FPRF Technical Services Newsletter

JUNE 3, 2009

VOLUME 4, NUMBER 2

"If I could remember the names of all these particles, I'd be a botanist."

— Albert Einstein

President's Column

In many countries PCR identification of species-specific, animal group-specific and plant DNA is employed as part of the audit program to ensure compliance with the feed ban in place for the control of bovine spongiform encephalopathy (BSE). DNA turned out to be a reliable tool for this aim, since DNA is a quite thermostable molecule able to resist severe heat treatments applied in the manufacturing of animal meals.

The detection limit of most ruminant primer sets ranged from 0.05 to 0.01% bovine meat and bone meal and 0.1 pg of bovine DNA. Last year, a new computer program known as "GSPRIMER" surfaced which will facilitate the development of PCR primers specific to multiple species. By analyzing all regions of mitochondrial DNA from target and non-target species species-specific primer sets have been developed for sheep, goats, swine, and group-specific primer sets for ruminants and animals susceptible to BSE. Similarly, at the beginning of 2009 a sensitive and specific real-time PCR assay was described for the quantification in raw and cooked meat products from donkey, pork and horses. Just last month, a new PCR method (Cer-194) was established for the detection of cervidae DNA in feedstuff's. The Cer-194 system prove to be effective detecting meat meal samples derived from 2 subfamilies, 4 genera, and 7 species including deer, bovine, ovine, camel, pig, rabbit, fish, and chicken. Likewise, a suitable method to detect fishmeal in ruminant feed has also been developed. The method is similar to the official method of "PCR detection of animal-derived DNA in feed", and it can detect fish DNA from species such as sardines, tuna, and salmonids.

Since most of the PCR results have demonstrated that the reliable determination of MBM from ruminants and other species has not been resolved, especially for low concentrations of MBM (0.1%) in feed, testing alone is not an effective solution to controlling prohibited materials in a feed operation.

Sergio F. Nates, Ph.D.

Country Focus (Indonesia) – Sergio Nates

Feeds in Indonesia account for 70% of the total production cost of livestock farming with animal feed production increasing at a rate of 8.4% annually in the past 5 years. In 2007, Indonesia's production of animal feed shrank 7.7 million tons from 9.9 million tons in 2006 as a result of bird flu. Production of day old chick (DOC) broilers in 2009 has been estimated to be below 1 billion, according to the chairman of Indonesia Poultry Breeder Association, Paulus Setiabudi.

According to the association of animal feed producers (GPMT), the country's animal feed industry could supply up to 5 million tons of 7 million tons requirement every year. In 2008, demand for animal feed was estimated to be at 8.13 million tons. Based on data from GPMT Indonesia has 42 animal feed factories in operation in 2008. The number declined from 50 earlier as 8 companies have stopped operation. The industry is still dominated by foreign investors such as Charoen Pokphand, Japfa Comfeed, Sierad Produce, CJ Feed, Gold Coin, and Sentra Profeed. Large feed factories are generally integrated with livestock farming and livestock product processing industries. Last March, Nutreco subsidiary Trouw Nutrition Indonesia opened a premix facility that has a capacity of approximately 15,000 tons of blends and premixes per year.

In 2000, Indonesia imported only 20 percent of their national beef needs. In 2008, it increased to 35 percent, or more than 70,000 tons of beef, mostly from Australia and New Zealand. Last month the Indonesian government announced that it would soon begin importing beef from Brazil, though the governenment plan is to achieve self-sufficiency in beef by the end of 2010.

R&D Update (Progress report)

08A-1 **Attractability and Palatability of Rendered Animal Proteins to blue shrimp, *Litopenaeus stylirostris* - Dr. Victor Suresh**

- Completed sourcing of all samples (poultry byproduct meal (petfood grade & feed grade), feather meal, blood meal, fishmeal, fish hydrolysate, krill meal and squid liver meal).
- The samples have been sent to Nestle Purina Analytical Laboratory, Saint Louis, Missouri for the following analyses: proximate composition, soluble protein, free amino acids, taurine, and nucleotides. Results of the following analyses are available: proximate composition, taurine, and nucleotides. Results of pending analyses will be available by the end of May.

- Samples have been sent to Keck Biotechnology Resource Laboratory, Yale University, New Haven, Connecticut for protein molecular weight profiling. They will be analyzed by the middle of May.

- All ingredients have been shipped to Brunei Darussalam for shrimp trials. Diets for the Phase I attractability and palatability assessments have been prepared. Palatability assessment is underway and will be completed by the middle of April. Attractability assessment will be completed in the latter half of May.

- Feeding trials will be started in the first week of June and completed by the end of July.

Progress Details:

(1) Procurement of samples: The following samples were sourced through FPRF from a supplier (source not revealed): poultry byproduct meal (petfood grade and feed grade), feather meal, and blood meal. IAI sourced the following samples: Peruvian fishmeal (CJ Indonesia); Fish hydrolysate (Sopropeche), krill meal (Aker Biomarine), and squid liver powder (CJ Indonesia).

(2) Shipment of samples: Bulk of the samples was shipped to Brunei Darussalam for storage and use in shrimp trials. About 300 g of each sample was shipped to Nestle Purina Analytical Laboratory, Saint Louis, Missouri and Keck Biotechnology Resource Laboratory, Yale University, New Haven, Connecticut for analyses.

(3) Analyses: Samples were analyzed for proximate composition, taurine and nucleotides at Nestle Purina Analytical Laboratory. Results are in Table 1 and show that marked differences occur in the nucleotide profile and taurine levels among the ingredients. Analyses are pending for soluble protein and free amino acids and expected by the end of April. Samples sent to Keck Biotechnology Resource Laboratory will be subjected to protein molecular weight profiling in mid-April.

(4) Diet preparation: Nine diets for the first set of comparisons of ingredients for attractability and palatability were prepared. The formulas are presented in Table 2. Diet preparation involved mixing the finely ground ingredients with 50% water, subjecting the wet mash to 105C for 5 minutes in an autoclave, and forming strands of feeds in a meat mincer. The feeds were then dried in a forced draft oven at about 50C for 6 hours.

(5) Palatability assessment is currently being carried out. Each day shrimp weighing 9-10 g are transferred from outdoor green water tanks to a clear water tank nearly 12 to 14 hours before the trial. The shrimp are not offered feed the in clear water. Six shrimp are randomly selected and transferred to the acclimatization chamber of the Y-maze glass aquarium filled with clear sea water. Shrimps are allowed to acclimatize for 30 minutes. One gram of test feed is placed in the feed chamber after the acclimatization period. The glass shutter is slowly removed two minutes after placing the feed. Shrimp are allowed consume feed for 30 minutes. After 30 minutes, all shrimp are removed from the tank. The uneaten feed is recovered completely with utmost care and placed on a pre-weighed piece of aluminum foil. Recovered feed (on the foil) is dried in convection drier until the feed is completely dry. The dried feed is cooled and allowed to absorb moisture from the

air at room temperature. Feed consumption within 30 minutes and adjusted for leaching losses and absorption of salt is considered as the index of palatability. A total of 9 observations are being made for each diet. Palatability results for comparison set 1 will be available in mid-April.

(6) Attractability assessment and comparison set 2 palatability trials will be conducted in latter half of May. Ideal shrimp size for conducting attractability assessment is 2-3 g. Due to biosecurity protocols at the laboratory, shrimp of 2-3 g size will be available only in the latter half of May.

(7) Grow-out feeding trials in microcosm tanks will be started in June and completed in July.

Table 1: Attractants in ingredients used in the trial

	Fishmeal	Fish Hydrolysate	Krill	Squid liver meal	Poultry byproduct meal, Petfood Grade	Poultry byproduct meal, Feed Grade	Feather meal	Blood meal
Nucleotides (ppb):								
Uridine	32	51	101	51	196	71	22	< 10
Cytidine	14	< 10	26	12	81	33	23	< 10
Inosine	16	516	312	1440	589	205	31	< 10
Guanosine	27	55	49	140	130	62	20	< 10
Adenosine	22	21	40	35	259	74	19	< 10
UMP	153	84	919	24	123	45	< 10	< 10
CMP	128	29	991	32	108	60	< 10	< 10
IMP	36	122	988	2230	182	88	22	< 10
GMP	274	58	798	67	71	41	< 10	< 10
AMP	130	292	2270	443	461	127	27	< 10
Taurine (ppm)	5046	7147	5381	7378	4463	2118	477	304

Noteworthy Article

Freeman SR, Poore MH, Middleton TF, Ferket PR. (2009) Alternative methods for disposal of spent laying hens: Evaluation of the efficacy of grinding, mechanical deboning, and of keratinase in the rendering process. Bioresource Technology – Available on Line May 15, 2009.

Besides the challenges of mortality and litter disposal, the poultry industry must find economical means of disposing of laying hens that have outlived their productive lives. Because spent hens have low market value and disposing of them by composting and burial is often infeasible, finding alternative disposal methods that are environmentally secure is prudent. The feasibility of grinding or mechanically deboning spent hens with and without prior mechanical picking was evaluated for the production of various proteinaceous by-product meals. The end products were analyzed for nutrient content and found to be high in protein (35.3-91.9% CP) and, with the exception of the feathers, high in fat (24.1-58.3%), making them potentially valuable protein and energy sources. After considering physical and economic feasibility, mechanical deboning was determined to be a logical first step for the conversion of spent hens into value-added by-product meals. Because the hard tissue fraction (primarily feathers, bones, and connective tissue) generated by mechanically deboning the hens presents the greatest challenge to their utilization as feedstuffs, attention was focused on technologies that could potentially improve the nutritional value of the hard tissue for use as a ruminant protein source.

Traditional hydrolysis of this hard tissue fraction improved its pepsin digestibility from 74% to 85%; however, subsequent keratinase enzyme treatment for 1h, 2h, 4h, or 20h after steam hydrolysis failed to improve the pepsin or amino acid digestibility any further (P>0.10). Enzyme hydrolysis did, however, increase the quantities of the more soluble protein fractions (A: 45.5, 46.6, 52.8, 51.6, and 55.8% of CP; B(1): 3.2, 9.8, 6.0, 4.6, and 4.1% of CP; B(2): 11.7, 18.1, 22.8, 29.6, and 22.0% of CP for 0, 1h, 2h, 4h, and 20h, respectively) and reduced quantities of the less soluble fractions (B(3): 30.2, 18.1, 10.8, 5.5, and 10.2% of CP; C: 9.4, 7.5, 7.6, 8.8, and 7.9% of CP for 0, 1h, 2h, 4h, and 20h, respectively). The protein digestibility of the steam hydrolyzed hard tissue fraction from the mechanical deboning of spent hens was found to be comparable to the digestibility of feather meal, but post-hydrolysis keratinase treatment did not improve feeding value for ruminants.

Fats and Proteins Research Foundation, Inc.801 N. Fairfax St., Suite 205 Alexandria, VA 22314

Phone: (703) 683-2914

Fax: (703) 683-2626

www.fprf.org