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Response of common carp (*Cyprinus carpio* L.) to supplemental DL-methionine and different feeding strategies ☆

L.C. Nwanna [a], A. Lemme [c], Abdallah Metwally [b,d], F.J. Schwarz [b,*]

[a] Department of Fisheries & Wildlife, Fed. Univ. of Technology PMB 704 Akure, Nigeria
[b] Department of Animal Sciences, Technical University Munich Freising Germany
[c] Evonik Industries, Health & Nutrition–Animal Nutrition Services, Rodenbacher Chaussee 4, 63457 Hanau, Germany
[d] Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Zagazig University, Egypt

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ABSTRACT

The common carp (*Cyprinus carpio*) is an agastric fish, meaning absorption of nutrients is highly dependent on the rate of feed passage in the gut. Two diets and 3 feeding strategies were used in a 2×3 factorial experiment, conducted to determine whether utilization of supplemental DL-methionine (DL-Met) interacts with feeding strategies. Diets 1 and 2 contained, respectively, no Met supplementation (Met deficient diet with Met level of 0.45% Met in DM) and supplemented with DL-Met (Met balanced diet with Met level of 0.86% Met in DM). The three feeding strategies included, hand feeding twice daily to apparent satiation (feeding strategy 1), pair feeding the same amount of feed as in strategy 1 continuously by means of an automatic feeder over 2×6 h periods (feeding strategy 2), and continuously feeding a fixed rate of 1.4% BW using an automatic feeder (feeding strategy 3). The fish, with a mean initial weight of 239 g, were fed for 82 days in a recirculation system. The results indicated that supplementation of DL-Met in the diet improved ($P<0.05$) final weight(483 vs. 424 g), daily weight gain, feed consumption, feed efficiency (1.60 vs. 1.92 g DM weight gain^{-1}) and protein digestibility (90.7 vs. 87.9%). Supplemented DL-Met also resulted in increased carcass protein and reduced carcass fat. Plasma levels of methionine and arginine were higher with supplemented dietary DL-Met, while plasma levels of lysine and serine were lower ($P<0.05$). Feeding strategy also affected performance and except for feed efficiency no interactions with dietary methionine supply were detected. Compared to feeding strategy 1 feeding strategies 2 and 3 improved ($P<0.05$) protein digestibility, whereas only feeding strategy 3 increased organic matter digestibility. Continuous feeding (strategies 2 and 3) increased ($P<0.05$) final weight, daily weight gain and improved feed efficiency. The positive impact of continuous feeding (strategies 2 and 3) compared to twice daily feeding was more pronounced with Diet 1 indicating that also at low feeding frequency supplemental DL-Met is effectively utilized for growth. Feeding strategy 3 was accompanied with a lower feed consumption in comparison to feeding strategies 1 and 2. Feeding strategy did not influence the chemical composition of the carp. The study established that DL-Met can be an effective nutrient for carp, that feeding frequency impacts nutrient utilization and that this effect is not restricted to DL-Met supplemented diets.

1. Introduction

For sustainable fish nutrition, more knowledge regarding the protein supply of the feed is required. Protein is generally the most abundant macronutrient in fish feed formulations. When reducing the protein content for e.g. ecological or economical reasons, the amino acid pattern nevertheless has to correspond to the amino acid requirement of the fish species and its growth stage. Additionally, the exchange of fishmeal for different plant proteins often results in a deficient supply of specific amino acids, thus requiring amino acid supplementation. Crystalline amino acids can be the "key components of cost-effective fish-feed formulations" (NRC, 2011). Lysine and methionine are generally the first limiting amino acids in aqua feeds (NRC, 2011). Methionine (Met) in particular is in low supply from plant protein sources such as soybean meal, peanut meal and copra meal (Mai et al., 2006), which makes its supplementation increasingly important for modern aqua feed formulation. Met is commonly supplemented as dry DL-methionine (DL-Met; 99% pure) (Chattopadhyay et al., 2006), where the D-isomer is probably used with a similar efficiency as the L-isomer (NRC, 2011).

Aquaculture nutritionists often doubt the effectiveness of supplemental amino acids compared to protein bound amino acids. This is

☆ Name of Institution where the work was carried out: Department of Animal, Sciences, Technische Universität München, Liesel-Beckmann-Strasse 6, D-85350 Freising-Weihenstephan Germany.

* Corresponding author at: Department of Animal Sciences, Technische Universität München, Liesel-Beckmann-Str. 6, D-85350 Freising-Weihenstephan Germany. Tel.: +49 8161 713696; fax: +49 8161 715367.
E-mail addresses: drlel@yahoo.com (LC. Nwanna), schwarzf@wzw.tum.de (FJ. Schwarz).

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because free amino acids are absorbed faster which may result in an unbalanced amino acid profile in the blood and at the sites of protein synthesis. This might affect amino acid metabolism to a great extent when feeding fish to apparent satiation once or twice daily than for continuous feeding (Ambardekar et al., 2009; Schuhmacher et al., 1997). Similarly, it is hypothesized that supplemental amino acids are partly excreted through the gill, kidney or leached into the water directly from the feed, reducing its efficient utilization by fishes (Li et al., 1999; Yamada and Yone, 1986; Zarate and Lovell, 1997). Another factor might be the degradation of the free amino acids by enterocytes and/or microflora in the gastrointestinal tract (Wu, 1998).

Cyprinids are very often involved in this critical discussion regarding the effectiveness of diet crystalline amino acids. Common carp is an agastric fish with no acidic hydrolysis of protein. Depending on the residence time of the feed in the gut, digestibility of protein and absorption of the amino acids may be very much dependent on feeding regiment. This study was therefore designed in a two-factorial to determine whether supplemental crystalline DL-Met improves the performance of common carp and whether feeding strategies, including those that prolong the residence of feed in the gut, can influence the utilization of supplemental DL-Met and therefore interact with the crystalline amino acid.

2. Materials and methods

2.1. Experimental diets

Two diets were formulated to contain 0.45% (Diet 1) and 0.86% (Diet 2) methionine on a dry matter (DM) basis, while maintaining cysteine at 0.40% in DM for both diets. The dietary composition (Table 1) was similar to those used by Schwarz et al. (1998) and mainly based on peas, soybean protein, gelatin and wheat starch. The basal diet (Diet 1) was deficient in methionine and was supplemented with 0.40% MetAMINO® (DL-methionine, Evonik Industries AG, Hanau, Germany) to obtain a methionine balanced diet according to Schwarz et al. (1998) and NRC (2011). Dietary ingredients were mixed in dry form and pelleted at 60 °C, using a 3 mm pellet die. Chromic oxide was added as an inert marker for nutrient digestibility determination. Diets were sieved in order to ensure that only pellets were fed. The pelleted feeds were stored in the cooling room at 6 °C throughout the feeding period. The experimental diets were analyzed for crude ash, crude protein, crude fiber and crude fat according the methods of AOAC (1995), while the amino acid contents were analyzed according to Llames and Fontaine (1994). In brief, dietary amino acid concentrations were determined by ion-exchange chromatography with postcolumn derivatization with ninhydrin. Amino acids were oxidized with performic acid, which was neutralized with Na metabisulfite (Llames and Fontaine, 1994; Commission Directive, 1998). Amino acids were liberated from the protein by hydrolysis with 6 N HCl for 24 h at 110 °C and quantified with the internal standard by measuring the absorption of reaction products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110 °C (Commission Directive, 2000). Tyrosine was not determined. Supplemented methionine was determined after prior extraction with 0.1 N HCl (Commission Directive, 1998).

Table 1
Gross and analyzed composition of experimental diets (g kg^{-1} DM).

Treatment	Diet 1	Diet 2
	Diet not supplemented with DL-Met	Diet supplemented with DL-Met
Ingredients		
Peas	372	372
Isolated soybean protein	221	221
Gelatin	100	100
Wheat starch	200	196
Fish meal	60.0	60.0
Vitamin–mineral premix[a]	45.0	45.0
Chromic oxide	2.00	2.00
DL-methionine	0.00	4.00
Analyzed composition (% DM)		
Dry matter (%)	92.7	92.6
Crude ash	5.22	5.31
Crude fiber	3.51	3.52
Crude lipid	6.77	7.12
Crude protein	39.4	39.9

[a] kg^{-1} diet: Vit. A 8050 IU, Vit. $D_3$2100 IU, Vit. E 300 mg, Vit. K_3 14 mg, Vit. C 294 mg, Vit. B_1 19.6 mg, Vit. B_2 30.1 mg, Vit. B_6 14.7 mg, Vit. B_{12} 0.02 mg, Ca-D-pantothenate 56 mg, Nicotinic acid 100.1 mg, Folic acid 5 mg, Inositol 400 mg, Cholinchloride 1000 mg, Biotin 1.0 mg, $NaH_2PO_4.H_2O$ 13.33 g, NaCl 0.66 g, $NaSO_4.10H_2O$ 1.67 g, $CaCO_3$ 4.39 g, $MgSO_4$ 1.76 g, $MgCl_2.6H_2O$ 1.05, KCl 3.1, $ZnSO_4$ 202 mg, $MnSO_4$ 43.1 mg, $CuSO_4$ 18.5 mg, KI 1.3 mg, $Na_2SeO_3.5H_2O$ 1.0 mg in all diets mixed with maize starch.

2.2. Growth experiment and feeding strategy

Two hundred and sixty common carp (*Cyprinus carpio* L) juveniles were purchased from a private fish farm in Southern Germany. The fish were acclimatized for about five months in twenty aquaria before the study started. Each aquarium contained 300 L of water which was supplied through recirculation systems with filtration processes. The system allowed for a complete (theoretical) water turn over in individual aquaria within 24 h. During the course of the experiment, the water temperature was maintained at 21.7 ± 0.7 °C, and the water was aerated using air-stone diffusers, which were adjusted to give dissolved oxygen levels of 6.5 ± 0.9 mg L^{-1} and pH of 7.6 ± 0.1. The photoperiod was conditioned for a period of 16 h of fluorescent light daily. Detachable glass tanks (large beakers) were fixed to each of the experimental aquaria for feces collection. The design was developed by Schwarz and Kirchgeßner (1982) and Kürzinger et al. (1986).

At the start of the experiment, all fish were weighed individually and 180 fish with an average weight of 239 ± 7 g were randomly distributed into 20 aquaria. Each aquarium contained 9 fishes. Half of the aquaria were fed the Met-deficient diet (Diet 1), whereas the other half received the diet supplemented with DL-Met (Diet 2). Three feeding strategies were applied. Feeding strategy 1 was assigned to eight aquaria (4 replicates per treatment), while feeding strategies 2 and 3 were assigned to six aquaria each (3 replicates per treatment). In strategy 1 carp were fed twice daily (8 am; 5 pm) by hand until apparent satiation(twice fd. by hand). The length of feeding time per meal was about 20 to 30 min each. Feed quantities were recorded in order to feed the same mean quantities of each treatment to carp over 2 × 6 h periods (continuously) by means of automatic band-feeders on the following day (strategy 2; pair fd., cont.). As shown in Table 4, feed consumption of carp of strategy 2 was similar to that of strategy 1 confirming a successful pair-feeding approach. In strategy 3 carp were fed at a rate of 1.4% of BW/d and feed was also provided by automatic feeders (2 × 6 h 1.4% of BW cont.). This amount of feed was adjusted fortnightly for each aquarium depending on the mean BW of the fish and an estimated growth rate for the next two weeks. Continuously fed carp (feeding strategies 2 and 3) were fed daily from 8 am to 2 pm and from 5 to 11 pm. Fish were fed six days per week (without Sunday). Every two weeks all carp were weighed on Monday morning before feeding. The experimental period lasted for 82 days including 70 feeding days. The growth performance of the fish was assessed using the following parameters: mean weight gain = final mean weight − initial mean weight); feed efficiency = weight of feed fed (dry wt.) (g)/fish weight gain (wet wt.).

2.3. Digestibility measurement

The diets contained 0.2% of chromic oxide (Cr_2O_3) as an indigestible marker. Feces were collected for 5 days after 65 days of the experiment