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Crude glycerin as dietary energy source for Nile tilapia

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ABSTRACT

Biofuels are a renewable and clean alternative to keep up with the increasing demand for energy. Biodiesel stands out among biofuels, but its production yields crude glycerin [CGL] as a result of the transesterification process. The increased availability and low cost of CGL encourage the assessment of digestibility of glycerin as dietary energy source, and growth, hematological parameters and carcass composition of Nile tilapia fed diets containing crude glycerin. The apparent digestibility coefficient of energy from dietary glycerin (indirect method, chromium III oxide as inert marker) was 79.41%, corresponding to 12.04 MJ kg^{-1}. Juvenile tilapia (7.73 ± 0.09 g) were stocked into 300-L, indoor plastic tanks (12 fish per tank), closed-loop recirculating system under continuous in a totally randomized experimental design ($n = 4$), and fed to apparent satiation for 90 days with isonitrogenous and isocaloric diets containing 0, 4, 8, 12 and 16% of CGL. At the end of feeding trial, fish were measured and weighted; blood samples of were drawn from the caudal vein of two fish per tank and two fish from each replicate were euthanized for analysis of carcass proximate composition. Data were subjected to one-way ANOVA ($\alpha = 0.05$) and optimum dietary crude glycerin level was determined by polynomial regression analysis. Weight gain, feed intake, feed conversion ratio, protein retention efficiency and specific growth rate were significantly affected by dietary CGL contents. Weight gain fitted a second-degree equation and the optimum inclusion level was estimated at 5.9%, but up to 12% dietary crude glycerin did not impair growth performance of fish. Increasing dietary CGL levels did not affect hepatosomatic, liposomatic and viscerosomatic indices, as well as survival rate, proximate composition of tilapia carcass and hematological parameters. Crude glycerin seems to be an interesting and safe source of energy for Nile tilapia.

1. Introduction

Because oil is the primary energy source consumed worldly, it has become a key factor for sustainable development. Several industrial and environmental concerns associated with the increasing oil exploitation have driven a quest for more sustainable energy substitutes. Biofuels, a renewable and clean alternative to the ever increasing energy use (Ayoub and Abdullah, 2012), can mitigate detrimental effects of exhaust emissions of industry and transportation alike. Biodiesel stands out among biofuels, but its production yields glycerin as a result of the transesterification process. In theory, the ratio for transesterification reaction requires 3 moles of alcohol for each mol of triglyceride to produce 3 moles of fatty acid ester and 1 mol of glycerol (Leung et al., 2010) corresponding to approximately 9% of total process yield (Van Gerpen, 2005; Fukuda et al., 2001). Therefore, growing biodiesel production will inevitably lead to large surpluses of glycerin worldwide.

Purification of crude glycerin [CGL] is a rather costly process. In addition, pure glycerin has already developed a saturated market, a compelling fact that turns crude glycerin into a potential, low cost alternative energy source for animal feeds (Ayoub and Abdullah, 2012; Leoneti et al., 2012). Crude glycerin has been successfully used in feeds for swine and poultry (Shields et al., 2011; Kroupa et al., 2011; Berenchtein et al., 2010; Mendoza et al., 2010; Min et al., 2010; Duttlinger et al., 2009; Lammers et al., 2008; Cerrate et al., 2006; Mourot et al., 1994). It has been demonstrated that levels up to 10% of dietary crude glycerin do not affect performance of channel catfish *Ictalurus punctatus* (Li et al., 2010) and Nile tilapia (Neu et al., 2013). Nevertheless, little is known about glycerin inclusion in Nile tilapia diets at levels above 10%. Lammers et al. (2007) highlights a potential decline in the ability of pigs to metabolize more than 10% of crude glycerin. Owing to the fact that diets containing more than 15% of crude glycerin have impaired growth in catfish (Li et al., 2010), further studies involving high levels of glycerin inclusion in Nile tilapia diets are needed.

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Crude glycerin may contain relatively low concentrations of methanol resulting from the chemical processing of biodiesel. Ingested methanol is converted into formic acid, which, in excess, seriously harms animals causing, for instance, blindness, nervous system depression, motor alterations and metabolic acidosis in poultry and mammal production (Lammers et al., 2008). However, such a problem can be overcome without effort in aquaculture since methanol is stripped from fish diets under high temperature processing, i.e., extrusion cooking. Moreover, inclusion of small amounts of glycerin in diet formulations was proven to improve pellet durability and machinery efficiency, reducing circa 50% of energy costs in feed processing (Groesbeck et al., 2008; Shields, 2009).

Nile tilapia is a rather important aquaculture commodity, the quest for surrogate dietary energy sources for fish farming purposes a true need, and the increased availability and low cost of CGL a reality. This work thus assessed the digestibility and effects of graded levels of dietary CGL on growth, hematological parameters and carcass composition of Nile tilapia.

2. Material and methods

2.1. Digestibility assay

Determination of apparent digestibility coefficient (ADC) of crude energy was performed by indirect method using chromic oxide III (Cr_2O_3) as inert marker (2 g kg^{-1}). A practical feed was formulated to meet the nutritional requirements of tilapia and used as the reference diet (RD; Table 1). Soybean meal and poultry meal were used as dietary protein sources. The test diet was obtained by replacing 15% of RD by crude glycerin (moisture: 207.2 g kg^{-1}; ether extract: 0.90 g kg^{-1}; glycerol: 836.3 g kg^{-1}; total chlorine: 49.1 g kg^{-1}; ash: 69.7 g kg^{-1}; sodium: 18.3 g kg^{-1}; crude energy: 15.16 MJ kg^{-1}). Feed ingredients were ground and sieved (0.9–mm), mixed with crude glycerin, moistened (10% water, *v:v*) and pelleted (4.0 mm granules). The diet was oven dried (forced air; 40 °C; 24 h) and stored at −20 °C until its use.

Juvenile Nile tilapia (66.53 ± 10.49 g) were stocked into six, 80-L cages (20 fish per cage), housed in two, 1000-L circular tanks and fed to apparent satiation four times a day (0800, 1100, 1400, 1600). One hour after last meal, cages were transferred to six 200-L, cylindrical, conical-bottomed tanks coupled to iced collection vials; feces were then collected by decantation, centrifuged (4 °C; 4250 *g*) and oven-dried (40 °C; 24 h). ADC of diets and crude glycerin were calculated according to Bureau and Hua (2006).

2.2. Feeding trial

2.2.1. Diets and animals

Juvenile Nile tilapia (7.73 ± 0.09 g) were stocked into 300-L, indoor plastic tanks (12 fish per tank) in a totally randomized experimental design ($n = 4$), set in a closed-loop, continuously aerated water cycling system and fed experimental diets formulated to be isonitrogenous and isocaloric containing graded levels of crude glycerin [CGL]: 0, 4, 8, 12 and 16%. Sodium chloride was added to the diets to balance CGL chloride and sodium contents as recommended by Rust (2002). Diets were formulated considering digestible energy contents of CGL determined in digestibility trial and findings of Pezzato et al. (2002) for the remaining ingredients, and processed and stored as already described (Table 2).

Fish were fed the experimental diets ad libitum in two daily meals (08h00m and 16h00m) for 90 days. At the end of the feeding period, fish were fasted for 24 h, anesthetized (eugenol, 0.5 mL L^{-1}) and weighed. Growth performance was evaluated by estimating the following parameters:

$$\text{Feed intake}(\text{FI, g}) = \frac{\text{total feed consumed (g)}}{\text{number of fish per replicate}}$$

$$\text{Weight gain}(\text{WG, g}) = \frac{W_F - W_I}{\text{number of fish per replicate}}$$

$$\text{Feed conversion ratio}(\text{FCR}) = \frac{\text{feed intake (g)}}{\text{weight gain (g)}}$$

$$\text{Protein retention efficiency}(\text{PRE}, \%) = \frac{\text{protein gain(g)}}{\text{protein intake(g)}}\text{x100}$$

$$\text{Specific growth rate (SGR, \%)} = 100 \text{ x} \left(\frac{\ln W_F - \ln W_I}{\text{days of feeding period}}\right)$$

$$\text{Hepatosomatic index}(\text{HSI}, \%) = 100\text{x}\frac{W_l}{W_b}$$

$$\text{Liposomatic index}(\text{LSI}, \%) = 100\text{x}\frac{W_{mf}}{W_b}$$

Table 1
Formulation and chemical composition of reference diet.

Ingredients	Contents g kg^{-1}
Soybean meal	538.0
Poultry meal	74.0
Corn	145.0
Corn starch	153.4
Cellulose	22.8
Soybean oil	20.0
Dicalcium phosphate	23.0
DL-methionine	3.6
Mineral and vitamin mix[a]	20.0
BHT[b]	0.2
Proximate composition (dry matter basis)	
Protein (g kg^{-1})	300.1
Lipid (g kg^{-1})	44.3
Ash (g kg^{-1})	67.4
Carbohydrates[c] (g kg^{-1})	588.2
Energy (MJ kg^{-1})	18.09

[a] Mineral and vitamin mix (Premix Nutrifish Guabi®, Campinas, SP, Brazil) per kg of product: Fe 15 000 mg; Cu 2500 mg; Zn 12 500 mg; I 375 mg; Mn 12 500 mg; Se 87.5 mg; Co 125 mg; vitamin A 2 500 000 IU; vitamin D3 600 000 IU; vitamin E 37 500 IU; vitamin K 3750 mg; vitamin C 50 000; vitamin B1 4000 mg; vitamin B2 4000 mg; vitamin B6 4000 mg; vitamin B12 4000 μg; pantothenic acid 12 000 mg; biotin 15 mg; folic acid 1250 mg; niacin 22 500 mg; BHT 15 000 mg.
[b] Butyl-hydroxy-toluene.
[c] Carbohydrates = 100 − (protein + lipid + ash).

Table 2
Formulation and chemical composition of diets.

Ingredients (g kg^{-1})	Crude glycerin (%)				
	0	4	8	12	16
Soybean meal	550.0	555.0	556.0	561.0	570.0
Corn gluten meal	60.0	60.0	60.0	56.0	56.0
Corn	155.0	130.0	120.0	120.0	70.0
Corn starch	115.0	85.0	40.0	0.0	0.0
Cellulose	32.8	44.6	60.5	61.3	64.2
Crude glycerin	0.0	40.0	80.0	120.0	160.0
Soybean oil	20.0	20.0	20.0	20.0	20.0
Salt	7.4	5.6	3.7	1.9	0.0
Dicalcium phosphate	36.0	36.0	36.0	36.0	36.0
DL-methionine	3.6	3.6	3.6	3.6	3.6
Mineral and vitamin mix[a]	20.0	20.0	20.0	20.0	20.0
BHT[b]	0.2	0.2	0.2	0.2	0.2
Proximate composition (dry matter basis)					
Protein (g kg^{-1})	332.1	330.0	324.5	323.2	331.9
Lipid (g kg^{-1})	51.2	52.0	47.8	48.7	48.4
Ash (g kg^{-1})	112.5	128.3	142.5	140.3	147.0
Carbohydrates[c] (g kg^{-1})	504.2	489.7	485.2	487.8	472.7
Gross energy (MJ kg^{-1})	16.6	16.5	16.2	16.1	16.2
Digestible energy (MJ kg^{-1})	13.0	13.0	12.99	13.1	13.2

[a] Mineral and vitamin mix (Premix Nutrifish Guabi®, Campinas, SP, Brazil) per kg of product: Fe 15 000 mg; Cu 2500 mg; Zn 12 500 mg; I 375 mg; Mn 12 500 mg; Se 87.5 mg; Co 125 mg; vitamin A 2 500 000 IU; vitamin D3 600 000 IU; vitamin E 37 500 IU; vitamin K 3750 mg; vitamin C 50 000; vitamin B1 4000 mg; vitamin B2 4000 mg; vitamin B6 4000 mg; vitamin B12 4000 μg; pantothenic acid 12 000 mg; biotin 15 mg; folic acid 1250 mg; niacin 22 500 mg; BHT 15 000 mg.
[b] Butyl-hydroxy-toluene.
[c] Carbohydrates = 1000 − (protein + lipid + ash).