

INTERRELATIONSHIP AMONG METHIONINE, CHOLINE, AND BETAINE
IN CHANNEL CATFISH, *Ictalurus punctatus*.

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee.

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INTERRELATIONSHIP AMONG METHIONINE, CHOLINE, AND BETAINE
IN CHANNEL CATFISH, *Ictalurus punctatus*

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THESIS ABSTRACT

INTERRELATIONSHIP AMONG METHIONINE, CHOLINE, AND BETAINES

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Methionine, choline, and betaine are the three dietary sources of methyl groups in fish diets. Hence, there may be an interrelationship among dietary methionine, choline and betaine as methyl donors. The present research was conducted to evaluate if there is an interaction between methionine and choline as well as betaine and choline in feed formulations for the channel catfish. A semi-purified basal diet was formulated to contain 28 g crude protein/100 g diet, of which 13.07 g crude protein/100 g diet originated from intact protein and 14.93 g/crude protein/100 g diet from free amino acids. DL-methionine was added to the basal diet to produce deplete, sufficient and replete levels of methionine (0.35%, 0.39%, and 0.43%); choline chloride was added to the basal diet to produce deplete, sufficient and replete levels of choline (200, 400, and 600 mg choline/kg of diet); and free

base betaine was added to the basal diet to produce sufficient and excess levels of betaine (0 and 1000 mg betaine/kg of diet) in eight test diets. Each diet was randomly fed to triplicate groups of fish (15 fish/each group) over a 13 week growth trial.

Weight gain in fish fed diet 5 (deplete methionine, and replete choline) was not significantly different from fish fed diet 6 (sufficient methionine and choline), but significantly higher than fish fed diet 1 (deplete methionine and choline). Weight gain of fish fed diets with deplete methionine increased as choline levels in the diets increased. Weight gain and feed consumption in fish fed diet 4 (replete methionine and deplete choline) were not significantly different from fish fed diet 1 (deplete methionine and choline), but significantly lower than diet 6 (sufficient methionine and choline). Weight gain of fish fed diets with deplete choline did not increased as methionine levels in the diets increased. Weight gain, feed consumption, and FCR in fish fed diet 7 (deplete choline and excess betaine) were not significantly different from fish fed diet 6 (sufficient choline and no betaine), but significantly higher than fish diet 2 (deplete choline and no betaine). Weight gain of fish fed diets with deplete choline increased as betaine levels in the diets increased

Based on results of the present study, we concluded that in the absence of sufficient methionine, choline or betaine can spare a portion of methionine requirement of channel catfish. Further, in the absence of sufficient choline, methionine can not spare choline requirement of channel catfish. Further, in the absence of sufficient choline, betaine can spare at least a portion of choline requirement of channel catfish.

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- Figure 3. Effect of choline and betaine on weight gain of channel catfish. Values of weight gain are means of three replicates. Means in the columns with different superscripts were significantly different ($p < 0.05$).. . . . 25

INTRODUCTION

Farming of channel catfish (*Ictalurus punctatus*) is the largest aquaculture industry in the United States. Channel catfish has long been a popular food item in the southeastern areas of USA. It grew from annual production of 16.5 million pounds in 1965 to annual production of 597 million pounds in 2001 (Tucker, 2000; Aquaculture Magazine, 2002). As the fish are usually stocked in earthen ponds at high densities, a nutritionally complete and economically viable diet is critical to the success of a commercial operation.

Primary nutrients necessary for healthy growth of fish include proteins, energy, fats, vitamins and minerals. Protein is the major organic material in fish tissue, making up about 65% to 75% of the total on a dry-weight basis (Avault, 1996). Protein is needed by fish throughout life for maintenance and growth. Total protein requirements for optimum growth by channel catfish ranges from 25% to 36% (National Research Council, 1977). Because proteins are composed of up to 20 amino acids, it is more precise to formulate feeds on the basis of amino acid requirements rather than a gross protein level. Amino acids can be classified as essential or nonessential. Essential amino acids (EAA) are those that fish can not synthesize or synthesize in quantities sufficient for physiological needs. Considerable research has been conducted to determine catfish requirements of 10 EAA that include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Robinson et al., 1980a, 1980b, and 1981; Harding et al., 1977;

Wilson et al., 1977, 1978, and 1980). Pathologies resulting from deficiency of EAA in fish included a reduction in weight gain, cataracts, scoliosis (lateral curvature of the vertebral column), and a derangement of mineral metabolism (National Research Council, 1993). The remaining amino acids are classified as nonessential because they can be synthesized by fish.

Energy intake is a basic requirement because maintenance of life processes takes priority over growth and other functions. Energy is not truly a nutrient, but a property of nutrients as it is released during metabolic oxidation of carbohydrates, fats, and amino acids. Energy levels should be balanced with protein as excess or deficient energy in relation to protein can lead to waste of protein, reduction of feed consumption, and deposition of large amount of body fat (National Research Council, 1993). The ratio of digestible protein to digestible energy (DP/DE) for maximum weight gain for channel catfish was recommended at 81 mg/kcal (Li and Lovell, 1992).

Lipids are a highly digestible source of concentrated energy, supply essential fatty acids (EFA), serve as a vehicle for absorption of fat-soluble vitamins, and provide precursors for steroid and other components (Robinson and Li, 1996). Essential fatty acids are fatty acids that cannot be synthesized by fish or can not be synthesized in sufficient quantities. Signs of EFA deficiency in fish included shock syndrome, reduced growth rate, reduced feed efficiency, and increased mortality (Sato et al., 1989). The quantitative EFA requirements of channel catfish was 1-2% linolenic acid (Sato et al., 1989).

Vitamins are organic compounds that are required in trace amounts from an exogenous source (usually the diet) for normal growth, reproduction and health. Vitamins

are classified as water soluble and fat soluble. Although the requirements of fish are small, deficiencies of these micronutrients can cause symptoms that included poor appetite, poor growth, anemia, scoliosis, fatty liver and tissue deformities (Lovell, 1989). The quantitative requirement for most of the vitamins for channel catfish have been summarized by Robinson (1989).

Minerals, which are inorganic elements, are required for various metabolic processes such as the formation of skeletal structure, electron transfer, regulation of acid-base equilibrium, and osmoregulation (National Research Council, 1993). Minerals can be classified as macrominerals or microminerals, depending on the amount required in the diet. In addition to minerals being absorbed from the diet they can be absorbed from the water. Mineral deficiency signs included reduced bone ash, muscle flaccidity, reduced hemoglobin, reduced bone mineralization, and reduced heart cytochrome oxidase activities (Robinson and Li, 1996). The quantitative requirements for a number of minerals has been determined for channel catfish (National Research Council, 1993).

In order to provide a nutritionally complete diet for fish at the least cost, considerable information must be known with regards to requirements of nutrients as well as interrelationships among nutrients. Considerable research has been conducted to determine individual nutrient requirement. Yet, little research has been done to evaluate interrelationships among nutrients. Hence, the objective of the present research was to expand our understanding of the interrelationships among selected nutrients in fish.

LITERATURE REVIEW

When two or more nutrients in a diet are interrelated, it becomes essential to consider how their presence and quantity influence the growth of fish when one or all of them are supplemented to the diet. Kidd et al.(1997) concluded that vertebrates are unable to synthesize methyl groups, and thus need to receive them in the diet. Because methionine, choline, and betaine are the three main dietary sources of methyl groups (Kettunen et al., 2001), there may be interrelationships among dietary methionine, choline and betaine. Before we consider these interrelationships, it is necessary to first have an understanding of the functions and important properties of methionine, choline, and betaine.

Methionine is an indispensable amino acid that is required by all animals including fish (Ketola, 1982). Methionine has three major metabolic functions: as an essential amino acid, which is required for protein synthesis; as a sulfur source, which is needed for synthesis of other sulfur-containing biochemicals; and as a methyl donor, which can provide methyl groups for use in methylation reactions (Mehler, 1986). The requirement for total sulfur amino acids can be met by either methionine alone or the proper mixture of methionine and cystine (Moon and Gatlin III, 1991). Cystine can spare as much as 40-60% of the methionine requirement of fish (Harding et al., 1977). Since methionine is often one of the most limiting amino acids in ingredients used for fish feeds (Harris,1980), it can influence cost of a diet formulation. Consequently, considerable works have been conducted with various species

(Table 1). Signs of methionine deficiency included poor growth, cataracts, and lens opacity (Rumsey et al., 1983; Keembiyethetty and Gatlin, 1993).

Choline is considered a vitamin in the diets of vertebrate such as poultry, cats, rats, dogs, and several species of fish. Choline has three major metabolic functions: as a component of phosphatidylcholine, which has structural functions in biological membranes and in tissue lipid utilization; as a precursor of neurotransmitter acetylcholine; and as a precursor of betaine, which serves as a source of labile methyl groups for methylation reactions such as the formation of methionine from homocysteine and creatine from guanidoacetic acid (National Research Council, 1993). Signs of choline deficiency in fish included poor growth and feed efficiency, anorexia, fatty livers, and hemorrhagic areas in kidney, liver, and intestine.(Ketola, 1976; Wilson and Poe, 1988). Dietary choline requirements have been estimated for several fish species (Table 2).

Betaine is a non-toxic amino acid derivative found widely distributed in nature (Kettunen et al., 2001). Betaine has three major metabolic functions: as a methyl donor, which can provide methyl groups for essential biochemical reactions (Scott, 1986; Sheard and Zeisel, 1989), as an osmolyte, which can allow accumulation of a sufficient muscle betaine reserve so as to reduce the consequences of osmotic stress in fish (Virtanen te al., 1989; Clarke tel al., 1994; and Castro et al., 1998), and as a feeding stimulant for several species of fish and crustaceans (Carr, 1976; Mackie and Mitchell, 1985, Virtanen et al.,1994; Coman et al., 1996; Dy Penaflorida and Virtanen, 1996; Knights, 1996; Harpaz, 1997;and Papatryphon and Soares, 2000).

Table 1. Methionine requirements of several fish species

Fish species	Protein of diet (%)	Requirement (% of)		Type of diet	Reference
		dietary protein	dry diet		
Channel catfish (<i>Ictalurus punctatus</i>)	24	2.34	0.56	Semi- purified	Harding et al., 1977
Tilapia (<i>Sarotherodon mossambicus</i>)	40	3.2	1.3	Practical	Jackson and Capper, 1982
Rainbow trout (<i>Oncorhynchus mykiss</i>)	35	3.0	1.1	Semi- purified	Rumsey et al., 1983
Tilapia (<i>Oreochromis niloticus</i>)	28	2.68	0.75	Semi- purified	Santigo and Lovell, 1988
Red drum (<i>Sciaenops ocellatus</i>)	35	2.69	0.94	Semi- purified	Moon and Gatlin, 1991
Hybrid striped bass (<i>Morone Chrysops</i> × <i>M. saxatilis</i>)	35	2.49	0.87	Semi- purified	Keembiyehetty and Gatlin, 1993

Table 2. Choline requirements of several fish species.

Fish species	Requirement (mg/kg diet)	Response criteria	Reference
Lake trout (<i>Salvelinus namaycush</i>)	1000	Weight gain	Ketola, 1976
Channel catfish (<i>Ictalurus punctatus</i>)	400	Weight gain, liver lipid content	Wilson and Poe, 1988
Rainbow trout (<i>Oncorhynchus mykiss</i>)	714-813	Weight gain, liver lipid content	Rumsey, 1991
Hybrid striped bass (<i>Morone saxatilis</i> × <i>M. chrysops</i>)	500	Weight gain, feed efficiency	Griffin et al., 1994
Channel catfish (<i>Ictalurus punctatus</i>)	429	Weight gain, feed efficiency, liver choline	Zhang and Wilson, 1999
Yellow perch (<i>Perca flavescens</i>)	593-634	Weight gain, feed intake	Twibell and Brown, 2000

Because methionine, choline, and betaine share a common physiological use as labile methyl donors, Du Vigneaud *et al* (1939; 1940; and 1950) and Klose and Almquist (1941) postulated that once the requirements for methionine and choline per se are met, the metabolic requirement for labile methyl groups can be met by either methionine or choline or betaine. In the poultry industry, the interrelationships among methionine, choline, and betaine has been a subject of controversy among research workers for many years. Emmert *et al.* (1996), Schutte *et al.* (1997), and McDevitt *et al.* (1999) concluded that both choline and betaine were inefficient at sparing methionine in diets fed to chicks. However, Gerry *et al.* (1948), Quillin *et al.* (1961), Pesti *et al.* (1980) and Barker *et al.* (1982) reported in the absence of sufficient methionine, either choline or betaine can spare methionine in diets fed to chicks. Studies on the interrelationships among methionine, choline, and betaine in fish are scarce. Because the price of methionine is higher than choline and betaine, it is important to use methionine for protein synthesis and to use choline or betaine as methyl donor in metabolism. A better understanding of the interrelationship among methionine, choline, and betaine might not only improve fish growth performance but also reduce the cost of feed. The present research was designed to evaluate if there is an interrelationship between methionine and choline as well as betaine and choline in feed formulations for the channel catfish.

MATERIALS AND METHODS

Channel catfish fingerlings were obtained from a private producer in Alabama, and maintained at the North Auburn Fisheries Research Unit (Auburn, Alabama). Upon receipt of the fish, they were dipped into a solution of potassium permanganate (5mg/l) for 5 minutes. Fish were then transferred into a semi-closed recirculating system which consisted of a series of 60-L aquaria. The system was equipped with a settling chamber biological filter, a rapid-rate sand filter, and a circulation pump. Aeration was provided via a regenerative air blower and submerged air diffusers.

Fish were acclimated to laboratory conditions for two weeks before initiation of the experiment. Prior to the experiment, fish were fed a commercial diet twice daily to satiation. After the acclimation period, 15 fish (5.77 g mean initial weight) were randomly selected and stocked into each of 24 aquaria, and the study was initiated. Experimental diets were randomly assigned to triplicate groups of fish. The diets were offered at a rate of approximate 4% of body weight daily divided into two equal feedings. Fish of each aquarium was weighed weekly, and the feed ration was adjusted accordingly.

Water temperature and dissolved oxygen (DO) were measured daily using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio). Total ammonia-nitrogen was determined using the Solorzano (1969) phenolhypochlorite method, and nitrite-nitrogen was estimated using the methods described by Boyd and Tucker (1992) every two

weeks. The pH was monitored daily using an electronic pH meter (pH pen; Fisher Scientific, Cincinnati, Ohio). The pH of the water was adjusted on an as need basis using sodiumbicarbonate. Seasalt was added to maintain a low level of chloride ions (approximate 0.2 mg/l).

The basal diet (Table 3) used in the present experiment was formulated to contain 28% crude protein and 6% lipid. Casein, gelatin and corn gluten meal provided 13.07 g crude protein/100 g diet, and a crystalline amino acid mixture provided 14.93 g/crude protein/100 g diet. Contribution of essential amino acids of basal diet was listed in Table 4. Total sulfur amino acid concentration in the basal diet was 0.53% (0.35% methionine and 0.18% cystine). The basal diet contained 0.35% methionine, thus meeting 90% of the dietary requirement of channel catfish for methionine (0.39%) as reported by Harding et al. (1977). The remaining dietary essential amino acid concentrations met or exceeded the essential amino acids requirements of channel catfish recommended by National Research Council (1993). The basal diet contained 200 mg/kg choline, thus meeting 50% of choline requirement of channel catfish (400 mg/kg) as reported by Wilson and Poe (1988) and Zhang and Wilson (1999). A choline free, but otherwise complete vitamin premix and nutritionally complete mineral premix were added to the diets.

DL-methionine was added to the basal diet to produce three concentrations of methionine (0.35%, 0.39%, and 0.43%); choline chloride was added to the basal diet to produce three concentrations of choline (200, 400, and 600 mg choline/kg of diet); and free base betaine was added to the basal diet to produce two concentrations of betaine (0 and

Table 3. Composition (as g/100g dry weight) of the basal diet.

Ingredient ^a	Amount
Casein (vitamin free)	4.00
Gelatin	1.00
Corn gluten meal	12.50
Amino acid mixture ^b	14.93
Wheat starch	35.00
Cellulose	20.32
Carboxymethyl cellulose	2.00
Corn oil	6.00
Vitamin premix, choline-free ^c	2.00
Mineral premix ^d	0.50
Calcium phosphate, dibasic	1.75

^a Obtained from U. S. Biochemical (Cleveland, OH).

^b Contained : L-cystine-HCl, 0.08; L-arginine-HCl, 0.70; L-histidine-HCl, 0.12; L-isoleucine, 0.15; L-lysine-HCl, 0.94; L-phenylalanine, 0.64; L-threonine, 0.09; L-tryptophan, 0.03; L-valine, 0.10; L-aspartic acid, 3.80; L-glutamic acid, 4.97; L-serine, 2.22; and L-proline, 1.09. L-Phenylalanine, L-aspartic acid, and L-glutamic acid were obtained from U. S. Biochemical (Cleveland, OH), and the remaining amino acids were obtained from ICN Biomedicals (Irvine, CA).

^c Contained (as g/kg): thiamin-HCl, 1.75; riboflavin, 1.00; pyridoxine-HCl, 1.82; pantothenic acid, hemicalcium salt, 10.92; nicotinic acid, 7.81; biotin, 0.10; folic acid, 0.27; vitamin B₁₂, 0.001; inositol, 20.00; L-ascorbic acid polyphosphate, 14.29; menadione sodium bisulfite, 0.96; vitamin A acetate, 0.62; vitamin D₃, 0.10; dl-alpha-tocopherol acetate, 10.53; alpha-cellulose, 929.843.

^d Contained (as g/kg): cobalt chloride, 0.04; cupric sulfate pentahydrate, 2.50; ferrous sulfate, 40.00; magnesium sulfate anhydrous, 138.62; manganous sulfate monohydrate, 6.50; potassium iodide, 0.67; sodium selenite, 0.10; zinc sulfate heptahydrate, 131.93; cellulose, 679.64.

Table 4. Contribution (as g/100g dry weight) of essential amino acids from basal diet.

Amino acid	Supplied by casein, gelatin, and corn gluten meal	Supplied by amino acid mixture	Total
Methionine	0.35	0.00	0.35
Cystine	0.18	0.08	0.26
Arginine	0.50	0.70	1.20
Histidine	0.30	0.12	0.42
Isoleucine	0.58	0.15	0.73
Leucine	1.80	0.00	1.80
Lysine	0.49	0.94	1.43
Phenylalanine	0.76	0.64	1.40
Threonine	0.47	0.09	0.56
Tryptophan	0.11	0.03	0.14
Valine	0.74	0.10	0.84

1000 mg betaine/kg of diet) in eight test diets (Table 5). Based on dietary requirement of channel catfish for methionine (0.39%) as reported by Harding et al. (1977), three methionine concentrations of 0.35%, 0.39%, and 0.43% were respectively regarded as deplete, sufficient, and replete concentrations of methionine for channel catfish in the present research. Based on the choline requirement of channel catfish (400 mg/kg) as reported by Wilson and Poe (1988) and Zhang and Wilson (1999), three choline concentrations of 200, 400, and 600 mg choline/kg were respectively regarded as deplete, sufficient, and replete concentrations of choline for channel catfish in the present research. Dry ingredients were homogenized in a mixer for 20 minutes and then hot water was added to mix again for 10 additional minutes. Before pelleting, diets were adjusted to pH 7.0 with saturated sodium hydroxide using methods reported by Wilson et al. (1977). A 5 g portion of the diet was homogenized with 50 ml of water and pH was determined. NaOH solution (6 N) was added to diet to establish pH level of 7.0. All diets were then pelleted using 3 mm die, dried at 40C in a forced-air oven for 24 hours and stored at -20 C.

At the termination of experiment, the fish were weighed 24 hours after the final feeding. Then the fish were immersed in a solution of tricane methanesulfonate (MS222; Argent Chemical, Redmond, WA) until euthanized. From each aquarium, fish were randomly collected and total body weight and liver weight per fish were recorded for calculation of hepatosomatic index ($HSI = \text{liver weight/body weight} \times 100$). Livers were then pooled by aquaria and frozen for subsequent analyses. Total liver lipid was determined for each pooled sample by homogenizing duplicate sub-samples in chloroform/methanol (2:1, v:v) using a

Table 5. Calculated concentrations of methionine, choline, and betaine in the eight dietary treatments.

Diet #	Methionine		Choline		Betaine
	(g/ 100g)	Requirement status*	(mg/kg)	Requirement status*	(mg/kg)
1	0.35	-	200	-	0
2	0.39	=	200	-	0
3	0.35	-	400	=	0
4	0.43	+	200	-	0
5	0.35	-	600	+	0
6	0.39	=	400	=	0
7	0.39	=	200	-	1000
8	0.39	=	400	=	1000

* -, =, and + were respectively used to represent deplete, sufficient, and replete concentrations.

modification of the methods described by Folch et al. (1957). Moisture was determined in duplicate on a portion of the composite liver sample by drying to a constant weight at 95C using the methods described by the Association of Official Analytical Chemists (1990).

Data from the present experiment was statistically analyzed as a completely randomized design using each aquarium as an experimental unit. All statistical analyses were performed using Statistical Analysis System (V 8.2, SAS Institute Inc. Cary, North Carolina, USA). One-way analysis of variance (ANOVA) was used to detect significant ($P < 0.05$) differences among treatment means, and then Duncan's Multiple Range Test was applied to determine significant difference between treatment means.

RESULTS

Averages and standard deviations for water quality variables were temperature, 30.6 ± 1.0 C; dissolved oxygen, 6.9 ± 0.7 mg/L; and pH, 8.0 ± 0.2 , total ammonia nitrogen, 0.012 ± 0.007 mg/L, and nitrite nitrogen 0.003 ± 0.001 mg/L.

Fish fed diet 1, 2, 3, 4, 5, and 6 were analyzed to evaluate the interrelationship between methionine and choline (Table 6). Weight gain of Fish fed diet 5 (deplete methionine and replete choline) and 6 (sufficient methionine and choline) were not significantly different from each other. Weight gain of fish fed diet 5 (deplete methionine and replete choline) was not significantly different from fish fed diet 3 (deplete methionine and sufficient choline), but was significantly higher than fish fed diet 1 (deplete methionine and choline). There were no significant differences in weight gain for fish fed diet 1 (deplete methionine and choline), 2 (sufficient methionine and deplete choline), 4 (replete methionine and deplete choline) and 3 (deplete methionine and sufficient choline). Feed consumption of fish fed diet 5 was significantly higher than fish fed diet 1 (deplete methionine and choline), but was significantly lower than fish fed diet 6 (sufficient methionine and choline). There were no significant differences in feed consumption for fish fed diet 1 (deplete methionine and choline), 2 (sufficient methionine and deplete choline), 4 (replete methionine and deplete choline) and 3 (deplete methionine and sufficient choline). No significant differences were found in FCR, survival rate, HSI, and liver lipid content for fish fed any

Table 6. Response of channel catfish offered the test diets containing various levels of methionine and choline over a 13 week growth trial¹.

Diet #	Methionine		Choline		Feed consumption (g/fish)	FCR ²	%			
	(g/100g)	Requirement status *	(mg/kg)	Requirement status *			% Weight gain ³	Survival	HSI ⁴	Liver lipid ⁵
1	0.35	-	200	-	34.2 ^c	2.5 ^a	239.3 ^c	100.0 ^a	1.30 ^a	6.58 ^a
2	0.39	=	200	-	34.9 ^{bc}	2.5 ^a	241.3 ^c	100.0 ^a	1.44 ^a	9.40 ^a
3	0.35	-	400	=	35.3 ^{bc}	2.4 ^a	259.7 ^{bc}	93.3 ^a	1.49 ^a	7.29 ^a
4	0.43	+	200	-	35.1 ^{bc}	2.5 ^a	241.7 ^c	97.7 ^a	1.70 ^a	7.05 ^a
5	0.35	-	600	+	37.2 ^b	2.4 ^a	280.3 ^{ab}	91.0 ^a	1.57 ^a	6.13 ^a
6	0.39	=	400	=	39.4 ^a	2.2 ^a	304.7 ^a	97.7 ^a	1.40 ^a	9.40 ^a
PSE ⁶					0.73	0.09	11.47	3.87	0.16	1.49
Pr > F					0.0028	0.2664	0.0082	0.5105	0.6358	0.3051

¹ Values are means of three replicates. Means in the same column with different superscripts were significantly different ($p < 0.05$).

² FCR = dry weight of feed offered/wet weight gain.

³ % Weight gain = (final weight - initial weight) \times 100/initial weight

⁴ HSI= Liver weight/body weight \times 100.

⁵ Expressed on a dry matter basis.

⁶ PSE=pooled standard error.

* -, =, and + were respectively used to represent deplete, sufficient, and replete concentrations.

of the diets.

Fish fed diet 2, 6, 7 and 8 were analyzed to evaluate the interrelationship between choline and betaine (Table 7). Weight gain of fish fed diet 7 (deplete choline and excess betaine) was not significantly different from fish fed diet 6 (sufficient choline and no betaine), but significantly higher than fish fed diet 2 (deplete choline and no betaine). Fish fed diet 8 (sufficient choline and excess betaine) had a significantly higher weight gain than fish fed all other diets. Feed consumption of fish fed diet 7 (deplete choline and excess betaine) was not significantly different from fish fed diet 6 (sufficient choline and no betaine), but was significantly higher than fish fed diet 2 (deplete choline and no betaine). There were no significant differences in FCR for fish fed diet 6 (sufficient methionine and choline), 7 (deplete choline and excess betaine), and 8 (sufficient choline and excess betaine). Fish fed diet 2 (deplete choline and no betaine) had a significantly higher FCR than fish fed all other diets. No significant differences were found in survival rate, HSI, and liver lipid content for fish fed any of the diets.

Table 7. Response of channel catfish offered the test diets containing various levels of choline and betaine over a 13 week growth trial¹.

Diet #	Choline		Betaine (mg/kg)	Feed consumption (g/fish)	FCR ²	%			
	(mg/kg)	Requirement status *				% Weight gain ³	% Survival	HSI ⁴	Feed consumption (g/fish)
2	200	-	0	34.9 ^c	2.5 ^a	241.3 ^c	100.0 ^a	1.44 ^a	8.25 ^a
6	400	=	0	39.4 ^{ab}	2.2 ^b	304.7 ^b	97.7 ^a	1.40 ^a	9.40 ^a
7	200	-	1000	37.9 ^b	2.2 ^b	295.3 ^b	97.7 ^a	1.57 ^a	9.47 ^a
8	400	=	1000	42.0 ^a	2.1 ^b	339.7 ^a	97.7 ^a	1.55 ^a	9.74 ^a
PSE ⁶				0.8571	0.05	8.54	2.02	0.1154	1.1567
Pr > F				0.0026	0.0023	0.0003	0.8018	0.6867	0.8078

¹ Values are means of three replicates. Means in the same column with different superscripts were significantly different ($p < 0.05$).

² FCR = dry weight of feed offered/wet weight gain.

³ % Weight gain = (final weight-initial weight) \times 100/initial weight.

⁴ HSI= Liver weight/body weight \times 100.

⁵ Expressed on a dry matter basis.

⁶ PSE=pooled standard error.

* -, =, and + were respectively used to represent deplete, sufficient, and replete concentrations.

DISCUSSION

Quite often there are interrelationships among nutrients. In steps of metabolism of methionine, choline and betaine (Figure 1), methionine and ATP synthesize S-adenosylmethionine (SAM), which is the principle methyl group donor in vertebrate tissue. When SAM donates a methyl group it forms S-adenosylhomocysteine that is transformed to homocysteine. In order to recycle methionine, choline is first converted into betaine, and then betine reacts with homocysteine to produce methionine and dimethylglycine in the presence of betaine-homocysteine methyltransferase (BHMT). In this step of metabolism, betaine donates a methyl group, forming dimthylglycine. Therefore, choline or betaine can reduce the amount of methionine required for methylation by providing the methyl groups for the regeneration of methionine from homocysteine. Because the physiological need for methionine often exceeds its availability in the diet, the methionine-sparing effect of the transmethylation cycle is very important (Krebs et al. 1976; Davies et al. 1992).

In the present experiment, weight gain of fish fed diets with deplete methionine increased as choline levels in the diets increased (Figure 2). Weight gain of fish fed diet 5 with deplete methionine and replete choline was not significantly different from fish fed diet 6 with sufficient methionine and choline, but significantly higher than fish fed diet 1 with deplete methionine and choline (Table 6). These results indicated that in the absence sufficient methionine, choline can spare a portion of methionine requirement of channel

Figure 1. Metabolism of methionine, choline and betaine (Mehler, 1986). Numerals indicate the following enzymes:(1) methionine adenosyltransferase; (2) various enzymes; (3) S-adenosylhomocysteine hydrolase; (4) choline dehydrogenase; (5) betaine-homocysteine methyltransferase.

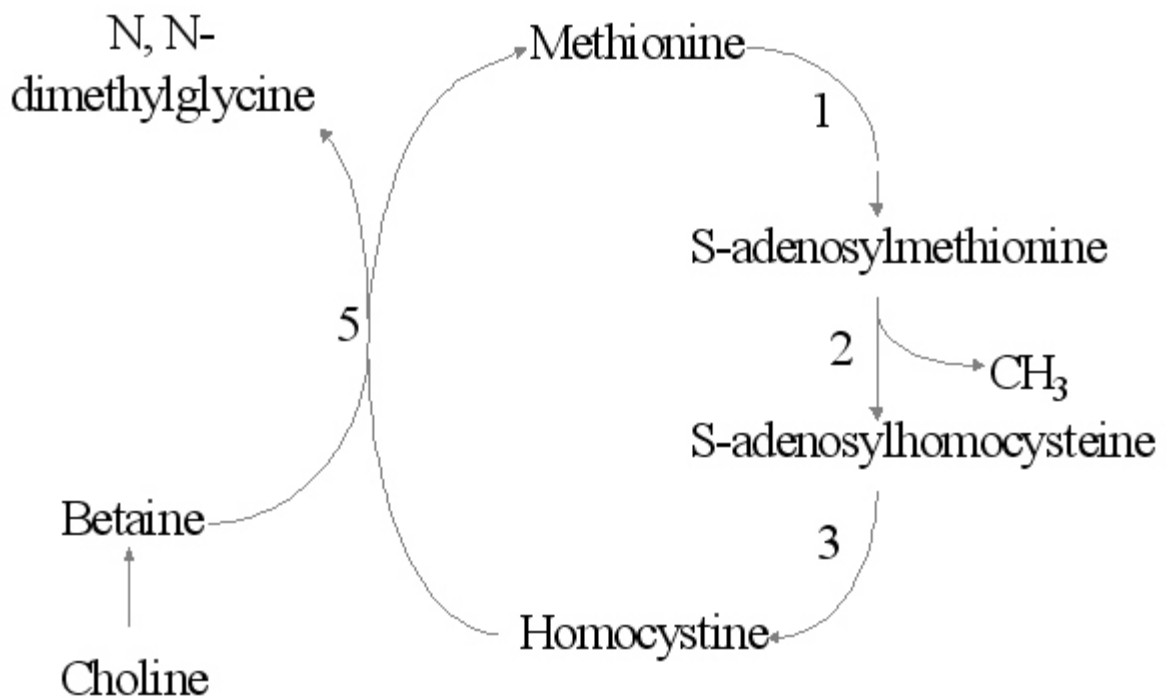
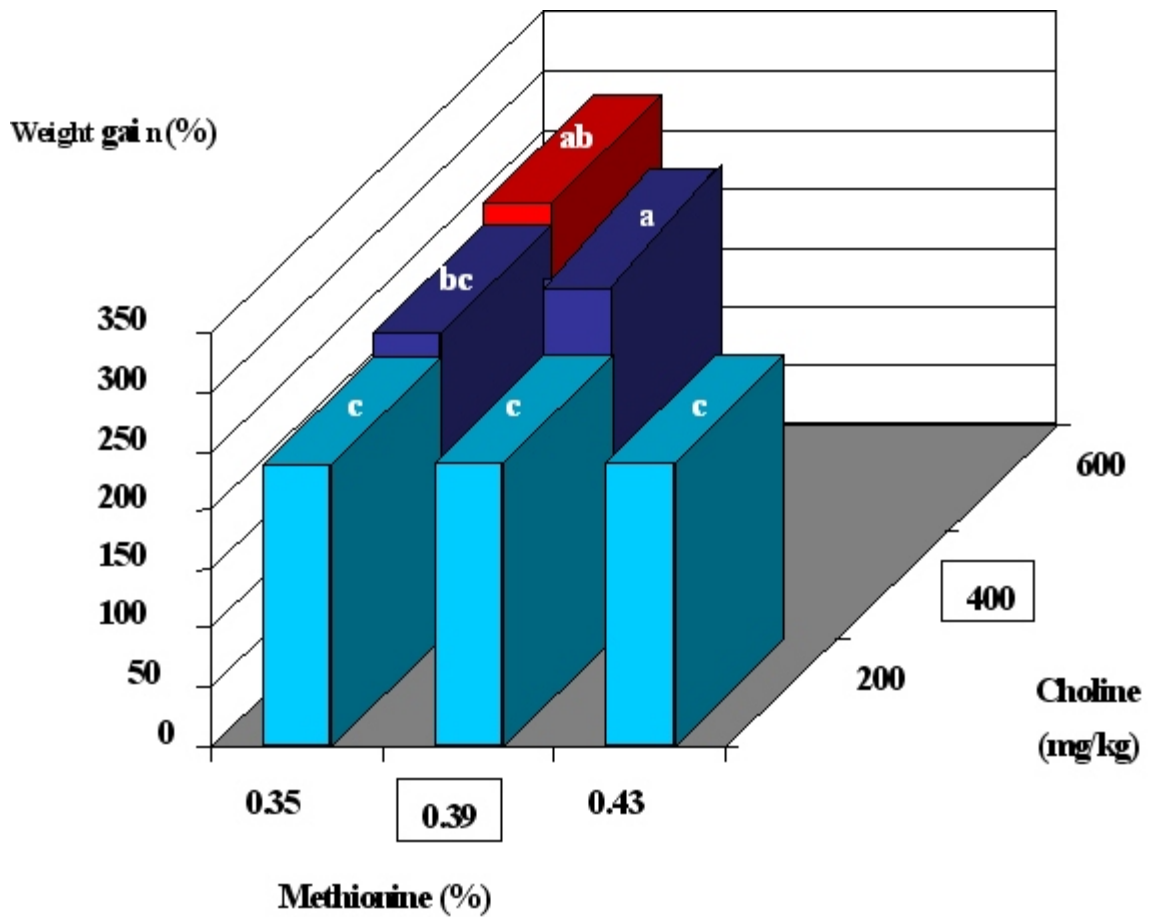


Figure 2. Effect of methionine and choline on weight gain of channel catfish. Values of weight gain are means of three replicates. Means in the columns with different superscripts were significantly different ($p < 0.05$).



catfish fed semi-purified diets. Choline have been shown to be capable of sparing methionine in the diet of chicks (Klose and Almquist, 1941; Gerry et al., 1948; Quillin et al., 1961; Pesti et al., 1980 and Barker et al., 1982). The studies in metabolism of methionine choline, and betaine , and the research in relationship between choline and methionine supported our conclusion that choline can spare a portion of methionine requirement of catfish.

In major steps of choline metabolism (Figure 1), choline has to first be converted to betaine in order for its methyl groups to be available in cellular reactions which require methyl groups. Oxidation of choline to betaine is considered one-way reaction (Scott, 1986; Sheard and Zeisel, 1989). Because choline can spare a portion of methionine requirement of catfish, betaine can also spare a portion of methionine requirement of catfish in the absence of sufficient dietary methionine. Similar to our results in catfish, Virtanen and Rossi (1995) reported that betaine might have a methionine sparing effect in chicks.

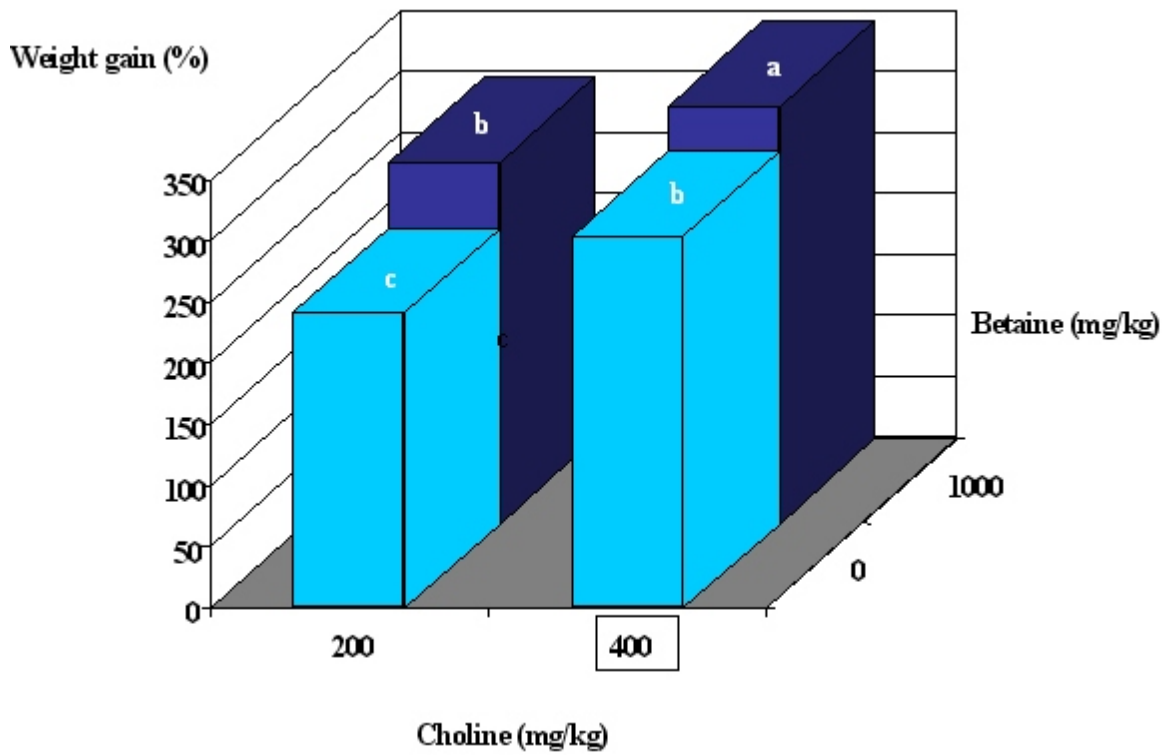
In the present experiment, weight gain of fish fed diets with deplete choline did not increase as methionine levels in the diets increased (Figure 2). Weight gain and feed consumption of fish fed diet 4 with replete methionine and deplete choline were not significantly different from fish fed diet 1 with deplete methionine and choline, but significantly lower than fish fed diet 6 with sufficient methionine and choline (Table 6). These results indicated that in the absence of sufficient choline, methionine can not spare choline requirement of channel catfish fed semi-purified diets. Jukes (1945) reported that because chicks appeared to be unable to methylate ethanolamine, chicks can not utilize methionine to spare choline unless monomethylethanolamine or dimethylethanolamine is

present. Similarly, Ketola (1976) and Rumsey (1991) concluded that because rainbow trout and lake trout can not methylate ethanolamine to methylethanolamine, choline can not be spared by methionine in fish. These studies supported our conclusion that methionine can not spare choline in channel catfish.

In the present experiment, weight gain in fish fed diets with deplete choline increased as betaine levels in the diets increased (Figure 3). Weight gain, feed consumption, and FCR of fish fed diet 7 with deplete choline and excess betaine were not significantly different from fish fed diet 6 with sufficient choline and no betaine, but significantly higher than fish fed diet 2 with deplete choline and no betaine (Table 7). These results indicated that in the absence of sufficient choline, betaine can spare at least a portion of choline requirement of channel catfish fed semi-purified diets. Rumsey (1991) concluded that half of the choline requirement of rainbow trout can be spared by betaine. Kasper *et al.* (2002) found that the dietary betaine can spare the entire choline requirement of juvenile tilapia fed chemically purified diet. These studies strongly support our conclusions that choline can be spared by betaine in catfish.

As graphically represented in Figure 3, we found that weight gain of fish fed diet 8 with sufficient choline and excess betaine was significantly higher than fish fed diet 6 with sufficient choline and no betaine. In addition to functioning as a methyl donor, betaine is known as flavor attractant and osmolyte for some species of fish and crustacean. Kasper *et al.* (2002) reported that a significant increase in feed consumption was used to evaluate betaine as a flavor additive. However, the function of betaine as flavor additive was not the focus of the present experiment. The feeding rate was restricted to approximate 4 % body

Figure 3. Effect of choline and betaine on weight gain of channel catfish. Values of weight gain are means of three replicates. Means in the columns with different superscripts were significantly different ($p < 0.05$).



weight of fish, and all 8 diets were observed to be eaten by fish within 3 minutes in the present study. The function of betaine as a flavor additive was restricted in the present study. Seasalt was added several times to the semi-closed recirculating system to maintain a low level of chloride ions in the present experiment. Because betaine can reduce osmoregulation stress in fish (Virtanen et al., 1989; Clarke et al., 1994), higher weight gain of fish fed diet 8 with excess betaine could be attributed to betaine's function as an osmolyte.

Based on results of the present study, we concluded that in the absence of sufficient methionine, choline or betaine can spare a portion of methionine requirement of channel catfish. Further, in the absence of sufficient choline, methionine can not spare choline requirement of channel catfish. Further, in the absence of sufficient choline, betaine can spare at least a portion of choline requirement of channel catfish.

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