FPRF Technical Services Newsletter

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President's Column

Nutrition is one of the key drivers in animal production, especially intensive animal production. In aquaculture, the high cost of diets makes it even more so. Using feed that is targeted to give maximum efficiency from the species grown can mean the difference between a profitable and a marginal operation.

On the other hand, studies have shown that commercial aquaculture feeds contain a mixed microflora, of which many are pathogenic or potentially pathogenic to fish or shrimp and humans. In addition, it has been suggested that the ingredients used in feed formulation partially determine the nature of its microflora. Moreover, cross contamination of feeds is possible and last week report on fish feed being contaminated with melamine-tainted "protein concentrate," imported from China is a good example of it.

Given the trend to replace expensive animal-derived proteins, such as fish meal, with less expensive animal proteins, the importance of good record keeping should be under the microscope as the use of rendered by-product meals increase drastically in aquaculture feeds.

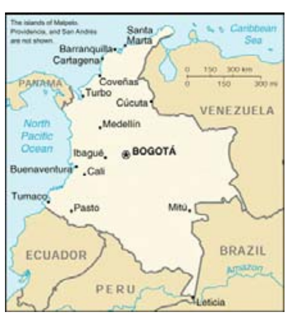
The Code of Practice on Good Animal Feeding "Codex Alimentarius" clearly establishes that "Records should be maintained and readily available regarding the production, distribution and use of feed and feed ingredients to facilitate the prompt trace-back of feed and feed ingredients to the immediate previous source and trace-forward to the next subsequent recipients if known or probable adverse effects on consumers' health are identified".

Sergio F. Nates, Ph.D.

"It is kind of fun to do the impossible."

—Walt Disney

Country Focus - Colombia (by Sergio Nates)



According to the U.S. Meat Export Federation, the current annual market for pork in Colombia is around 170,000 metric tons, imports account for 8,000 to 10,000 mt and most imports are from the United States (36 percent), Chile (35 percent) and Canada (25 percent). Colombia ranks third in the Central and South American region as a destination for U.S. pork exports

Likewise, prior to the first case of U.S. BSE case, the United States sent livers, lungs and stomachs to Colombia. Beef production is a traditional industry in Colombia and annual per capita beef consumption (37.4 lbs) is relatively high in comparison with other Latin American countries. The cattle herd numbers approximately 24 million head and annual beef production is around 700,000 mt. The herd is primarily zebu and average slaughter age is 4 to 4.5 years. The current annual market for beef in Colombia is around 680,000 mt.

On the other hand, in the last 20 years, domestic production of chickens has increased nearly fourfold, while chicken and egg consumption per capita has tripled. Today, poultry is the second largest source of protein, accounting for 40 percent of total meat consumption and 10.5 percent of agricultural gross domestic product.

In terms of food safety regulation, in 1997, the government approved a food safety regulation to be enforced by the newly established National Institute for Food and Drug Surveillance (INVIMA). This rule substantially increases standards for fresh products and shifts the emphasis from inspection of final product to process control. The rule requires companies to document compliance with Good Manufacturing Practices (GMPs). It also embraces the minimum standards defined by the Codex Alimentarius commission. In 2002, the government approved a regulation that recommended adoption of Hazard Analysis Critical Control Point (HACCP), established parameters for certification of HACCP plans, and defined rules for quality assurance labels. In addition, since March 6, 2001, Colombia has enforced Resolution N° 0347/2001 by which the use of meat meal, blood meal, bone meal, meat and bone meal of mammalians is prohibited for ruminant feeding (MMBM-ban).

Rendering

Bovine raw material is rendered for feed, including confiscated and condemned materials. Animals that died in the pastures are buried on site. Animals that died during transport or are diseased do not enter rendering. Bovine material is not rendered together with raw material from other species (sheep, goats and poultry), but no information is known whether MBM is produced in the same rendering plants. Therefore it has to be assumed that the MBM produced contains raw material from ruminants and other species. Details on the rendering process parameters that are applied in the country are available for both BM and MBM.

R&D Update (Progress report)

05A-1 ***Nutritive Value of Rendered Animal Proteins and Fats for Aquaculture Species & Targeted Uses for these Ingredients in AquaFeeds (by Dominique P. Bureau)***

Objectives:

The objectives of this study are to:

1) Compile and review all information on the nutritive value of rendered animal protein and fat ingredients for various aquaculture species published in the scientific and grey literature and summarize these findings into an informative and attractive technical document easily accessible to feed manufacturers, renderers, and other industry stakeholders.

2) Review and summarize information on nutritional requirements and feed formulation of key aquaculture species, identify how rendered ingredients can find their usefulness in these formulations.

3) Propose a framework to improve our ability to make accurate recommendations applicable to different species and production environments,.

4) Identify and test targeted uses for rendered animal protein ingredients in aquaculture feeds.

Summary of Project Results / Progress to date:

1) Database of Scientific Papers on Rendered Animal Proteins

A visiting research scientist, Dr. E.R. El-Haroun, compiled more than 350 scientific documents on the nutritive value of rendered animal proteins and fats for various aquaculture species published in the scientific and grey literature over the past three decades. More references are added daily. All references were inputted in a reference management database (e.g. Procite) and electronic and paper copies of the articles have been made and are kept on file. The reference database and copies of the papers will be accessible through the internet through the website of the Fish Nutrition Research Laboratory (http:/www.fishnutrition.uoguelph.ca) in a few months. Members of the FPRF will be able to request copies of the various papers free of charge. We have already provided numerous references on several occasions to the NRA Hong Kong Office.

2) Review of Scientific Information on Rendered Animal Proteins

Dr. El-Haroun initiated a review of all published information on the digestibility of rendered animal proteins (> 60 studies) for various species. He has assembled these data in a table format. These tables require some formatting and revision. The finalized tables will be incorporated in the technical document that will be published at the end of this project. Drs. Bureau and El Haroun has worked on the elaboration of several “chapters/sections” for the technical document and is currently working on detailed table of content. Preparation of the technical document is in progress and on schedule.

3) Development of Models of Nutrient Utilization and Requirement

Dr. Katheline Hua did a significant amount of work on the development of nutrient digestion, utilization, and requirement for various fish species. Dr. Hua has used her expertise in nutrient modelling and feed formulation to integrate available information on nutrient requirement and utilization by fish and develop a framework to improve our ability to make accurate recommendations on how to best utilize rendered products in aquaculture feeds. This part of the project has proven very difficult despite Dr. Hua tireless efforts to develop a “nutrient-flow” model (See Abstract #1). We are currently resorting to constructing a more traditional “factorial” model of nutrient requirement. However, this model is elaborate and more realistic than current factorial (bioenergetics) models since it will take into account growth trajectory and nutrient deposition across life stages, physiological state of the animal, and numerous interactions between nutrients (see Abstract #2).

4) Target Uses for Rendered Animal Protein Ingredients in Aquaculture Feeds.

This part of the project has focused on two main applications/targeted uses.

A. Rendered Animal Proteins as Source of Highly Bioavailable Amino Acids

We conducted two studies on lysine bioavailability in blood meal from various origins. In an initial trial, Dr. El Haroun observed that the bioavailability of lysine in flash-dried or spray-dried blood meals was significantly higher than that of free lysine (L-lysine HCl). This study demonstrated the high nutritive value of this ingredient as source of high available amino acids. Dr. El-Haroun devoted significant amount of time to the preparation of a scientific manuscript on this study. This manuscript was published by the journal “Aquaculture”. A reprint was forwarded to the FPRF President a few weeks ago. A technical article on this study was published in the October 2006 issue of Render Magazine.

In order to validate the observation of the first trial, Dr. El-Haroun carried out a second growth experiment (12 weeks) to compare the bioavailability of lysine in spray-dried blood meal with that of free lysine. The results from this second trial again quite clearly demonstrated that the bioavailability of lysine in blood meal is better than that of free lysine (results not shown since statistical analyses are not completed). Amino acids in well-processed blood meals are highly digestible and appear to be more efficiently utilized than synthetic amino acids by fish. This difference in lysine bioavailability between blood meal and synthetic lysine is significant enough for fish feed manufacturer

B. Rendered Animal Ingredients as Sources of “Minor” Nutrients of Animal Origin

Rendered animal ingredients are good sources of several “minor” nutrients, many of which have been overlooked in feed formulation in the past due to the use of high levels of fish meal and fish oil in fish feeds. Some of these nutrients, such as, cholesterol and taurine, are almost exclusively found in ingredients of animal origins. An increasing number of studies indicate that some of these nutrients are essential, or conditionally essential, to fish or crustaceans. This is the case for cholesterol, arachidonic acid, myo-inositol, and, possibly, also taurine.

Cholesterol is generally not considered to be an essential nutrient for fish. However, recent studies have suggested that fish fed plant-based diets may benefit from cholesterol supplementation. Meat and bone meals and other rendered products could be cost-effective sources of cholesterol for fish fed high plant protein diets since these ingredients contain significant amounts of cholesterol (Table 1).

A growth trial was carried out to examine whether rainbow trout fed plant-based diets (low cholesterol, < 10 mg/100g diet) would benefit from cholesterol supplementation (0, 30, 60. 90 mg/100 g diet). The trial was conducted by Nevada Young, a visiting MSc student from the University of Saskatchewan. The trial was recently completed and the results are not fully analyzed. Due to very interesting preliminary results, another trial was initiated to determine if the results of this first trial are repeatable. The results of both trials suggest that rainbow trout may benefit from dietary cholesterol supplementation when fed all-plant diets. An interesting interaction between n-3 polyunsaturated fatty acids and cholesterol on weight gain and survival of the fish was also observed. More details will be provided in the next progress report (Sept. 2007).

Table 1. Cholesterol content (mg/100g) of various animal protein ingredients obtained from various rendering plants and fish feed manufacturers.

Ingredients	**Cholesterol (total)**
	mg/100g
Fish Meal, Menhaden	237
Fish Meal, Herring	302
Blood Meal, Avian, Disc Dried	407
Blood Meal, Mammalian, Flash-Dried	255
Blood Meal, Bovine, Ring-Dried	241
Feather Meal, Steam Hydrolyzed	90
Meat and Bone Meal, 43% CP	98
Meat and Bone Meal 56% CP	100
Meat and Bone Meal 56% CP	107
Poultry By-Products Meal, 65% CP	168

List of publications related to this project:

Bureau, D.P. and K. Hua. 2007. Models of nutrient utilization by fish and potential applications for fish culture operations, pp xxx-xxx. In: France J. and E. Kebreab (Eds.) Animal Nutrition Modelling. Centre for Nutrition Modelling, University of Guelph, Guelph, Ontario, Canada (*in press*).

Bureau, D.P. 2006. Rendered products in aquaculture feeds, pp. 179-194. In: Meaker, D.L. (Ed.) Essential Rendering. All About the Animal By-Products Rendering Industry. National Renderers Association, Alexandria, VA.

El-Haroun, E.R. and D.P. Bureau. 2007. Comparison of the bioavailability of lysine in blood meals of various origins to that of L-lysine HCL for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 262, 402-409.

Bureau, D.P. 2006. Feed issues in the aquaculture industry, pp. 181-196. Proceedings of the 27th Western Nutrition Conference, 19-20 September 2006, Winnipeg, Manitoba.

Bureau, D.P. 2006. Animal by-products utilization in aquaculture, pp.11-125. Proceedings of the 42nd Eastern Nutrition Conference, 11-12 May 2006, Guelph, Ontario.

Bureau, D.P. and K. Hua. 2006. Future directions in feed formulation for waste reduction, pp. 48-52. Proceedings of the Canadian Freshwater Aquaculture Symposium,17-20 October 2004, Quebec City, QC, Canada. Aquaculture Association of Canada Special Publication No. 11, St. Andrews, NB, Canada.

Bureau, D.P. and P. M. Encarnacao. 2006. Adequately determining the amino acid requirements of fish: the case example of lysine. In : Cruz-Suarez, L.E., D. Ricque-Marie, M. Tapia-Salazar, M.G. Nieto-Lopez, D.A. Villareal Cavazos, A.C. Puello Cruz, and A. Garcia-Ortega (Eds.) Avances en Nutricion Acuicola VIII. VIII Simposium Internacional de Nutricion Acuicola. 15-17 November 2006. Mazatlan, Sinaloa, Mexico. Universidad Autonoma de Nuevo Leon, Monterrey, Nuevo Leon, Mexico. ISBN 970-694-333-5.

Bureau, D.P. 2005. Aquaculture nutrition: The need for a transition from bioenergetics to nutrient-flow models. Annual Meeting of the Mexican Association of

Animal Nutrition (AMENA), 25-28 October 2005, Puerto Vallarta, Jalisco, Mexico.

Bureau, D.P. 2005. *Utilización de harinas de origen animal en la nutrición de peces* [Use of animal proteins in fish nutrition]. Proceedings of the 2nd Foro Internacional de acuicultura, 1-3 December 2005, Hermosillo, Sonora, Mexico.

Bureau, D.P. 2006. Predicting efficiency of conversion of feeds into aquatic biomass: Easier said than done! Seminar Series of the Instituto Investigación de Oceanologica, FCM-IIO, 28 April 2006. Universidad Autónoma de Baja California, UABC, Ensenada, BC, Mexico.

Chowdhury, M.K. and D.P. Bureau. 2006. A rapid appraisal approach to identify feed ingredients for low-input cage aquaculture. International Aquafeed, 9 (1): 14-19, Jan-Feb 2006.

El-Haroun, E.R. and D.P. Bureau. 2006 Tech Topics: Bioavailability of lysine in various blood meals. Render Magazine, October 2006, 24-25.

Hua, K. and D.P. Bureau. 2006. Animal proteins: good phosphorus sources. Render Magazine, August 2006, 16-17.

Mazumder, M. and D.P. Bureau. 2006. Managing solid waste outputs from cage culture operations with emphasis on copper and Zinc. Martin Mills Cage Grower Meeting, 12 April 2006, Parry Sound, Ontario.

Bureau, D.P. 2005. Advanced concepts in fish nutrition and feed formulation. National Renderers Association-sponsored lecture presented in Jakarta and Surabaya, Indonesia, 8-12 August 2005.

Bureau, D.P. 2005. Formulating more cost-effective aquaculture feeds. International Aquafeed, 7 (3): 6-9.

Abstract #1

Adaptation of a Swine Nutrient-Flow Model to Represent Nutrient Utilization for Growth in Rainbow Trout

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Models that accurately describe growth and nutrient utilization of fish can provide means of developing strategies to improve the economical and environmental sustainability of aquaculture operations. Models used currently in aquaculture are based on bioenergetics or empirical equations. These models are not sufficiently flexible to be applied to a wide range of conditions encountered in fish culture. There is a need for more mechanistic approaches to predict growth and nutrient utilization by fish.

The nutrient-flow/growth model developed by Birkett and de Lange (2001) is an interesting mechanistic model that has been used successfully in swine production. This model explicitly describes the flows of the energy-yielding nutrients and their metabolites at the whole animal level. In this model, amino acid (AA) metabolism is represented based on AA requirements for physical body maintenance functions (integumental losses, endogenous gut losses, minimal urinary N excretion), inevitable catabolism, protein deposition (Pd) in viscera and carcass, catabolism of AA supplied in excess of requirements, and excretion of total urinary N. The upper limit to Pd (Pdmax) is defined from empirical observations. Utilization of energy-yielding nutrients, including AA that are not used explicitly for Pd, body lipid deposition (Ld) or supplying energy (in the form of ATP equivalents) is represented

based on biochemical conversions. The partitioning of retained body energy, between Pd and Ld, is ruled by the minimum ratio of the body lipid (L) mass to protein (P) mass (minLP), which is related to digestible energy intake through an empirical equation. Live weight gain is then calculated from deposition of protein, lipid, water and ash.

The pig growth model was adapted to fish through parameterization and various modifications consistent with its framework. The parameters were empirically derived from studies conducted with rainbow trout. New parameters, such as the effect of water temperature on metabolism and a limit on the amount of glucose that can be efficiently utilized, were incorporated in the model to account for the determinant effect of water temperature on fish metabolism and limited capacity of fish to utilize carbohydrates. The model was then calibrated based on cost of protein and lipid deposition in rainbow trout as determined by Azevedo et al. (2005).

The model was evaluated through comparing model simulations with data from numerous feeding trials carried out with rainbow trout. The results suggest that despite repeatedly calibrating and adjusting parameters, the predictions of the model were not highly realistic, especially for larger fish (> 500 g). It became apparent that some concepts cannot be transposed from swine to fish without significant modifications. The core of nutrient-flow model is the rule of partitioning protein and lipid retention at different degrees of maturity. In fish, the relationship between maturity and the partitioning of Pd and Ld is more complex than in swine. Evidence also suggests that amino acid metabolism differ significantly between pig and fish. For example, in fish the maximum efficiency of utilization of essential AA for Pd was found to be affected by intake level and some of the non-protein energy-yielding nutrients in fish diets. The efficiency of Pd and its relationship to Ld in rainbow trout was also observed to be driven by strong endogenous regulations, such as body size and life stage. The partitioning rules at the basis of the pig growth model apparently cannot be easily adapted to fish.

Despite an apparent failure in successfully adapting the model to fish, this modeling exercise offered an interesting opportunity to improve our understanding of nutritional and endogenous determinants of fish growth.

Abstract # 2.

PREDICTING FEED EFFICIENCY OF RAINBOW TROUT: TRANSITIONING FROM BIOENERGETICS MODELS TO APPROACHES BASED ON PROTEIN ACCRETION.

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Several factors are known to affect feed efficiency (live weight gain/feed intake) in fish. These include factors such as, body weight, diet composition, feeding or performance level, nutritional history, and environmental factors (e.g., water temperature). The combined effects of several of these factors complicates comparison of feed efficiency values obtained in different studies, farms, and, even, different production cycles. Bioenergetics models, such as the Fish-PrFEQ model, have been developed to predict feed requirement and feed efficiency as a function of various factors. These bioenergetics models have proven very useful but they present significant limitations.

Bioenergetics models predict a linear decrease in feed efficiency with decreasing feed allocation. As feed allocation is decreased, a greater proportion of the metabolizable energy (ME) intake is spent on “maintenance”, which results in less energy being deposited per unit of ME consumed. A number of studies have, nonetheless, shown that the effect of feeding level on feed efficiency is much less significant than predicted by bioenergetics models. Dramatic restriction in feed allocation (e.g. feeding at 25-50% of maximum voluntary feed intake) resulted in no significant effect (or very minor effect) on feed efficiency in salmonids. Fish fed a maintenance ration (ration resulting in zero energy gain) had positive N gain (deposited protein), gained weight, and showed a feed efficiency of about 1. Protein

accretion appears to be the main factor driving weight gain in fish.

Feed efficiency of fish has been observed to decrease significantly as weight of the animal increases. Larger fish contain more dry matter (mainly lipids but also protein) and, contain more energy per unit of live weight than smaller fish. One unit of weight gain represents a larger "energy requirement" for a larger fish compared to a smaller one. Models of carcass energy content are at the core of current bioenergetics models and these models predict relatively well the differences in feed efficiency observed between fish of different weights. However, studies have indicated that different salmonid species can have very similar "energy requirements" but very different feed efficiencies. Differences in feed efficiency appear to be more closely related to efficiency of protein (amino acids) utilization than energy utilization in the different species studied so far. The decrease in feed efficiency observed in fish of increasing weight appears to be closely related to a decrease in nitrogen retention efficiency (NRE, N gain/digestible N intake). These observations suggests that predicting feed utilization on the basis of the "energy requirement", as it is done by bioenergetics models, is not very rational.

A model explicitly representing the determinant effect of protein accretion and NRE on weight gain was developed for rainbow trout by combining results from several studies conducted in recent years. The model borrows many of the elements of the Fish-PrFEQ bioenergetics model but the main component of the model are the estimates of protein mass and NRE of rainbow trout as a function of body weight (BW) and digestible protein (DP) to digestible energy (DE) ratio of the diet. These are estimated from experimental data and are calculated as follows:

NRE (%) = 66.5-0.98*DP/DE-0.016*BW (R^2=0.77)

Protein mass (g/fish) = 0.122*BW$^{1.051}$ (R^2=0.99)

A validation of the model was done by comparing model simulation with experimental data from fish fed two extruded commercial-type feeds and growing from 6 to 130 g BW at 15.4°C for 84 days. The results suggest that the model simulation agreed significantly better with experimental observations than estimates obtained using current bioenergetics model.

The ACREC Update
Clemson University Animal Co-Products Research and Education Center (ACREC) by Dr. Annel K. Greene

The proposal below is one that was not approved by the board at our last meeting at Clemson (April 5th, 2007). However, it is an interesting approach to a problem that can be recurrent in many places around the world and I have taken the liberty to include it in this newsletter.

Carcass preservation in a natural disaster: exploratory study of potential means of effectively preventing decomposition and disease transmission

Project Summary

During natural disasters such as hurricanes, earthquakes, tsunamis, domesticated and wild animals can perish. In the ensuing cleanup of debris, the carcasses of these animals can pose both odor and disease transmission issues. Rendering the carcasses would be ideal; however, with hampered transportation and utilities, it can be difficult to transfer these carcasses to rendering plants in a timely manner to prevent significant decomposition. In the aftermath of Hurricanes Katrina and Rita, more than 40,000 cattle were reported killed in Louisiana alone. Without a means to collect and transport these carcasses to rendering facilities, these carcasses rotted on the ground emitting odors and attracting rodents and insects. Thousands more cattle were killed in Mississippi when Hurricane Katrina made landfall.

In the event of certain infectious animal disease outbreaks, animal health officials will destroy huge numbers of animals to contain disease spread. In a large outbreak, animal carcasses can overwhelm rendering capabilities. To the residents in an area stricken by a natural disaster, the huge number of animals killed in a natural disaster poses health and safety issues. To the rendering industry, the inability to preserve the carcasses for later processing represent lost starting material and lost profits.

The following project is designed as a preliminary exploration into methods of economically treating animal tissues during natural disasters to 1) preserve the material for later rendering; 2) to prevent odor problems; 3) to prevent disease spread.

Project Description

1. **Objective(s)**

   - *Test different means of treating animal carcass tissues to preserve and destroy microorganisms.*

2. **Review of Related Research/Literature Review**

   Food microbiologists often utilize "hurdle technology" to preserve foods which involve a series of microbial inhibition mechanisms. Acidification, drying, salting, fermentation, freezing, refrigeration, and other means are often used to prevent the deterioration of animal tissues for food use. However, not all of these means are practical for a whole carcass lying in an inaccessible area after a natural disaster.

3. **Experimental Procedures**

   Various methods of direct acidification, fermentation and salting with NaCl and KCl will be tested for preservation of animal tissues. Also, ancient methods of embalming tissues will be investigated for potential use in this project. In all cases, samples of tissues will be treated and then tested under typical summer conditions of heat and humidity for deterioration and deleterious microbial growth over a one month period. Samples of different organs and muscle tissues will be collected from the Clemson University Meats Laboratory for testing.

4. **Projected benefits and economic opportunities resulting from the project**

   A method of preserving animal carcasses during a natural disaster would allow renderers the opportunity to recover tons of marketable fats and proteins while preventing disease transmission and odor problems from the animal tissues.

Noteworthy Article

Suyama K, Yoshioka M, Akagawa M, Murayama Y, Horii H, Takata M, Yokoyama T, and Mohri S. 2007. Prion inactivation by the Maillard reaction. Biochem. Biophys. Res. Commun. 356(1): 245-8.

Since variant Creutzfeldt-Jakob disease (vCJD) has been suspected to be attributable to the infectious agents associated with bovine spongiform encephalopathy (BSE), it is important to prevent the transmission of pathogenic forms of prion protein (PrP(Sc)) through contaminated feeding materials such as meat and bone meal (MBM). Here, we demonstrate that the Maillard reaction employing a formulation of glucose in combination with sodium hydrogen carbonates effectively reduced the infectivity (approximately 5.9-log reduction) of a scrapie-infected hamster brain homogenate. In addition to a bioassay, a protein misfolding cyclic amplification (PMCA) technique, in which PrP(Sc) can be amplified in vitro, was used as a rapid test for assessing PrP(Sc) inactivation. The PMCA analysis also indicated that the PrP(Sc) level in the infected material significantly decreased following the Maillard reaction. Therefore, the Maillard reaction can be employed for the decontamination of large amounts of byproducts such as MBM.

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