0	100	150	200		L	Q	LF
Sucrose ¹	3.0465	3.7886	3.7891	3.3492	8.217	0.042	<0.001	0.487
Glucose ²	1.5576	1.6207	1.7066	1.6451	3.873	<0.001	0.006	0.084
Fructose ³	1.0423	1.0758	1.0927	1.0895	7.120	0.017	0.477	0.975

SSF - solid state fermentation; L and Q - linear and quadratic order effects of the inclusion of enzyme complex SSF in the diet; LF - lack of fit.

¹ Quadratic effect ($P<0.05$): $\hat{Y} = 3.041 + 0.01396X - 0.0000617X^2$; $r^2 = 0.99$.

² Quadratic effect ($P<0.05$): $\hat{Y} = 1.553 + 0.001425X - 0.000004439X^2$; $r^2 = 0.87$.

³ Linear effect ($P<0.05$): $\hat{Y} = 1.0463 + 0.000256X$; $r^2 = 0.90$.

Conclusions

The inclusion of 150 ppm of enzyme complex SSF in pellet diets improves the performance of Nile tilapia in function of the higher bioavailability of nutrients.

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