Optimization of tilapia nutrition by understanding the mechanistic basis of amino-acid/peptide transport in their gut

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Project award year: 2015

Three year research project

Abstract:

Tilapias are the most important fish cultured in Israel and represent one of the fastest growing aquaculture industries in the US and world-wide. Despite the growing interest in these fish, and the importance of nutrition in the production process, little research has been conducted on the relationship between ionic / acid-base regulation and fish nutrition. Ion dependent peptide and amino-acid transporters that were first discovered in mammals were subsequently also found in fish. However, not much is known about these transporters in fish and their role in the regulation of ion and amino acid transport represents a critical knowledge gap. The goals of this project are to understand the role and function of gastrointestinal tract (GIT) transporters in nutrient absorption, and develop dietary means to enhance feed utilization efficiency leading to better productivity.

Our previous work has established the infrastructure and workflows for analysis of key elements in this pathway in tilapia (*Oreochromis niloticus* and *O. mossambicus*), including pipelines for transcriptome and proteome analyses, gene expression analyses of specific intestinal transporters, validation of antibodies for transporter(s), etc. Our previous studies and preliminary results have demonstrated a strong effect of dietary mineral supplementation on tilapia growth, and an equally strong effect of water salinity on the expression of tilapia peptide and amino-acid transporters. The primary objective of the proposed research is to characterize parameters that regulate and influence the activity and function of peptide and amino-acid transporters in the tilapia GIT. For that purpose we propose to work in two parallel directions, extensive characterization of peptide transporters that have already been identified in our preliminary work, and a systems-level transcriptomic and proteomic screening. We expect that information gained in this research will provide a detailed rationale for designing novel diets for enhancing growth rate in fishes, and reducing nitrogen waste, through improved feed utilization.

Summary Sheet

Publication Summary

PubType	IS only	Joint	US only
Abstract	0	1	0
Abstract - Presentation	0	1	0
Reviewed	3	0	0

Training Summary

Trainee Type	Last Name	First Name	Institution	Country
Ph.D. Student	Con	Pazit	ARO	Israel
Ph.D. Student	Hamar	Jens	UC Davis	USA
Ph.D. Student	Larken	Root	UC Davis	USA
Ph.D. Student	Levitan	Bryn	UC Davis	USA
Ph.D. Student	Li	Johnathon	UC Davis	USA

BARD collaboration

The two PI (Cnaani and Kultz) met in July 2017 to discuss and coordinate the collaboration and the sharing of samples. Pazit Con, a graduating student from Avner Cnaani's lab went for 4 months to UC-Davis to learn cell lines manipulations and develop cells that express single PepT gene. Avner Cnaani visited in UC-Davis to follow the progress and the work of the graduate student. The two PI (Cnaani and Kultz) met again in July 2019 to discuss and summarize the results and coordinate future and ongoing research.

Tissue samples from two experiments conducted in Israel were sent to UC-Davis for proteomic analysis. Tilapia cell lines were sent from UC-Davis to Israel.

BARD Project No. IS-4800-15 R, Final Scientific Report**Optimization of tilapia nutrition by understanding the mechanistic basis of amino-acid/peptide transport in their gut**

Researchers: Avner Cnaani, Dietmar Kueltz, Sheenan Harpaz

Achievements

In a comparative salinity challenge between two tilapia species, *O. niloticus* and *O. mossambicus*, we characterized the effect of water salinity on gene expression and enzymatic activity along the gastrointestinal tract. In both species, we observed a spatial distribution along the intestine, with the two PepT1 variants in the proximal intestinal sections and PepT2 in the distal intestinal sections. This segment-specific expression of the PepT variants corresponds to the reduction in nutrient availability along the intestine, and to their biochemical properties, with PepT1 being a high capacity/low affinity transporter and PepT2 being a low capacity/high affinity transporter. A salinity-dependent increase in the PepT gene expression was previously recorded in *O. mossambicus* and a similar pattern was also seen in this study. However, *O. niloticus* showed a different response, with decreased expression of PepT1b in the anterior intestine (Chourasia et al., 2018. *Comp Biochem Physiol A Mol Integr Physiol*, 218:16-23). The salinity-related impaired growth and feed efficiency observed in *O. niloticus* may be related to this reduction in expression of the intestinal nutrient transporter. It is important to characterize and understand this system in *O. niloticus*, as this is the world's most widely cultured fish species. We have sequenced the transcriptome of these fish by Illumina sequencing and now analyze it for both, differential gene expression and allele specific expression. In addition, tissue samples from these two species, from both freshwater and saltwater, were sent from Israel to UC-Davis for proteome analysis. However, these transcriptome and proteome analyses are still in progress.

An increase in dietary NaCl content affected the mRNA levels of the peptide transporters, with higher expression in the group fed salt-enriched diet for all three transporters. Also, strong correlations were found between expression levels of V-H⁺-ATPase and peptide transporters, in fish receiving both diets. This is a further indication for the V-H⁺-ATPase role as the driving force behind the proton gradient that enable PepTs activity. Comparing the fish receiving the two different diets, those consuming dietary NaCl supplementation had both higher expression levels

of peptide transporters and higher protein digestibility values. However, within each dietary treatment, in contrast to our expectation, we found that there is a negative correlation between the PepT2 expression and protein digestibility (Hallali et al., 2018. *PLoS One*, 13:e0202351). One explanation for this phenomenon could be that in fish that are highly efficient in protein digestion, the majority of the di/tri-peptides were absorbed in the anterior and middle intestine, resulting in low concentration in the posterior intestine, which led to down-regulation of the PepT2 gene expression. Yet, under such conditions we would expect a positive correlation between PepT1 expression in the proximal intestinal sections and protein digestibility, such a correlation was not found. Other explanations might be differences in the intestinal lumen conditions that affect transporters efficiency rather than expression, or that protein digestibility relies more on free amino-acids absorption rather than on di/tri-peptide.

The larval developmental stages is a critical period in fish life, with long lasting effect on its fitness and performances. Therefore, it is important to understand the function and development of the digestive system during this period. In order to better understand the role of peptide transporters in the development of the tilapia intestine we characterized their expression levels along the larvae development period. In this analysis we found for the first time different expression pattern between PepT1a and PepT1b, implying that these two paralogs have different role, that has not yet revealed. All three PepT genes were expressed in very early developmental stage, when the larvae feed on the yolk-sac and more than a week before they can eat. Moreover, we have demonstrated that these transporters are active and absorb di-peptides in the primary gastrointestinal tract of pre-feeding tilapia larva (Con et al., 2019. *Front Physiol*, 10:808). It is not clear yet what is the role of PepT in the developing intestine, whether it is preparation for later function or actual digestion and absorbance of proteins from endogenic or microbial origin.

Pazit Con, an Israeli graduate student, traveled to UC-Davis to initiate a project aiming to differentially express the three peptide transporters in tilapia cell line. Thus, create a tool to enable the biochemical characterization of each of the three peptide transporters separately (PepT1a, PepT1b, PepT2). This TmB cell-line, originating from the bulbus arteriosus of Mozambique tilapia, doesn't endogenously express anyone of the three peptide transporters. All three peptide transporters were fully sequenced, cloned and inserted into plasmids. Tilapia's TmB cells were transfected with the plasmid. Gene expression analysis demonstrated that we created three lines, each one express different peptide transporter. Immunohistochemistry analysis demonstrated that

these transporters are indeed localized on the membrane of the transfected cells. Absorbance assay using fluorescence tagged di-peptides (AMCA) demonstrated that the transporters are active and take di-peptides into the cells. We are now calibrating a method to measure and quantify the peptide transport, in order to characterize the transporter's biochemical properties.

We have generated spectral libraries and cDIA (curated data-independent acquisition) assays utilizing curated target lists of transitions for reliable and highly precise quantitative proteomics of thousands of proteins in OmB cells and tissues of intact tilapia, include different sections of the intestine. These assays enable high-throughput systems-level quantitation of proteome dynamics in response to recombinant PepT expression in the cell lines and in response to salinity/nutritional challenges of intact fish. A dual luciferase reporter assay has been established and validated for tilapia OmB cells that allows identification of enhancer sequences by enhancer trapping. This assay are now used for identifying cis- and trans-acting elements in intestinal genes that are up- and down-regulated by salinity in *O. mossambicus* and *O. niloticus*. In addition, gradual salinity acclimation experiments have been performed with the two species under conditions where a) rate of salinity increase until reaching 90% CSmax is comparable, and b) time of acclimation until reaching 90% CSmax is comparable. The three segments of intestine have been dissected and are now being analyzed using quantitative proteomics (using the cDIA assays). Samples from the salt-enriched diet feeding experiment were sent to the lab in UC-Davis for proteomic analysis, thus, we will have all the growth and digestibility data combined with gene expression and protein expression. These analyses are still in process and expected to be published as joined paper later this year.

List of publications resulted from this grant:

- Con P, Nitzan T, Slosman T, Harpaz S, Cnaani A, 2019. Peptide transporters in the primary gastrointestinal tract of pre-feeding Mozambique tilapia larva. *Front Physiol*, 10:808.
- Hallali E, Kokou F, Chourasia TK, Nitzan T, Con P, Harpaz S, Mizrahi I, Cnaani A, 2018. Dietary salt levels affect digestibility, intestinal gene expression, and the microbiome, in Nile tilapia (*Oreochromis niloticus*). *PLoS One*, 13:e0202351.
- Chourasia TK, D'Cotta H, Baroiller J-F, Slosman T, Cnaani A, 2018. Effects of the acclimation to high salinity on intestinal ion and peptide transporters in two tilapia species differing in their salinity tolerance. *Comp Biochem Physiol A Mol Integr Physiol*, 218:16-23.

Publications for Project IS-4800-15 R

Stat us	Type	Authors	Title	Journal	Vol:pg Year	Cou n
Published	Reviewed	Con P, Nitzan T, Slosman T, Harpaz S, Cnaani A	Peptide transporters in the primary gastrointestinal tract of pre-feeding Mozambique tilapia larva	<i>Frontiers in Physiology</i>	10 : 808 2019	IS only
Published	Reviewed	Hallali E, Kokou F, Chourasia TK, Nitzan T, Con P, Harpaz S, Mizrahi I, Cnaani A	Dietary salt levels affect digestibility, intestinal gene expression, and the microbiome, in Nile tilapia (<i>Oreochromis niloticus</i>).	<i>PLoS ONE</i>	13 : e0202351 2018	IS only
Published	Reviewed	Chourasia TK, D'Cotta H, Baroiller J-F, Slosman T, Cnaani A,	Effects of the acclimation to high salinity on intestinal ion and peptide transporters in two tilapia species differing in their salinity tolerance.	<i>Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology</i>	218 : 16- 23 2018	IS only
Published	Abstract - Presentati on	L Root, D K?ltz, A Cnaani, P Con	Intestinal proteome response to salinity stress in three tilapiine spp	<i>The FASEB Journal</i>	32 (1) : 586.3 2018	Joint
Published	Abstract	LT Root, P Con, A Cnaani, D Kultz	Comparative intestinal proteome response to salinity stress in three tilapiine spp.	<i>NTEGRATIVE AND COMPARATIVE BIOLOGY</i>	58 : E408 2018	Joint