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CONSULTANCY REPORT

INDO-USAID SUB-PROJECT

ON

CONVERSION OF BIODEGRADABLE ANIMAL WASTES FOR LIVESTOCK FEED

PROJECT NO.: 386-0000-C-00-5039-00

SUBMITTED BY

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I. SUMMARY OF CONSULTING REPORT

Microbiology research conducted by the centers participating in the INDO-USAID CBAW Sub-Project have made considerable advancements toward the assurance that animal wastes when properly processed are safe for feeding. Studies have revealed that there is no more health risk associated with the feeding of poultry litter than the feeding of commercially available composite feed or feed ingredients. Although mycotoxin moulds were found in both poultry litter and in commercial poultry feeds, the incidence of mycotoxin was considerably higher for the feed sources. Studies conducted at the CBAW centers and by the microbiology trainees during training in the U.S.A. have confidently established that the deep stacking process used to treat poultry litter is adequate to render the litter safe from enteric pathogens.

Cattle waste has been processed by ensiling it with wheat straw either treated with various levels of urea or not treated. Urea treatment of wheat straw when combined with cattle waste and ensiled improves the crude protein content of the waste mixture, but there is concern that the elevated pH of the mixture might render the ensiling process less effective to eliminate pathogens. However, studies indicate that coliform bacteria are eliminated by the process. Studies should be conducted with specific bacterial pathogens inoculated into the waste to determine unequivocally that the urea treatment of straws, when combined with cattle waste and ensiled, satisfactorily kills enteric pathogens.

The Central Toxicological Laboratory at Hisar has tremendous capabilities with respect to equipment and personnel to conduct analyses to determine the safety of by-product feeds. It is important to the mission of the INDO-USAID Sub-Project that the participating research centers at Ludhiana, Hisar, Trichur and Bombay make use of the Toxicological Laboratory to ensure that animal by-products, under development as feed sources at the centers, are safe from toxicants and toxic microbial metabolities.

Microbial analyses have been conducted to evaluate the ensiling process to render prawn waste safe from enteric bacterial pathogens. Attempts to isolate Salmonella and Campylobacter from prawn waste silage have not succeeded, indicating that the waste is free from these bacterial pathogens. Inoculation studies are suggested with specific bacterial pathogens to ensure that the ensiling process for treatment of prawn waste is adequate to kill enteric, bacterial pathogens. During the past year the scientists discovered that the omission of water from the blended mixture of prawn waste with other ingredients resulted in a better fermentation of the ensiled waste. Lower pH values are now achieved (about 4.8) which has improved the microbial safety of the waste.

A chitin digesting bacterium has been isolated and tentatively identified as Curtho sp. Preliminary studies showed that the bacterium improved the degradation rate of prawn waste. Studies are planned to add the culture or an enzyme preparation of the culture to prawn waste during the ensiling process to determine if the procedure will improve the digestibility of prawn waste fed to cattle.

Rumen contents from slaughtered buffaloes and male cattle has been successfully ensiled to ensure that the waste is safe from pathogens. However, the addition of urea to increase the crude protein content of the rumen contents raises some concern relative to the safety of the process conducted at higher pH values. However, ensiling for long periods of time, 21 days or more, appears to be sufficient to eliminate most bacterial pathogens even if the pH does not decline below 5.0 during ensiling. At high pH values, 8.0 and above, which occurs with rumen contents treated with urea, the urea treatment appears to be adequate to eliminate enteric bacterial pathogens. To verify these observations, it will be necessary to monitor faecal coliform bacteria and to conduct inoculation studies with specific bacterial pathogens.

I. INTRODUCTION

The purpose of the consultancy assignment was (1) to participate in the annual workshop of the All-India Coordinated Research Project (AICRP) on by-products utilization and the INDO-USAID Sub-Project on Conversation of Biodegradable Animal Wastes for Livestock Feed (CBAW), (2) to review progress of research on pathogenic microorganisms in biodegradable animal wastes and processing to destroy pathogens, and (3) to advise and assist scientists at the participating centers to initiate research programs to ensure that animal wastes used as feed are safe from pathogenic microorganisms. The consultant visited all the centers participating in the CBAW Sub-Project. During this assignment, the consultant presented seminars at all the participating research centers except Bombay Veterinary College, and also presented an invited paper at the International Animal Nutrition Worker's Conference for Asia at the Pacific, September 23-28, 1991. A copy of the paper presented at the International Conference is appended (Annex A). Also, appended is the consultant's itinerary (Annex B) during his stay in India.

The consultant visited with scientists involved with the INDO-USAID Sub-Project at each of the following participating research centers:

Punjab Agricultural University, Ludhiana
Haryana Agricultural University, Hisar
Kerala Agricultural University, Mannuthy
Bombay Veterinary College, Bombay.

During his trip to Haryana Agricultural University, the consultant also visited the Central Toxicological Laboratory.

The consultant arrived in New Delhi, India, September 13, 1991, with two other consultants and departed New Delhi, October 9, 1991.

The consultancy report on microbiological research at the participating research centers is submitted here under the following major headings:

I. Summary of Consulting Report
II. Introduction
III. CBAW Workshop
IV. On-site visits to participating centers
V. Recommendations
VI. Annexures

III. CONVERSION OF BIO-DEGRADABLE ANIMAL WASTE WORKSHOP (CBAW)

The CBAW workshop was held September 21-22, 1991 at the Southern Regional Station, National Dairy Research Institute at Bangalore. The purpose of the workshop was to review the research progress on the conversion of animal waste and other agricultural by-products to animal feed. The workshop was a joint meeting of representatives of the All-Inda Coordinated Research Project on Agricultural By-products and representatives of the INDO-USAID Sub-Project on the Conversion of Bio-degradable Animal Wastes for Livestock Feed. The consultants participated in all phases of the workshop, but their charge was to review the progress and accomplishments of the research centers associated with the INDO-USAID Sub-project.

IV. VISITS OF PARTICIPATING INDO-USAID RESEARCH CENTERS

A. Punjab Agricultural University (PAU), Ludhiana

The scientist who will conduct studies to determine the microbial safety of animal wastes used as feed ingredients has received training in the U.S.A. and is now actively engaged in research at PAU.

Laboratory facilities, equipment and supplies are available to conduct laboratory and field studies to assess the microbial safety of processed animal wastes used as feed resources.

1. Research Conducted

a. Inoculation studies to assess deep stacking of poultry litter to eliminate pathogens (Study conducted by trainee at Auburn University)

Cultures of pathogenic bacteria (*E. coli*, *Salmonella* and *Listeria*) were inoculated into poultry litter to achieve approximately 100 million viable bacteria per gram of litter for each culture. The inoculated litter was placed into each of three stacks of poultry litter (about 25 tons each). Three stacking treatments were evaluated. One stack was covered with polyethylene, one was not covered and the third stack was wetted with water to form a crust about 2.5 cm. thick. At time intervals during deep stacking, the inoculated litter was analyzed for viable pathogens. *E. coli* and *Salmonella* inoculated into the litter of all stacks were eliminated from the litter by day 14, and *Listeria* was eliminated by day 28. All litter stack treatments were effective in eliminating the pathogens after the litter was stacked 28 days.

b. Ensiling poultry litter with corn

Poultry litter and corn grain were mixed in the following ratios 87.5:12.5, 75:25, 50:50 and adjusted to 40% moisture. The three mixtures were ensiled in plastic bags for 14 days. During ensiling, lactic acid bacteria increased in numbers from about 10^5/g of litter mixture at day 0 to over 10^8/g after ensiling for 14 days. Further evidence of a successful fermentation of the litter was evident in the production of lactic acid. No lactic acid was evident in the litter mixtures on day 0 (prior to ensiling), but after 14 days of ensiling the level of lactic acid increased to 3.8% of dry matter in the 87.5:12.5 litter-corn mixture, and up to 6.2% in the 50:50 mixture. Initially, the mixtures contained about 10^5 viable coliforms and faecal coliforms per gram each, and after 14 days of ensiling all coliforms were eliminated.

c. Inoculation studies to demonstrate elimination of bacterial pathogens from ensiled mixtures of poultry litter, corn grain and green forage

Three mixtures containing the following levels of litter, corn grain and alfalfa forage were ensiled in plastic bags for 14 days: 87.5:6.25:6:25, 75:12.5:12.5, and 50:25:25, respectively. After ensiling 14 days, the pH of the mixtures were 5.7, 5.6 and 5.3, respectively. *E.coli* and *Salmonella* inoculated into the mixtures at 10^8 viable bacteria per gram of silage were all eliminated after the three mixtures were ensiled for 8 days. However, *Listeria monocytogenes* inoculated into the mixtures was still viable in the silages after ensiling for 14 days. Additional work is needed to ensure that the ensiling of poultry litter with corn grain and forage is properly conducted to eliminate *Listeria monocytogenes*. Either the level of litter in the mixture should be reduced to 30% of the total dry matter, and/or to evaluate longer ensiling periods.

d. Deep stacking poultry layer litter

One-year old, built-up, poultry layer litter was deep stacked in approximately 4-ton lots for 4 weeks. The litter was sampled prior to deep stacking (day 0) and again after deep stacking for 4 weeks. There was no change in pH of the litter (pH 7.4) during deep stacking. However, coliforms bacteria were eliminated and the yeast and mould count was decreased 100-fold. Faecal coliform and Salmonella bacteria were not present in the litter initially, therefore, it was not possible to determine conclusively if the process would have eliminated faecal coliforms and Salmonella. However, since coliforms were eliminated, it appears highly likely that E.coli and Salmonella, if present in the litter, also would have been eliminated. The litter from this study was used in a feeding trial at PAU.

e. Identification of fungi and mycotoxins in poultry litter

A study was conducted at PAU to assess the incidence of pathogenic fungi and mycotoxins in poultry litter. This work was conducted in conjunction with the deep stacking study described above under item (d).

As indigenous mould counts were determined on the deep stacked litter, a representative number of mould colonies were selected for identification. Some 100 mould colonies have been identified to date. Also, 146 mould colonies isolated from poultry feed have been identified. The predominant moulds in poultry feed, based on incidence of isolation, were Aspergillus flavus (45%), Mucor spp. (18%), and Aspergillus niger (10%). In poultry litter Penicillium spp. was dominant (49%), followed by Aspergillus flavus (18%) and other unidentified Aspergillus spp. (14%). The major issue associated with moulds in poultry feed, or poultry litter intended to be used as a poultry feed ingredient, is the presence of mycotoxins in these feed stuffs.

Poultry are sensitive to mycotoxins, especially to aflatoxin which was shown in the above study to be present in both poultry feed and poultry litter.

Assays were conducted on 177 samples of composite feed (43 samples), poultry litter (65 samples) and feed ingredients (69 samples) for aflatoxin. Composite feed had the highest percentage of samples containing aflatoxin, which represented 70% of the samples, 45% of the feed ingredients contained aflatoxin, and 12% of the poultry litter samples had 25 ppb or more of aflatoxin. This study, showed that composite feed and feed ingredients are potentially at greater risk from aflatoxin than poultry litter.

f. A fungal process is being developed to upgrade the feed value of poultry litter. Various genera of fungi, including Pleurotus, Coprinis and Trichoderma are being evaluated for the conversion on non-protein nitrogen in poultry litter to fungal protein. Studies have shown that up to 90% of the uric acid of poultry litter can be metabolized by fungi. This project has potential for improving the feed value of poultry litter for non-ruminants.

2. Recommendations

Microbiological research at PAU, to assess the efficacy of the deep stacking and ensiling processes necessary to render poultry litter safe from pathogens, has made significant contributions to ensure that litter is safe for animal feeding. A notable example is that poultry litter is less of a potential risk from aflatoxin than conventional feed ingredients. However, additional safety studies need to be conducted as listed below:

1. Evaluate efficacy of sun drying and mechanical drying of poultry litter on incidence of enteric pathogens, fungi and mycotoxins. These two methods of processing litter appear to have potential for processing litter for poultry feed.

2. Explore the possibility of evaluating the two drying methods described above for elimination of viruses especially those viruses of poultry that might pose a potential health risk when litter is fed to poultry. The microbiologist trained in the U.S.A. was not provided expertise in this area, however, this expertise does exist in the Veterinary College at PAU.

3. Research on solid state fermentation of poultry litter should continue as this process has good potential of upgrading the feed value of poultry litter for non-ruminants.

B. Haryana Agricultural University (HAU), Hisar

The microbiologist attached to the Central Toxicological Laboratory, who trained in the U.S.A. to conduct pathogen studies on animal waste, returned from the U.S.A. only 30 days prior to this visit. His primary task since returning to HAU has been to set up facilities to conduct microbiological studies and ordering culture media and other supplies required for his research. However, work will begin immediately as facilities are available. Special types of culture media are on order, but in the meantime the scientist will attempt to borrow media from other laboratories on campus in order to start his research immediately.

1. Research conducted

The principal aim of the HAU scientists is to develop technologies applicable for the utilization of cattle waste as a feed ingredient for ruminants. Ensiling the waste with other by-products and/or with conventional feed ingredients is the processing method of choice.

a. Ensiling cattle waste with urea-treated straw

Studies have been conducted on ensiling cattle waste with wheat straw either treated with various level of urea or non-treated, and cattle waste ensiled with wheat straw and green fodder with and without molasses. Preliminary studies have indicated that wheat straw treated with urea increased the crude protein content of the straw, but when the treated straw was blended and ensiled with cattle waste, sufficient acidity was not produced in the silage to decrease the pH below 5.0. A lower pH is advisable to eliminate pathogens and to preserve the silage, especially to reduce crude protein loss through ammonia volatilization.

However, a study has indicated that urea-treated silages, with pH values above 5.0, are not a source of coliforms or faecal coliforms. The addition of green fodder and/or molasses improved the fermentation process for ensiled cattle waste mixed with urea-treated wheat straw. More research needs to be done on these ensiled mixtures, especially on the fate of pathogens such as Salmonella. Since the pathogens might only occasionally be isolated from the cattle waste, it is recommended that mixtures be inoculated with pathogens to ensure their presence prior to the ensiling process.

The microbiologist has been advised to begin work immediately to determine if Salmonella, total coliform and faecal coliform bacteria are present in mixtures of cattle waste ensiled with urea-treated wheat straw. Following 21 days of ensiling, all the mixtures had a pH in the range of 7.9 to 8.2. Although the ensiled mixtures were held frozen for some time, the microbiologist at HAU has been advised to analyze the mixtures. If coliforms or Salmonella are found in any of the samples, this would indicate that they were present in the mixtures prior to ensiling, and they survived the ensiling process. Such results would indicate a definite pathogen problem with feed mixtures composed of cattle waste and urea-treated wheat straw. To further explore this potential problem, studies have been planned by the HAU scientist team which will enable the microbiologist to monitor the ensiling process more closely. This should precede the scale-up fermentation trials planned for 1992 when the silage will be used to conduct digestibilities trials with adult cattle.

b. <u>Degradation of animal waste silages by ruminal cellulolytic bacteria</u>

Preliminary work has been conducted to propagate three ruminal cellulolytic bacteria in synthetic laboratory media, but due to poor growth responses of the bacteria in the media, the study has not progressed to the stage to determine whether the bacteria will improve digestibility of animal waste silages when fed to cattle. The lack of viability or, in this case, poor growth of micro-organisms is not uncommon. The following recommendations are suggested to solve the problem:

1. Obtain new cultures and prepare several subcultures of each bacterium for storage under lyophilized or other appropriate storage conditions. Therefore, in the future, as working stock cultures fail to perform satisfactorily, new stock cultures can be revived and used.

2. If the new cultures fail to grow actively in pure culture, then either the media or the technique used to cultivate the anaerobic bacteria is at fault.

3. If new cultures are obtained, it is strongly recommended that the cultures be transferred at least two times before they are shipped to HAU.

4. The gas used to maintain anaerobic conditions might be contaminated. If the cultures were grown satisfactorily with the gas previously, but now fail to grow from the same gas cylinder, then the source of gas can be ruled out as the problem.

5. Since it will be difficult and time-consuming to isolate and identify new cultures, active cultures should be obtained from some source.

2. Recommendations

Inoculation studies should be performed. Cattle waste ensiled with urea-treated wheat straw has a high pH, even after 21 days of ensiling. Generally, low pH values are necessary to destroy pathogens. Preliminary studies indicate that coliforms are destroyed in the waste, but more studies should be performed to determine if ensiling at high pH values are effective in eliminating pathogens such as E.coli and Salmonella.

The scientist trained in the U.S.A. to conduct research on ruminal cellulolytic bacteria at HAU will require more time to organize his program.

Recommendations to improve activity of the ruminal cellulolytic bacteria are listed under **B.1.b.**

C. Central Toxicological Laboratory (CTL), Hisar

The laboratory has only recently become functional after receipt of essential equipment. Relative to the microbiological safety of animal waste as feed, the laboratory will perform mycotoxin assays to determine if health hazards are imminent with regard to toxic mould metabolites. This is vital if poultry litter and other animal wastes will be developed into safe animal feeds. Poultry litter is especially of concern because mycotoxin producing moulds have been identified in litter at PAU. However, the incidence of mycotoxin (aflatoxin) was lower in poultry litter than in feed ingredients or composite feed for poultry.

1. Research Conducted

a) Blood enzyme analyses

Analyses have been performed on blood samples collected from cattle fed animal waste diets. Slightly elevated levels of blood enzymes were noted for some of the animals indicating a stress condition, but the results are inconclusive and the source of stress could not be determined.

b) Drug residues and mycotoxins

Analyses have not been performed for drug residues or mycotoxins as yet in animal waste-derived feeds. Plans have been developed to initiate this work in the coming year.

c) Evaluation of three deep stacking methods for the elimination of pathogenic bacteria inoculated into poultry litter (Study conducted by trainee at Auburn University)

Poultry litter and other types of relatively dry animal wastes are generally more economical to process by the deep stacking method. The process is effective in eliminating pathogens due to spontaneous heat generated by the stacked material. Excessive heating (greater than 60 C) of poultry litter, although beneficial to destroy pathogens, can reduce the available crude protein in litter. Three methods of deep stacking poultry litter were evaluated to moderate heating during the stacking process and to determine if all methods were effective in eliminating Salmonella typhimurium, E. coli and Listeria monocytogenes inoculated into the litter. The pathogens were inoculated into litter to achieve about 100 million viable organisms per gram of litter. The inoculated litter was placed into each of three litter stacks : (1) covered with polyethylene sheeting, (2) not covered and, (3) aerated with perforated pipes. The highest temperature occurred in Treatment 3 (71 C) and the lowest in Treatment 1 (58 C). Insolubilization of litter nitrogen was correlated with temperature, which was highest for Treatment 3 and lowest for Treatment 1. All the pathogens were eliminated from the litter after deep stacking for 14 days. Total coliform and faecal coliform bacteria indigenous to the litter also were eliminated by day 14.

Studies conducted in the laboratory showed that enteric pathogens such as E. coli, when inoculated into litter and subjected to heat for various periods of time, were killed within a few hours. E. coli inoculated into poultry litter at relatively high levels was eliminated from the litter following heating at 60 C for 5 hours.

2. Recommendations

The Central Toxicological Laboratory at Hisar has tremendous capabilities in terms of equipment and personnel available to conduct analyses to determine safety of by-product feeds. Therefore, it is important to the mission of the INDO-USAID Sub-Project that the participating research centers at Ludhiana, Hisar, Trichur and Bombay make use of the Toxicological Laboratory, to ensure that animal by-products under development as feed resources at the research centers are safe from toxicants and toxic microbial metabolites. Two concerns, relative to the safe use of animal waste as feed, have surfaced during this consultancy mission. To address these concerns, the services of the toxicological laboratory will be required as described below:

1. Poultry litter is widely used in the USA as a low cost feed ingredient for cattle but not for dairy cattle. In India, litter has more potential as feed for dairy cattle. Poultry, especially broiler poultry, are fed diets containing medicinals to promote efficient poultry production. Some of the medicinals are excreted in the faeces, therefore, they are likely to be present in the poultry litter. With cattle, it is recommended that poultry litter be withheld from cattle diets 15 days prior to slaughter to ensure that any unapproved residues in the tissues of the animals have cleared before the carcasses are marketed for food. There is no opportunity to withdraw litter containing diets from lactating dairy cattle prior to marketing the milk. Therefore, studies should be conducted to ensure that feeding poultry litter to producing dairy cattle does not cause concern for illegal levels of drug residues in milk. A controlled study should be conducted to determine if there are potential drug residues problems associated with the feeding of poultry litter to milk producing cattle and buffaloes.

2. A similar potential problem exists relative to mycotoxins in poultry litter fed to dairy cattle. Although studies at PAU have shown that composite feed and feed ingredients have a higher incidence of mycotoxin than poultry litter, studies, nevertheless, should be conducted to ensure that milk of cows fed poultry litter does not cause concern for illegal levels of mycotoxin in the milk.

3. Pathogen inoculation studies should be performed to evaluate the ensiling method used to process cattle waste for feed. Cattle waste combined with urea-treated straw is more difficult to ferment than non-treated straw due to the high pH created by the urea treatment. Inoculation studies should be conducted especially on ensiled, urea-treated wastes.

D. Kerala Agricultural University (KAU), Mannuthy

The scientist who will conduct microbiological studies to determine the microbial safety of animal wastes as economical sources of feed for livestock has received training in the USA, and has since returned to KAU to conduct research. An on-site visit of the laboratory facilities at KAU by the consultant revealed that facilities, equipment and supplies are adequate for the microbiologist to conduct his work. The animal by-product most readily available as a potential animal feed in Cochin, Trichur and other coastal cities in Kerala is seafood wastes. Studies by the scientists at KAU have shown that prawn waste has potential for use as animal feed. The microbiologist has conducted pathogens studies to assess the microbial safety of prawn waste silage as a feed for livestock.

1. Research Conducted

Because prawn waste contains relatively high levels of moisture, which would make it uneconomical to dry by mechanical means, the most feasible method of processing the waste is ensiling. This process is currently being evaluated by the microbiologist, and studies that have been completed are discussed below.

a) Prawn waste ensiled with paddy straw and tapioca flour

A mixture of 45 parts prawn waste, 45 parts paddy straw and 10 parts tapioca flour were ensiled in duplicate treatments for 2 months. Prior to ensiling and after ensiling, five replicates of each ensiling treatment were analyzed for *Salmonella* and *Campylobacter*. These pathogenic bacteria were not detected in either the pre-ensiled or post-ensiled prawn waste. Although the absence of these pathogens indicates that the prawn silage is safe from these pathogens, their absence in the pre-ensiled material provides little insight if the ensiling process is adequate to eliminate the pathogens if they were present in the material prior to ensiling. For this reason, the prawn waste mixture should be inoculated with the pathogens, and

after the mixture is ensiled for at least 10 to 15 days, attempts should be made to recover any viable pathogens. At least two successive recovery attempts should be made during ensiling to ensure that the ensiling process has eliminated the pathogens.

Other microbial analyses also were conducted on the ensiled prawn waste. Of the 10 samples analyzed, only 3 of the samples had less than 100,000 viable total coliform bacteria per gram of silage, and 7 samples had over one million coliforms per gram. Generally a successful ensiling process will eliminate coliform bacteria. A possible explanation for failure to eliminate the bacteria might be due to insufficient acidity produced during the ensiling process. Prior to ensiling the pH of the mixture was 8.8 and after ensiling the pH was 5.2. The pH should drop below 5.0 after ensiling, and ideally below pH 4.5 to eliminate coliforms as well as enteric pathogens such as Salmonella and Campylobacter. In the study described below, the problem has been addressed.

b) Ensiling prawn waste, rice bran and tapioca flour

From the previous study, described above, two changes were made in the ensiling mixture which increased the acidity of the silage. Rice bran was used in place of rice straw and the moisture content of the ensilage mixture was less. In previous studies water was added to the mixture to achieve 50% moisture. In this, and in subsequent studies, no water was added to the mixture prior to ensiling. This procedure resulted in a lower moisture content of the mixture (about 40%) which improved the fermentation process and made the ensiled material less cakey. This innovation in the ensiling of prawn waste has improved the microbial safety of the waste. In earlier ensiling studies, the pH of the mixture did not decrease to 5.0 or lower, which is essential to eliminate pathogenic bacteria. With the lower moisture content in the prawn silage, a pH of 4.8 was achieved.

A study was conducted employing the modifications in the ensiling process as described above. A mixture of prawn waste (45 parts), rice bran (45 parts) and tapioca flour (10 parts) were ensiled in plastic bags placed in steel barrels. At 0-, 3- and 6- week intervals, the silage was analyzed for Salmonella and Campylobacter bacteria. All samples were negative for the pathogens prior to ensiling and after the two ensiling intervals.

c) Evaluation of prawn waste ensiling process to eliminate inoculated pathogens (study conducted by trainee at Auburn University)

Three levels of prawn waste (50, 55 and 60%) were combined with 20% ground shelled corn and 15% molasses. Cottonseed hulls were blended into the mixture to balance the ingredients to 100% in each of the three mixtures. All mixtures had approximately 50% moisture. The mixtures were inoculated with Salmonella, E. coli and Listeria monocytogenes to achieve about 100 thousand viable bacteria per gram of each culture in each of the three mixtures. The mixtures were ensiled in gas impermeable plastic bags at 32 C for 12 days. Prior to ensiling and after 0, 3, 6 and 12 days of ensiling the silages were analyzed.

After 12 days of ensiling all the mixtures had 11% or more of lactic acid (dry basis) which indicated that the prawn waste mixtures had satisfactorily fermented. Further evidence of a successful fermentation was indicated by a satisfactory decrease in pH of the mixtures. All of the silages had pH values of 4.4 or less by day 12 of the ensiling process, and indigenous faecal coliform bacteria were reduced from 100 thousand per gram, initially in the mixtures, to less than one per gram after 12 days of fermentation. The three pathogenic bacteria inoculated into the mixtures also were eliminated after day 3 of ensiling. These results indicate that prawn waste can be properly ensiled to eliminate pathogenic bacteria.

d) Screening and isolation of chitinolytic and ligninolytic bacteria

A chitinolytic bacterium has been isolated which will be used to treat prawn waste to improve its digestibility by cattle. A bacterium, tentatively identified as Curtho sp., has been isolated from the gut of a fish which has chitin digesting activity. Preliminary studies in the laboratory showed that the bacterium improved the degradation rate of prawn waste. Future studies are planned to add the culture, or an enzyme preparation of the culture, to prawn waste during the ensiling process to determine if the procedure will improve the digestibility of the waste by cattle. A long term objective is to use chitinolytic organisms to convert prawn waste into feed suitable for non-ruminant animals such as swine and poultry.

Studies also are underway to isolate ligninolytic organisms which will be used to improve the utilization of lignocellulosic materials such as paddy straw. As yet, no progress has been reported on these studies.

2. Recommendations

Prawn waste prior to ensiling should be inoculated with pathogens. The viability of the cultures during the ensiling process should be monitored. Based on the acidity that has been achieved in the ensiled waste at KAU, the silage should be safe from pathogens, however, this needs to be verified for the specific process employed by the KAU scientists. The most important criteria to monitor relative to microbial safety are faecal coliform bacteria, specific pathogen inoculations and pH of the ensiled animal waste product. Other tests should be conducted only after these analyses have been performed.

The scientist assigned to the project to conduct pathogen studies has been relocated to the Fisheries College in Cochin, some 60 to 70 km away from the Trichur Center. This arrangement potentially can hamper progress by the microbiologist to assess the safety of ensiled prawn waste.

Studies on the isolation, characterization, optimization and practical application of chitinolytic and ligninolytic organisms, to improve the utilization of animal waste as feed, potentially can contribute to the economic development of animal wastes as a marketable feed. These studies should be initiated soon.

E. Bombay Veterinary College (BVC), Bombay

The scientist trained in the U.S.A. has completed his training and is currently conducting research to promote the safe use of animal by-products as feed.

1. Research Conducted

The principal animal by-product available in Bombay, which has potential for use as a low-cost feed for ruminant animals, is rumen contents or paunch material from slaughtered buffaloes and male cattle. Studies conducted at the BVC to convert the abattoir waste into animal feed have focused primarily on rumen contents.

a) Ensiling paddy straw alone and paddy straw combined with rumen contents

Paddy straw was adjusted to 65% moisture and ensiled with and without 5.3% urea added on a dry matter basis. The two mixtures were ensiled 12 days and analyzed after 0,6, 9 and 12 days of ensiling. Total coliform bacteria initially were present at about 100,000 bacteria per gram in the mixtures, and they were eliminated after the mixtures were ensiled for 6 days. The effect of the urea was not evident because all coliforms were eliminated in all silages on the first sampling period following ensiling. However, the effect of urea on microbial growth was evident on the anaerobic bacterial spore count. The ensiling process had litter effect on the spore count of paddy straw ensiled alone. With urea added, anaerobic bacterial spores were eliminated from the treated straw after ensiling for 6 days.

A similar trend was observed for paddy straw ensiled with rumen contents with and without 5.3% urea added on a dry matter basis. Total coliforms were eliminated in both of the mixtures, whereas, anaerobic spores were eliminated only from the silage containing 5.4% urea.

Although pH of the mixtures was not measured, it is logical to assume that the urea was converted to ammonia which increased the pH to 8.0 or higher. Ammonia is toxic to micro-organisms which was probably responsible for the elimination of anaerobic bacteria spores from the urea treated silage. It is reasonable to conclude from the studies that rumen contents ensiled with paddy straw either with or without the addition of urea eliminated total coliform bacteria. With the addition of urea, spore forming, anaerobic bacteria also were eliminated. It is the intention of the scientists at BVC to incorporate urea with the rumen contents to increase the crude protein level of the material. This practice will improve the feed value of the material and greatly contribute to the microbial safety of the product as feed. However, more studies are recommended, particularly the monitoring of faecal coliform bacteria and inoculations with specific bacterial pathogens, to determine the safety of the ensiled mixtures.

b) <u>Ensiling squeezed rumen contents treated with urea</u>

Rumen contents were squeezed to reduce the moisture contents of the material to 65%. Urea was added at three levels (0, 4 and 5.35% of dry matter) to the rumen contents and ensiled for 21 days. During ensiling, total coliforms and anaerobic spore forming bacterial counts were monitored at 0, 5, 8, 14 and 21 days. About 24,000 total coliforms per gram of product were initially present, but after the product was ensiled 5 days, total coliform bacteria were eliminated from the silage irrespective of the urea treatment. Anaerobic spore forming bacteria were eliminated from the silages only when urea was added to the rumen contents. These results indicate that ensiling rumen contents, either treated or not treated with urea, eliminates total coliform bacteria. Inoculation studies with specific bacterial pathogens should be conducted to properly evaluate the safety of the ensiled rumen contents.

c) Rumen contents ensiled with blood and paddy straw

Rumen contents were combined with blood at the following ratios on an as-is basis (1:0, 1:1 and 2:1). Paddy straw was added to the mixtures to achieve 40, 50, 60 and 70% levels. Molasses was added to all 12 treatments at a level of 5%, and moisture was adjusted to 65% in all treatments. Total coliform bacteria were present initially in all treatments at about 200,000 bacteria per gram of material. After ensiling for 21 days, the pH of all mixtures was below 5.0, except for those containing 70% paddy straw. Total coliform bacteria were eliminated in all 12 treatments irrespective of the level of blood or paddy straw incorporated in the mixture. These results indicate that the ensiling process is adequate to eliminate total coliform bacteria in rumen contents ensiled with blood and paddy straw. Because total coliform bacteria were eliminated, other bacteria with similar characteristics, such as faecal coliforms and Salmonella, should also be eliminated. However, more studies should be conducted to verify this assumption.

2. Recommendations

The scientist trained to conduct pathogenic microbiology research should work closely with the animal nutritionists to evaluate the microbial safety of ensiled rumen contents for animal feed. The pH values of the material should be monitored several times during the ensiling process to establish some relationship between pH, duration of ensiling and the elimination of pathogens. Acid production during the ensiling process, usually to achieve pH values below 5.0, is recommended to ensure the elimination of enteric bacterial pathogens from the silage.

However, acid production will not be sufficient in urea treated rumen contents to kill pathogens. Microbiological data, however, indicate that total coliforms and anaerobic spore forming bacteria are eliminated by the addition of urea to ensiled rumen contents. Therefore, the following recommendations are advised :

1. Determine pH of all rumen content mixtures before and after ensiling for various periods of time.

2. Monitor faecal coliform bacteria in the mixtures before and after ensiling. Total coliforms should be eliminated in the silages, but it is more important that faecal coliforms are eliminated.

3. Conduct inoculation studies with specific bacterial pathogens to demonstrate unequivocally that the pathogens are eliminated by the ensiling process.

4. Determine the pH of urea-treated rumen contents before and after ensiling for various periods of time, and determine if the elevated pH and ensiling time directly affect the microbial safety of the ensiled material.

V. RECOMMENDATIONS

A. General Recommendations

Research on pathogenic micro-organisms should continue at a rapid pace to ensure that animal by-products used as feed for animals are safe from bacterial pathogens. In most cases, sufficient research has been conducted to determine acceptable combinations of waste that need to be blended with other feed sources to produce nutritious by-product feeds. Also, methods of processing the waste, to preserve its nutrient content and to improve its safety status, have generally been accomplished. However, microbiological studies have not been performed to adequately assess the microbial safety of animal waste feeds nor the adequacy of the methods used to process the waste. Therefore, it is recommended that all participating research centers conduct inoculation studies with specific bacterial pathogens to determine if the processing method being used is adequate to eliminate pathogens.

B. Specific Recommendations

a) Microbial safety studies at the Punjab Agricultural University should be focused on the following research agenda :

1. Conduct microbial safety studies specifically related to the use of poultry litter as feed.

2. Evaluate the deep stack, sun drying, and mechanical drying methods for processing poultry litter to ensure that the litter is safe from enteric pathogens, including inoculating the litter with specific bacterial pathogens such as *E. coli* and *Salmonella* prior to processing.

3. Because poultry litter has more potential for use as a feed for milk producing animals than for beef, more studies should be conducted on the fate of specific mycotoxins in poultry feed, levels found in built-up poultry litter, the effect of litter processing, and finally, if mycotoxins can be detected in the milk of cattle and buffalo fed litter.

b) Studies at Haryana Agricultural University should be concentrated on the microbial safety of feeding cattle waste ensiled with urea-treated wheat straw. Cattle waste, if adequate carbohydrate is available, usually develops adequate acidity to eliminate most enteric bacterial pathogens. When waste is treated with urea to increase its crude protein content, the ensiled waste usually does not generate sufficient acidity to kill pathogens. However, studies indicate that pathogens are eliminated when urea-treated wastes are ensiled. Perhaps, high pH levels also are destructive to pathogens. This needs to be studied to establish a critical pH for urea-treated wastes which will eliminate pathogens. Controlled studies should be conducted with specific bacterial pathogens inoculated into the waste prior to ensiling the urea-treated material.

c) The Central Toxicological Laboratory at Hisar serves as a central analytical laboratory for the participating research centers. The services of this laboratory should be utilized to determine if mycotoxins in poultry feed are excreted by poultry and accumulate in the poultry litter. The fate of these needs to be determined if poultry litter will be used as feed for milk producing cattle and buffaloes.

d) <u>Studies at Kerala Agricultural University</u> should continue to assess the microbial safety of prawn waste ensiled with paddy straw and tapioca flour. Inoculation studies need to be conducted with specific bacterial pathogens to determine the adequacy of the ensiling process to ensure that the waste is safe from bacterial pathogens. The microbiologist trained to conduct the pathogen studies has been transferred to the Fisheries College in Cochin. This transfer might delay work on pathogenic bacteria.

d) <u>Pathogen studies at Bombay Veterinary College</u> should continue to assess the microbial safety of ensiled mixtures of rumen contents, blood, paddy straw and molasses. Inoculation studies need to be conducted to ensure that the ensiling process is adequate to eliminate specific pathogens from rations containing paunch contents and blood.

A N N E X U R E S

Health Aspects of Feeding Processed Biodegradable Animal Wastes to Farm Animals

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The nutrients contained in animal wastes and other types of by-products have been shown in numerous reports to have feed value for animals, especially ruminants. Early reports on nutrients contained in animal wastes and their potential value as feed ingredients appeared in the late 1940's and early 1950's. Due to interests in sustainable agricultural practices and the potential impact that wastes can have on the environment, especially when they are not managed as a resource, recycling wastes as feed ingredients has become an economically viable alternative waste management method. The principal incentive to utilize animal wastes as feedstuffs is the economic advantage that can be gained by using low-cost sources of nutrients.

Although animal wastes have been used as feed ingredients for many years, the principal deterrent to the practice has been the concern that wastes are unsafe because they might serve as sources of pathogenic organisms or harmful chemical agents. The Food and Drug Administration (FDA) in the USA issued a policy statement in 1967 concerning the practice of feeding animal wastes to animals (5). Although the statement specifically was directed at the feeding of poultry wastes, the statement was broadly applied to all species of animal waste intended to be used as feed ingredients. The FDA stated that because there was

little or no data available relative to the risk assessment to animals fed waste, or to humans who consume the products of the animals, the practice of feeding wastes to food producing animals would not be condoned by the FDA. The policy statement was issued following a review by a committee formed by the U.S. Department of Agriculture. The committee recommended the need for research to develop methods of processing poultry litter and other animal wastes to improve the safety status of these by-products as feed ingredients. Although the safety status of animal wastes and other types of by-products cannot be guaranteed, which is also applicable to conventional feed ingredients, research since 1967 has made great strides toward making animal wastes and other by-products safe as feed ingredients.

Methods of Processing Wastes for Feed

To ensure that animal wastes are safe for feeding, the wastes must be processed. Processing has several purposes. First, the process must be demonstrated to eliminate pathogenic microorganisms, restrict their growth and/or prevent them from producing harmful toxins. Pathogenic, enteric bacteria, such as Salmonella, should be eliminated by the process. In some cases it is not practical to eliminate the organism, i.e. Clostridium botulinum, when the process creates conditions unfavorable for the organism to grow and produce toxin. Since Clostridium botulinum is widely dispersed in the environment, even in conventional feedstuffs, and it produces spores that are highly

resistant to adverse conditions, it is not practically feasible to eliminate the bacterium from feedstuffs (11). The organism is, for all practical purposes, harmless unless conditions are favorable for its growth. Drying or fermenting waste creates unfavorable conditions for the growth of C. botulinum.

Another purpose of processing wastes is to conserve the nutrients in the wastes. The natural tendency of wastes, if adequate moisture is available, is to degrade through microbial activity. At completion, essentially all the metabolizable components of the waste have been eliminated. This process is encouraged for the disposal of wastes, but should be avoided for the processing of wastes for use as animal feed. To conserve nutrients in animal wastes, the process must reduce the moisture content (drying) or maintain the moisture at a low level (closed vessels), preferrable below 15% moisture, to discourage microbial attack on the waste components. If wastes contain high levels of moisture making them uneconomical to dry, microbial activity can be discouraged through the use of approved chemicals. Organic acids such as lactic, acetic or propionic can be added to reduce the pH of the waste to 4.5 or lower. At pH 4.5 or lower, microbes which metabolize proteinaceous materials are inhibited. Inorganic acids, i.e. phosphoric and sulfuric, also can be used to retard microbial activity of wet wastes. Wastes also can be fermented to increase the acidity naturally. To accomplish this, the waste should be blended with ingredients that contain fermentable carbohydrate, usually grain, molasses, or some carbohydrate by-product. Moisture content of the mixture must be

adjusted to about 40%, by adding dry ingredients (chopped hay or straw) to reduce the moisture content of the mixture, or by adding water to increase the moisture content. Too little moisture prevents the microbial production of lactic acid, and too much moisture encourages the undesirable butyric acid fermentation. Corn and sorghum silages typically have 65 to 70% moisture, but by-product silages, which contain animal wastes or other types of by-products, are best fermented at 40% moisture (1, 14). The lactic fermentation process as applied to the processing of animal feedstuffs is typically called ensiling. The fermentation process has been used successfully for many years to preserve food for man and feed for ruminant animals. Agricultural and industrial by-products that have been successfully processed into ruminant animal feed by the lactic acid fermentation process include all types of animal wastes, paunch contents from slaughtered ruminants, and seafood processing wastes. All types of wastes can be processed by fermentation provided that the waste contains adequate carbohydrate (in excess of 2% dry basis), the moisture is 40%, and the waste is maintained under anaerobic conditions. All these conditions are essential for the lactic fermentation process. This process has several benefits. It retains and preserves the nutrients in the wastes, renders wastes safe from pathogens, improves the aesthetic characteristics of wastes, improves animal palatability of wastes, and it improves the nutrient content of wastes through the production of microbial products that can serve as dietary nutrients for animals.

Relatively dry wastes, such as poultry litter, are best processed by the method called deep stacking. The process involves stacking litter in large piles to reduce surface area and to minimize the loss of litter nutrients through volatilization. The major dietary nutrient in litter for ruminants is nitrogen, which is found in litter in the form of uric acid (principal excretory nitrogen), urea, ammonia, wasted feed and feathers. This nitrogen is susceptible to loss through volatilization and insolubilization. Prior to the deep stacking process, litter should be analyzed, and only litter that is deemed acceptable for feeding should be selected for use as a feed ingredient. As a general rule, litter should contain at least 19% crude protein, less than 25% of the litter nitrogen should be insoluble, and the litter should not contain in excess of 28% ash. In Alabama (USA), a survey of 106 litter samples collected across the state indicated that the average crude protein was 24.9%, insoluble nitrogen was 15%, and ash averaged 24.7% (16). Research and demonstrations relative to the effects of litter management practices on the nutrient content of litter have been pursued by the principal author for the past five years. The purpose of the research was to evaluate practical methods of litter management to maximize its value as a feed resource. Listed below are some guidelines for managing poultry litter as a feed resource:

1. Remove poultry mortalities daily from the poultry house. Do not feed litter that contains dead poultry.
2. Keep litter dry in the poultry house and after removal.

3. Nutrient content of litter improves in the poultry house with increased number of broods reared on the litter.

4. Avoid soil contamination of litter during removal from dirt floor poultry houses.

5. After removal from the poultry house, stack litter on a concrete pad in a barn.

6. Cover the litter with 6 mil polyethylene plastic and seal at the bottom of the litter stack. Covering is done to reduce access of the litter to air to avoid overheating of the litter. When litter contains in excess of 25% moisture and the litter stack is not covered. excessive heating occurs (>60 C), which causes nitrogen loss through volatilization and insolubilization.

7. Allow the litter to be deep stacked under cover for 20 days. After this time, the litter will have reached its maximum heating temperature, and any pathogens in the litter will have been killed. Destruction of pathogens in deep stacked litter is attributed to ammonia released from the litter and by heat generated during the deep stacking process.

Deep stacking is widely used in the southeastern USA for processing broiler litter as a feed ingredient. The process is economical and easily accomplished by cattle producers.

Studies are underway to investigate the marketing of poultry litter as a feed ingredient through commercial channels similar to the marketing of feed grains. The process which seems to be best for this mode of marketing litter is pelleting. This process also employs heat to ensure that the litter is free of pathogens. Although pelleting is considerably more costly than deep stacking, it improves the handling characteristics of the litter, which is essential for commercial marketing of litter as a feed ingredient.

Poultry litter per se does not undergo the lactic fermentation process. It is too dry and lacks readily available fermentable carbohydrate. If litter is to be processed by the lactic fermentation process, small amounts of litter can be mixed with fresh, green chopped forage. Due to the high buffer capacity of litter, the ratio of litter to forage should not exceed 1 part litter to 7 parts forage on a wet basis. Higher levels of litter are likely to ferment, but sufficient acidity (pH <4.5) will not be produced to ensure the elimination of pathogens or to preserve the litter/forage silage.

Health aspects of Feeding Wastes

Several health-related issues have been raised relative to the feeding of wastes to animals. These concerns are listed in Table 1. Most of the concerns relative to potential hazards associated with non-biological entities, such as drug residues, pesticides, etc, can be controlled by avoidance of waste which contains excessive levels of the agents. In general, processing the waste cannot be relied upon to eliminate these potential hazards. Therefore, prudence must be exercised in the selection of waste that is safe to use as feed. A more detailed discussion of non-biological agents and their potential hazards in animal waste intended for use as feed was published earlier by the author (8, 13). Processes, such as fermentation, chemical addition, deep stacking and pelleting, are effective to ensure that waste-derived feeds are safe from pathogens. All wastes

intended to be used as feed should be processed to ensure safety from pathgens.

Table 1. Health Risk Assessment of Issues Pertaining to the Feeding of Wastes to Animals

Pathogenic microorganisms	Drug residues
Microbial toxins	Hormones
Mycotoxins	Coccidiostats
Parasites	Pesticides
Viruses	Heavy metals
Arsenicals	Trace elements

Fermentation of Wastes

If adequate carbohydrate is available, the moisture is adjusted to 40%, and the waste is held under anaerobic conditions, lactic acid-producing bacteria will proliferate and produce lactic acid. Provided that sufficient acid is produced (pH <4.5), usually within 10 to 15 days, pathogenic bacteria such as Salmonella (12), Listeria (7), pathogenic E. coli (7), mycobacteria (tubercle bacilli) (15), and Brucella abortus (6) will be eliminated from the ensiled waste. The effects of temperature and pH on the viability of Salmonella in an ensiled mixture of bovine waste, corn grain and corn silage are shown in Table 2 (12). All 27 cultures of Salmonella were eliminated from the mixture after ensiling at 35 C for 4 days. At lower temperatures, one or more of the cultures survived. Generally the temperature of the ensiled waste mixtures is not a critical issue because heat is generated during the ensiling process. Studies conducted during the winter months have shown that sufficient heat is generated during the ensiling of wastes to

achieve adequate acidity to eliminate Salmonella. The elimination of Salmonella from ensiled wastes is due principally to acids that are produced during the ensiling processs. Other factors may also contribute to the elimination of pathogens, such as bacterial competition, production of bactocins, and anaerobiosis that occurs during the ensiling process. Since acidity is easily monitored as an indication of successful fermentation, this method is advocated to achieve safety fron pathogens. A pH of 4.5 or less should be achieved in the ensiled waste. Usually this is obtained within 3 to 4 days, and the waste should be ensiled for at least 10 days prior to use as a feed ingredient.

Table 2. Effects of Temperature and pH on the Viability of Salmonella in an Ensiled Mixture of Bovine Waste, Corn Grain and Hay

Viability after 4 days ensiling	Temperature			
	5C	15C	25C	35C
No. cultures survived[a]	21	25	1	0
Percent of cultures	78	93	4	0
pH, initial	4.8	4.8	4.8	4.8
pH, 4 days	4.6	4.4	4.0	4.1

[a] 27 different cultures of Salmonella were inoculated into a mixture of 45 parts corn grain, 15 parts corn silage and 40 parts bovine manure prior to ensiling.

Animal waste alone usually will not ensile properly to achieve a pH of 4.5 or less. However, the waste will ensile if carbohydrate is added and the moisture is adjusted to 40%. This principle is exemplified by the ensiling of bovine waste with

corn grain and corn silage (Table 3). The mixture achieved pH 4.5 after it was ensiled for 3 days, whereas the pH of the waste alone held for 3 days declined to pH 6.0 (12). Twenty-seven cultures of Salmonella inoculated into the waste mixture were eliminated in 3 days, but 25 of the cultures were still viable in waste alone held 3 days.

Table 3. Viability of Salmonellae in Manure and in an Ensiled Manure-Feed Mixture

	Recovery after 3 days incubation at 25 C	
	Manure	Manure-Feed[a]
No. cultures survived[b]	25	0
Percent recovered	92	0
pH, initial	6.8	6.5
pH, 3-day	6.0	4.5

[a] 45 parts corn grain, 15 parts corn silage and 40 parts bovine manure.

[b] 27 cultures of Salmonella were inoculated into the manure and manure-feed mixture prior to ensiling.

Differences occur in the viability of pathogens inoculated into ensiled wastes from different species of animals. However, the principle of pH 4.5 or less and a minimum ensiling time of 10 days is still necessary to eliminate pathogens. The wastes of two animal species (beef cattle manure and poultry litter) were used to prepare mixtures for ensiling (Table 4). The amount of waste of each animal species and the amount of ground corn grain used to prepare the two silages were similar. Moisture content of each silage was adjusted to 40%. As a control silage, chopped whole corn plant (71% moisture) was ensiled. All three rations

were inoculated with one culture each of E. coli, Salmonella, and nine cultures of Mycobacterium (tubercle bacilli) prior to ensiling. The rations were ensiled in heat-sealed polyethylene bags at 25 C for 15 days.

Table 4. Composition of Bovine Manure and Poultry Litter Silages[a)]

Ingredient	Wet Weight (%)
Bovine Manure Silage	
Bovine manure (75% moisture)[b)]	60
Ground bermudagrass hay	20
Ground corn grain	19
Limestone	1
Poultry Litter Silage	
Poultry litter (39% moisture)	55 (64.7)[c)]
Ground corn grain	20 (23.5)
Ground bermudagrass hay	5 (5.9)
Cane molasses	5 (5.9)
Water added	15

[a)] Blended ingredients contained 40% moisture

[b)] Fresh waste

[c)] Percent composition without added water

The pH of the bovine manure silage and corn silage decreased to 4.1 and 3.8, respectively, after 5 days of ensiling (Table 5). E. coli, Salmonella and nine cultures of Mycobacterium were eliminated from the rations after they were ensiled for 5 days (15). Although the Mycobacterium cultures survived for some time after the rations attained pH 4.5, the principle of microbial safety was still applicable because both pH 4.5 or less and a

minimum ensiling time of 10 days or longer must be met to achieve safety.

Table 5. Effect of Ensiling Bovine Manure, Poultry Litter and Corn Plant on the Survival of Bacterial Pathogens Inoculated into the Silages

Silage	Days ensiled	pH	Inoculated Cultures[a]		
			E. coli	*Salmonella*	*Mycobacteria*
Bovine manure (40% moisture)	0	6.3	+	+	9
	2	4.5	-	-	9
	4	4.1	-	-	4
	5	4.1	-	-	0
Corn silage (71% moisture)	0	5.0	+	+	9
	2	4.0	+	+	9
	4	3.9	-	-	6
	5	3.8	-	-	0
Poultry litter (40% moisture)	0	8.2	+	+	9
	5	5.5	+	+	9
	10	5.5	+	+	9
	15	5.7	-	-	9

[a] One culture of *E. coli* and *Salmonella* and nine cultures of *Mycobacterium* were inoculated to the samples to achieve about 10^8 viable bacteria per gram of sample.

Because poultry litter has both a high buffer capacity and a high pH, it is difficult to achieve a pH of 4.5 or less when poultry litter is ensiled. Mixing litter with 20% ground corn grain and 5% molasses improves the fermentation of the litter, but the pH of the mixture, even after 15 days of ensiling, does not drop below 5.5 (Table 5). *Salmonella* and *E. coli* were eliminated after the litter was ensiled 15 days, at which time the silage achieved pH 5.5 to 5.7, but all nine cultures of mycobacteria inoculated into the mixture were still viable.

Therefore, to ensure the microbial safety of poultry litter silage, and all types of by-product containing silages, the silages must be ensiled to achieve a pH of 4.5 or less and continue to be ensiled for 10 days or more. Studies conducted by the author have shown that poultry litter can be successfully ensiled with chopped whole corn plant provided that the ratio of litter to forage is maintained at 1:7 (as-is basis) or below (9).

Deep Stack Process for Poultry Litter

Because poultry are known carriers of enteric, pathogenic bacteria, such as Salmonella, it can be assumed that poultry litter also might be contaminated with the bacteria. However, litter that has been held for some time, either in the poultry house or after the litter has been removed from the house, is less likely to harbour the bacteria than freshly voided feces. Studies with poultry litter inoculated with 100,000 to 500,000 E. coli per gram showed that litter was not conducive to the survival of the bacterium (Table 6). After the litter was held for 2 days at ambient temperature, the viable count of E. coli in the litter decreased to an average of 20 organisms/g of litter (10). The average reduction for E. coli in the litter was log 4.1. This indicates that if litter containing 10,000 viable E. coli/g was held without treatment, the E. coli count would decrease after 48 hours to about 1 viable E. coli/g of litter. Naturally contaminated litter is not likely to contain more than 100 viable E. coli/g. Therefore, a 4.1 log reduction would reduce the viable count of E. coli in naturally contaminated

litter to log -2.1 (log 2 - log 4.1) or about 1 viable *E. coli*/100 g of litter. Because *E. coli* and *Salmonella* share the same environmental habitat and have similar growth characteristics, their survival patterns in litter should be similar. The natural die-off of *E. coli* in litter is probably associated with the alkalinity of litter and the release of ammonia from nitrogenous components in poultry litter, such as uric acid and urea. When poultry litter is processed, a further reduction in pathogen numbers occurs. Heat processing litter, either by mechanical drying, pelleting, or deep stacking the litter, has been demonstrated to be an effective means of eliminating *E. coli* and *Salmonella* from litter (13). The combined effects of the natural die-off of enteric bacteria in poultry litter and the die-off associated with processing the litter are adequate to render the litter safe from enteric pathogens such as *E. coli* and *Salmonella*. It is important, however, to take appropriate measures not to contaminate the litter after it has been processed.

Table 6. Viability of *E. coli* in Raw Litter (Viable Count/g litter)

E. coli culture	Viability at 0 h[a]	48 h
2 - 5b	3.0×10^5	20
1 - 7b	1.0×10^5	16
ATCC 11246	5.0×10^5	24

[a] Counts at 0 h represent number of *E. coli* inoculated into raw litter.

Perspectives on the Safety of Wastes Used as Feedstuffs

Following the statement issued by the FDA in 1967 that feeding wastes would not be condoned, several issues relative to the safety of feeding wastes to animals have been addressed (4, 8, 13). In 1980, the FDA evaluated requested comments and published data on safety of feeding wastes and concluded that the practice of feeding wastes was conducted at a local level rather than over broad areas of the country. Since the distribution of litter and other animal wastes as feed did not involve interstate marketing, the FDA concluded that jurisdiction over marketing of animal wastes as feed was the responsibility of state governments. With these remarks, the FDA rescinded its policy statement of 1967 (2).

Although there have been no published reports of health problems associated with feeding wastes to animals, except feeding poultry litter with high copper levels to sheep (3), safeguards must be followed to ensure that animal wastes and other types of by-products intended to be used as feedstuffs are properly managed to minimize potential health risks associated with their use.

Safeguards for use of wastes as feedstuffs include:

1. Do not feed litter (especially with high copper levels) to sheep.

2. Do not feed waste known to contain pesticides or drugs that are not approved for the animals to be fed the waste.

3. Process the waste (ensiling, deep stacking, etc) to ensure that pathogens have been eliminated.

4. In the USA, it is not recommended to feed animal waste, such as poultry litter, to lactating dairy cattle.

There is no opportunity for withdrawl of the waste as a feedstuff from the diet of the animals prior to marketing the milk.

5. Withdraw animal waste from the diets of meat producing animals at least 15 days prior to slaughter. Any residues that might have been in the diet will have an opportunity to clear the tissues of the animals prior to slaughter.

6. Avoid potential risks of chemical residue build-up in tissues of livestock fed animal waste by feeding such diets to animals that are not destined for immediate marketing. In the southern USA, very little poultry litter is fed to feed lot beef cattle that will be marketed in the near term for slaughter. Generally, litter is fed to stocker beef cattle that are purchased at about 180 kg, fed to about 340 kg, and then moved to a feed lot for fattening. Litter also is fed to beef brood cows, usually before and after calving. Both stocker cattle and brood cows are not destined for near term slaughter, therefore, any residues in their tissues have ample time for clearance.

Although poultry litter and other types of by-products have been successfully used as low-cost feed ingredients for the past 35 years, safeguards must continue to be improved on the feeding of waste products to food-producing animals to ensure a safe food supply for mankind.

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ANNEX B

Itinerary for CBAW Consultants

Dr. J.P. Fontenot,	Profesor/Lead Consultant Virginia Polytechnic Institute & State University, Blacksburg, VA, USA
Dr. B.G. Ruffin,	Extension Specialist, Auburn University, Auburn, AL, USA
Dr. T.A. McCaskey,	Professor of Microbiology, Auburn University, Auburn, AL, USA

Sept. 13 (Fri)	Arrive	New Delhi	LH-760	12:40 a.m.
	Stay :	Taj Palace Hotel, New Delhi		
14 (Sat)	Delhi/Ludhiana		By Road	(Car)
	Visit CBAW Project, PAU, Ludhiana			
	Stay :	PAU Guest House, Ludhiana		
17 (Tue)	Ludhiana/Hisar		By Road	(Car)
	Visit CBAW Projects at HAU, Hisar			
	Stay :	HAU Guest House, Hisar		
20 (Fri)	Hisar/Delhi		By Road	(Car)
	Delhi/Bangalore		IC-803	1715/1950
21 (Sat) } 22 (Sun) }	Attend CBAW Annual Meetings at NDRI, Bangalore			
23 (Mon) } to } 28 (Sat) }	Attend International Animal Nutrition Workers' Conference for Asia & Pacific Hold separate meetings with CBAW researchers			

	29 (Sun)	Bangalore/Cochin	IC-537	1010/1100
		Cochin/Trichur	By Road	(Car)
		Stay : Elite Int'l. Hotel, Trichur		
	30 (Mon)	Visit CBAW Project at KAU, Trichur		
Oct.	02 (Wed)	Trichur/Cochin	By Road	(Car)
		Cochin/Bombay	IC-192	1145/1330
	03 (Thur)	Visit CBAW Project at BVC, Parel, Bombay		
		Stay : Searock Sheraton Hotel, Bombay		
	05 (Sat)	Bombay/Delhi	IC-808	1630/1825
	07 (Sun) } 08 (Tue) }	Report Preparation and de-briefing at USAID/ICAR		
	09 (Wed)	Depart New Delhi for U.S.A.		