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Evaluation of Glycerol from Biodiesel Production as a Feed Ingredient for Channel Catfish, *Ictalurus punctatus*

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Abstract

Glycerol is the main by-product of biodiesel production from vegetable oils and animal fats. It has been evaluated as an energy source for several farm animals. A study was conducted to examine the effects of various levels of glycerol in channel catfish, *Ictalurus punctatus*, diets. Fish with mean initial weight of 6.8 ± 0.1 g were stocked in 110-L flow-through aquaria and fed practical diets containing 0, 5, 10, 15, and 20% glycerol for 9 wk. There were no significant differences in feed consumption, weight gain, feed efficiency ratio, and liver lipid level among fish fed diets containing 0, 5, and 10% glycerol. However, fish fed diets containing 15 and 20% glycerol had reduced weight gain, feed efficiency, and liver lipid content. Survival was not affected by dietary glycerol levels. Blood glucose level was significantly higher in fish fed 5% glycerol than fish fed other diets. Fillet protein and fat generally decreased and fillet moisture increased as dietary glycerol level increased. It appears that channel catfish can utilize about 10% glycerol in the diet without adverse effects on feed consumption, weight gain, feed efficiency ratio, hemoglobin, hepatosomatic index, and liver lipid.

Because of government energy policies, biofuel production in the USA has increased rapidly in the past few years, which has resulted in an increasing supply of its by-products. Glycerol or glycerin, the main by-product of biodiesel production from vegetable and animal oils and fats, is the carbohydrate fraction that comprises about 10% by weight of typical triglycerides. The U.S. biodiesel industry is projected to produce more than 600 million kg of glycerol in the next 10 yr (Niles 2006). Glycerol is "generally recognized as safe" when used in accordance with good manufacturing or feeding practice (CFR 2003). Pure glycerol is currently used in cosmetics and hygiene products. Finding alternative uses of this surplus by-product could reduce biodiesel production cost.

Glycerol has been evaluated in poultry, swine, and cattle diets as an alternative energy source (Simon et al. 1996; Schröder and Südekum 1999; Lammers et al. 2007a, 2007b). About 10–20% glycerol can be used in feeds for these animals. Menton et al. (1986) examined glycerol as a source of dietary energy for rainbow trout, *Oncorhynchus mykiss*, in low-energy (fat) diets. They found that the use of glycerol at levels of up to 12% (by replacing part of wheat middlings) in low-energy diets did not affect fish growth, feed efficiency, and carcass composition, but fish fed diets containing glycerol had lower feeding activity. They suggest that rainbow trout do not efficiently utilize glycerol as an energy source. There has been no report on the use of glycerol in channel catfish, *Ictalurus punctatus*, diets. The present study was conducted to examine the use of various levels of glycerol as a carbohydrate/energy source in channel catfish diets.

Materials and Methods

Five 32% protein, practical diets (Table 1) were formulated to contain 0, 5, 10, 15, and 20% glycerol from biodiesel production. All known nutrient requirements of channel catfish were satisfied (NRC 1993). The diets

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TABLE 1. *Ingredient composition (as fed), proximate composition, and pellet retention (20 min submersion in water) of experimental diets.*

	Glycerol (% of diet)				
	0	5	10	15	20
Ingredients					
Soybean meal, 48%[1]	44.30	45.01	46.35	47.65	48.95
Cottonseed meal, 41%[1]	15.00	15.00	15.00	15.00	15.00
Meat, bone, blood meal[2], 65%[1]	3.00	3.00	3.00	3.00	3.00
Corn grain	33.01	27.25	20.96	14.65	8.35
Glycerol	0.00	5.00	10.00	15.00	20.00
Other ingredients[3]	4.65	4.65	4.65	4.65	4.65
Proximate analysis[4] (%)					
Dry matter	95.0 ± 0.0	92.6 ± 0.0	92.0 ± 0.0	90.6 ± 0.2	89.2 ± 0.2
Crude protein[5]	32.0 ± 0.1	32.4 ± 0.1	32.8 ± 0.1	33.0 ± 0.0	33.7 ± 0.1
Crude fat[5]	3.15 ± 0.02	2.72 ± 0.09	2.39 ± 0.02	2.20 ± 0.01	2.04 ± 0.08
Dry matter retention (%)[6]	56.0	64.4	69.3	61.0	62.8

[1]Percentage crude protein.

[2]From pork products.

[3]Includes 1.0% dicalcium phosphate, 0.05% vitamin premix, 0.05% ascorbyl monophosphate, 0.10% trace mineral mix, 2% carboxymethyl cellulose as pellet binder, and 1.5% catfish offal oil. Vitamin and trace mineral premixes were the same as described by Robinson and Li (2007).

[4]Values represent the mean ± SD ($n = 2$, two batches per diet).

[5]Expressed as 90% dry matter basis.

[6]Standard error of mean was 3.15. No significant differences among diets ($P = 0.12$).

were prepared as sinking pellets according to procedures described previously (Li et al. 1993) and dried in a forced air oven at 70 C for 1.5 h to evaporate any methanol (boiling point is 65 C) residue of the glycerol. The dried feed pellets were stored at −20 C until used. The glycerol was obtained from Scott Petroleum Corporation (Greenville, MS, USA). Other feed ingredients were obtained from the Delta Western Feed Mill (Indianola, MS, USA) and were from commercial sources. Because glycerol is in the liquid form, it may leach out in the water. Therefore, a dry matter retention test was conducted on all diets. A 30-g diet sample was added in a dip net with 1-mm mesh and placed in a 110-L glass aquarium with flowing water (flow rate: approximately 1 L/min) and continuous aeration for 20 min at 30 C. Then the wet diet pellets were removed from the net and transferred to a crucible and dried in a mechanical convection oven (Precision, Winchester, VA, USA) at 105 C for 12 h. Three replicates were used for each diet. Dry matter retention of the diet was determined as follows:

$$\text{Dry matter retention}(\%) = (\text{dry weight of diet after 20 min immersion in water})/(\text{diet weight} \times \text{dry matter of diet}) \times 100$$

Juvenile channel catfish were obtained from the United States Department of Agriculture's Agricultural Research Service, Catfish Genetics Research Unit, Stoneville, MS, USA. Forty fish were stocked into each of 25, 110-L flow-through, glass aquaria at the National Warmwater Aquaculture Center (NWAC), Mississippi State University, Stoneville, MS, USA. Five aquaria were randomly allotted to each of five diets. The aquaria were supplied with well water (flow rate: approximately 1 L/min) and continuous aeration. Water temperature and oxygen were monitored in the system once daily using a YSI oxygen meter (Yellow Springs Instruments, Yellow Springs, OH, USA) and maintained at 30 ± 1 C and >5 mg/L, respectively. A diurnal light:dark cycle was regulated at 14:10 h.

Before initiation of the experiment, the fish were conditioned for 2 wk and fed diet 1 (the control diet) once daily to apparent satiation at 0800 h. After conditioning, all fish were pooled and graded to a uniform size, and 20 fish were restocked in each aquarium. Initial fish weight averaged 6.8 ± 0.1 g/fish (mean $\pm$ SD). Fish were fed to apparent satiation (in about 40 min) once daily for 9 wk. Satiation was achieved by first feeding an amount of diet based on percentage of fish body weight (less than satiation), followed by feeding several times from a preweighed diet container. Feed consumption was monitored and recorded at each feeding. Dead fish, if any, were removed daily from the aquarium and weighed. Aquaria were cleaned weekly.

Fish in each aquarium were counted and group weighed every 3 wk. At the end of the feeding period, diet consumption and weight gain per fish, feed efficiency ratio (FER), and survival were determined. FER was determined as follows:

$$\text{FER} = ([\text{final fish weight, g/tank}] - [\text{initial fish weight, g/tank}] + [\text{weight gain of mortality, g/tank}])/(\text{total diet fed, g/tank})$$

Three hours after the last feeding, five fish from each tank were anesthetized with 100 mg/L tricaine methanesulfonate (MS-222; Argent Chemical Laboratories, Redmond, WA, USA). Approximately 0.4 mL of blood from each fish was collected into a 1-mL heparinized syringe with a 21-gauge needle. Blood glucose and hemoglobin concentrations were determined with a Stat Profile CCX blood analyzer (Nova Biomedical, Waltham, MA, USA).

After the final fish number and weight were determined, five fish from each aquarium were euthanized by an overdose (500 mg/L) of MS-222. Liver and fillet samples were removed from these fish, weighed, pooled by aquarium, and stored at -20 C for subsequent analyses. Liver samples were homogenized by means of a tissue homogenizer, and lyophilized with a Freezone Freeze Dry System (Labconco, Kansas City, MO, USA) for 16–18 h for fat analysis. The fillet samples were homogenized into a paste by means of a Grindomix GM-200 Knife Mill (Retsch GmbH, Haan, Germany). Part of the sample was lyophilized for protein and fat analyses. Proximate analyses were performed in duplicate on the composite samples with methods described by Association of Official Analytical Chemists (AOAC) (2000). Crude protein of diet and fillet samples was analyzed by combustion method with an FP-2000 protein determinator (Leco Corporation, St. Joseph, MI, USA), crude fat by ether extraction with the Soxtec System (Foss North America, Inc., Eden Prairie, MN, USA), and moisture by oven drying with a mechanical convection oven (Precision, Winchester, VA, USA).

Data were subjected to one-way ANOVA and the Fisher's protected least significant difference (LSD) procedure (Steel et al. 1997) with the Statistical Analysis System (SAS) version 9.1 software (SAS 2004). Aquaria were the experimental units and variation among aquaria within a treatment was used as the experimental error in tests of significance. Linear and quadratic regressions were also performed on all variables against dietary glycerol level using the contrast statement of SAS. A significance level of $P \leq 0.05$ was used.

Results

Dry matter retention of diet after immersion in water for 20 min ranged from 56.0 to 69.3% (Table 1) and was not statistically different among diets. Feed consumption, weight gain, FER, and fillet (muscle) yield decreased as dietary glycerol level increased (linear and quadratic regressions were significant) (Table 2). The ANOVA showed no significant differences in feed consumption, weight gain, and FER among fish fed diets containing 0, 5, and 10% glycerol. Fillet yield was significantly lower in fish fed 20% glycerol than that in fish fed lower levels of glycerol. Survival was 99-100% and was not significantly different among treatments.

Blood glucose level increased as dietary glycerol increased from 0 to 5% and then decreased (linear and quadratic regressions were significant) (Table 3). Blood hemoglobin

TABLE 2. *Mean[1] feed consumption, weight gain, feed efficiency ratio (FER), survival, and edible tissue weight of juvenile channel catfish fed diets containing various levels of glycerol for 9 wk.*

Glycerol (%)	Feed consumption[2] (g/fish)	Weight gain[3] (g/fish)	FER[2] (gain/diet)	Survival (%)	Fillet yield[4] (%)
0	62.8[a]	49.2[a]	0.783[a]	100.0	27.2[a]
5	59.8[ab]	46.1[a]	0.771[a]	100.0	27.7[a]
10	61.4[ab]	46.8[a]	0.762[a]	99.0	27.2[a]
15	57.1[b]	41.5[b]	0.727[b]	100.0	27.2[a]
20	44.0[c]	28.9[c]	0.656[c]	99.0	26.4[b]
Pooled SEM	1.7	1.3	0.008	0.6	0.3
Regression P value (R^2)					
Linear	<0.01 (0.57)	<0.01 (0.69)	<0.01 (0.76)	0.33 (0.04)	0.03 (0.17)
Quadratic	<0.01 (0.17)	<0.01 (0.15)	<0.01 (0.13)	1.00 (0.00)	0.05 (0.14)

[1]Means represent average values of five tanks per dietary treatment. Means within each column followed by different letter were different ($P \leq 0.05$, the Fisher's protected least significant difference procedure).

[2]Based on 90% dry matter of the diet.

[3]Initial weight was 6.8 ± 0.1 g/fish ($n = 25$).

[4]Percentage of fillet weight relative to whole fish weight.

level was unaffected by dietary glycerol level. Hepatosomatic index (HSI) increased and liver lipid level decreased linearly as dietary glycerol level increased. The ANOVA demonstrated that fish fed 5% glycerol had higher blood glucose than fish fed the control and higher levels. Fish fed diets containing 15 and 20% glycerol had a significantly higher HSI than fish fed diets containing 5 and 10% glycerol, but HSI of fish fed the control diet was intermediate, not significantly different from other diets. Liver lipid was significantly lower in fish fed 15 and 20% glycerol than fish fed lower levels. Fillet protein and fat decreased and fillet moisture increased linearly as dietary glycerol level increased (Table 4). The ANOVA showed significant differences in fillet fat and moisture levels, but no significant differences in fillet protein levels among treatments.

TABLE 3. *Mean[1] blood glucose and hemoglobin, hepatosomatic index, and liver lipid of juvenile channel catfish fed diets containing various levels of glycerol for 9 wk.*

Glycerol (%)	Blood glucose (mg/dL)	Blood hemoglobin (g/dL)	Hepatosomatic index[2] (%)	Liver lipid[3] (%)
0	69.2[b]	6.98	2.13[ab]	5.82[a]
5	88.2[a]	6.82	2.01[b]	6.34[a]
10	66.9[b]	6.98	2.08[b]	5.99[a]
15	62.6[b]	6.79	2.34[a]	5.30[b]
20	60.1[b]	6.75	2.32[a]	5.13[b]
Pooled SEM	3.0	0.12	0.08	0.23
Regression *P* value (R^2)				
Linear	<0.01 (0.28)	0.19 (0.08)	<0.01 (0.26)	<0.01 (0.28)
Quadratic	0.03 (0.07)	0.78 (0.00)	0.21 (0.05)	0.06 (0.10)

[1]Means represent average values of five tanks with five fish per tank. Means within each column followed by different letter were different ($P \leq 0.05$, the Fisher's protected least significant difference procedure).

[2]Percentage of liver weight relative to whole fish weight.

[3]On dry matter basis.

TABLE 4. *Mean*[1] *fillet protein, fat, and moisture concentrations of juvenile channel catfish fed diets containing various levels of glycerol for 9 wk.*

Glycerol (%)	Fillet protein (%)	Fillet fat (%)	Fillet moisture (%)
0	17.5	4.21[a]	76.9[b]
5	17.5	3.59[ab]	77.5[b]
10	17.8	3.34[b]	77.4[b]
15	17.8	2.99[bc]	77.8[ab]
20	17.8	2.48[c]	78.2[a]
Pooled SEM	0.1	0.22	0.3
Regression *P* value (R^2)			
Linear	0.02 (0.22)	<0.01 (0.63)	<0.01 (0.38)
Quadratic	0.30 (0.04)	0.88 (0.00)	0.92 (0.00)

[1]Means represent average values of five tanks with five fish per tank. Means within each column followed by different letter were different ($P \leq 0.05$, the Fisher's protected least significant difference procedure).

Discussion

Results from the present study indicate that channel catfish can utilize about 10% glycerol in the diet, which is similar to or lower than the results reported for other animals. Menton et al. (1986) reported that the addition of 12% glycerol in low-energy (low fat) diets did not affect weight gain of rainbow trout, but feeding activity appeared slower than fish fed the control diet. Several studies on broiler chickens found that 20% glycerol can be used in the diet without affecting growth, feed consumption, and FER (Campbell and Hill 1962; Lin et al. 1976; Simon et al. 1996). Schröder and Südekum (1999) reported that dairy cows could use 10% glycerol in the diet without affecting feed intake and nutrient digestibility. Lammers et al. (2007a) also demonstrated that 10% glycerol did not affect the growth performance in nursery pigs.

Glycerol is a precursor for synthesis of triglycerides and phospholipids in the liver and adipose tissue. In the liver, glycerol is converted to glucose through the gluconeogenesis pathway and provides energy for cellular metabolism (Emmanuel et al. 1983). It can also be converted to glyceraldehyde-3-phosphate, which enters the glycolytic pathway and eventually the Krebs cycle to produce ATP (Lin et al. 1976; Rosebrough et al. 1980). In rainbow trout, the addition of 6–12% glycerol in the diet elevated the plasma glucose level, but did not affect the liver glycogen concentration (Menton et al. 1986). In the present study, channel catfish fed 5% glycerol had higher plasma glucose level than fish fed the control, but as dietary glycerol increased to 10% and above, plasma glucose level generally decreased. This response cannot be easily explained, but circulating glucose levels are also influenced by other carbohydrates, such as starch, and other nutrients in the diet.

The digestible energy of experimental diets was not reported because digestibility of glycerol is unknown for channel catfish. Pure glycerol contains 4.3 kcal gross energy/g, similar to that of corn starch (4.2 kcal/g). However, it is not clear how efficiently channel catfish digest, absorb, and metabolize glycerol. Energy digestibility of glycerol has been reported to be about 75% in broiler chickens (Simon et al. 1996). Lammers et al. (2007b) reported a glycerol energy digestibility of 93% for nursery pigs and 100% for market pigs. In mammals, glycerol is readily absorbed in the intestine, but at a rate of about one fourth of glucose (Lin 1977). In American eel, *Anguilla rostrata*, glycerol is mainly converted to glucose in the liver with lesser amounts metabolized into lipid, carbon dioxide, and glycogen (Renaud and Moon 1980). In rainbow trout, Menton (1985) demonstrated, through intraperitoneal injection of radiolabeled glycerol, that a portion of the glycerol was absorbed into the blood and tissues. In the present study, liver and fillet fat levels generally decreased as dietary glycerol levels increased, indicating that channel catfish are not efficient at metabolizing glycerol into lipid. In addition, the low body fat levels in fish fed high glycerol diets might have been attributed to the lower dietary fat levels because of corn (containing about 3.5% oil) being replaced by glycerol, as well as to the lower body weight of the fish because of less feed consumed.

In the present study, the HSI was higher in fish fed 15 and 20% glycerol than fish fed 5 and 10% glycerol. The reason for this is not clear especially considering that fish fed the control diet without glycerol had an intermediate HSI.

Menton et al. (1986) reported no significant differences in the HSI of rainbow trout fed 0 and 12% glycerol (1.0 vs. 1.4%).

Cerrate et al. (2006) found that broiler chickens fed diets containing 2.5 and 5% glycerol had higher breast yield than birds fed the control diet. Dietary glycerol levels of up to 12% did not appear to affect dressed yield in rainbow trout (Menton et al. 1986). The present study shows that dietary glycerol levels of up to 15% do not affect fillet yield of channel catfish, but a diet containing 20% glycerol results in lower fillet yield.

During the biodiesel production process, methanol is typically added to the oil to convert it to fatty acid methyl esters (biodiesel) and glycerol in the presence of a catalyst (sodium or potassium hydroxide). Crude glycerol may contain methanol, which is toxic to animals at high levels. However, this is not the case for catfish feeds because they are extruded and dried at high temperatures (130–150 C). If there is some methanol present in the product, it should be evaporated during the feed manufacturing process.

Because glycerol is in liquid form, some of it may leach out when the feed pellet comes into contact with water. The dry matter retention test of diet pellets showed no differences among diets (ranging from 56 to 69% after 20 min immersion in water), indicating that glycerol is well incorporated into the pellet. However, it should be noted that the diets used in the present study were sinking pellets. The effect of glycerol on the water stability of extruded (floating) pellets remains to be determined.

In summary, it appears that channel catfish can utilize about 10% glycerol in the diet without adverse effects on feed consumption, weight gain, feed efficiency, hemoglobin, HSI, and liver lipid. Fish fed 10% glycerol had lower fillet fat than fish fed the control diet without glycerol. Glycerol may be used as a carbohydrate or energy source in channel catfish. At current prices it is economical to use glycerol to replace part of corn in channel catfish diets. Further studies on the glycerol digestible energy value for channel catfish are warranted.

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