THE SOY RESEARCH TRIFECTA

WEBINAR | JULY 1 | 2:00 PM CDT

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SOY AQUACULTURE
ALLIANCE

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SOY AQUACULTURE ALLIANCE | US SOY

Trout-Grains Project USDA

SOY AQUACULTURE ALLIANCE WEBINAR

Breeding Rainbow Trout for a Changing Feed Landscape

More than two decades of selection for growth on plant-based diets

Jacob W. Bledsoe, Ph.D.
University of Idaho | Aquaculture Research Institute
Hagerman Fish Culture Experiment Station

Collaborative breeding program:
USDA Agricultural Research Service
University of Idaho

USDA United States Department of Agriculture
Agricultural Research Service

Soy Aquaculture Alliance Webinar | July 1, 2026

THE CHALLENGE

Aquafeeds changed faster than the fish

TRADITIONAL FEEDS

High reliance on marine protein

CHANGING FORMULATIONS

Greater use of soy and other plant proteins

PRODUCTION CONSTRAINTS

Growth, efficiency, intestinal health, water quality

High soybean meal inclusion challenged performance and gut health in carnivorous salmonids.

THE BREEDING IDEA

Do not only adapt the diet — adapt the fish to the diet

FEED INNOVATION

- Ingredient selection
- Processing and formulation
- Supplementation

GENETIC IMPROVEMENT

- Evaluate families on the target diet
- Select the strongest performers
- Repeat across generations

BETTER FISH — FEED COMPATIBILITY

Feed formulation and selective breeding are complementary tools.

PROGRAM HISTORY

Twenty-four years of continuous selection built the Hagerman strain

Dr. Ken Overturf

2002	EARLY YEARS	LATER YEARS	EXPANDED RESEARCH	2026
Collaborative breeding program initiated under the leadership of Dr. Ken Overturf (USDA-ARS)	Growth on fishmeal-free plant-protein diets; Continued optimization of soy-based diet formulations	Increasing soy inclusion and dietary challenge	Gut health, metabolism, immunity, nutrient utilization	Newest brood candidates selected, under common garden

OVERLAPPING YEAR CLASSES | GENERATION INTERVAL: 2 YEARS

Three-year-old males from one selected year class are crossed with females from the alternate selected year class.

Families → **Selection diet "challenge"** → **Measure growth** → **Select brood** → **Cross selected year classes**

SELECTION ENVIRONMENT

Selective pressure applied through “challenge” formulation

0%
fishmeal

49%
soybean meal + soy protein concentrate

41.3%
crude protein

19.6%
crude lipid

INGREDIENT INCLUSION (%)

Soybean meal	Soy protein concentrate	Corn protein concentrate	Other ingredients
25% SBM	24% SPC	10.23% corn protein concentrate	Other ingredients include 17% fish oil

Fishmeal-free, plant-protein selection diet

PRODUCTION RESPONSE

The growth advantage translates to about one month less time to market size on a plant-meal diet

CONTROLLED GROWTH COMPARISON ON PLANT-MEAL DIET

Market size

Commercial control

~1 month

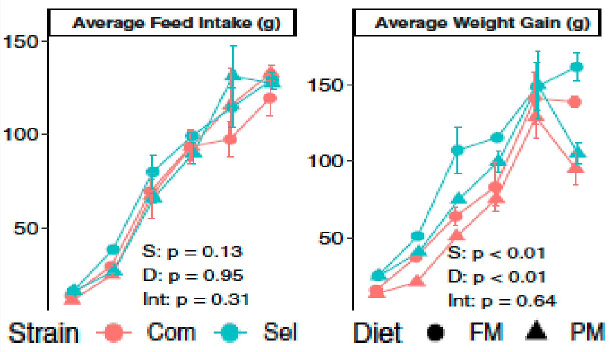
Market Size Achieved

Hagerman selected

SEVEN-MONTH PLANT-MEAL TRIAL | INITIAL WEIGHT ≈ 5 g

498 g	**388 g**
Selected strain gain	Commercial strain gain

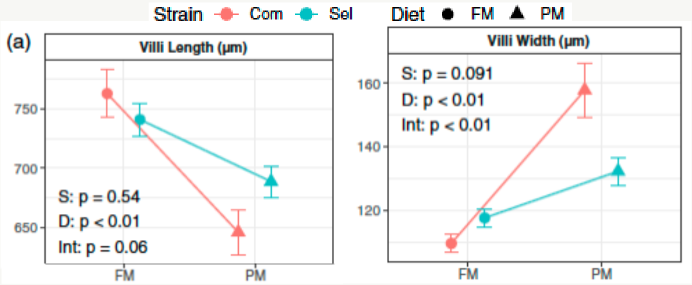
Greater growth and improved feed conversion!



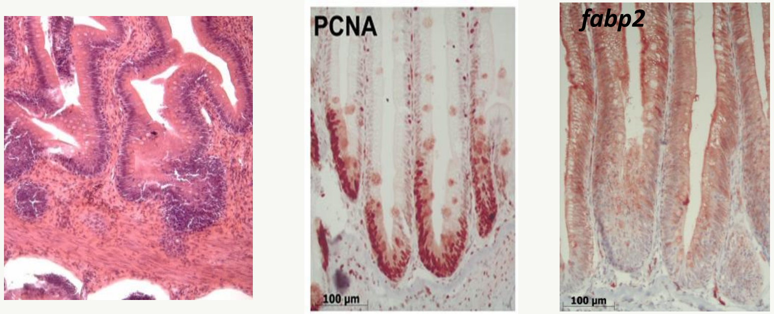
Bledsoe et al., 2025 | Controlled program comparisons; approximate market-time interpretation

INTESTINAL PHENOTYPE

Selection changed the intestinal response to a high-soy diet

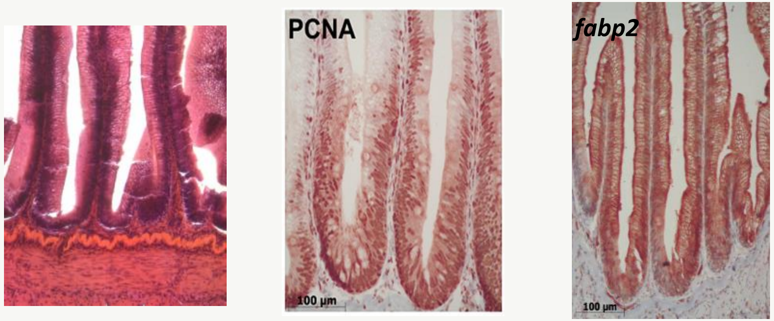


COMMERCIAL STRAIN + PLANT-MEAL DIET



- Shortened and widened villi; Lamina propria and submucosal changes
- Inflammatory-cell infiltration, reduced cellular proliferation
- Reduced nutrient absorption and transport

HAGERMAN SELECTED + SAME DIET



- Intestinal architecture maintained
- Limited inflammatory response, maintained cellular proliferation
- SBMIE-resistant phenotype under study conditions

Only the commercial strains develop characteristic SBMIE during long-term trials (≈ 6 months)!

Bledsoe et al., 2025 | Blaufuss et al., 2020

CORRELATED PHYSIOLOGICAL GAINS

Mechanistic studies reveal a more resilient physiological and immune phenotype

SUSCEPTIBLE COMMERCIAL FISH

High-soy plant-meal diet

Intestinal damage

Innate immune + Toll-like signaling

Cellular stress + DNA-damage response

Systemic inflammatory signal
Greater disease susceptibility

HAGERMAN SELECTED FISH

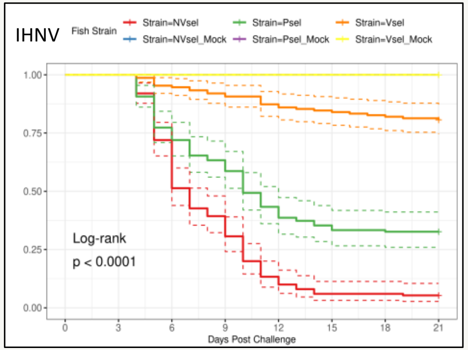
High-soy plant-meal diet

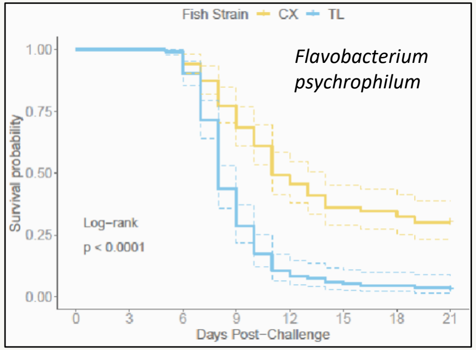
Intestinal architecture maintained

Limited inflammatory signatures

More stable physiological response
Stronger non-specific immunity

Nlrp1 Candidate plasma marker associated with SBMIE-related inflammation





Bledsoe, unpublished | Bledsoe et al., 2025

University of Idaho Aquaculture Research Institute

SOY AQUACULTURE ALLIANCE | US SOY

THE NEXT PHASE

Preserve the gains – and build a more complete performance phenotype

PRESERVE

- Maintain the closed selected population
- Retain accumulated plant-diet performance
- Continue UI-USDA-Industry collaborations

EXPAND

- Consider carefully selected external germplasm
- Traits of interest: disease, temperature, lipid metabolism, etc.
- Dietary ingredients and supplementation

REFINE

- Feed formulation and growth performance
- Non-lethal markers + digital phenotyping
- Overall production performance

Established selected population → **Broader genetics + selection traits** → **Physiology + digital phenotyping** → **Improved fish-feed performance**

Feed innovation and genetic improvement work best together!

Future aquafeeds will benefit from fish genetically equipped to utilize them.

PHYSIOLOGY-BASED RESEARCH TO UNDERSTAND SBMIE AND MAXIMIZE SOY NUTRIENT VALUE FOR FISH

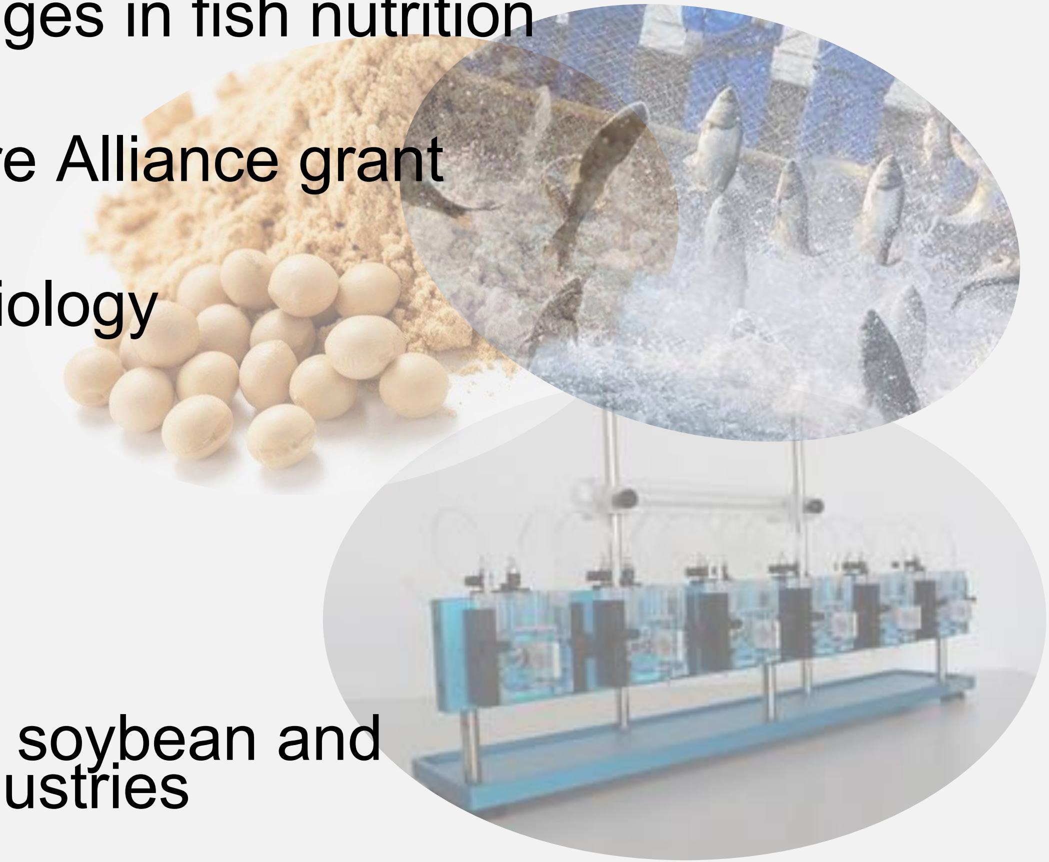
Subramaniam M.*[1], Powell M.S.[1], and Small B.C.[1]

[1]*Hagerman Fish Culture Experiment Station, Aquaculture Research Institute, University of Idaho*

University of Idaho
Aquaculture Research Institute

OUTLINE

- Current challenges in fish nutrition
- Soy Aquaculture Alliance grant
- Digestive Physiology
- Objectives
- Results
- Implications for soybean and aquaculture industries



CURRENT CHALLENGES IN FISH NUTRITION

Challenges

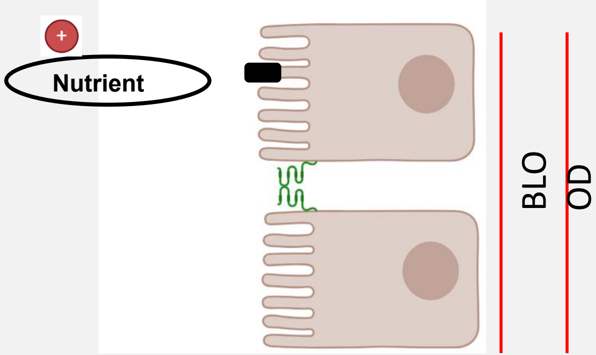
- Feed $$
- Limiting factor: Protein
- Alternative protein ingredients
  - Plants – soy
  - Insects
  - Single-cell organisms

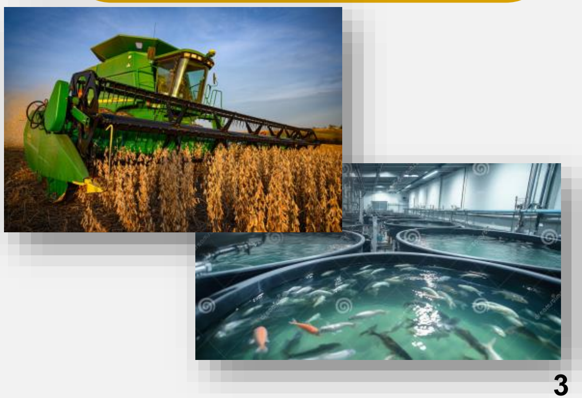
Knowledge Gaps

- How does soy affect nutrient utilization?
- Fish health and welfare?
- Effects of anti-nutritional factors?
- Effects on gut function ultimately affects growth and health of production fish

Implications

- Applications to soybean industry
- Implications for fish feed and aquaculture production
- Increase soy inclusion for salmonids





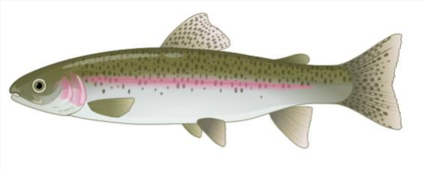
SOY AQUACULTURE ALLIANCE GRANT

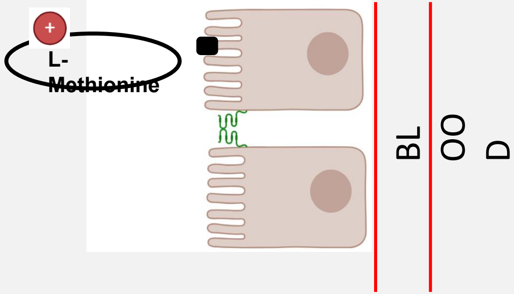
Alternative protein source = Soy-based feed ingredients

High inclusion levels for salmonids □ hinder growth and affect digestive function

Methionine is a limiting amino acid – crucial to investigate absorption in commercial fish species and its potential impedance by ANFs

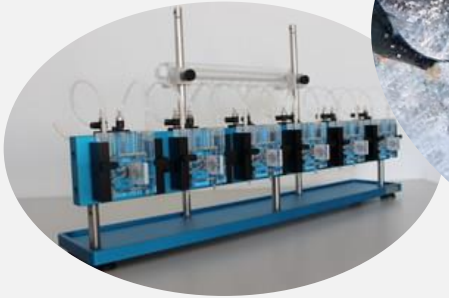
Present the first definitive molecular characterization on the impacts of SBMIE and targeted ANFs on nutrient absorption and gut barrier function in Atlantic salmon and rainbow trout



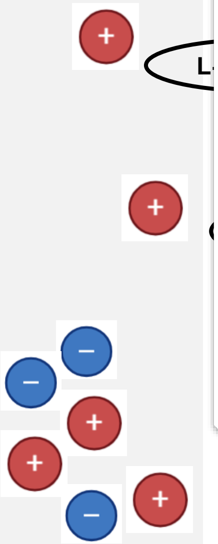


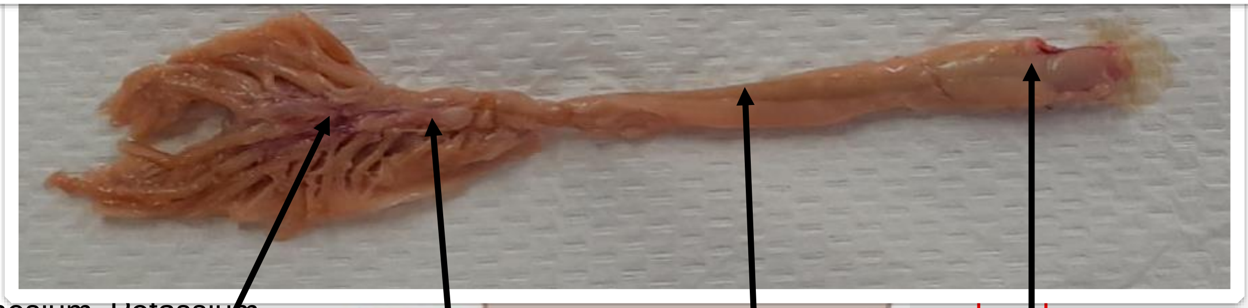
DIGESTIVE PHYSIOLOGY

- Understanding the underlying mechanisms on how soy-based ingredients affect growth performance and health
  - Start with the digestive tract (gastrointestinal tract, GIT) characterize nutrient digestive and absorptive mechanisms in response to soy-based ingredients
  - Take advantage of these mechanisms to offer solutions/suggestions to different industry areas such as feed formulations, feed processing, and mitigation strategies that result in increased inclusion levels of soy in fish feed
- How can we measure intestinal function? Ussing chambers



- Molecular perspective:





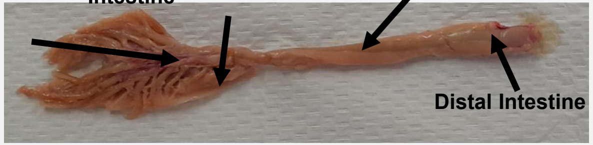
it current, Isc

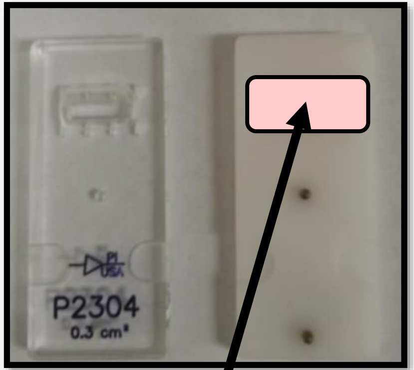
elial electrical
TEER ($\Omega \cdot cm^2$)

+ = Sodium, Magnesium, Potassium

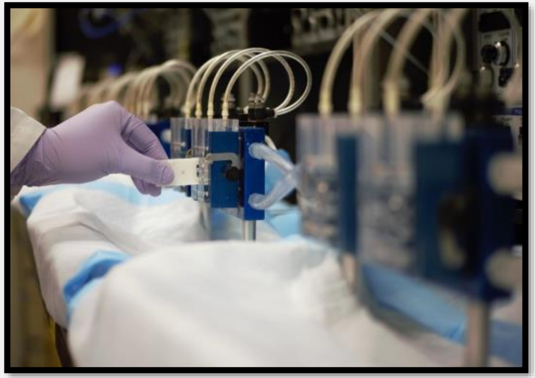
− = Chloride, Phosphates

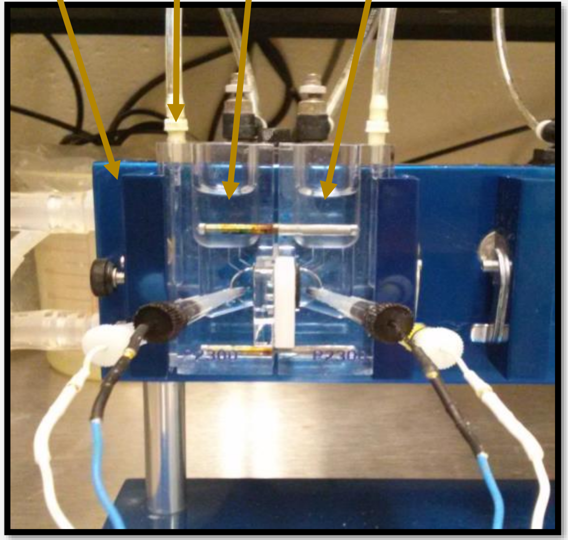
Ussing Chambers





Area of exposure: 0.3cm^2





Teleost Buffer at pH 7.9
Temperature: ~15^{o}C - 15.5^{o}C
Gas input: 99%O_2/1%CO_2

OBJECTIVES

Objectives:

1) To characterize baseline gut barrier function and methionine transport along the mid-intestine of Atlantic salmon and rainbow trout, and in response to isolated anti-nutritional factors (lectin, saponin, and β-conglycinin) using Ussing chambers, supported by qPCR.

2) To assess changes to methionine transport and gut barrier function in response to a soy-based and fishmeal-based diet in Atlantic salmon and rainbow trout using Ussing chambers, supported by qPCR.

SHORT-CIRCUIT CURRENT AND INTESTINAL INTEGRITY

- Sodium-dependent methionine transport:
  - Short-circuit current (Isc, $\mu A/cm^2$) □ Ussing chamber electrogenic data

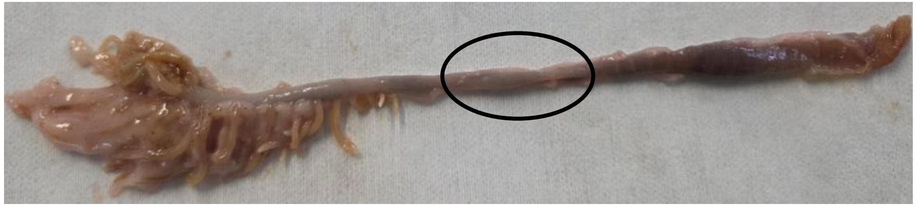
- Total methionine transport
  - Total methionine flux □ Liquid chromatography-mass spectrometry

- Gut barrier function
  - Transepithelial electrical resistance (TEER) □ Ussing chamber data

MATERIALS AND METHODS

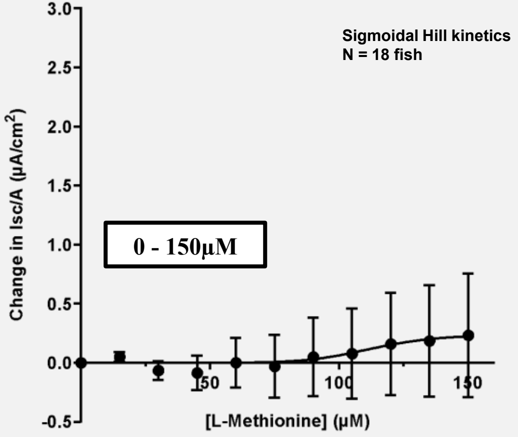
Rainbow Trout

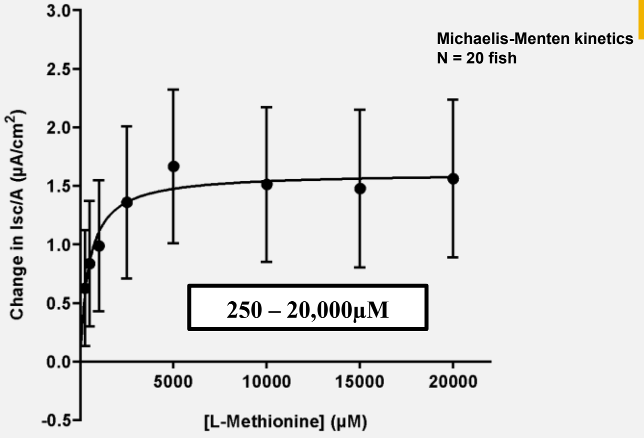
Atlantic Salmon



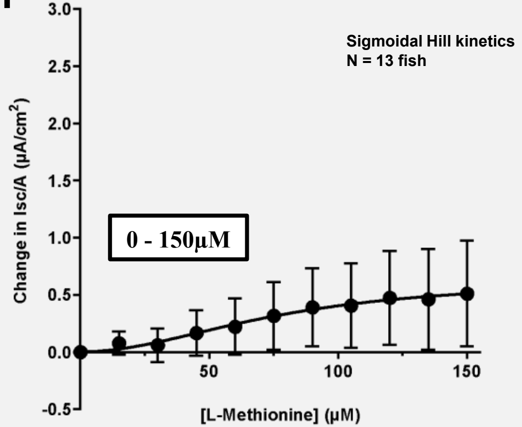
KINETIC CHARACTERIZATION OF NA-DEPENDENT METHIONINE TRANSPORT

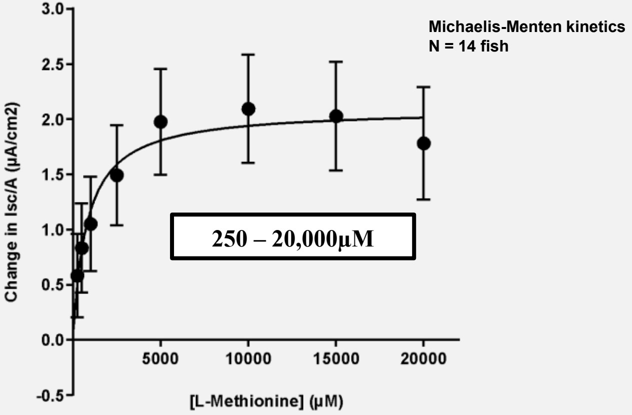
Rainbow trout





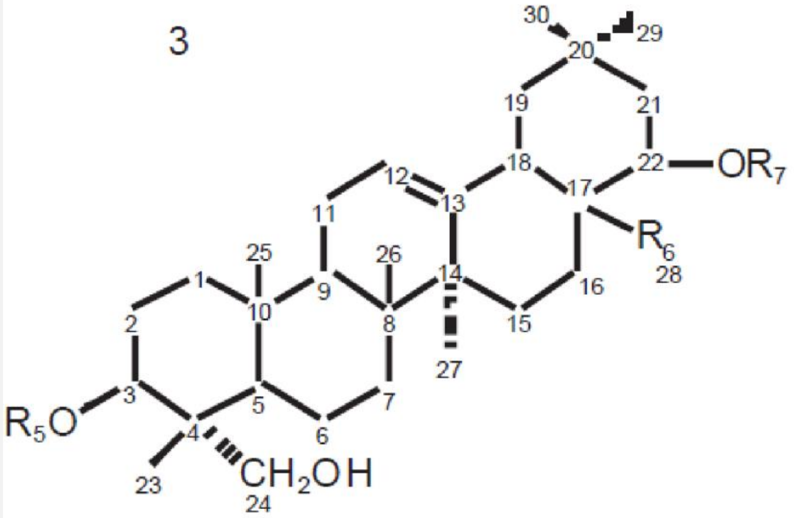
Atlantic Salmon





SAPONIN

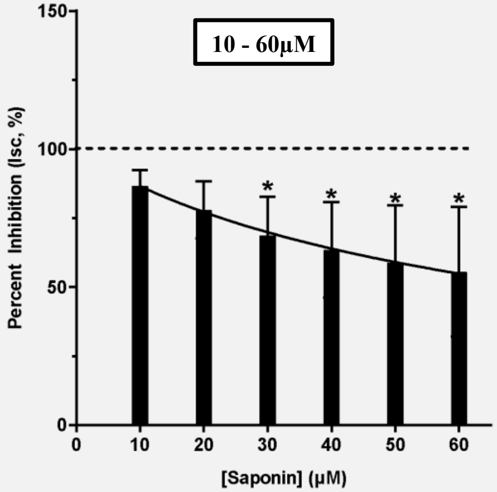
3

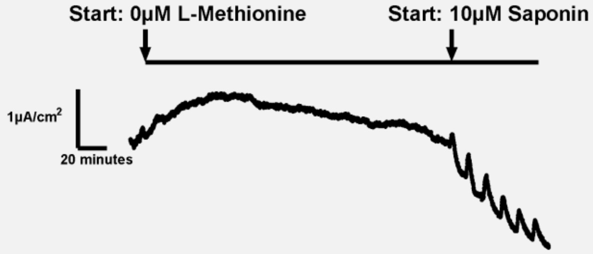


B. Levavi-Sivan et al. / Comparative Biochemistry and Physiology, Part C 140 (2005) 79–86

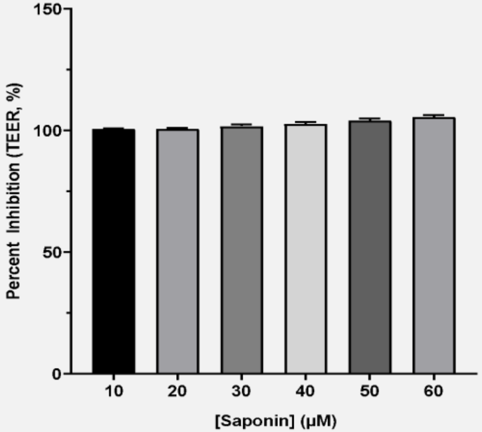
Rainbow Trout

Isc





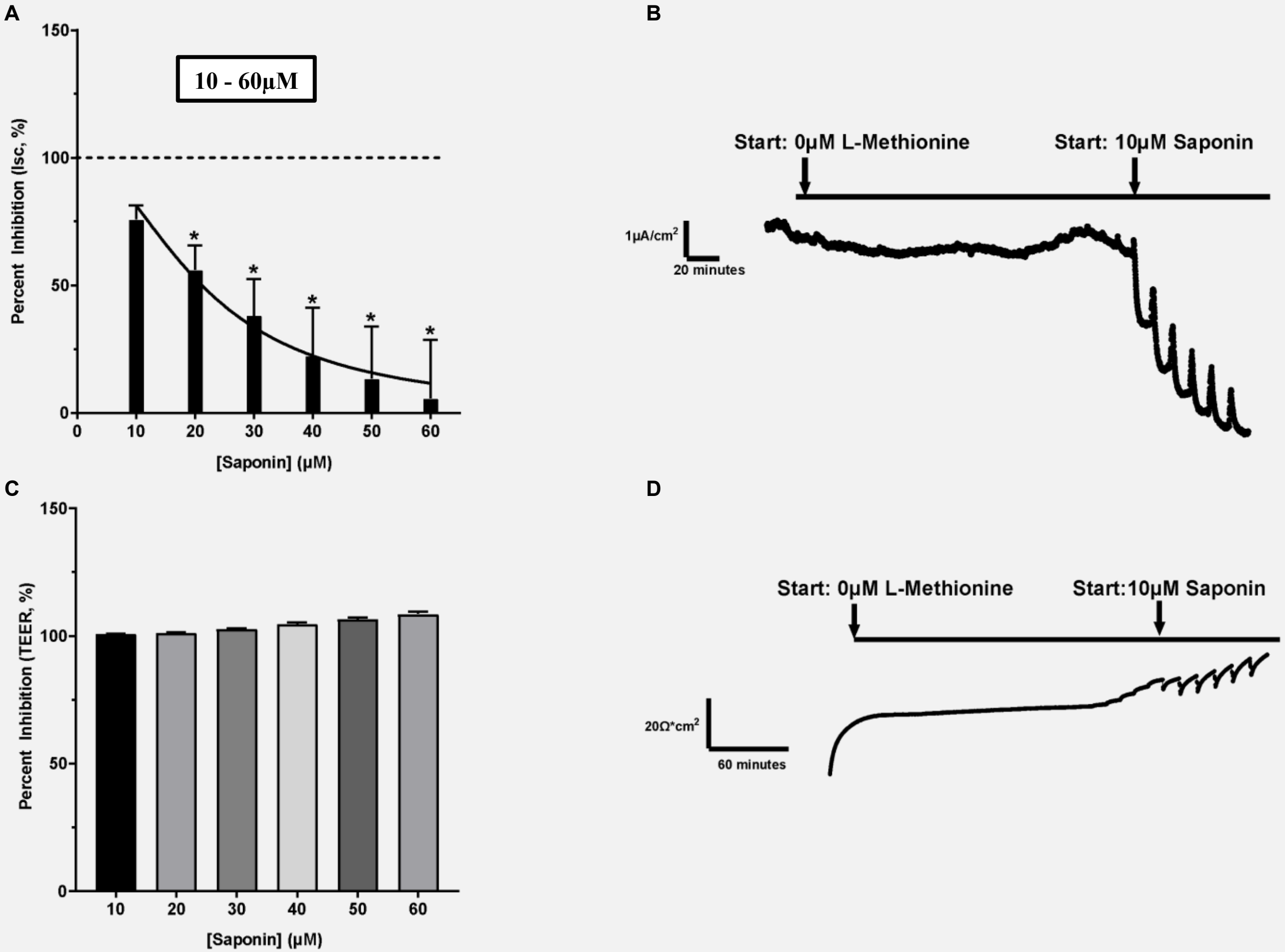
TEER



Atlantic Salmon

Isc

TEER



IMPLICATIONS FOR SOYBEAN AND AQUACULTURE INDUSTRIES

Challenges

- Limit feed costs using sustainable alternatives
- Alternative protein ingredients □ Soy
  - Increase inclusion of soy in fish feed
  - Mitigate impacts of anti-nutritional factors (ANFs)

Knowledge Gaps

- How do ANFs affect nutrient utilization?
  - Fish health and welfare?
- Isolate and eliminate specific ANF components

Deliverables

- Identify and characterize ANF effects on nutrient absorption
- Isolate and eliminate ANFs
- Increase soy inclusion in fish feed for salmonids

ACKNOWLEDGEMENTS

Dr. Brian Small (University of Idaho; PI/Supervisor)

Dr. Madison Powell (University of Idaho; Co-PI)

Wetlab Technicians

Funding Agents:

Soy Aquaculture Alliance

University of Idaho

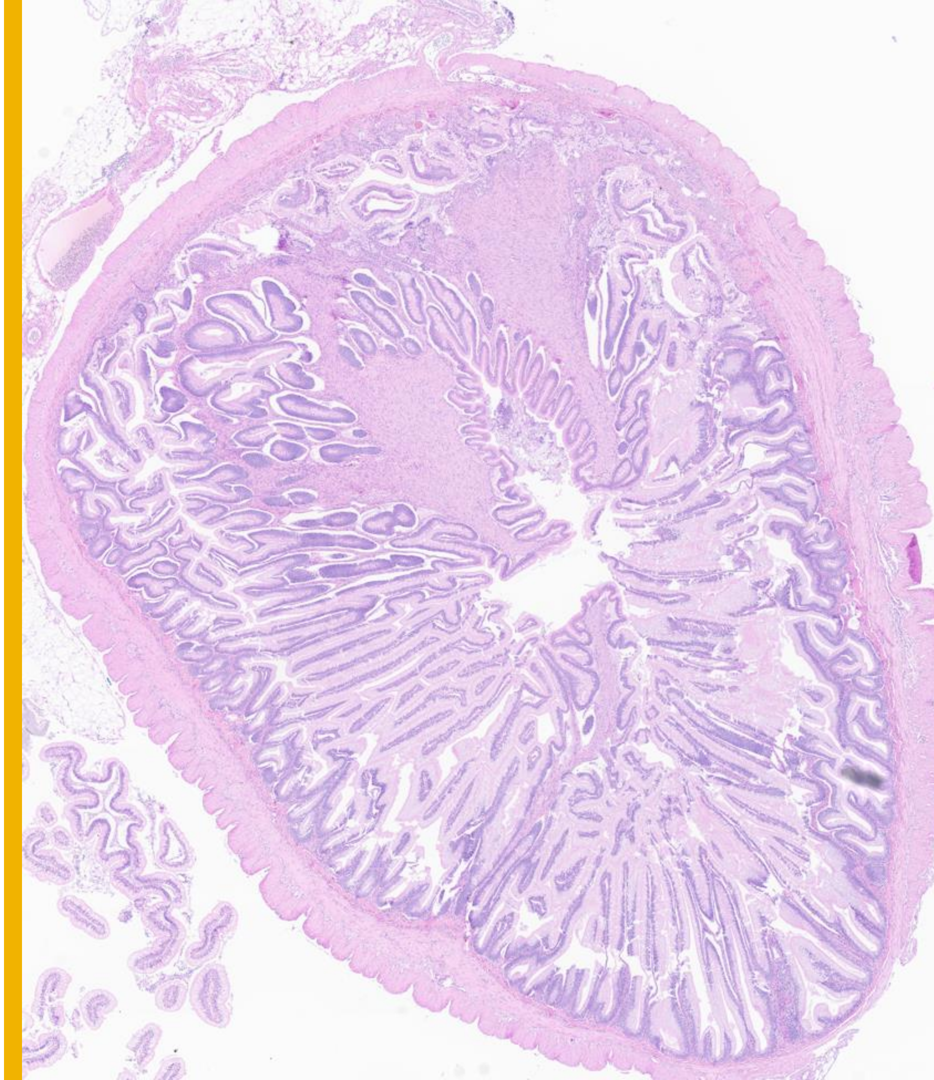
DIGITAL PATHOLOGY

Machine Learning for Rainbow Trout Intestinal Histopathology

Automating enteritis screening and building toward standardized trait-level assessment tool for fish nutrition

Jacob W. Bledsoe, Ph.D.
University of Idaho | Aquaculture Research Institute
Hagerman Fish Culture Experiment Station

Supported by the Soy Aquaculture Alliance



WHY AUTOMATE HISTOLOGY?

Histopathology is highly informative — but remains a research bottleneck

STRUCTURE
Tissue architecture

ABSORPTION
Epithelial condition

INFLAMMATION
Cellular response

PERFORMANCE
Dietary interpretation

HISTOLOGY PROVIDES

- Direct assessment of intestinal inflammation
- Tissue architecture and epithelial condition
- Biological interpretation of dietary response

MANUAL INTERPRETATION REQUIRES

- Specialized expertise
- Substantial time
- Consistent trait definitions
- Review across many fish and studies

Digital pathology could increase throughput and consistency while retaining expert biological interpretation.

STUDY FOUNDATION

A curated dataset and expert rubric translated trout histology into model-ready traits

182

analysis units

13,629

analysis-ready image tiles

INFLAMMATION + CELLULAR RESPONSE

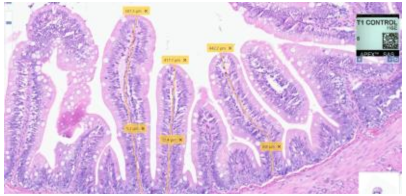
- ✓ Mononuclear infiltration
- ✓ Eosinophilic granular cells
- ✓ Goblet cells

TISSUE ARCHITECTURE

- ✓ Mucosal-fold architecture
- ✓ Lamina propria thickness
- ✓ Submucosa / stratum compactum

EPITHELIAL + MORPHOMETRIC

- ✓ Supranuclear vacuolization
- ✓ Villus length
- ✓ Villus width



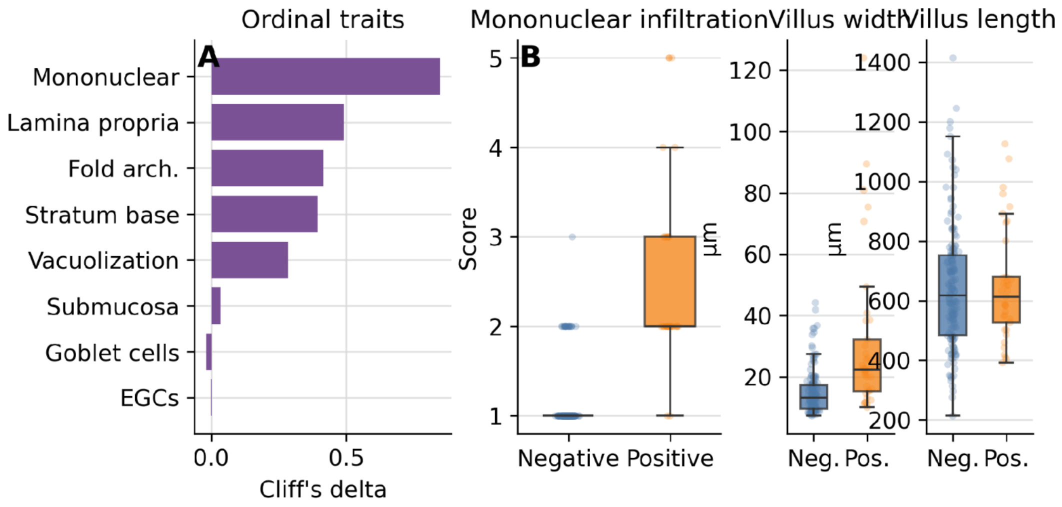
H&E whole-slide images • expert-labeled analysis units

The rubric supported binary, ordinal, and quantitative model targets.

Expert pathology input: Dr. Salvatore Frasca, VMD, Ph.D., DACVP
Aquatic and Investigative Pathology

BIOLOGICAL SIGNAL

Mononuclear infiltration showed the strongest biological association with enteritis



Spearman ρ

0.729

Strong monotonic association

Cliff's δ

0.846

Large separation between groups

FDR-adjusted p

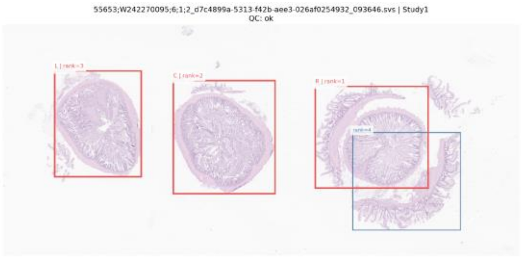
9×10^{-22}

Highly significant relationship

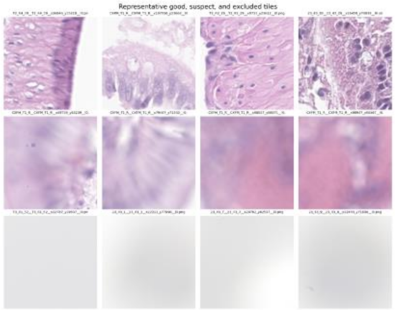
Inflammatory-cell infiltration provided the clearest biological target for model development.

Whole-slide images were converted into fish-level predictions

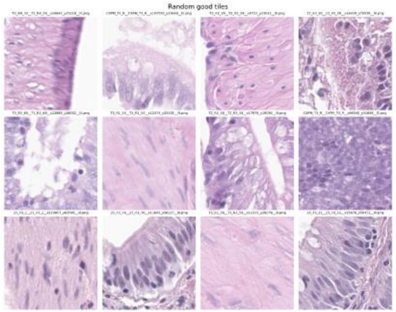
WHOLE-SLIDE IMAGE ❯ ROI EXTRACTION ❯ QUALITY CONTROL ❯ IMAGE TILING ❯ MODEL TRAINING ❯ TILE-LEVEL PREDICTION ❯ ANALYSIS-UNIT AGGREGATION



Detect tissue regions



Review image quality



Generate analysis-ready tiles

Weakly supervised ResNet18 models • analysis-unit labels • reproducible processing • public code foundation

The binary enteritis model identified every enteritis-positive validation sample

VALIDATION CONFUSION MATRIX

OBSERVED \ PREDICTED	Negative	Positive
Negative	22	15
Positive	0	9

SENSITIVITY

100%

SPECIFICITY

62%

AUROC

0.86

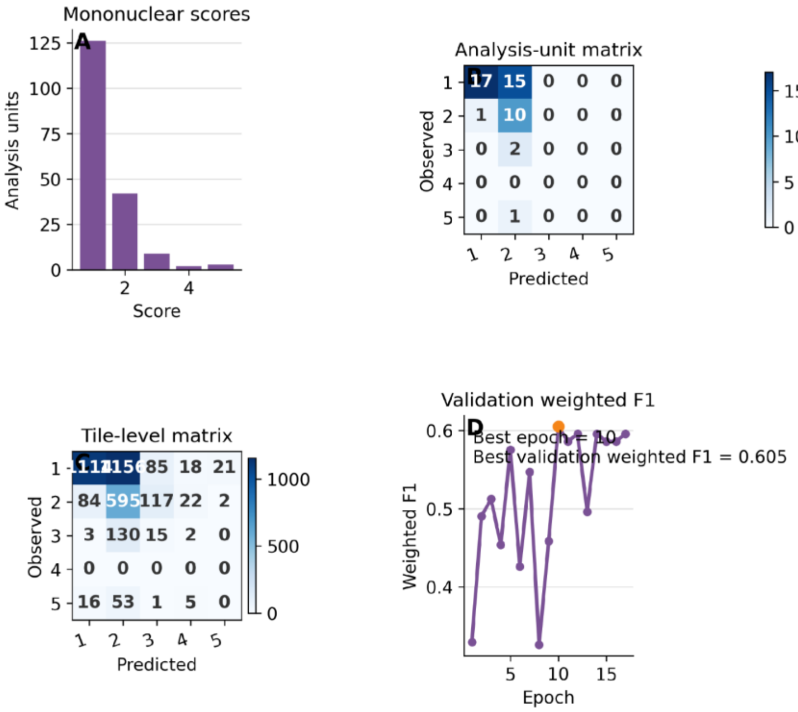
TRUE POSITIVE SAMPLES

9 of 9

The model favored sensitivity over specificity — appropriate for screening and prioritizing expert review.

Research screening prototype — not a standalone diagnostic.

Detailed trait severity prediction more difficult than binary enteritis screening



ACCURACY	WEIGHTED F1	MACRO F1
58.7%	0.605	0.302

1. Predictions concentrated in common mild categories
2. Severe pathology was underrepresented
3. Class imbalance limited five-level separation; thresholding limited binary mononuclear cell model despite strongest learning
4. **Local inflammation was more learnable than broad architecture**

Inflammatory signal was detected, but all five severity levels were not reliably distinguished due to class imbalance in training data

WHAT THE PROJECT TAUGHT US

Inflammation was easier to learn than large-scale intestinal architecture

LOCAL TILE-SCALE FEATURES

Inflammatory cells
Local cellularity
Epithelial texture

High-resolution tiles retain cellular detail that can support pattern recognition.

WHOLE-TISSUE CONTEXT

Fold architecture
Villus dimensions
Regional morphology

Small tiles may lose spatial relationships needed to describe large-scale structure.

1. **Mononuclear infiltration was the strongest correlate of enteritis.**
2. Weakly supervised models recognized inflammatory visual patterns.
3. Large-scale architectural traits were harder to reconstruct from small tiles.
4. Villus width showed a stronger association with enteritis than villus length.

Future models should include segmentation, greater supervision, and more sophisticated thresholding.

FROM PROTOTYPE TO RESEARCH TOOL

The project established the workflow; broader validation must establish generalizability

WHAT THE PROJECT PRODUCED

- ✓ Expert-developed histopathology rubric
- ✓ Curated digital-slide dataset
- ✓ Automated ROI extraction and tiling
- ✓ Binary and ordinal candidate models
- ✓ Public computational pipeline to build on

WHAT IS STILL REQUIRED

- → Multiple expert pathologists
- → Balanced mild and severe phenotypes
- → External scanners and laboratories
- → Independent feeding trials
- → Additional trout strains and salmonid species
- → Models preserving or segmenting whole-slide spatial context

The current models are research prototypes—not standalone diagnostic or commercial tools.

Public release: github.com/jbledsoe/Trout-Intestinal-Histopathology-ML

FUTURE DIRECTION

The next opportunity is standardized measurement of subtle intestinal phenotypes

Improved soybean ingredients, feed formulations, and fish genetics have reduced the frequency and severity of pronounced enteritis in many contemporary studies.

INITIAL TARGET
Enteritis positive / negative

FUTURE TARGET
Multidimensional intestinal phenotype

- Mononuclear infiltration
- Lamina propria thickness
- Mucosal-fold architecture
- Supranuclear vacuolization
- Goblet-cell response
- Villus length + width
- Submucosal changes

✓ Compare ingredients + processing
✓ Evaluate additives + supplementation
✓ Detect subtle formulation differences
✓ Quantify strain-by-diet interactions
✓ Standardize endpoints across trials

1. Automated screening of trout intestinal histology is feasible.
2. Inflammatory-cell infiltration was the clearest machine-learnable signal.
3. The larger opportunity is standardized measurement of trait-level differences.

Digital pathology can complement expert review and make intestinal histology more scalable and quantitative.

THANK YOU FOR JOINING US

EASTON KUBOUSHEK

Interim Executive Director
Soy Aquaculture Alliance

JACOB BLEDSOE, PH.D.

Assistant Professor and Extension Specialist in Aquaculture
University of Idaho

MARINA SUBRAMANIAM, PH.D.

Postdoctoral Research Scientist
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QUESTIONS?

SOY AQUACULTURE ALLIANCE

WEBINAR AVAILABLE ONLINE POST-EVENT:
soyaquaculture.com