Improvement of Cottonseed Meal Protein with Supplemental Lysine in Feeds for Channel Catfish

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ABSTRACT. Two studies were conducted to evaluate cottonseed meal (CSM) with and without supplemental lysine as a substitute for soybean meal (SBM) in catfish feeds. One study was conducted in aquaria under controlled environmental conditions with fingerling channel catfish, and the other was conducted with fish raised in 6-m^3 net pens suspended in a 1.6-ha earthen pond. In each study, solvent extracted CSM was substituted for SBM on a nitrogen basis. Supplemental lysine was added to the feeds in which CSM replaced 50 and 100% of the SBM because they were estimated to be deficient in available lysine. In the aquarium study, 0, 25, 50, and 100% of the SBM was replaced with CSM. The same feeds used in the aquarium study were used in the net pen study, except that the 25 substitution was not used. Fish raised in aquaria were fed 4% body weight divided into two equal daily feedings, and those raised in net pens were fed to satiation once daily. Data presented herein demonstrate that channel catfish can effectively utilize supplemental amino acids (even if offered in a single daily feeding) and that CSM can be used to replace 100% of the SBM in catfish feeds if supplemental lysine is used. It appears that about one-half of the SBM can be replaced with CSM without the use of supplemental lysine.

INTRODUCTION

Soybean meal (SBM) is the primary protein source used in a typical commercial catfish feed. Other plant protein concentrates such as cottonseed meal (CSM) and peanut meal (PM) have been used sparingly in catfish feeds, primarily because these meals are deficient in the indispensable amino acid, lysine. In addition, CSM contains free gossypol, which is toxic to certain animals (Martin 1990). However, several studies have demonstrated that protein quality is the primary factor limiting the use of CSM in catfish feeds, rather than free gossypol toxicity (Dorsa et al. 1982; Robinson and Rawles 1983; Robinson et al. 1984; Robinson and Daniels 1987; Robinson and Brent 1989). Presently, the level of CSM used in commercial catfish feeds is limited to about 15 to 20% of the feed (Robinson and Brent 1989). It may be possible to use higher levels if the protein quality of the feed can be improved by amino acid supplementation.

Data from laboratory studies in which fingerling channel catfish were fed a lysine supplemented ration divided into three equal feedings per day indicated that growth and feed efficiency were improved by amino acid supplementation of deficient proteins (Robinson et al. 1980). Although their data demonstrated that fingerling channel catfish utilized synthetic lysine supplemented into a lysine-deficient feed when the fish were fed multiple feedings, no studies have been published that demonstrate the utilization of synthetic amino acids by channel catfish fed once daily, as is typical in commercial catfish culture. Multiple feedings may be necessary for efficient utilization of supplemental

amino acids by fish. Thus, it is not known if the protein quality of CSM can be improved by lysine supplementation for channel catfish fed a single daily feeding. The present study was designed to evaluate substitution of SBM with CSM or CSM and lysine in channel catfish feeds.

MATERIALS AND METHODS

Experimental Diets and Design

The experimental feeds used in both aquarium (Table 1) and net pen studies (Table 2) were formulated to contain 32% crude protein and 2.7-2.8 kcal/g digestible energy. Protein levels were verified by analysis. Digestible energy values were based upon values reported for channel catfish (National Research Council 1983; Robinson 1989). The feeds used in the aquarium study were prepared in the feed manufacturing laboratory located at the Delta Branch Experiment and Extension Center, Mississippi Agricultural and Forestry Experiment Station, Stoneville, Mississippi. The feeds used in the net pen study were extruded by a commercial feed mill (Delta Western, Indianola, Mississippi [1]). The control feeds (Tables 1 and 2) contained SBM (48% crude protein) as the primary protein source and were typical of commercial catfish feeds used for grow-out. CSM (41% crude protein) was substituted for SBM on a nitrogen basis in the experimental feeds. Since SBM and CSM contain different levels of nitrogen, the level of wheat midds (aquarium study) or corn (net pen study) was adjusted to compensate for these differences. In the aquarium study, CSM was substituted for 0, 25, 50, and 100% of SBM. The 25 substitution was not included in the net pen study. Two additional treatments were included in both studies: feeds in which 50 and 100% of the SBM was substituted with CSM were supplemented with crystalline L-lysine HCl (BioKyowa Inc., St. Louis, Missouri) to meet the minimum requirement for lysine for channel catfish, which is 5.0% of the dietary protein (Wilson et al. 1977; Robinson et al. 1980). Lysine HCl (78.8% active) used as the lysine supplement was assumed to be 90% available to channel catfish. The CSM used in the study was a solvent extracted meal obtained from a local oilseed mill. CSM's from this source typically contain about 400 to 800 mg/kg free gossypol, which when mixed into the experimental feeds provides a level of free gossypol well below the 900 mg/kg free gossypol reported to be detrimental to channel catfish growth (Dorsa et al. 1982). Assay of a sample of the CSM used in this study showed that it contained 216 mg/kg free gossypol.

Prior to initiation of the aquarium study, the fish underwent a 2-week conditioning period during which they readily adjusted to a conditioning feed (same as control feed, Table 1) and standardized environmental conditions. The study was conducted in 110-1 flow-through aquaria with flow rates of approximately 900 ml/minute. Water temperature ranged from 28 to 30°C and a diurnal light:dark cycle was regulated at 14:10.

[1] Use of trade or manufacturer names does not imply endorsement.

TABLE 1. Percentage composition (as fed) of experimental feeds for aquarium study.

	Treatment					
Ingredient	Percent soybean meal replaced with cottonseed meal					
	0 (control)	25	50	100	50 + lysine	100 + lysine
Soybean meal, 48% [1]	42.5	32.5	23.2	0.0	23.2	0.0
Cottonseed meal, 41% [1]	0.0	12.4	24.8	53.8	24.8	53.8
Menhaden fish meal, 61% [1]	4.0	4.0	4.0	4.0	4.0	4.0
Meat and bone meal, 50% [1]	4.0	4.0	4.0	4.0	4.0	4.0
Wheat midds	37.8	35.4	32.2	26.5	32.2	26.3
Carboxymethycellulose	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin C premix [2]	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin premix [3]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix [4]	4.0	4.0	4.0	4.0	4.0	4.0
Lysine HCl, 78.8% [5]	0.0	0.0	0.0	0.0	0.3	0.8
Cod liver oil	2.0	2.0	2.0	2.0	2.0	2.0
Choline chloride	2.2	2.2	2.2	2.2	2.2	2.2
Available lysine [6]	1.70	1.55	1.41	1.06	1.67	1.64

[1] Percent protein

[2] Provides 100 mg/kg ascorbic acid as ascorbyl polyphosphate

[3] Meets or exceeds vitamin requirements of channel catfish, with the exception of choline (National Research Council 1983)

[4] Provides trace minerals required by channel catfish (National Research Council 1983)

[5] Estimated by be 90% available to catfish

[6] Based on amino acid availability data for channel catfish (Wilson et al. 1982)

At the start of the experiment, 20 fish (average weight = 7.2 g; range = 7.0-7.2 g) were stocked into individual aquaria. There were 4 aquaria per treatment. The experimental feeds were fed at 4 of total body weight per day divided into two equal feedings. Each group of fish was weighed biweekly, and the daily ration was adjusted accordingly. During the 12-week study, one replicate was lost in the treatment in which all of the SBM was replaced with CSM and lysine because the water was inadvertently left off during the night.

A second experiment was conducted in 6-m^3 net pens (made from 10 mm mesh knotless polypropylene) suspended in a 1.6-ha earthen pond located at the Delta Branch Research and Extension Center. The experiment was conducted in net pens to reduce the environmental variability that is typically observed in feeding trials where treatments are assigned to individual ponds. Twenty-four net pens (four per treatment) were each stocked with 400 fish (average weight = 54 g), a density several times higher than that used in a typical commercial catfish pond (12,500/ha) but less than that used in high density cage culture (500/m2). Based on pond size, the stocking rate was 6,OOO/ha. Two replicates

were lost due to physical damage to the net pens by animals, which allowed the fish to escape: one in each of the treatments in which 50% of the SBM was replaced with either CSM or CSM and lysine.

Beginning on May 10, 1989, fish were fed the experimental feeds to satiation once daily until October 25, 1989. Water temperature and dissolved oxygen were measured in the pond around the cages throughout the night and prior to feeding each day. An electrical paddle-wheel aerator was used to provide emergency aeration when the dissolved oxygen level dropped to 4 mg/l. Well water was added to replace water lost to evaporation and seepage.

TABLE 2. Percentage composition (as fed) of experimental feeds for net pen study.

	Treatment					
Ingredient	Percent soybean meal replaced with cottonseed meal					
	0 (control)	50	100	50 + lysine	100 + lysine	
Soybean meal, 48% [1]	49.2	24.4	0.0	24.4	0.0	
Cottonseed meal, 41% [1]	0.0	29.9	60.0	29.9	60.0	
Menhaden fish meal, 61% [1]	4.0	4.0	4.0	4.0	4.0	
Meat and bone meal, 50% [1]	4.0	4.0	4.0	4.0	4.0	
Wheat midds	3.8	3.8	3.8	3.8	3.8	
Corn grain	35.8	30.7	25.0	30.4	24.2	
Vitamin premix [2]	0.1	0.1	0.1	0.1	0.1	
Mineral premix [3]	0.1	0.1	0.1	0.1	0.1	
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0	
Lysine HCl, 78.8% [4]	0.0	0.0	0.0	0.36	0.8	
Catfish oil	2.0	2.0	2.0	2.0	2.0	
Available lysine [5]	1.76	1.40	1.05	1.65	1.63	

[1] Percent protein

[2] Meets or exceeds vitamin requirements of channel catfish, with the exception of choline (National Research Council 1983)

[3] Provides trace minerals required by channel catfish (National Research Council 1983)

[4] Estimated by be 90% available to catfish

[5] Based on amino acid availability data for channel catfish (Wilson et al. 1982)

The fish were harvested October 25-26. Fish from each net pen were counted and collectively weighed. Five fish were randomly sampled from each net pen (20/treatment), and were then manually skinned, gutted, decapitated, and filleted to determine dressout percentage. Five fillets obtained from fish from each net pen were randomly selected and frozen at - 80°C for subsequent proximate and free gossypol analyses.

Sample Analyses

Individual fillets were homogenized at room temperature using a Vitishear homogenizer (The VirTis Co. Inc., Gardiner, New York). Nitrogen was determined as described by the method of Hach et al. (1985) and crude protein calculated (N x 6.25). Fat was determined using Soxtec system HT extraction apparatus (Tecator Inc., Herndon, Virginia) by extracting approximately 0.5 g of freeze- dried fillet for 45 minutes with ethyl ether. Ash and moisture were determined on homogenized fillets by methods described by the Association of Official Analytical Chemists (1983). Free gossypol levels were determined on the edible portion of fish (two/treatment) fed feeds containing CSM by the Mississippi State Chemical Laboratory at Mississippi State University, using AOAC method Ba 7-58 (Association of Official Analytical Chemists 1983). All analyses were conducted in duplicate.

Statistical Analysis

Data were analyzed with the Statistical Analysis System, SAS- 79 (Helwig and Council 1979), using the General Linear Models procedure. Analysis of variance and Duncan's multiple range tests were used to determine statistical differences (Steel and Torrie 1960). Results were considered significant at the 0.05 level.

RESULTS

Weight Gain, Feed Conversion Ratio (FCR), and Survival

Weight gain, FCR, and survival data for fish raised in aquaria are presented in Table 3. Fish fed a feed in which the SBM was completely replaced with CSM had a significantly lower percentage weight gain

TABLE 3. Mean weight gain, feed conversion ratio (FCR) and survival of fingerling channel catfish fed feeds in which soybean meal was replaced with cottonseed meal or cottonseed meal and lysine (aquarium study). Means sharing the same letter are not significantly different. [1]

Treatment	Weight Gain	FCR	Survival
Percent soybean meal replaced	(%)		(%)
0 (control)	396 ab	1.72 bc	95 a
25	437 a	1.63 c	100 a
50	396 ab	1.72 bc	99 a
100	326 c	1.98 a	99 a
50 + lysine	421 ab	1.69 bc	100 a
100 + lysine	387 b	1.77 bc	99 a

[1] The pooled SEM for weight gain, FCR and survival were 4.24, 0.01 and 0.20, respectively.

and a higher FCR than fish fed the other experimental feeds. There were no other significant differences in percentage weight gain or FCR among fish fed other experimental feeds. The addition of lysine to the feed in which SBM was completely replaced by CSM significantly improved fish weight gain and FCR.

Survival was 95 or greater in all treatments. The same trends were observed in fish raised in net pens (Table 4): there was a depression in weight gain and an increase in FCR in fish fed the feed containing 0% SBM. When the feed in which CSM completely replaced SBM was supplemented with lysine, weight gain and FCR were significantly improved. Fish fed the feeds in which 50% of the SBM was replaced with CSM with and without supplemental lysine had significantly higher weight gains than fish fed the other experimental feeds. Survival of fish raised in net pens was 95% or greater.

TABLE 4. Mean weight gain, feed conversion ratio (FCR) and survival of fingerling channel catfish fed feeds in which soybean meal was replaced with cottonseed meal or cottonseed meal and lysine (net pen study). Means sharing the same letter are not significantly different. [1]

Treatment	Weight Gain	FCR	Survival
Percent soybean meal replaced	(%)		(%)
0 (control)	272 b	1.61 bc	97 a
50	300 a	1.53 cd	96 a
100	227 c	1.84 a	96 a
50 + lysine	300 a	1.46 d	95 a
100 + lysine	263 b	1.66 b	97 a

[1] The pooled SEM for weight gain, FCR and survival were 4.24, 0.01 and 0.20, respectively.

Dressout, Free Gossypol, and Proximate Composition

There were no significant differences in percentage dressout among fish fed the various feeds containing CSM as compared to fish fed the control feed (Table 5). Free gossypol levels in edible tissue were below detectable limits (2.0 mg/kg) in fish fed feeds containing CSM. Except for a higher percentage protein and a lower percentage fat in fish fed the feed containing 0 SBM supplemented with lysine, there were no other significant differences in proximate composition of fish fed feeds containing CSM as compared to fish fed feeds containing no CSM.

TABLE 5. Mean dressout and proximate composition (dry weight) of fillets from channel catfish fed feed in which soybean meal was replaced with cottonseed meal or cottonseed meal and lysine (net pen study). Means sharing the same letter are not significantly different. [1]

	Dressout (%)		Proximate composition (%)			
Treatment	Whole body	Fillet	Protein	Fat	Ash	Moisture
Percent soybean meal replaced						
0 (control)	61.7 ab	39.6 abc	66.6 bc	26.5 b	4.6 a	76.1 a
50	61.4 ab	39.8 ab	65.5 c	27.5 a	4.9 a	76.5 a
100	61.0 b	38.8 c	64.7 c	29.0 a	4.8 a	76.3 a
50 + lysine	62.3 a	40.4 a	69.9 b	24.6 ab	4.9 a	76.5 a
100 + lysine	61.1 b	39.0 c	70.2 a	23.4 c	4.9 a	76.6 a

[1] The pooled SEM for percentage dressout of whole body and fillet were 0.17 and 0.14, respectively. The pooled SEM for percentage protein, fat, ash and moisture were 0.47, 0.14, 0.05 and 0.16, respectively.

DISCUSSION

The direct toxic effects of free gossypol does not limit the usefulness of CSM in catfish feeds, at least using CSM typically available in the Mississippi Delta (Robinson and Brent 1989). However, free gossypol lowers the protein quality of CSM by binding to lysine during heat processing of CSM (Kuiken 1952), which reduces lysine availability. Lysine availability of CSM to channel catfish is reported to be about 66% (Wilson et al. 1982). Lysine availability from CSM may be even lower in extruded (floating) catfish feeds, since the extrusion process involves high temperatures, which may bind additional free gossypol to lysine. Data reported by Robinson and Brent (1989) indicated that extrusion processing of catfish feeds lowered free gossypol levels. In contrast, no differences in free gossypol levels were found in a recent study (unpublished) conducted at the Delta Branch Research and Extension Center in which a catfish feed mix containing CSM was assayed [using the same process as that used by Robinson and Brent (1989)] prior to and after extrusion. Nevertheless, using the amino acid availability data of Wilson et at. (1982) and the lysine requirement reported for channel catfish (Wilson et at. 1977; Robinson et at. 1980), feeds in which 50 and 100% of the SBM was replaced with CSM should have been deficient in available lysine. However, a lysine deficiency was reflected only in fish fed the feed in which SBM was completely replaced with CSM. The reason a lysine deficiency was not observed in fish fed a feed in which one-half of the SBM was replaced with CSM is not readily explainable. Natural food organisms, which can provide a source of nutrients, were not a likely contributing source of lysine in the present study because the fish were raised in net pens and thus could not forage for prey. It is possible that the available lysine level of the CSM used in the present study was higher than anticipated.

It is apparent from this study and the study of Robinson and Brent (1989) that up to 25-30% CSM can be used in catfish feeds without detrimental effects. If higher levels of CSM are to be used in channel

catfish feeds, protein quality must be improved by using supplemental lysine. The efficiency with which channel catfish utilize supplemental amino acids is still debatable. Two general schools of thought prevail: one argument is that channel catfish grow slowly on amino acid-supplemented feeds and that these types of feeds must be fed several times a day to be effective. The contrasting view is that amino acid-supplemented feeds are utilized effectively by channel catfish even when offered in a single daily feeding. Amino acid research reported by Wilson and Robinson (1982) demonstrated that channel catfish can utilize feeds based primarily on free amino acids, but less effectively than feeds with similar amino acid profiles that were prepared from highly digestible protein sources. Robinson et al. (1980) demonstrated utilization of supplemental lysine by channel catfish fed a lysine-deficient feed (supplemented with lysine) 3 times a day. Although unpublished data from studies conducted at Mississippi State University indicated that channel catfish use supplemental lysine when fed once daily, until the present study there has been no clear indication that channel catfish fed once daily effectively use supplemental lysine. Data from the present study demonstrate that lysine is utilized when used to supplement a CSM-based feed deficient in lysine. The significance of this finding is that supplemental lysine can be used to improve the quality of lysine-deficient feeds fed to channel catfish raised under a management scheme reflective of that used in commercial catfish culture, i.e., where fish are fed a single daily feeding until they are of harvestable size.

Data from the present study and previous studies (Robinson and Daniels 1987; Robinson and Brent 1989) show that accumulation of free gossypol in edible tissue of catfish fed feeds containing CSM for extended periods is not a problem. In the present study, free gossypol levels in edible tissue of channel catfish fed feeds containing up to 60% CSM were below detectable limits of 2 mg/kg.

There were no negative effects on percentage dressout or proximate composition of fish fed feeds containing CSM. There was an unexplained increase in percentage protein and a reduction in percentage fat in fish fed a feed containing 50% CSM and lysine. Although a high-protein, low-fat carcass is desirable, it is unlikely that fish fed such a feed would consistently be higher in protein and lower in fat.

Previous studies which have evaluated CSM as a potential catfish feed ingredient indicate that CSM can be used to replace part of the soybean meal typically used in channel catfish feeds (Dorsa et al. 1982; Robinson and Rawles 1983; Robinson et al. 1984; Robinson and Daniels 1987; Robinson and Brent 1989). Based on those studies, the level of CSM currently used in catfish feeds usually does not exceed 15 to 20%. However, based on data presented herein, it appears that up to 30 CSM can be used without adding lysine and that 100% of the SBM can be replaced with CSM if supplemental lysine is used to improve protein quality.

CONCLUSIONS

Three main conclusions may be drawn from the present study: One, channel catfish fed a daily ration supplemented with lysine effectively utilized lysine. Two, CSM can be used to replace 100% of SBM in catfish feeds if supplemental lysine is also added. Three, it appears that up to 30% CSM can be used in channel catfish feeds without adding lysine.

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