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Effects of feeding the repartitioning agent L644,969 on growth and body composition of blue catfish, *Ictalurus furcatus*, fed diets containing two protein levels reared in cages

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Abstract

Juvenile blue catfish with an average initial weight of 110 g, were stocked into 3.5-m³ floating cages at a rate of 200 fish/cage and fed one of four diets. Diets contained either 27 or 36% protein and either 0 or 3 mg/kg of the beta-adrenergic agonist (BAA) L644,969. There were three replicates per diet. Fish were fed all they would consume in 40 min for 94 days. There was no significant effect of protein, L644,969, or their interaction ($P > 0.05$) on final individual weight, percentage weight gain, survival, and specific growth rate (SGR). No difference ($P > 0.05$) in whole body composition was found between protein levels; however, fish fed diets containing L644,969 had a higher ($P < 0.05$) fillet dressout and a lower ($P < 0.05$) percentage of abdominal fat than fish fed control diets. The fish fed 3 mg L644,969/kg diet had 15% more protein and 28% less lipid in the fillet than fish fed control diets ($P < 0.05$). Fish fed diets containing L644,969 also had a significantly higher ($P < 0.05$) percentage of protein and a lower percentage of lipid in viscera than fish fed diets without the BAA. These results indicate that blue catfish reared to 485 g in cages can be fed as much as they will consume of a diet containing 27% protein without adversely affecting growth, and that the addition of 3 mg L644,969/kg to diet containing either 27 or 36% protein reduces lipid deposition in muscle and visceral depots while increasing protein accretion in muscle.

Keywords: *Ictalurus furcatus*; Feeding and nutrition —fish, metabolism

1. Introduction

Beta-adrenergic agonists (BAA) are a group of compounds that act as repartitioning agents in intermediary metabolism by redistributing nutrients from lipid stores to muscle

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synthesis, thereby improving body composition in the animal. Since muscle proteins undergo continual degradation and turnover, regulation of the rate of muscle protein degradation may alter the rate of muscle growth. At least seven BAA compounds are known to increase protein deposition. These include: cimaterol (American Cyanamid, Princeton, NJ), clenbuterol (American Cyanamid), isoproterenol, ractopamine (Eli Lilly, Greenfield, IN), salbutamol, BRL47672, and L644,969 (Merck, Sharp, and Dohme, Rahway, NJ). L644,969 is a biodegradable compound whose chemical name is 6-amino- α -(R)((((1R)-methyl-3-phenylpropyl) amino) methyl)-3-pyridinemethanol dihydrochloride-hemihydrate. It has been demonstrated to increase muscle accretion through hypertrophy and decreased muscle protein degradation in lambs and steers (Gwartney et al., 1991; Wheeler and Koohmaraie, 1992).

While there are numerous reports on the effects of BAA on livestock (Gwartney et al., 1991; Mitchell et al., 1991; Wheeler and Koohmaraie, 1992; Uttaro et al., 1993), there is only one published report on the effects of BAA on fish. Mustin and Lovell (1993) reported that channel catfish fed a 36% protein diet containing 20 ppm ractopamine gained more weight than fish fed a diet without ractopamine. Further, ractopamine-fed fish had lower percentages of mesenteric and muscle fat, and a higher percentage of muscle protein compared to fish fed a diet without ractopamine. These results would indicate that channel catfish show a similar response to ractopamine as mammalian livestock. However, since the current commercial catfish diets contain between 28–32% protein, it may be that the effects of BAA reported by Mustin and Lovell (1993) may be less when commercial diets are fed. Mitchell et al. (1991) reported that ractopamine had a more profound effect on carcass composition in swine fed a lower (12%) protein diet compared to swine fed a diet containing 18% protein.

Cage culture allows for the rearing of fish in ponds that would be difficult to harvest by seine. However, cage-reared channel catfish have a higher percentage of muscle fat than do pond-reared fish (Webster et al., 1993). Consumers are eating meat and fish with more reduced levels of lipid than 5–10 years ago. Reducing levels of body lipid in both pond- and cage-reared fish, and increasing muscle weight, thereby increasing dressing yield, may be desirable to producers and consumers. The objective of the present study was to evaluate the effects of L644,969 at two protein levels on growth and body composition of juvenile blue catfish reared in cages.

2. Materials and methods

Experimental conditions and animals

Blue catfish (*Ictalurus furcatus*) juveniles (average individual weight \pm SE of 110 ± 1.0 g; Meadows Catfish Haven, Buffalo, TX) were stocked on 20 June 1994 into twelve 3.5-m³ floating cages moored over the deepest area (4 m) of a 1.0-ha pond (average depth, 2.0 m) located at the Agricultural Research Farm, Kentucky State University, Frankfort, KY, USA. Two hundred juveniles were hand-counted and randomly stocked into each cage. Fish were fed one of four extruded diets for 94 days. Diets were formulated to contain either 25% or 35% crude protein, and were isocaloric (2.5 kcal of digestible energy/g of diet) (Table 1). Within each protein level, diets either contained 0 (control) or 3 mg of

Table 1
Ingredient and chemical composition of diets

	Protein level	
	27%	36%
<i>Ingredient (%)</i>		
Menhaden fish meal ^a	10.00	14.00
Soybean meal (44% protein)	23.00	44.00
Wheat flour	23.00	6.00
Corn meal	38.50	32.50
Menhaden fish oil ^b	3.00	1.00
Premix ^c	1.45	1.45
Ascorbic acid	0.05	0.05
Dicalcium phosphate	1.00	1.00
<i>Chemical analysis</i>		
Dry matter (%)	90.5	89.8
Protein (%) ^d	27.4	36.2
Lipid (%) ^d	5.2	5.9
DE ^e	2.50	2.51

^aMenhaden fish meal (67% protein) was shipped from Virginia and purchased from a commercial feed mill (Farmer's Feed, Inc., Lexington, KY).

^bBHT was added at a rate of 0.02%.

^cPremix was identical to that in Webster et al. (1993).

^dMoisture-free basis.

^eDE = digestible energy in kcal/g of diet; based on estimated values of the diet ingredients for channel catfish (NRC, 1983).

L644,969/kg of diet. The L644,969 was supplied by Merck and Co., Inc. (Rahway, NJ). Diets were extruded by a commercial feed mill (Integral Fish Foods, Inc., Grand Junction, CO) for use in this study. The L644,969 was mixed with 2.5 kg of wheat flour, then mixed with the rest of the wheat flour, and then added to the other ingredients to make 455 kg of diet. Diets 2 and 4 supplied the BAA, while the control diets (diets 1 and 3) had 2.5 kg of wheat flour added without the BAA. Fish were fed once daily (09.00) all they would consume in 40 min. There were three replications per treatment.

Diets were analyzed for crude protein, fat, moisture, and amino acid composition. Crude protein was determined by the macro-Kjeldahl method, crude fat was determined by the acid-hydrolysis method, and moisture was determined by drying samples to constant weight (AOAC, 1990). Digestible energy (DE) values were calculated from the diet ingredients for channel catfish (NRC, 1983). Diets were stored in plastic-lined bags in a freezer (-10°C) until fed.

Each cage had a frame made of polyvinylchloride tubing with a removable lid and was constructed of 10-mm polyethylene mesh. A panel of polyethylene mesh (0.2-mm mesh, 20 cm high) was installed around the top of the inside of each cage to prevent loss of floating diet. Cages were anchored to a floating dock, and the distance between cages was 2 m.

Temperature and dissolved oxygen (DO) were monitored twice daily (08.00 and 16.30) outside the cages, at a depth of 0.75 m, with a YSI model 57 oxygen meter (Yellow Springs

Instruments, Yellow Springs, OH). If DO was graphically predicted to decline below 4.0 mg/l, aeration was provided with an electric paddlewheel (5 HP; S and N Sprayer Co., Inc., Greenwood, MS). Weekly measurements of pH were recorded with an electronic pH meter (pH Pen, Fisher Scientific, Cincinnati, OH). Total ammonia–nitrogen, nitrite, and alkalinity were measured weekly with a DREL 2000 spectrophotometer (Hach Co., Loveland, CO).

Average monthly morning water temperatures (\pm SE) ranged from $21.0 \pm 1.0^\circ\text{C}$ for September to $25.7 \pm 1.0^\circ\text{C}$ for July, whereas average monthly afternoon water temperatures ranged from $22.0 \pm 1.0^\circ\text{C}$ for September to $26.7 \pm 1.0^\circ\text{C}$ for July. Morning DO levels averaged 6.1, 5.5, 7.3, and 8.2 mg/l for June, July, August, and September, respectively, whereas afternoon values were 7.6, 7.6, 9.4, and 10.8 mg/l for those respective months. Total ammonia–nitrogen averaged 0.09 ± 0.05 mg/l, nitrite averaged 0.004 ± 0.002 mg/l, alkalinity averaged 111 ± 31 mg/l, and pH averaged 8.3 ± 0.4 during the study, and were within acceptable values for growth of channel catfish (Boyd, 1979).

Prior to the start of the study, due to hauling stress, an infection of *Aeromonas hydrophila* occurred and heavy mortality resulted. Fish were treated with 2 mg/l of nitrofuracin and fed the 36% protein control diet coated with a nitrofuracin/cod liver oil mixture. Mortalities were not replaced. All mortality ceased after 7 days of treatment and no subsequent mortality occurred during the duration of the study. Fish were harvested on 22 September 1994 and were not fed for 18 h before harvest.

Data collection

Total number and weight of fish in each cage were determined at harvest. Ten fish were randomly sampled from each cage and individually weighed (g), killed by decapitation, skinned by hand, and dressing variables were determined (dressing percentage, abdominal fat, viscera weight, fillet weight, and frame weight). Liver was not included in the viscera weight. All body components are reported as a percentage of total body weight.

Fillet (muscle), frame, and viscera (with liver included) of two samples, comprised of three fish per sample, from each cage were homogenized separately in a blender and analyzed for protein, lipid, moisture, and ash. Protein was determined by the Kjeldahl method, lipid was determined by ether extraction, ash was determined by a muffle furnace, and moisture was determined by drying in an oven at 100°C to constant weight (AOAC, 1990). Amino acid composition of fillets was analyzed by a commercial laboratory (Woodson-Tenent Labs, Dayton, OH).

Statistical analysis

Data were analyzed as a 2×2 factorial using the SAS General Linear Models procedure (Statistical Analysis Systems, 1988) for significance among protein level, dietary L644,969 inclusion, and their interaction (Zar, 1984). Duncan's multiple range test was used to determine differences among means ($P = 0.05$). All percentage and ratio data were transformed to arc sin values prior to analysis (Zar, 1984).

3. Results

Growth, survival, and feed conversion

No significant ($P > 0.05$) interaction effects were found and data were reported by protein and dietary L644,969 levels. No differences ($P > 0.05$) in final individual weight, percentage weight gain, survival, and specific growth rate (SGR) were found when data were

Table 2
Individual weight, weight gain, survival, feed conversion ratio (FCR), and specific growth rate (SGR) of juvenile blue catfish fed diets containing two protein levels and two levels of L644,969^a

	Individual weight (g)	Weight gain (%)	Survival (%)	FCR ^b	SGR ^c
<i>Protein (%)</i>					
27	481.3 ± 9.9	269.2 ± 4.7	75.7 ± 1.6	1.63 ± 0.03 ^a	1.37 ± 0.02
36	487.5 ± 10.9	274.7 ± 10.0	74.3 ± 2.2	1.50 ± 0.03 ^b	1.38 ± 0.02
<i>L644,969 (mg/kg)</i>					
0	493.0 ± 5.6	276.8 ± 6.1	75.0 ± 1.3	1.57 ± 0.01	1.40 ± 0.01
3	475.8 ± 12.6	267.0 ± 8.9	75.0 ± 2.5	1.56 ± 0.06	1.36 ± 0.03

^aValues are means ± s.e. of three replications. Means within a column by protein level or L644,969 having different superscripts were significantly different ($P < 0.05$).

^bFCR = feed conversion ratio: total diet fed (g)/total wet weight gain (g).

^cSGR = specific growth rate (%/day): $(\ln W_t - \ln W_0) / T \times 100$, where W_t is weight of fish at time t , W_0 is weight of fish at time 0, and T is the culture period in days.

analyzed by individual diet, protein level, and addition of L644,969 and averaged 485 g, 272%, 75.0%, and 1.38%/day, respectively (Table 2). When analyzed by protein level, fish fed diets containing 27% protein had a higher ($P < 0.05$) FCR, 1.63, than fish fed diets containing 36% protein, 1.50. However, no difference in FCR was found when L644,969 was added.

Whole body composition

When analyzed by protein level, no significant differences in the percentage of whole body comprised of fillet, frame, abdominal fat, liver, and viscera were found and averaged 21.3, 62.9, 2.2, 1.4, and 6.0%, respectively (Table 3). Fish fed diets containing L644,969 had a significantly higher fillet dressout, but lower body weight percentages of frame and abdominal fat compared to fish fed diets without L644,969 (Table 3). No differences occurred in percentage liver and viscera among treatments.

Table 3
Percentage of whole body comprised by fillet, frame, abdominal fat, liver, and viscera of juvenile blue catfish fed diets containing two protein levels and two levels of L644,969^a

	% of whole body				
	Fillet	Frame	Abdominal fat	Liver	Viscera
<i>Protein (%)</i>					
27	21.0 ± 0.5	63.0 ± 0.8	2.2 ± 0.1	1.4 ± 0.1	6.0 ± 0.3
36	21.6 ± 0.6	62.8 ± 0.8	2.1 ± 0.1	1.4 ± 0.0	6.0 ± 0.2
<i>L644,969 (mg/kg)</i>					
0	20.2 ± 0.2 ^a	64.4 ± 0.4 ^b	2.4 ± 0.1 ^b	1.3 ± 0.1	5.7 ± 0.2
3	22.4 ± 0.4 ^b	61.4 ± 0.4 ^c	2.0 ± 0.1 ^c	1.4 ± 0.0	6.3 ± 0.3

^aValues are means ± s.e. of three replications of 10 fish. Means within a column by protein level or L644,969 having a different superscript are significantly different ($P < 0.05$).

Table 4
Percentage moisture, protein, lipid, and ash in fillet (muscle), frame, and viscera of blue catfish fed diets containing two protein levels and two levels of L644,969^a

	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Fillet (muscle)				
<i>Protein (%)</i>				
27	74.7 ± 0.7 ^a	69.2 ± 3.2 ^a	27.5 ± 3.9 ^b	4.3 ± 0.1 ^a
36	73.9 ± 0.5 ^b	67.8 ± 0.4 ^b	30.1 ± 0.7 ^a	4.0 ± 0.1 ^b
<i>L644,969 (mg/kg)</i>				
0	72.8 ± 0.4 ^b	63.8 ± 2.1 ^b	33.4 ± 2.4 ^a	3.8 ± 0.1 ^b
3	75.8 ± 0.4 ^a	73.2 ± 1.5 ^a	24.2 ± 2.2 ^b	4.4 ± 0.1 ^a
Frame (head and spine)				
<i>Protein (%)</i>				
27	69.9 ± 0.3	49.6 ± 1.2	41.8 ± 1.7	7.7 ± 0.7
36	70.2 ± 0.3	51.0 ± 0.3	40.6 ± 0.6	7.7 ± 0.2
<i>L644,969 (mg/kg)</i>				
0	69.5 ± 0.3 ^b	49.5 ± 0.8	42.0 ± 1.2	7.6 ± 0.3
3	70.6 ± 0.5 ^a	51.1 ± 0.8	40.3 ± 0.3	7.8 ± 0.5
Viscera				
<i>Protein (%)</i>				
27	46.1 ± 0.8	15.4 ± 0.7	75.7 ± 0.6	1.5 ± 0.1
36	46.0 ± 1.3	15.9 ± 0.6	75.3 ± 0.3	1.7 ± 0.1
<i>L644,969 (mg/kg)</i>				
0	48.1 ± 0.6 ^a	14.7 ± 0.3 ^b	77.1 ± 0.4 ^a	1.5 ± 0.1
3	44.1 ± 2.0 ^b	16.6 ± 1.1 ^a	73.9 ± 0.3 ^b	1.7 ± 0.2

^aValues are means ± s.e. of three replications with two samples of 3 fish per replicate. Means within a column by protein level or L644,969 with different superscripts are significantly different ($P < 0.05$).

Fillet composition

Fish fed diets containing 36% protein had significantly higher percentages of moisture and lipid, and lower percentages of protein and ash in fillets than fish fed diets containing 27% protein (Table 4). Fish fed diets containing L644,969 had significantly higher ($P < 0.05$) percentages of protein (73.2%) and ash (4.4%), and lower percentages of lipid (24.2%) and moisture (24.2%) than fish fed diets without L644,969 added (Table 4).

Fish fed diets containing 27% protein had a higher ($P < 0.05$) level of alanine in the fillet than fish fed diets containing 36% protein. However, all other amino acid levels were not different ($P > 0.05$). Fish fed diets containing L644,969 had higher ($P < 0.05$) levels of cystine and methionine in the fillet than fish fed diets without L644,969; however, all other amino acid levels were not different.

Frame and viscera composition

There were no differences in frame (head and spine) found among treatments when analyzed by protein level or addition of L644,969, except for percentage moisture for data analyzed by L644,969 (Table 4).

Percentages of moisture, protein, lipid, and ash in viscera were similar among fish fed the four diets, or when analyzed by protein level (Table 4). Fish fed diets containing L644,969 had significantly lower percentages of moisture (44.1%) and lipid (73.9%) and a higher percentage of protein (16.6%) than fish fed diets without L644,969 (Table 4). No difference in percentage ash was found between treatments.

4. Discussion

Data indicate that blue catfish reared in cages can be fed a diet containing 27% protein and 10% fish meal without adversely affecting weight gain. This is in agreement with our previous study (Webster et al., 1994) which indicated that channel catfish reared in cages could be fed a diet containing 27% protein without negatively impacting growth compared to fish fed diets with 32, 37, and 42% protein.

Beta-adrenergic agonists have been shown to reduce fat deposition and increase protein accretion in muscle in broilers, steers, lambs, and swine (Mitchell et al., 1991). Unlike growth hormone, which produces an increase in both tissue and organ growth, BAA increase protein deposition in skeletal muscle, but does not increase the size of organs (Reeds and Mersmann, 1991). In the present study, the percentage of whole body weight comprised of liver and viscera were similar among treatments, while the percentage comprised of fillet was 10% higher in fish fed diets containing L644,969 compared to fish fed control diets. Further, there was an increase of 13% in percentage protein and a decrease of 28% in lipid in fillet of fish fed diets containing L644,969. The increase in fillet yield and improved composition (higher protein, lower lipid) may be of commercial importance.

There are conflicting reports on the effect of BAA on weight gain in animals. Several workers have reported that ractopamine did not affect daily gain in swine (Hancock et al., 1987; Ott et al., 1989; Gwartney et al., 1991; Mitchell et al., 1991; Uttaro et al., 1993). However, others have reported up to a 15% increase in weight gain in swine (Crenshaw et al., 1987; Veenhuizen et al., 1987; Watkins et al., 1988; Williams et al., 1994). Mustin and Lovell (1993) reported that channel catfish fed a diet containing 20 mg ractopamine/kg diet had higher weight gains than fish fed a diet without ractopamine. In the present study, no increase in weight gain was observed in blue catfish fed diets containing 27 and 36% protein, or when fed diets containing 3 mg L644,969/kg diet.

There are conflicting reports as to when the greatest response of feeding BAA in terms of reducing body lipid and increasing protein deposition may occur. It has been reported that BAA gives the greatest growth response within the first 21 days of feeding, whereas after 30 days of use, improvements in growth performance decrease. However, improvements in carcass composition increase as the duration of use increases (Williams et al., 1994). Since blue catfish were fed >90 days, it may be that the duration of feeding was sufficiently long enough to offset a short-term growth response.

It does not appear that the two protein levels fed in the present study altered the effects of L644,969. Mustin and Lovell (1993) reported that ractopamine affected growth and body composition in channel catfish fed a diet containing 36% protein. This is higher than commercial channel catfish diets, which contain between 28–32% protein. They stated that the effects of ractopamine may be less in a lower protein diet. However, results from the

present study indicate that L644,969 is effective in reducing body lipid and increasing muscle protein at dietary protein levels of 27 and 36% and thus, may be effective under commercial production conditions.

While effects of BAA on muscle protein accretion have been demonstrated, the mechanism for increased protein levels is uncertain. Some proposed mechanisms include: an increase in protein synthesis (Bergen et al., 1989; Anderson et al., 1990), decreased protein degradation (Reeds et al., 1986; Bohorov et al., 1987), or a combination of both (Koochmaraie et al., 1991). Some reports have indicated that the effects of BAA are diphasic, that is, an initial reduction in protein degradation followed by an increase in synthesis (Wheeler and Koochmaraie, 1992). Others have stated that there is an increase in protein deposition due to decreasing lipogenesis and increasing lipolysis in adipose tissue, and increasing protein synthesis in muscle (Adeola et al., 1990; Williams et al., 1994).

It has been postulated that the reduced rate of fat deposition may partly be due to a diversion of amino acid catabolism that leads to ATP synthesis and partly from an increase in the overall rate of energy expenditure (Reeds and Mersmann, 1991). Thus, amino acid composition of treated animals may differ from that of control animals. If BAA have a specific action for muscle tissue, the contribution of essential amino acids to the increase in protein deposition may be higher. However, in the present study, only levels of cystine and methionine showed small differences between fish fed diets containing L644,969 and fish fed control diets. Thus, amino acid diversion may not be the mechanism for the reduction in fat deposition.

It has been reported that ractopamine improves feed efficiency by increasing growth rate and reducing feed intake (Jones et al., 1988; Watkins et al., 1988) or by increasing utilization efficiency by increasing muscle mass and repartitioning feed energy to production of lean growth, not lipid deposition (Williams et al., 1994). Wheeler and Koochmaraie (1992) reported improved feed efficiency in steers fed a diet containing L644,969. Mustin and Lovell (1993) could not calculate feed conversion since a sinking diet was used and made measurement of feed consumption difficult. Reliable feed consumption data was obtained in the present study since a floating diet was fed. In the present study, L644,969 had no effect on FCR. This is consistent with the data that no increase in weight gain or in amount of diet consumed were observed in the present study.

Feeding L644,969 did not affect weight gain, but did reduce abdominal and muscle lipid, increase muscle protein content and increase in fillet dressout in blue catfish at two protein levels. These effects are similar to those found in land animals and channel catfish. Since protein levels of the diets used in the present study were within the range of that used by commercial producers, it may be that feeding BAAs has potential application in commercial catfish production. However, at present, no BAAs are approved by the Food and Drug Administration for use in fish feeds.

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