UNIVERSIDAD DE ANTIOQUIA 1803

Revista Colombiana de Ciencias Pecuarias

Indispensable amino acid deprivation does not cause rapid amino acid depletion in fish body and this principle has a potential to be used as the new strategy in nutrition

La deprivación de aminoácidos indispensables no causa depleción rápida de aminoácidos en peces y este principio tiene aplicación potencial como nueva estrategia de nutrición

Konrad Dabrowski[1]; Yongfang Zhang[1,2]; Murat Arslan[1,3]; Bendik Fyhn Terjesen[1,4]

[1]School of Natural Resources, Ohio State University, Columbus, OH 43210, USA
[2]Ohio State University Interdisciplinary Program in Nutrition, Columbus, OH 43210, USA
[3]Ataturk University, Ispir, Turkey
[4]AKVAFORSK, Institute of Aquaculture Research AS, N-6600 Sunndalsøra, Norway

Introduction

Degradation of body proteins occurs not only during fasting but it is a continuous process of renewal of proteins, including those degraded proteosomally immediately after synthesis which apparently accounts for 30% of all newly formed (18), as well as those "storage" proteins that are hydrolyzed at much slower rate by cytoplasmic proteases. If this concept is applied to acute deficiency in indispensable amino acids in food, then such imbalances may in fact become imperfections physiologically overridden by continuous flow of amino acids returned to synthesis sites.

Studies conducted in animals have shown that an amino acid imbalanced diet reduced feed intake (10, 16) and growth of animals (5, 8). In addition, studies have also demonstrated that rats could recover from such deficiency when fed with feed containing the specific IDAA they were lacking (12). Recently, Hao *et al*, (2005) argued that mammals recognize dietary IDAA deficiency in the brain's anterior cortex that is signaling diet rejection (7). However, there was no information available on how IDAA deficient diets affect fish feed intake, feed utilization and differences in feed acceptance in a short term (within hours) and during a long period (within several weeks) until recently. We presented evidence that fish may in fact differ from a mammalian model, and a generalization over the anorectic response in the case of lacking IDAA did not apply (3).

Amino acid balanced diets in monogastric animals must be composed of various protein sources to provide the right proportion of all IDAA. Compared to fish meal, the major feed protein sources, such as plant proteins and meat and bone meal are deficient in methionine, whereas oilseeds are deficient in lysine. Therefore, these ingredients may not be used as the sole protein source in formulated, high-protein content (45-55%) diets. Under practical farming conditions, feed formulations that include plant proteins may result in disproportionate amounts of IDAA and IDAA deficiencies. IDAA deficient diets affected animals adversely, including depression of food intake and growth, change of feed intake behavior, development of lesions and result in low survival rates (8). Previous studies showed that feed intake of rats decreased significantly after they were fed a threonine deficient diet in comparison to control group (10, 11). The level of plasma histidine and threonine decreased rapidly after ingesting histidine or threonine deficient diets respectively, while the level of plasma total IDAA other than histidine and threonine increased significantly. There was no significant change in the plasma dispensable amino acid (DAA) concentrations after rats consumed histidine or threonine deficient diets. Liver and muscle histidine or threonine concentrations decreased after rats were fed histidine or threonine imbalanced diets (14). Feurte *et al*, (1999) also reported that plasma threonine concentrations significantly decreased between 30 to 60 minutes after rats ingested a threonine devoid diet (4).

The mechanism of interdependence of behavioral response (food rejection) and physiological indicators (concentration of IDAA in tissue) is being addressed systematically in mammals. Studies showed that a threonine-imbalanced diet significantly decreased threonine concentrations in the anterior piriform cortex (APC) of the brain (5). This correlated with rats rapidly rejecting IDAA deficient diet i.e. within 15 min after ingestion of threonine-imbalanced diet. Recent studies by Koehnle *et al* (2003) confirmed that rats recognized threonine deficient diet within the first meal and reduced the first meal duration and rejected the threonine deficient diet within 12-16 min (9). It has been long recognized, however, that animals as ancient as spiders (6) optimize proportions of IDAA in their diets by adapting specific strategy of nutritional polyphagy. By diversification of prey (protein and amino acid composition), predatory insects arrive at selecting nutrients not only by quantity but also most importantly by quality (13).

Weekes *et al* (2006) suggested an alternative method of induced amino acid imbalance in mammals by abomasal infusion of free amino acid solution lacking one or more indispensable amino acids (20). This is the first attempt to our knowledge that defined response in blood amino acid following prolonged deficiency in healthy animal. However, using fish model may prove to provide enormous advantage in respect to opportunity to follow the dietary impact on the "whole animal level", whole body free amino acid concentrations.

Questions

1) ¿Will withdrawal of the set of 5 indispensable amino acids (out of 10 IDAA) from fish diets, named here either (-)Arg (deficiency in Arg, Thr, Val, Leu and Met) or (-)Lys diets (deficiency in Lys, His, Ile, Phe and Trp), result in corresponding decrease or traceable amount of these amino acids in fish body?
2) ¿Will "switching" (or mixing) of (-)Lys and (-)Arg diets in a series of separate meals allow complete recovery of FAA in fish body or "compensatory" response, i.e. higher levels of FAA than in control group of fish fed a balanced diet?

Materials and methods

Two experiments were conducted in our laboratory to examine the effects of consuming IDAA-imbalanced diets on fish (2). A cichlid fish from Lake Nicaragua, midas (*Amphilophus citrinellum*) was used as experimental animal. Midas is well known for its great diversity of trophic adaptations (1) and is widely used in ornamental fish industry. Four diets were used in the first experiment: a) casein-gelatin basal diet, b) free amino acid (FAA)-based diet, c) (-)Lys diet with deficiency in Lys, His, Ile, Phe and Trp, and d) (-)Arg diet with deficiency in Arg, Thr, Val, Leu and Met. Amino acids listed as "deficient" were completely withdrawn from the amino acid mixture (17) and only marginal amounts were provided as components of casein-gelatin (4.5%). Each dietary treatment had three replicates (tanks) with 100 juvenile midas per tank replicate. Fish were fed four times per day at two hours interval. In the second experiment, the following diets were used: a) FAA-based balanced diet, AND b) FAA-based imbalanced diet ((-)Arg and (-)Lys) with different "feeding strategies". The frequency and interval of feeding were the same as in the first experiment. In treatment 1 (FAA group), fish were fed FAA-based balanced diet each day throughout the experiment. In treatment 2 [(-)Lys(-)Arg group], fish were fed 4 meals of (-)Lys, (-)Arg, (-)Lys, and (-)Arg diets each day throughout the experiment. In treatment 3 [(-)Lys(-)Lys group], fish were fed 2 meals of the (-)Lys diet as first two meals in the morning and then 2 meals of the (-)Arg diet in the afternoon. And in treatment 4 [(-)Lys/(-)Arg group], fish were fed four meals of the (-)Lys diet and the (-)Arg diet on alternate days.

Results

Our feeding experiment 1 showed that the feed intake of fish when fed to satiation did not show significant differences on day 1 and 2 among a protein-based, FAA, (-)Lys and (-)Arg groups (2). In experiment 2, the feed intake of midas on day 1 and day 2 in the FAA group was significantly higher than that of (-)Lys(-)Arg group, (-)Lys(-)Lys group and (-)Lys/(-)Arg group (2). On day 31, the feed intake of midas in the FAA group was significantly lower than (-)Lys(-)Arg group, (-)Lys (-)Lys group and (-)Lys/(-)Arg group. In summary, our results indicated that midas did not decrease feed intake when fed with an amino acid imbalanced diet compared with amino acid balanced diet. Secondly, fish increased their feed intake of amino acid imbalanced diets although they had lower weight gain and lower feed conversion rates. The most significant finding was that the weight gains improved significantly over the duration of the experiment when imbalanced/ complimentary diets were offered.

Free amino acids in the whole body of midas in experiment 1, analyzed according to Terjesen *et al* (2004) (19) and Dabrowski *et al* (2005) (3), increased significantly from immediately after the meal (35 min) to 6 h after the feeding, in major part due to DAA concentrations increase, whereas IDAA either decreased (in control, FAA-based diet fed fish, from 8.45 to 6.87 mM/kg) or remained essentially the same (in protein-based diet fed fish, 3.0 mM/kg). These findings strongly suggest that fish were able to maintain a "nutritional composure" and continue to send the signal to the neural receptors that no major change in substrates for protein synthesis occurred. That would explain why, in comparison to mammals, the rejection of the following meals of the imbalanced diet did not take place.

Discussion

We were able to demonstrate that in the teleost cichlid fish the anorectic response does not occur when the IDAA imbalance diet was offered. It remains to be answered if this phenomenon is characteristic for other teleosts and if there is an ontogentic component involved in anorectic responses to IDAA lacking diets. If confirmed, that imbalanced/ complimentary diets feeding strategy results in up-regulation of dietary amino acid utilization and consequently enhanced growth rates of fish (see Figure 1), then this strategy may have direct implications in feeding fish. It is important to realize that in many respects this strategy may follow diurnal variation in "natural" diet (see Figure 2). Most of the fish are opportunistic feeders/predators and this will eliminate the element of "monotony" as compared to feeding with pelleted, homogeneous diet composition, in the current practices.

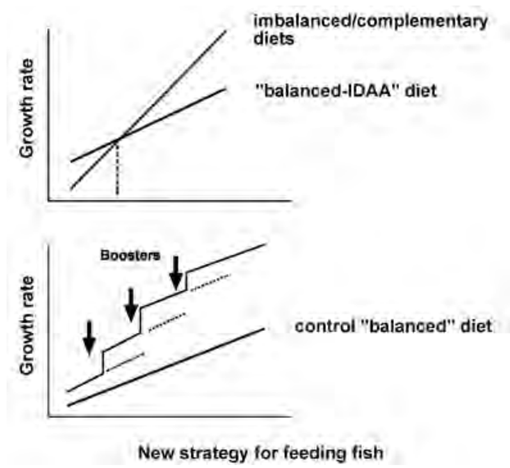

Figure 1. New strategies in feeding imbalance/complimentary diets to fish that are characterized by no anorectic response to deficiencies of IDAA.

Figure 2. Natural strategy in fish feeding in the wild depicts the fact of frequent encounter with dietary items that are nutritionally deficient. As the sum of several meals per day, nutritionally complete ration (drawing by Kornelia Dabrowska).

It appears that midas response to (-)Lys or (-)Arg diets was similar to cats (15), and fish do not have neurological receptors of dietary IDAA deficiency. However, feed intake of fish in response to IDAA deficient diets is markedly different from rats. Evidence, that the IDAA utilization (via changes in V_{max} or K_m processes) efficiency increased during dietary amino acid imbalances despite a decrease in substrate (AA concentration in blood) for muscle and liver protein synthesis, has been documented in dairy cows (20). Therefore, contrary to the conventionally used approach in aquaculture feeding, imbalances coupled with interchangeable diet compositions may have stimulatory effect on protein yield and fish growth. This strategy can also be used in "booster" diets (see Figure 1) where highly enriched feeds can be offered intermittently and therefore decreasing the risk of specific nutrients degradation, increasing efficiency of absorption and deposition.

Acknowledgments

This study was supported by the project from USAID Grant No. LAG-G-00-96-90015-00 through the Aquaculture Collaborative Research Support Program (ACRSP), Oregon State University, OR, USA.

References

1. Barluenga M, Meyer A. The midas cichlid species complex: incipient sympatric speciation in Nicaraguan cichlid fishes? Mol Ecology 2004; 13:2061-2076.
2. Dabrowski K, Arslan M, Terjesen BF, Zhang Y. The effect of dietary indispensable amino acid imbalances on feed intake: Is there sensing of deficiency and neural signaling present in fish? Aquaculture 2007; 268:136-142.
3. Dabrowski K, Terjesen BF, Zhang Y, Phang JM, Lee KJ. A concept of dietary dipeptides: a step to resolve the problem of amino acid availability in early life of vertebrates. J Exp Biol 2005; 208:2885-2894.
4. Feurte S, Nicolaidis S, Even PC, Tome D, Mahe S, *et al* Rapid fall in plasma threonine followed by increased intermeal interval in response to first ingestion of a threonine-devoid diet in rats. Appetite 1999; 33:329-341.
5. Gietzen DW. Neural mechanisms in the responses to amino acid deficiency. J Nutr 1993; 123:610-625.
6. Greenstone MH. Spider feeding behavior optimizes dietary essential amino acid composition. Nature 1979; 282:501-503.
7. Hao S, Sharp JW, Ross-Inta CM, McDaniel BJ, Anthony TG, *et al* Uncharged tRNA and sensing of amino acid deficiency in mammalian piriform cortex. Science 2005; 307:1776-1778.
8. Harper AE, Benevenga NJ, Wohlhueter RM. Effects of ingestion of disproportionate amounts of amino acids. Psychol Rev 1970; 50:428-558.
9. Koehnle TJ, Russell MC; Gietzen DW. Rats rapidly reject diets deficient in essential amino acids. J Nutr 2003; 133:2331-2335.
10. Koehnle TJ, Russell MC, Morin AS, Erecius LF, Gietzen DW. Diets deficient in indispensable amino acids rapidly decrease the concentration of the limiting amino acid in the anterior piriform cortex of rats. J Nutr 2004; 134:2365-2371.a
11. Koehnle TJ, Stephens AL, Gietzen DW. Threonine imbalanced diet alters first meal-microstructure in rats. Physiol Behav 2004; 81:15-21.b
12. Markison S, Thompson BL, Smith JC, Spector AC. Time course and pattern of compensatory ingestive behavioral adjustments to lysine deficiency in rats. J Nutr 2000; 130:1320-1328.
13. Mayntz DR, Raubenheimer D, Salomon M, Toft S, Simpson SJ. Nutrient-specific foraging in invertebrate predators. Science 2005; 307:111-113.
14. Peng Y, Tews JK, Harper AE. Amino acid imbalance, protein intake, and changes in rat brain amino acids. Am J Physiol 1972; 222:314-321.
15. Rogers QR, Wigle AR, Laufer A, Castellanos VH, Morris JG. Cats select for adequate methionine but not threonine. J Nutr 2004; 134(8 Suppl): 2046S-2049S.
16. Russell MC, Koehnle TJ, Barrett JA. The rapid anorectic response to a threonine imbalanced diet is decreased by injection of threonine into the anterior piriform cortex of rats. Nutr Neurosci 2003; 6:247-251.
17. Santiago CB, Lovell RT. Amino acid requirements for growth of Nile tilapia. J Nutr 1988; 118:1540-1546.
18. Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, *et al* Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature 2000; 404:770-774.
19. Terjesen BF, Park K, Tesser MB, Portella MC, Zhang Y, *et al* Lipoic acid and ascorbic acid affect plasma free amino acids selectively in the teleost fish pacu (*Piaractus mesopotamicus*). J Nutr 2004; 134:2930-2934.
20. Weekes TL, Luimes PH, Cant JP. Responses to amino acid imbalances and deficiencies in lactating dairy cows. J Dairy Sci 2006; 89:2177-2187.