

# Director's Digest

FATS AND PROTEINS RESEARCH FOUNDATION, INC.



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#297

## Fats and Proteins Research Foundation, Inc.

### Executive Summary of Current Projects

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By: Gary G. Pearl, D.V.M.\*

**“Development of formulated organic soil amendments for managing populations of soilborne pests, including weeds, nematodes, fungi and bacteria.”**

**Dr. George Lazarovits - Agriculture and Agri - Food Canada**

This work has been progressing since 1994. The project was initiated with 4 basic objectives.

1. To evaluate the efficacy of organic amendments, derived from the animal processing industries to reduce populations of soilborne plant pathogens: Verticillium dahliae and Streptomyces scabies. This objective has been completed and verified the efficacy of meat and bone meal for this purpose. Results have been presented in previous progress reports and have been published in referred journals (Conn & Lazarovits 1999 Can. J. Plant Path 21:81-92 and Lazarovits et. al. 1999 Can. J. Plant Path 21: 345-353.

2. To identify the mechanisms by which amends reduce inoculum levels of pathogens in soil. This objective has been completed. Results have been presented in previous progress reports. Those reports have detailed ammonia (NH<sub>3</sub>) and nitrous acid (HNO<sub>2</sub>) toxicity as mechanisms by which organic amendments kill a broad range of soilborne pathogens and pests. Several publications are in the final stages of editing and soon to be submitted to referred journals. Since the October 1999 report toxicity evaluations of NH<sub>3</sub> and HNO<sub>3</sub> indicate that HNO<sub>3</sub> is 10 to 100 times more toxic than NH<sub>3</sub> for a range of pathogens and pests.

3. To identify and isolate key microorganisms associated with the release of bioactive components.

Two groups of bacteria were isolated for inclusion into MBM formulations,  $\text{NH}_3$  producers (ammonifiers and  $\text{HNO}_2$ -producers (nitrifiers)). Previous progress reports detailed the isolation and quantification of  $\text{NH}_3$  production from MBM by several bacteria isolated from soil. These bacteria were tested in formulations of meat and bone meal in soil (see results of objective 4). Several bacteria were also obtained from the American Type Culture Collection, as they were known to be responsible for nitrification in most soils. When added to amended soil the bacteria failed to produce  $\text{HNO}_2$ . We attribute this to their intolerance of soil pH of less than 7, which was the case for the soil we used. We are currently isolating acid tolerant nitrifiers (pH 5). Since no published methodology for isolating these organisms is available we are experimenting with various media and protocols for selection of these agents. The high toxicity of  $\text{HNO}_2$  to soil pathogens indicates that addition of nitrifiers that can increase this product will provide the greatest boost in efficacy of a formulated product.

4. Develop and test formulations of custom amendments for effects on crop development and crop health.

These studies aim to utilize information obtained in Objectives 1- 3. Considering the economics of production of high value crops, formulations consisting of between 4 to 10 t/ha of MBM-like products likely will be considered as affordable. Rates of 10 to 20 t/ha are likely economical for higher value vegetable and fruit crops as replacements for methyl bromide. Their use however, may be restricted to arid environments where leaching of nutrients to groundwater is not a concern.

Studies are in progress involving creation of formulations by blending microbial and pH-modifying agents with MBM. Alkali generating agents and  $\text{NH}_3$ -producing organisms are to be used in formulations for soils of neutral to high pH and low organic matter. Agents tested to date that have increased the efficacy of amendments include activated sewage sludge,  $\text{CaO}$ , and  $\text{Ca}(\text{OH})_2$ . Acid generating agents and  $\text{HNO}_2$ -producing organisms are to be used in formulations directed for soils of acid pH and low calcium carbonate content. For such formulations we tested  $\text{FeSO}_4$ ,  $\text{H}_2\text{SO}_4$ , citric acid, and elemental sulfur. Of these reagents, elemental sulfur seems the most promising. Rates as low as 100 kg/ha were found to be sufficient to reduce soil pH and are currently being tested to increase the efficacy of amendments. Elemental sulfur is also commercially available in large quantities and at a relatively low cost.

We report here the affect of adding  $\text{NH}_3$ -producing bacteria to MBM amended soil on the accumulation of  $\text{NH}_3$  in soil. Five bacterial isolates that were found to vigorously produce  $\text{NH}_3$  in meat and bone meal amended liquid suspensions, were used for this study. A pasteurized sand-soil mixture was amended with MBM (to 0, 0.5, and 1% w/w) and the bacterial isolates were added to reach inoculum loads of 107 to 1 08 bacteria/g soil. Treatments were analyzed at day 0, 1, 2, 3, 5, and 7 for pH and  $\text{NH}_3$  concentrations. Of the five isolates, three, B96, BMB38, and BNS28, increased soil pH and ammonia concentrations at either rate of MBM tested. Soil pH was significantly higher in the bacteria-amended MBM treatments by day one. In these treatments pH kept increasing until day 5. Of the isolates tested, strain B96 provided the largest

increase in soil pH and ammonia accumulation. Five days after application, pH in B96 treatment reached 8.8, compared to 8.4 in the 0.5% MBM non-inoculated treatment. The concentration of NH<sub>3</sub> in the B96 treatment was 15.3 mM, which is well above the toxic levels required to kill many soil-borne plant pathogens and pests. In comparison, the 0.5% MBM non-inoculated treatment had maximum NH<sub>3</sub> concentration of 9 mM.

Addition of MBM to 1% (w/w) resulted sharply increasing soil pH from 6 to 8.4 within 2 days regardless of the bacterial isolate tested. Soil pH continued to rise and reached maximum levels at 5 days with isolates B96 and BMB32, being pH 9.2 and 9.0 respectively. In comparison, control soil with 1% MBM had a pH of soil at five days of 8.8. Ammonia levels peaked at 5 days with B96 and BMB38 treatments, being 48.0 mM and 31.7 mM respectively. In comparison, in the 1% MBM non-inoculated treatment the concentration of NH<sub>3</sub> was 33.9 mM. No apparent difference in soil pH or NH<sub>3</sub> concentration was evident between isolates at 7 days.

The results indicate that 2 of the isolates tested have the potential to promote accumulation of NH<sub>3</sub> within 5 days of addition of MBM to soil. Studies of longer duration are in progress testing these and other isolates in MBM amended non-pasteurized soils to promote NH<sub>3</sub> toxicity to plant pathogens including *Verticillium dahliae*, *Streptomyces scabies*, *Rhizoctonia sp.*, and *Phythium ultimum*.

The results of studies leading to an understanding of the modes of action (NH<sub>3</sub> and HNO<sub>2</sub> toxicity) to pathogens and factors that control the accumulation of the toxins in amended soil have been summarized for a patent application. Results of studies leading to development of amendment formulations will be included in the patent application.

**Conclusions:** These studies indicate that meat and bone meal and related products when applied to soil can be an effective broad spectrum and practical alternative to chemical fumigants in many soils. However, the rate of meat and bone meal must be matched to the soil properties of an individual field, if these products are to be used as an alternative to chemical fumigants. Further the rate of amendment must be reduced to be cost affective and minimize nitrogen leaching to groundwater. Formulation of rendered products is therefore required. This will involve modifying the amount of organic N added to soil depending upon soil properties, and the incorporation of organisms and pH modifying agents to optimize the production of the toxins (ammonia or nitrous acid). For soils containing low levels of organic matter ammonia generation will be the mechanism of choice whereas, for soils lacking carbonates, nitrous acid will be more effective.

## **“Can Prepartum Dietary Tallow Be Used to Increase Body Condition and Prevent Fatty Liver in Dairy Cows?”**

**Dr. James Drackley - University of Illinois**

### **Final Report in Final Stages**

It was proposed that supplemental fat fed to dairy cows during the dry period would help to restore body condition (body fat reserves) to thin cows and help to prepare cows for subsequent milk production. In a previous experiment funded by FPRF, we found that cows fed a diet supplemented with choice white grease had less fatty liver development at calving, which would

indicate a more desirable metabolic adjustment to lactation. However, total nutrient intake was decreased by the high-fat diet, so that we could not directly attribute the metabolic changes to the fat supplementation. The objectives of the current project were:

- 1) to determine if the decreased liver fat at calving that we observed previously in cows supplemented with animal fat (grease) during the dry period was due to the fat supplementation or to nutrient restriction from decreased feed intake;
- 2) to determine if dry period feeding strategy affects lactation performance and incidence of health problems around calving; and
- 3) to determine if body condition can be added safely to dairy cows during the dry period by adding animal fat to the dry-period diet. The experiment was conducted with 60 Holstein cows, divided into five dietary treatment groups. The experimental diets were fed throughout the last 60 days before expected calving, and then all cows received one lactation diet after calving. Cows were fed either a fat-supplemented diet or an unsupplemented diet of equal energy content, both for ad libitum (free choice) intake and at a restricted intake. A fifth dietary treatment was a higher-energy diet with supplemental fat. Cows fed either diet at restricted intake during the dry period had greater feed intakes after calving and less liver fat at calving. Although supplemental fat caused some smaller changes in metabolism, we conclude that nutrient intake during the dry period has a much greater effect on metabolic adjustments to milk production than does fat supplementation. Fat supplementation to dry cows or to cows during the transition from pregnancy to lactation may have merits in certain situations, but it does not appear that fat supplementation before calving will benefit all dairy cows.

#### **“Further Evaluation of the Effects of Processing Temperature and Ash Content on Protein Quality of Animal Meals for Poultry.”**

**Dr. Carl Parsons, University of Illinois**

Two studies were conducted. In the first study, experiments were conducted to evaluate the effects of different processing pressures, temperatures and times on the digestibility of amino acid (AA) in meat and bone meal (MBM) when the pressure-processing was done after typical rendering (Experiments 1 and 2) or during the initial rendering process of raw materials (Experiment 3). In the second study, experiments were conducted to determine the effect of ash concentration on amino acid (AA) composition, true AA digestibility and protein efficiency ratio (PER; weight gain per unit of protein intake) in MBM samples varying in ash.

In the first experiment of study one, a small scale experimental cooker was used to pressure-process a commercial MBM at 0 psi for 20 min, 15 psi for 20 min, 15 psi for 30 min, 30 psi for 20 min, 30 psi for 30 min, 45 psi for 20 min or 60 psi for 20 min. In the second experiment, a commercially processed MBM was subjected to pressure-processing in a commercial feather meal cooker at 45 psi for 20 min or 60 psi for 20 min. In the third experiment, raw materials from a beef slaughter plant were pressure-processed at 30 psi for 20 min or 60 psi for 20 min. True digestibilities of the amino acids in the MBM samples were determined using the precision-fed cecectomized rooster assay.

Processing pressure and time had no consistent effect on CP, ash or fat content of the MBM samples in all three experiments. Increasing pressure during processing reduced MBM Cys

concentrations in Experiments 1 and 2. True digestibilities of most AA were significantly decreased by all pressures in Experiments 1 and 2. The reductions were generally largest for Lys and particularly Cys, as the severity of pressure increased. For example in Experiment 1, Cys digestibility decreased from 65 to 50 to 15% and Lys digestibility decreased from 76 to 68 to 41% as the MBM was processed at 0, 30 and 60 psi, respectively, for 20 min. Pressure processing during the initial rendering of the raw materials in Experiment 3 significantly reduced ( $P<.05$ ) the digestibility of most AA at 60 psi, but the decrease was much less than those observed in Experiments 1 and 2.

In the second study, commercially-rendered meat and bone meal (MBM) samples differing in ash content, due to air-classification or high bone content, were obtained from two sources. The ash content of the latter samples varied from 16 to 44% ash. Additional samples of MBM varying in ash from 9 to 63% were obtained by chloroform floatation or lab screening of a beef crax sample. True AA digestibility of MBM samples was determined using the precisionfed cecectomized rooster assay (4 roosters per MBM sample). To further evaluate protein quality, PER chick growth assay was conducted wherein chicks were fed 10% CP diets containing a MBM as the only source of dietary protein from 8 to 18 days of age.

In the second study, an increase in Ala, Pro, Gly and Arg as a percent of CP was observed in all MBM samples as ash percent increased, with Pro and Gly accounting for most of the increase. In contrast, the levels (% of CP) of all essential AA other than Arg decreased as ash level increased. For example, Lys concentrations per unit of CP decreased from 5.3 to 4.0% as ash increased from 9 to 63%. There was no effect of ash content on AA digestibility of MBM varying in ash from 9 to 44%. Digestibility of AA was reduced in MBM samples containing 60 to 63% ash (all bone). The PER of MBM markedly decreased from 3.34 to 0.72 as ash increased from 16 to 44%. Additional treatments showed that most of the effects of ash on PER were not due to differences in dietary Ca and P levels.

The results of study one indicate that pressure-processing of MBM decreases the digestibility of AA for poultry. The results of study two indicate that protein quality of MBM is reduced as ash content increases. The latter reduction in protein quality is almost entirely due to a decrease in analyzed essential AA per unit of CP, not a decrease in the digestibility of AA.

### **“Diet by Fat Interactions in Dairy Cattle.”**

**Dr. Ric Grummer - University of Wisconsin**

This project has several objectives that involve different corn silage and alfalfa silage ratios when fed in conjunction with tallow, choice white grease. Including fat in exclusive corn silage based diets had negative effects on production and rumen fermentation regardless of the source or level of supplemental fat in an initial experiment. Comparing either 0% or 2% tallow additions to rations in which all forage was either silage, 2/3 corn silage - 1/3 alfalfa silage or 1/2 corn silage - 1/2 alfalfa silage resulted in the fat supplemented cows having a lower DMI but producing more milk with less milk fat content relative to non-supplemented cows. The results suggest there are no beneficial effects of replacing corn silage with alfalfa silage when tallow is supplemented at 2% of diet DM.

**“Meat and Bone Meal as a Major Dietary Ingredient for Salmonids Under Several Growth Regimes.”**

**Dr. Gerald Schelling - University of Idaho**

**Project Completed**

Feeding experiments were conducted to examine the potential of replacing fishmeal protein (FM) with a standard, good quality meat and bone meal protein (MBM) in 25% increments for growing rainbow trout. With the objective of making an overall evaluation of growth performance and carcass composition, semi-purified diets were used to provide FM protein: MBM protein of 100:0, 75:25, 50:50, 25:75, and 0:100 as the sole dietary protein in isonitrogenous and isocaloric diets. Two hundred and twenty five rainbow trout (mean weight 120 g) were allotted to five treatments with three replicates in a randomized block design. The tanks were five cubic feet; water flow = 20.51 L/min; temperature = 15°C. The fish were hand fed to satiation twice daily and were weighed on d 0, 21, 42 and 63. Daily gain, daily feed intakes, and feed/gain ratios were determined for growth performance. A sub-population of fish were sacrificed on d 63 for body composition determination by whole carcass proximate analysis. In the 100:0 diet, fish gained 3.8 g/d and the relative percentage gains for the series of diets with increasing MBM protein were 100, 90, 83, 85, and 59% (59 lower,  $P < .05$ ). The feed/gain ratios were .94, .98, 1.04, 1.09 and 1.46 (1.46 less efficient,  $P < .5$ ), respectively. There were no marked differences ( $P > .05$ ) in dry matter and protein content of the whole body carcass fed the dietary treatments. The 0:100 diet resulted in reduced growth ( $P < .05$ ) and therefore, had somewhat more carcass fat ( $P < .05$ ). This work indicates that FM protein in semi-purified diets for rainbow trout can be replaced with 25 and 50% MBM protein with only 5 and 10% loss in F/G, and even up to 75% with a 15% loss in F/G.

**“Combinations of Rendered Animal Protein Ingredients as Protein Sources for Salmonid Diets.”**

**Dr. Dominique Bureau - University of Guelph**

**Project Completed**

Two growth trials were conducted using spray-dried blood meal (BM), feather meal (FEM), meat and bone meal (MBM), poultry by-products meal (PBM) as major protein sources in the diet of rainbow trout. In the first trial, increasing levels of BM (6, 12%) or PBM (0, 10, 20, 30%) replaced fish meal and corn gluten meal in the diet. PBM and BM provided up to 40% of the total digestible protein of the diet. For the second trial, eight diets were formulated to contain following combinations: FEM+MBM, FEM+PBM or MBM+PBM replacing half the fish meal and the totality of the soybean meal (control containing 40% fish meal and 13% soybean meal, experimental diets containing 20% fish meal and no soybean meal). Rendered animal protein ingredients provided about 2/3 of the total digestible protein of the diet in this trial. Some of the experimental diets were supplemented with either L-lysine or DL-methionine. The fish were fed the experimental diets for 16 weeks in the first trial or 12 weeks in the second trial. Faecal samples were collected for the experimental diets of the first trial to measure apparent digestibility.

High growth rates and feed efficiencies were achieved for all diets in the two trials. Apparent digestibility coefficients (ADC) of protein and energy of the diets containing high levels of PBM and BM were high and confirm the high ADC measured for PBM and BM in previous studies in our laboratory. There were no significant differences in the growth rate, feed efficiency, nitrogen and energy retention efficiencies (N or E gain/digestible N or E intake) of the fish fed the eight diets in the first trial. This suggests that both BM and PBM have high nutritive values and that they can be used at fairly high levels in rainbow trout diets. In the second trial, growth rate of the fish fed the diet containing the FEM+PBM combination was not statistically different from the growth rate of fish fed the control diet. Growth rates of the fish fed diets containing FEM+MBM or MBM+PBM combinations were significantly lower than that of the fish fed the control diet. Digestible nitrogen retention efficiencies of all the experimental diets were significantly lower than that of the control diet. Supplementation of diets with either L-lysine or DL-methionine had no effect on the performance of the fish. This suggests that diets containing high levels of combination of feather meals, poultry by-products meal, and meat and bone meals can support high growth performances and feed efficiencies. However, better definition of the nutritive value of these ingredients is required to be able to formulate diets that support performance levels matching those obtained with high fish meal diets.

**“Effects of High & Low Levels of UIP fed Prepartum on Postpartum Production and Health on Holstein Cows During Heat Stress.”**

**Dr. William Tucker - Mississippi State University**

This project is behind the completion schedule due to the volume of statistical analysis being evaluated. Fifty-seven cows calved on this project to evaluate the influence of undegraded intake protein (UIP) during extreme heat stress. Progress reports previously supplied have inferred several positive responses.

Thus far, we can hypothesize that feeding higher levels of undegradable intake protein (UIP) during the postpartum period will increase levels of fat corrected milk at freshening to 3 weeks postpartum. In general, the High UIP cows had lower rectal temperatures near parturition and weeks eight through twelve. In addition the consumption of high quality protein appears to influence physiological function as determined by significantly lower somatic cell counts. Plasma calcium and respiration rate at or near parturition. Higher DMI was obtained near peak milk production for cows fed higher levels of UIP.

Additional analysis will be conducted to ascertain further knowledge of UIP in prepartum diets. Completion of this project and summarization of data generated will provide a unique understanding of protein nutrition for dry cows during times of excessive heat and humidity. Practical implications include the possibility of manipulating dietary UIP content to improve cow performance at times of elevated environmental temperatures and humidity with respect to the southern U.S.

**“Does an Unhydrolyzed Vegetable Sucrose Polyester Affect Performance or Pigmentation in Broiler and Laying Hens?”**

**Dr. Bobby Damron - University of Florida**

Two phases of the laying hen portion of the study utilizing UVP at 0, 2000, 4000, 6000, 8000 and 10,000 ppm as supplied from snack chips have been completed. The initial study resulted in no significant differences found for any of the elevation criteria which included: egg production, daily feed intake, feed efficiency, egg weights, body weight change, egg specific gravity, manure moisture, yolk color and hatchability. An analysis of the second study is not complete.

The first of two broiler trials have also been completed and a duplicate study is scheduled to begin March 21, 2000. A 42-day broiler study will be conducted in litter-floored pens, starting with day-old sexed chicks obtained from a commercial broiler hatchery. Seven replicate pens, each starting with 24 chicks, will receive each dietary treatment for a 42-day feeding period. Dietary treatments will be 0., .5, 1, 1.5, 2.0 or 2.5% UVSP added to the starting and finishing diets. Feed and water will be provided ad lib through tube feeders and bell-type waterers. Infrared heat lamps will be utilized for brooding.

At 42 days of age, birds will be group-weighed by sex and pen, and litter samples taken from three locations in each pen away from feeders and waterers and blended for moisture determination. On the day prior to weighing, eight birds from each pen will have their shank pigmentation evaluated using a MinoltaChromo Meter. Evaluation responses for broilers will include average body weight, feed efficiency, mortality, shank pigmentation scores and litter moisture value.

**“Modeling Ruminal Metabolism of Tallow to Increase Fat usage in Dairy Rations.”**

**Dr. Tom Jenkins - Clemson University**

Previous work has suggested that the negative effects associated with lipids on fiber digestion were related to the concentration of free unsaturated fatty acids (UFFA) in the rumen. Work described in the progress report of this project proposes an equation as follows:

Fiber Digestibility Depression (%) =  $0.0125 (\text{UFFA})^2 - 0.2003 (\text{UFFA}) + 1.38$  Tallows differing in IV (47, 50 and 53) are being tested by the model in an attempt to quantify how actual concentrations of different lipid types and sources contributes to free unsaturated fatty acids in the rumen. The tallows have been shown to fit the model via an in vitro study. The three tallows had little influence in depressing fiber digestion and also did not appreciably increase UFFA. Tallow has a considerably lower rate of lipolysis than soil in the invitro cultures of this investigation. Although this effect did not impact biohydrogenation of saturated fatty acids appreciably, it may explain previous reports that tallow has fewer negative effects on ruminal digestion and fermentation compared to other fats, this hypothesis will be under scrutiny in the progress of this project.

**“Routine Techniques for Monitoring the Nutritional Value of Animal Meals.”**

**Dr. Theo van Kempen - North Carolina State University**

To prevent the large variation in nutritional quality of animal meals from becoming a bottleneck for the incorporation in feeds, techniques are needed which allow for the rapid quantification of



the actual nutritional value of the animal meals. The objective of this proposal is to develop such techniques. In brief, the in vitro digestible amino acid content of animal meals will be quantified, and the samples analyzed will subsequently be used to develop either Near Infrared Reflectance Spectroscopy, Fourier Transform Infra Red spectroscopy, or solid-state NMR calibrations relating spectral data to digestible amino acids. These calibrations can then be used to accurately predict the nutritional value of an animal meal sample in 2-20 minutes with a variable cost that is not much more than the labor required for the handling of the sample.

Close to 200 meat and bone meal samples have been obtained from members of the FPRF. In addition a number of samples assayed in vivo have been obtained to assist in the validation experiment. An enzyme digestive procedure being developed will serve as the in vitro reference assay.

### **“Influence of level of intake on the comparative value of Supplemental fat in finishing diets for feedlot cattle.”**

**Dr. Richard Zinn - University of California - El Centro**

This project was a replacement for one designed to study the effect of UVSP additions to the ration of feedlot cattle. However supplies of UVSP was not sufficient to complete the original approved study. This project is important in optimizing fat supplementation in feedlot cattle.

Trial 1. One hundred crossbred yearling steers with an average initial weight of 446 kg were used in a 100-d finishing trial. Steers will be blocked by weight and randomly allotted within weight groupings to 20 pens (5 steers/pen). Dietary treatments consisted of a steam-flaked corn-based finishing diet supplemented with 0, 1.5, 3 or 4% yellow grease. Steers were implanted with Synovex-Plus at the start of the trial. Fat supplementation increased ADG (13%, quadratic effect,  $P < .05$ ), DMI (3%, quadratic effect,  $P < .05$ ), feed efficiency (8%, linear effect,  $P < .05$ ), and dietary NE (linear effect,  $P < .05$ ). Optimal response in ADG, feed efficiency and dietary NE occurred with 3% supplemental fat, consistent with our hypothesis (Zinn, 1994) the NE value of supplemental fat declines quadratically with increasing total fat intake.

Trial 2. The sample collection aspect of this trial related to metabolism studies is completed. Samples are currently being analyzed. Problems related to the need for equipment replacement has prevented the completion of the analyses.

### **“Feeding Value of Unhydrolyzed Vegetable Sucrose Polyester in Starter - Grow Swine and Duck Diets.”**

**Dr. B. G. Harmon - Purdue University**

Unhydrolyzed Vegetable Sucrose Polyester (UVSP) is currently being used as a “non-digestible fat” source for human snack foods. A trial has been completed to evaluate its use and energy contribution to the rations of swine and ducks. A second verification trial is being completed and is in its final stages. A final report is pending.

**“Estimating Efficiency of Protein Accretion in Cattle Fed by-Product Proteins.”**

**Dr. Don Beermann - Cornell University**

**(Currently - University of Nebraska-Lincoln)**

**Project Completed**

In the first study a mixture of four animal by-product protein sources was formulated using the 1996 Beef NRC model to achieve a “balance” of individual amino acids to complement the corn-based, soybean meal-supplemented diet. The mixture consisted of 34% pork meal, 25% fish meal, 20.5% blood meal and 20.5% feather meal. This mixture was added to the basal diet to 0, 2.5, and 5.2% of diet DM to make up three treatments. A fourth diet containing 1.75% urea on a DM basis and no soybean meal made up the fourth treatment. Results indicate that the 1996 Beef NRC model accurately predicts protein and amino acid requirements in young rapidly growing steers. When steers are fed adequate protein in diets formulated by the 1996 NRC, feeding a small amount of a balanced animal by-product protein mixture increased N retention 7%, but when fed at 5.2% of the diet, a negative effect on N retention and efficiency of retention were observed. Feeding the by-product protein mix at 5.2% appeared to cause excess total protein intake and caused a reduced efficiency of protein gain. Feeding urea as the only protein supplement was able to achieve a similar efficiency and level of protein gain measured as N retention.

We concluded that feeding this mixture of animal by-product proteins in diets that do not provide adequate total protein or N intake significantly increases both efficiency and rate of protein gain. If proper formulation has been achieved to meet the animal’s requirements, however, replacement of soybean meal or urea with the by-product mixture may not significantly improve either efficiency or rate of protein gain.

In the second experiment rate of protein gain paralleled the two levels of urea supplementation. The higher level produced fifty percent higher rate of protein gain because the level of protein intake was about fifty percent higher as well. The twice-daily estradiol injection had no effect on rate of protein gain at either low or moderate levels of protein intake. Results of this experiment suggest that estradiol injections may have to be maintained for periods longer than fourteen days to see measurable effects on protein gain, or that a higher level of protein intake is required to facilitate observance of the stimulatory effect on protein gain.

**“Ileal Digestibility of Amino Acids, Phosphorus and Calcium in Meat and Bone Meal for Growing Pigs and Chicks.”**

**Dr. Gary Cromwell - University of Kentucky**

**Project Completed**

This project was initiated with three basic objectives.

1. To determine the ileal digestibility of calcium, phosphorus, and amino acids in meat and bone meal from two sources that vary in ash content for pigs.
2. To determine the ileal digestibility of calcium, phosphorus, and amino acids in meat and bone meal processed at different processing conditions for pigs.

3. To determine phosphorus bioavailability and ileal digestibility of calcium, phosphorus and amino acids in meat and bone meal for broiler chicks.

The results of the research with meat and bone meal that was conducted at the University of Kentucky over the past three years indicate that meat and bone meal is an excellent source of amino acids, calcium and phosphorus for swine. Although the research was less extensive with poultry than with swine, similar conclusions can be drawn from this research.

Averaged across all of the studies, the P in meat and bone meal was 87% (based on slope ratio studies of bone traits), 90% (based on total tract, true digestibility studies), and 97 to 99% (based on ileal and total tract apparent digestibility studies) as bioavailable as the P in monosodium phosphate for pigs. For chicks, these values for meat and bone meal were 82% (based on slope ratio) to 87% (based on ileal apparent digestibility) of the availability of P in monosodium phosphate, but they were based on only one study. In the pig studies, the lysine, threonine, tryptophan, and methionine (the four most limiting amino acids) in diets in which meat and bone meal supplied all of the supplemental Ca and P were 99% as digestible as the amino acids in corn-soybean meal diets in which the P and Ca were supplied by monosodium phosphate and calcium carbonate. For chicks, these amino acids were 95 to 99% as digestible in the diets containing meat and bone meal as in the corn-soybean meal diets.

Particle size of meat and bone meal did not consistently affect the bioavailability of P for swine, but chicks appeared to utilize the P better in the large vs. small particle sized product. In pigs, the P in high-ash meat and bone meal of cattle origin seemed to be more bioavailable than that in low-ash meat and bone meal of pork origin. Subjecting meat and bone meal to excessive processing pressure did not negatively affect the bioavailability of P, and may have actually improved it. Particle size, source, or processing pressure of meat and bone meal had little effect on ileal digestibility of dietary amino acids in either pigs or chicks.

The new information generated from these studies and the studies summarized in our 1999 report to the Fats and Proteins Research Council gives us a better understanding of the nutritional value of meat and bone meal in nonruminant feeding. This new information is available to the scientific community as a result of abstracts published in the Journal of Animal Science, shown at the end of this report, and oral presentations given at the Midwestern Section and Annual Meetings of the American Society of Animal Science.

Information generated from this research should be of benefit to the swine, poultry, and feed industries. Greater quantities of meat and bone meal and other animal by-products are likely to be available for use in swine and poultry feeds because of the recent ruminant-to-ruminant feeding ban. The use of meat and bone meal in swine and poultry diets could increase as this new information is made available to nutritionists, who can now formulate diets that will more precisely meet the animal's requirement without having excessive nutrients. Excesses in dietary nutrients cause excessive nutrient excretion that ultimately contributes to environmental pollution.

### **“Availability of Amino Acids in Pork Meat and Bone Meal for Ruminants.”**

**Dr. Terry Klopfenstein - University of Nebraska - Lincoln**

#### **Project Completed**

Thirteen, commercially available, porcine meat and bone meal products from both independent renderers and commercial packing plants were evaluated in a lamb digestion study for crude protein, undegradable intake protein, metabolizable protein, apparent nitrogen digestibility, true nitrogen digestibility, and digestibilities of individual amino acids. Twenty-nine lambs were used in three periods to allow a minimum of 5 observations for each meat and bone meal sample.

The ranges observed for the variables tested were: crude protein, 53.5 to 65.5%; undegraded intake protein, 41.5 to 63.0% of crude protein; metabolizable protein, 19.3 to 40.0% of crude protein; ash, 21.3 to 29.3% of DM; apparent nitrogen digestibility, 61.5 to 64.8%; true nitrogen digestibility, 75.5 to 87.8%. Digestibility of individual amino acids varied considerably among products tested. These data indicate that digestibility of cystine varied over the widest range and should be given special attention. Low cystine digestibility likely indicates inadequate hydrolysis of hair.

The results of this research indicate that significant variability exists among commercially available porcine meat and bone meal products. Although these results indicate all of the porcine MBM products have relatively similar CP contents and adequate protein digestibilities, the range in MP values and amino acid digestibilities indicates the products likely have large differences in feeding value for ruminants.

### **“Further Definition of the Utilization of Bypassed and Intestinal Digestion of Amino Acids in Meat and Bone Meal.”**

**Dr. Neal Merchen - University of Illinois**

On the basis of past research funded for Dr. Merchen, this project is a cooperative study supported primarily by the Council for Agriculture Research of Illinois. The meat and bone meal used in the study has been coordinated by FPRF. The study is a validation basis animal performance of the values previously determined for MBM especially the Intestinal Digestibility of Available Amino Acids as determined by the In Situ/Precision Fed Rooster Assay.

This project has the objectives of:

- 1) Evaluating the ability of porcine meat and bone meal (pMBM) to provide absorbable amino acids (AA) to the small intestine of growing cattle when fed alone or in combination with other protein sources; and
- 2) To evaluate growth performance of steers fed the same diets used to fulfill Objective 1.

A source of pMBM that was assessed to be of high quality based on previous screening was obtained with the assistance of the Fats and Proteins Research Foundation. To address Objective 1, six Angus cross steers (average weight 630 lbs.) that had cannulas in the rumen, proximal duodenum, and terminal ileum were fed five diets in a 5 X 6 Latin square arrangement of treatments. Diets were based on ammoniated corn cobs, ground corn, alfalfa hay, and molasses. Urea was added to all diets to ensure that all contained adequate amounts of available N. Dietary treatments consisted of different supplemental protein sources. Diets were supplemented with 1)

soybean meal (SBM, **control** diet), 2) **pMBM** alone, 3) **pMBM** in combination with SBM (**pMS**), 4) **pMBM** in combination with corn gluten meal (CGM, **pMC**), and 5) **pMBM** in combination with SBM and CGM (**pMSC**). Digestion of N and amino acids were measured at each of the sites (duodenum, ileum, total tract) in the GI tract.

Total amount of N reaching the duodenum and bacterial N synthesis were not different among the treatments. However, when **pMBM** was fed in combination with CGM or with CGM and SBM, the amounts of total essential amino acids, total nonessential amino acids, and total amino acids entering the small intestine were increased compared to other diets. A somewhat more striking finding was that the digestibility of the amino acids entering the small intestine was substantially higher when the **pMBM** was fed alone (approximately 76%) than when any of the other four diets were fed (average approximately 63%). Thus, it appears that the amino acids from **pMBM** that escape breakdown in the rumen are highly digestible in the small intestine of the animal.

The growth study in which the same diets were fed that were used in the digestion study described above has been completed. Those data are now being summarized and statistically analyzed. The graduate student (Mr. Fernando Orias) who has been conducting this work as part of his Ph.D. dissertation project will be completing his dissertation and taking his final exam within the next month. Copies of those portions of his thesis that address use of porcine MBM will be forwarded to the FPRF.

**Implications of the Work-** The findings discussed above establish that porcine MBM is superior to SBM and slightly less effective than CGM in escaping ruminal degradation and in providing highly digestible amino acids to the small intestine of growing steers.

Porcine MBM appears to provide significant amounts of lysine to the small intestine but is less effective than CGM in providing methionine. Our data suggest that porcine MBM should serve as an efficacious protein source in ruminant diets and that it compares favorably to other animal protein sources and is superior to soybean meal as a source of intestinally digestible amino acids for cattle.

### **“Effects of Supplemental Fat on Growth Performance and Quality of Beef From Steers Fed Barley (and Potato Product) Based Finishing Diet.”**

**Dr. Mark Nelson - Washington State University**

One hundred sixty-eight crossbred beef steers (317 kg ± 2.6) are being fed barley-based finishing diets containing 15% potato byproduct DM to determine the effects of fat and forage level on growth performance, and appearance, eating quality and fatty acid (including conjugated linoleic acid) composition of beef. The steers were blocked by weight and randomly assigned, within block, to a randomized complete block design with a 2 X 3+1 factorial arrangement of dietary treatments. Main effects are level of alfalfa hay (3.5 or 7% of DM) and level of yellow grease (0, 3, or 6% of DM) with the added treatment of 7% alfalfa hay and 6% tallow. Diets contain at least 13.5% CP, .5% Ca, .3% P, .6% K, .2% S, .1% Mg, 8.8 mg Tylan/kg, 33 mg Rumensin/kg, 2,200 IU vitamin A/kg and provide 417 IU vitamin E/d.

Dietary treatment did not affect DM intake (7.9 kg/d  $\pm$  .3) or average daily gain (1.4 kg/d  $\pm$  .1). However, a quadratic level of grease by forage level interaction was detected ( $P < .10$ ) for feed-to-gain. Level of forage increased feed-to-gain in 0% yellow grease diets but not in 3 or 6% yellow grease diets. Intake of DM, gain and feed-to-gain of steers fed 6% tallow did not differ ( $P > .0$ ) from steers fed 6% yellow grease and 7% forage.

**“Effect of Bile Salt Supplementation on Fat Digestion in Early Weaned Pigs.”**

**Dr. B.G. Harmon - Purdue University**

**Dr. G.M. Hill - Michigan State University**

**Dr. D.C. Mahan - Ohio State University**

This project was completed as an hypothesis that bile salts via its emulsifying properties would enhance the absorption and utilization of fat in the young pig. Animal scientists have been perplexed for a number of years as to why the young pig fails to utilize fat additions from added sources to the equal of that supplied via sows milk. In this study bile salt supplementation to the diets of young pigs concurrently with added fat (lard) up to 7% supplemental fat did not enhance performance.

**“Thermal death Time Values for Rendered Animal Products.”**

**Dr. Annel Greene - Clemson University**

This project is in progress to define the time required to kill specified bacteria at given temperatures. Raw materials of beef, pork, beef bones, pork bones, beef offal, mixed species offal, poultry and feathers have been supplied to the Clemson Laboratory. The thermal viability of eight microorganisms are being investigated: Listeria monocytogenes, Escherichia coli, Enterobacter, Campylobacter, Staphylococcus aureus, Salmonella, Clostridium perfringes and Bacillus stearothermophilus. This industry driven approach to studying thermal death time values for our rendered products will be useful in addressing the questions of origins of foodborne illnesses.

Eight mixtures of raw rendering materials were examined in heat penetration studies in a Lovelass still retort using a TechniCAL CALPlex 32 Datalogger, Ecklund needle thermocouples and TechniCAL CALSoft data collection software. the eight mixtures of rendered products were designated: Treatment A (primarily beef bones), Treatment B (primarily shop fat and bones -beef and pork bones, beef offal), Treatment C (primarily beef tallow with some mixed species fat), Treatment D (cattle offal), Treatment E (feathers), Treatment F (poultry offal), Treatment G (whole ground chicken), and Treatment H (50% whole ground chicken + feathers [v/v]).

Thermal conductivity studies indicate that beef tallow (Treatment C) and feathers (Treatment E) had the fastest heat penetration whereas mixtures containing large amounts of bone material had the slowest rates of heat penetration. Standard deviations were large as would be expected on such non-uniform products as raw rendered products. Work is continuing inoculated pack studies to determine process lethality.

**“Literature Review of the Energy Values of Tallow and Other Characterized Animal Fats.”**Dr. Tom Jenkins - Clemson University

This project consisted of an intense literature review of those data reporting digestibility values ( $NE_L$ ) for tallow and other fats for the lactating dairy cow. A very thorough and analyzed report has been made available and provided to several reviewers including the FPRF Research Committee. The data demonstrates considerable variation in both fatty acid composition and digestibility. Some studies show digestibilities to be linear while others quadratic with increasing intake. Evaluation of the wide variation in tallow fatty acid composition might imply that some sources mistakenly labeled grease as tallow. The terms “tallow” and “grease” have been used loosely and points out the extreme need to provide complete analytical information on the fat sources used to include complete fatty acid profiles as obtained via gas chromatograph chromatograph technique. The studies used in this review contained 21 scientific publications where “tallow” was fed but fatty acid compositions were not reported.

**“Prevalence of Selected Foodborne Pathogens in Rendered Products.”**

**Dr. Fred Troutt - University of Illinois**

This prevalence study of four potential foodborne bacteria and determination of coliform counts in raw material supplied to renderers and subsequent to the rendering process is progressing on schedule. Seventeen rendering establishments in seven states are cooperating and participating in the study. Winter samples have been obtained from all facilities. Laboratory procedures for all of the microbiological isolations and identifications have been established according to scientific accepted practices. Preliminary results of the microbiological examinations have identified a very high contamination rate for all of the organisms in raw material (range 45-82%). This project has extreme importance and relevance to the entire rendering industry.

**“ELISA vs. Traditional Bacteria Plating Procedures for Enumeration of Salmonella in Heat-treated Rendered Products.”**

**Dr. Annel K. Greene - Clemson University**

After reviewing literature on commercially available ELISA kits, we have chosen seven kits to test for quantifying Salmonella content of rendered materials. These kits were chosen based on availability, BAM and AOAC approval, sensitivity, detection of actual bacterial cells, current use in the food industry and ease of use. These kits will be examined for potential use in screening rendered products for Salmonella. We will continue to search for additional kits that offer potential advantages for use in the rendering industry. Naturally contaminated meat meal will be obtained from Ralston Analytical Laboratory for use in this project.

**“Rendered Non-marine Animal By-products as Shrimp Feed Components.”**

**Tacon/Dominy - The Oceanic Institute**

Aliquots of three quality commercial meat and bone products have been supplied for the project. Particle size analysis and proximate composition have been obtained. Experimental diets have been formulated and in final stages of analysis. These rations will be used in a digestibility

feeding trial. A second experiment will be aimed at determining the maximum level of meat and bone meal which can be used within practical shrimp feeds as a dietary replacement for fishmeal without compromising shrimp performance.

### **Biodiesel Multi Feedstock - Phase III**

#### **Mr. John Kinast - Institute of Gas Technology**

The primary objective of this study was to determine if biodiesel from different feedstocks exhibit different emissions performance when tested on a consistent basis. By consistent basis is meant at one laboratory on one engine in a controlled study. To meet this objective we have carefully quantified emissions from seven different biodiesel fuels, from a variety of source materials, on one engine. The EPA heavy-duty transient test (40 CFR Part 86 Subpart N) was employed for all tests. Fuels tested neat include soy methyl ester, canola methyl ester, pork lard methyl ester, edible and inedible beef tallow methyl esters, low free fatty acid (1%) yellow grease methyl ester, and high free fatty acid (10%) yellow grease methyl ester. Several of the fuels were also tested as B-20 blends with certification diesel fuel. Regulated emissions (NOX, CO, THC, and PM) were determined, as well as the volatile organic fraction of PM. Several PM samples were also analyzed for sulfate. A minimum of three replicate tests were performed for each fuel and testing of biodiesel fuels was bracketed with tests using certification diesel. The study design allows a statistical comparison to be made of emissions from the various biodiesels and blends versus emissions from certification diesel. Neat biodiesels were analyzed for C, H, and O content, cetane number, and gross heating value. The most important observations of this study are that PM emissions are to a very good approximation independent of biodiesel feedstock or chemical structure, but that NOX emissions vary. All neat biodiesels reduced PM and increased NOX with a high level of statistical significance. The PM emissions reduction relative to certification diesel fuel appears to depend only on the oxygen content of the fuel. For unsaturated biodiesels derived from soy and canola the NOX increase was roughly 0.5 g/bhp-h. For fuels produced from more saturated feedstocks (the remaining fuels) the NOX increase was much lower, roughly 0.1 g/bhp-h. NOX emissions are higher for fuels produced from unsaturated feedstock such as soy or canola, in comparison to saturated feedstocks such as lard, tallow, or grease. One difference between these fuels is cetane number and it appears that higher cetane, more saturated biodiesels, produce lower NOX. Energy content or (Btu) based fuel economy was identical for certification diesel and biodiesel fuels. VOF averages about 5.5% for certification diesel, but increases to an average of 21.4% for the diesel. Because PM from fuel combustion is much lower for biodiesel, sulfate as a fraction of total PM is higher, in the 3-4% range. There is no significant difference in the VOF or sulfate emissions for biodiesel from different sources.

### **NCWM Premium Diesel & Lubricity Testing**

#### **Jim Peeples - Falls Church, VA**

The National Conference on Weights and Measure (NCWM) a regulatory body has been instrumental in promoting a "Premium Diesel" standard definition for states to enforce consumer protection. It is important that specifications include lubricity. During the lengthy evaluation of each performance criterion including cetane, detergency, cold temperature performance, accelerated stability, Btu content and lubricity it was determined that the current test methods are



unreliable and cannot accurately be correlated to a minimum level of protection. Thus this has presented the necessary "round robin" testing procedures for lubricity from proceeding. It is not expected that this work will proceed as currently structured and lubricity will not be a part of the initial ASTM standards.

### **ASTM Cetane Testing and Specifications Project Cooperative Study FPRF/NBB**

FPRF has provided biodiesel produced from a mixed feedstock and from tallow for cetane testing by the Diesel National Exchange Group. This information is necessary to establish ASTM (American Society for Testing Materials) final specifications for biodiesel. This work has been completed. A tentative ASTM Specification has been approved. Final balloting is to take place in the summer of 2000. A lubricity guideline is currently included but not expected to pass the ASTM Committee due to the concerns expressed in the Lubricity Project Report.

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## **FPRF NEW PROJECTS — APRIL 2000**

- 00A-1 Pre-proposal  
Viral Inactivation via Rendering Processes  
Dr. Jeff Zimmerman - Iowa State University
- 00A-2 Availability of Energy in Rendered By-Product Used in Turkey Rations  
Dr. Jeffre Firman - University of Missouri
- 00A-3 Treatment of Rendering Plant Wastewater with Ozone to Reduce Biochemical Oxygen Demand, Chemical Oxygen Demand, Color, Malodor Emissions and Bacterial Content  
Dr. Annel Greene - Clemson University
- 00A-4 Highly Nutritive, Cost Effective Feeds for Sustainable Marine Fish Culture in China  
Dr. Wang Yan - Shanghai Fisheries University
- 00A-5 Life Cycle Assessment of Recycled Grease Biodiesel  
Co: NREL/FPRF  
Dr. Shane Tyson - NREL  
Dr. Gary Pearl - FPRF
- 00A-6 Evaluation of the Effects of Dietary Fat, Ractopamine and CLA on Growth Performance and Pork Quality in Genetically Lean Gilts  
Dr. Allan Schinckel - Purdue University
- 00A-7 Improving the Feeding Value for Saturated Fats for Lean Genotype Swine: Effects of Tallow and Supplemental CLA  
Dr. Jack Odle - North Carolina State University
- 00A-8 The Use of Meat and Bone Meal in Diets fed to Hybrid Striped Bass  
Dr. Paul Brown - Purdue University