Lysozyme Transgenic Goat Milk Regulates Expression of Peptidoglycan Recognition Protein 3 and 4

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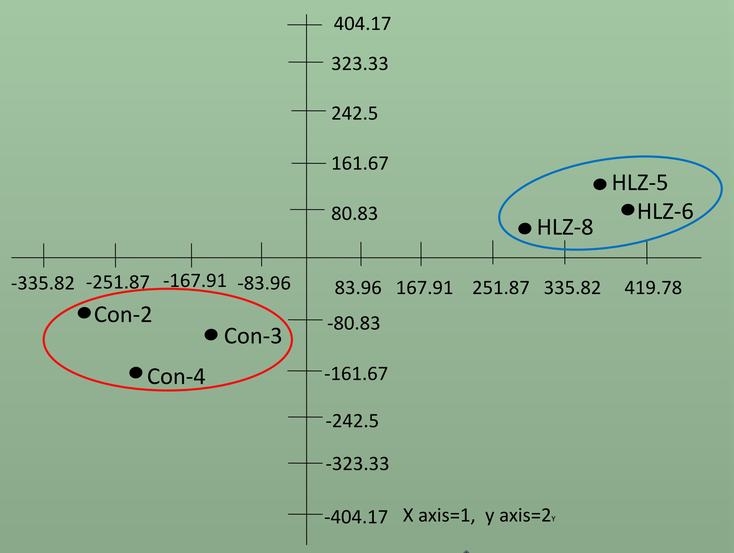
Introduction

A significant contributor to the formation of bacterial populations in the gut of mammals is host secreted immune effectors such as the antimicrobial enzyme lysozyme. Intestinal microbial imbalances lead to immune dysregulation, nutritional deficiencies and chronic inflammatory diseases, therefore the relationship between host secreted antimicrobials and maintenance of the microbiome is an important area of study. Since human milk has 1,500-3,000 times greater lysozyme levels than the milk of dairy animals, we have genetically engineered goats to produce an increased concentration of human lysozyme (hLZ) in their milk to 67% of the level of human milk. We hypothesize that hLZ-rich milk will be able to impact host intestinal microbial populations and in turn, act as a treatment/preventative agent for diarrheal illnesses. Using a pig model, consumption of hLZ milk resulted in microbiota modulation, changes to architecture of the small intestine and the alleviation of the symptoms of *E. coli*-induced diarrhea, but the mechanism by which hLZ asserts these effects has yet to be determined.

Peptidoglycan recognition proteins (PGLYRPs) are innate immune effector molecules implicated in the early establishment and maintenance of the commensal microbial populations. In humans and pigs there are four PGLYRPs with PGLYRP-3 and 4 having highest expression throughout the gastrointestinal tract. PGLYRPs modulate the immune response by binding bacteria peptiodglycan and killing them as a result of activation of their protein-sensing two-component system. Preliminary results indicated that PGLYRP-3 expression is upregulated in the presence of hLZ milk. Due to PGLYRPs function and localization, the effect of hLZ on PGLYRP-3 and 4 expression and secretion is being investigated as a mechanism by which hLZ asserts its effects. These studies are the basis of understanding how hLZ elevating PGLYRPs can help establish the microbiota in early life and potentially restore equilibrium after intestinal injury.

hLZ Goat Milk Shifts Microbiota of Pigs



Bacteroidetes — hLZ enriched biomarkers of gut health — Bifidobacteriaceae, Lactobacillaceae, Bacteroidetes

Firmicutes — hLZ reduced disease associated bacteria — Streptococcaceae, Clostridiaceae

Figure 1: Six week old pigs were fed either control or hLZ milk twice a day (n=3). At the end of two weeks feces was analyzed by 16S rRNA gene sequencing to determine bacterial populations present. Principle component analysis demonstrated significant differences between groups ($p \leq 0.05$).

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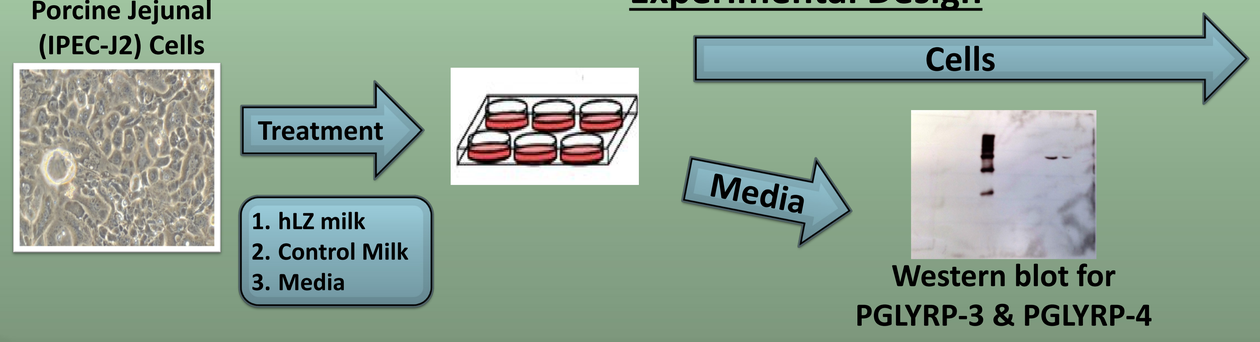
Experimental Design



RNA Seq Showed Increase in PGLYRP-3 Expression

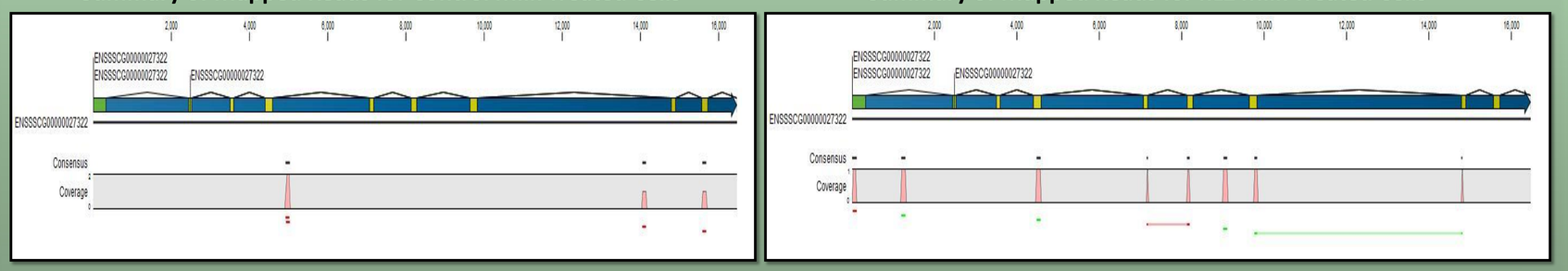


Figure 2: IPEC-J2 cells were treated with either hLZ or control milk for 8 hours after which RNA was isolated for RNA seq analysis. Results indicate a significant increase in PGLYRP-3 expression by 3.98 fold in cells treated with hLZ milk compared to those treated with control milk ($p=0.006$). Unpublished data generated by Lydia Garas.

The ability of hLZ Goat Milk to Regulate PGLYRP-3 and 4 Expression is Time Dependent

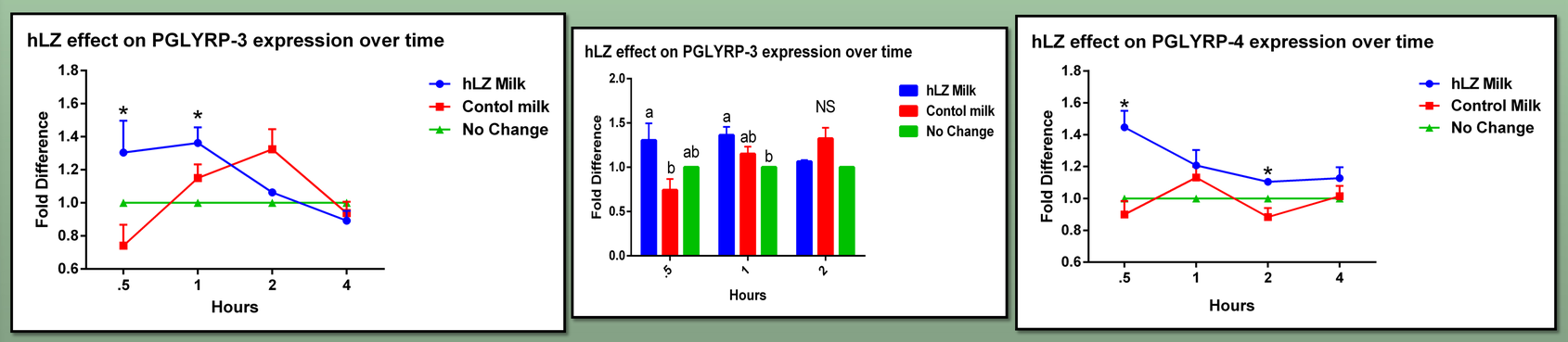


Figure 3: IPEC-J2 cells were treated with either hLZ or control milk for different periods of time. After treatment cells were harvested and qRT-PCR performed. Fold-changes in gene expression were analyzed by ANOVA and differences between groups determined by the Tukey test with $p \leq 0.05$ considered significant (*). One hour time point represents two separate experiments with n=9, half hour and two hour time points n=5. Data is presented as mean ± SEM.

Effect of Purified Lysozyme on Expression of PGLYRP-3/4

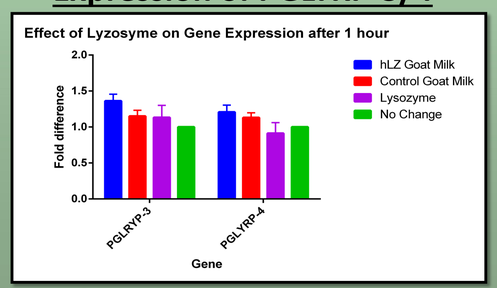


Figure 4: IPEC-J2 cells were treated with either hLZ or control milk or pure lysozyme. After treatment cells were harvested and fold-changes in gene expression were determined via qRT-PCR. Control and hLZ represent two separate experiments with n=9, lysozyme preliminary data with n=3. Data presented as mean ± SEM.

Secretion of PGLYRP-3/4

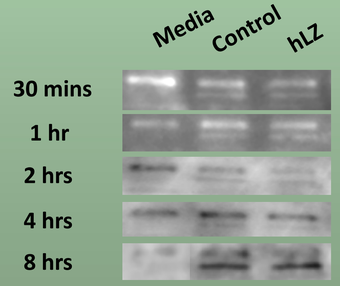


Figure 5: IPEC-J2 cells were treated with control or hLZ milk. After respective time, media was collected and western blot was preformed with an antibody that binds both PGLYRP-3 and 4.

Conclusions

- hLZ goat milk increases PGLYRP-3 and 4 expression in a time dependent manner.
- PGLYRP-3 and 4 is secreted into the media of cells treated with hLZ or control milk in very low concentrations.

Future Directions

- Determine if lysozyme's liberation of peptidoglycan from commensal bacterial species induces greater PGLYRP3/4 expression.
- Determine if there is a synergistic anti-inflammatory effect of lysozyme and PGLYRPs in response to bacterial challenge.
- Determine relationship between expression and secretion of PGLYRP3/4.