Effects of dietary methionine on feed utilization, plasma amino acid profiles and gene expression in rainbow trout (Oncorhynchus mykiss) - DTU Orbit (11/08/2019)

Aquafeed formulation has evolved dramatically in response to shortages in marine raw materials, driven in part by the sustainable management of the wild stocks and an increased demand for nutrient-dense diets. Aquaculture of carnivorous species such as salmonids relies on extruded feeds with optimal protein and energy ratio to maximize the growth performance. To support the increasing demands, aquafeds contain increasing contributions of protein products from alternative origin. Plant raw materials can be suitable substitutions for fish meal, benefiting from a high availability, low cost and similar nutritive properties. The major limitation in using plant derived protein, at least when using high quality protein concentrate, is the amino acid profiles of plant protein, which differs from that of fish meal. Their inclusion in aquafeed results in a product deficient in essential amino acids (EAA) compared to dietary requirements. Supplementation with amino acids in crystalline form (CAA) is a common practice to balance the dietary amino acid profile to achieve high growth performances. However, complete substitution of fish meal using plant proteins and CAAs often results in poorer growth performances. The reason for this is often suggested to be related to difference in amino acid uptake kinetics during digestion, resulting in a temporal mismatch in amino acid availability, resulting in poorer at protein synthesis site. In addition to their role as building blocks in protein synthesis, amino acids also serve as substrates for synthesis of metabolic intermediates, and increasing evidence shows that amino acids also function as signaling factors in the regulation of intermediary metabolism and growth related pathways.

The present thesis comprises four supporting papers, based on two laboratories studies, investigating the effect of dietary amino acid level and form on i) growth performances, ii) plasma amino acid profiles and iii) on the expression of genes involved in hepatic metabolic and growth-related pathways in rainbow trout (Oncorhynchus mykiss). The results from the first study are presented in Paper I and show that the protein source itself (fish meal or plant based) does not affect the plasma EAA profiles, but rather that plasma EAA levels reflect the dietary level. Supplementation with histidine, lysine and threonine in crystalline form to a plant based diet was, on the other hand, found to result in their concentrations as well as the expression of specific hepatic genes (G6PD, PEPCK, FBPase, G6Pase, ALT1, GDH and GLS01) responded in a linear manner (P<0.05) to gradual increase of dietary crystalline methionine; and were associated with a decrease in nitrogen excretion and relative liver mass. Additionally, the dietary form of methionine significantly affected postprandial plasma methionine levels. The study further showed that the nitrogen excretion resulting from feeding an AA deficient diet was higher than for the fish meal control diet. Supplementation of the plant meal diet with crystalline amino acids tended to improved nitrogen utilization, almost equaling the results obtained for the fish meal control diet.

The relationship between dietary methionine level and form (free, coated and bound), and plasma amino acid profiles was further investigated in Paper II by applying statistical modeling to a large dataset (504 individuals and 20 variables). Using generalized additive models, it was shown that i) dietary methionine level and form explained 74 % of the variance in methionine plasma concentrations observed during digestion, and ii) that the dietary form of methionine and concomitant changes in methionine plasma concentrations significantly affected the plasma concentrations of several other essential AAs (arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine and valine). Linear models revealed a positive relationship (R2=0.9) between plasma concentrations of the three branched chain amino acids (BCAAs; isoleucine, leucine and valine) during digestion of meals differing in dietary methionine levels.

Results from Paper III showed that dietary level and form (crystalline or protein-bound) of methionine affected the expression of hepatic genes related to i) the somatotropic axis and ii) protein turnover. For this purpose seven diets were fed to juvenile rainbow trout under control condition. The diets were formulated to differ only in methionine content (ranging from 0.6 to 1.29 % dry matter), supplied either in crystalline or protein-bound form. The transcript levels of the growth hormone receptor I (GHR-I) and insulin-like growth hormone I (IGF-I) increased linearly with dietary methionine content (P<0.01), which was reflected in the overall growth performances. In addition, the expressions of four components of the somatotropic axis investigated were significantly (P<0.05) affected by dietary methionine. The apparent capacity for hepatic protein degradation decreased with increasing dietary methionine level in a more or less linear manner. In comparison, the methionine source appeared to have limited effect on the expression pattern of protein degradation enzymes. The results suggest that methionine availability, influenced by dietary content or form, modulates the expression of genes involve in the GH/IGF response and protein turnover, further affecting growth performances.

Paper IV presents the results of the effects of dietary methionine level and form (free or bound) on hepatic intermediary metabolism using the same diets as in Paper III. The diets were fed to rainbow trout for 6 weeks, followed by sampling for i) hepatic gene expressions, ii) hepatosomatic index (HSI), iii) postprandial ammonia excretion, and iv) plasma methionine levels. The expression of several genes coding for enzymes involved in lipid metabolism (FAS, G6PD, CPT1a), gluconeogenesis (FBPase) and amino acid catabolism (ALT1, GHD and GLS01) responded in a linear manner (P<0.05 to 0.005) to gradual increase of dietary crystalline methionine; and were associated with a decrease in nitrogen excretion and relative liver mass. Additionally, the dietary form of methionine significantly affected postprandial plasma methionine concentrations as well as the expression of specific hepatic genes (G6PD, PEPCK, FBPase, G6Pase, ALT1, GHD and GLS02; P <0.05). Nitrogen excretion was found to be consistently higher for fish fed diets supplied with crystalline methionine than fish fed protein-bound methionine (P <0.05). This study is the first to demonstrate that expression of several genes related to intermediary metabolism respond in a dose-response manner to increasing levels of dietary methionine, and that the dietary source of methionine affect hepatic metabolism at the transcriptional level.

Dietary methionine, possibly in concert with other amino acids, appears to be a key regulatory factor in the expression of several genes involved in growth and intermediary metabolism. Furthermore, the gene expression seem to be significantly affected by crystalline methionine supplementation, as a possible explanation to the different utilization of CAA supplemented plant based diets, compared to conventional fish meal based diets.