Ref. No. OTA-89-4 (8503)

No.

# NATIONAL DRUG AND FOOD QUALITY CONTROL LABORATORY PROJECT IN THE REPUBLIC OF INDONESIA

## -REPORT OF A SURVEY ON EXPERIMENTAL ANIMAL DIET-

March 1985

Medical Cooperation Department

Japan International Cooperation Agency

MCF
JR
84-22

# NATIONAL DRUG AND FOOD QUALITY CONTROL LABORATORY PROJECT IN THE REPUBLIC OF INDONESIA

## -REPORT OF A SURVEY ON EXPERIMENTAL ANIMAL DIET-

March 1985

Medical Cooperation Department

Japan International Cooperation Agency

# Foreword

One of the main objectives of this technical cooperation project which has been in progress since April 1, 1983, with the aim to protect the people of Indonesia from inferior drugs by strengthening the functions of the National Quality Control Laboratory is to conduct full-scale animal experiments for the first time in Indonesia.

The key to a successful outcome of animal experiments including toxicity and pyrogen tests at the laboratory equipped with a most up-to-date animal house scheduled to be completed in March 1985 with grant-aid cooperation lies in the breeding and raising of experimental animals. Moreover, in order to obtain effective experimental results, the quality of animals has to be maintained at least at a certain level by providing them with appropriate diet.

However, members of the NQCL staff had no experience in animal breeding and the results of analyses of nutritional levels and contaminants of the experimental animal diet on the market were not available. A request for advice on animal raising from the stage of planning was thus made by the Indonesian scientists to its Japanese counterpart.

This report contains the results of a survey conducted by a team of experts sent by JICA in September 1984 upon the receipt of the request and the work of diet analysis, evaluation and design was assigned to the Clea Japan, Inc., through the appropriation of the funds for the Domestic Support System and Appropriate Technological Development at JICA.

It is hoped that this report will contribute to the successful implementation of the Indonesian Drug and Food Quality Control Project. We wish to record our sincere thanks to Dr. Akira Takanaka, Head of Pharmacology Division, National Institute of Hygienic Sciences Biological Safety Research Center, and other agencies concerned, for their valuable advice and guidance.

March 1985

Yutaka Hasegawa, M.D.
Director,
Medical Cooperation Department,
JICA

Contents

Appendices

# Chapter I Process and Steps of Diet Analysis and Survey in the National Drug and Food Quality Control Laboratory Project in the Republic of Indonesia

## 1.1 Process and necessity of practice of diet analysis and survey

A success in raising laboratory animals is a prerequisite for leading animal experiments in pharmacological and toxicological research to success in the project of National Drug and Food Quality Control Laboratory in the Republic of Indonesia and for intensifying the function of a laboratory in the republic to carry out assays and tests. In Indonesia few people are experienced in raising laboratory animals and the National Drug and Food Quality Control Laboratory (NQCL, or PPOM in Indonesian) is staffed with few experts in this regard. Under these circumstances the project must be started from making a policy to procure or produce diet suitable for raising and breeding of laboratory animals. It is necessary to collect much data and to conduct surveys in order to understand the particular purpose of production and the characteristics of diet for laboratory animals.

To meet this necessity, an activity was performed to collect data and a survey conducted by interview in 1984, so that diet for laboratory animals might be produced successfully in Indonesia. In this manner efforts were initiated with gathering information as their principal aim.

In the first attempt to produce diet for laboratory animals in Indonesia, the topics of discussion were to secure a source of fibers used as diet for rabbits and guinea pigs and to produce fine-particled powder and premix. While every effort was made to settle these problems, the writer was provided with information on rabbits and rabbit diet in Indonesia by Mr. Sasaki, of P.T. Otsuka Indonesia, by the courtesy of Dr. Takanaka (Appendix 1).

According to the information, rabbits raised in P.T. Otsuka were produced in the Batu area (around 25 kilometers from Malang) of the Java Island, Indonesia, weighing 1.5 ∿ 2.7 kg. They have been fed such vegetables as Kang kung and carrot, self-made diet (produced by P.T. Otsuka), and "Consentrat TU" (produced by EKA Poultry Industrial Enterprise). The results of a comparative feeding experiment performed in P.T. Otsuka served as a very good reference for the planning of the project. While conducting a survey in Japan, the writer was fortunate enough to meet with Mr. Takagi, of P.T. Bina Satwa, which is affiliated with the Nippon Formula Feed Co.,

Ltd., and registered in Jakarta, Indonesia. Mr. Takagi happened to return home in Japan. He was good enough to provide information on the condition of raw materials for animal diet in Indonesia. According to him, there is no trouble about cereals and imported oil cake, but attention must be paid to raw materials produced in Indonesia. Especially, fish meal causes many troubles. Moreover, careful investigation must be made on hay which is in a particular condition in this country.

Taking the information given by Mr. Takagi into consideration, discussion was made on how to conduct diet analysis and survey. It was concluded that a survey had to be conducted on the following items:

i) To collect information on the breed, ability, and hematological properties of rabbits raised in Indonesia

ii) To survey the condition of raw materials available for the production of diet for laboratory animals

iii) To survey diet producers in Indonesia

iv) To survey such technical capability as required for diet analysis

v) To examine raw materials for the production of hay and premix

vi) To examine the possibility for domestic production and production on a consignment basis

1.2 Steps for practice of diet analysis and survey

What are the requirements for diet for laboratory animals? They are mentioned in §58.90 Animal Care (g) of Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies (GLP) prescribed by the U.S. Food and Drug Administration (FDA). They read as follows:

(g) Feed and water used for the animals shall be analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed or water are not present at levels above those specified in the protocol. Documentation of such analyses shall be maintained as raw data.

The "contaminants" mentioned in GLP are aflatoxins (total), As, Cd, DDTs, dieldrin, estrogenic activity (DES equiv.), Hg, lindane, malathion, Pb, PCBs, and Se, for example, mentioned by J.P.Bercz, of the U.S. Health Effects Research Laboratory, in this report on the "Temporal Variability of

Toxic Contaminants in Animal Diets." The numerical data given by the U.S. Environmental Protection Agency (EPA) and the National Center for Toxicological Research (NCTR) may serve as references for the standards of these contaminants (Appendix 3).

As mentioned above, diet for laboratory animals is controlled by the purpose of a given experiment and a person in charge of the experiment. It is not sufficient for the selection of diet for laboratory animals to take simply the food habit and nutrient requirements of a given animal species and the palatability of a given diet into consideration.

In planning diet analysis and survey in Indonesia, it had been assumed necessary as a prerequisite for this analysis to carry out an analysis on products and raw materials of animal diet available in Indonesia on the basis of information collected in Japan.

The information required for this analysis includes residual agricultural chemicals found in raw materials for animal diet, mycotoxins, oxidation, and microbial contamination. Particular attention must be paid to residual agricultural chemicals. Therefore, efforts were made mainly to collect data on these chemicals. Analysis on the other items was made by anticipation from the data obtained from the preliminary analysis performed in Japan.

The results of the preliminary (or primary) analysis are mentioned below in section 1.3. In this section, discussion is made on residual agricultural chemicals.

In his paper printed in the Tropical Agriculture, Volume 15, No. 2, Hattori presented a list of agricultural chemicals authorized to be imported into Indonesia. Aldrin, BHC, dieldrin, DDT, malathion and parathion are shown in this list. It is also reported that the crop of rice is doubled or tripled by the introduction of agricultural chemicals, the cost of which is covered by the increase in crop, in Indonesia (Appendix 4).

Hayashi et al. clarified that the larvae of house flies produced in Indonesia tended to be lower in susceptibility to malathion than those produced in Japan.

In Indonesia, adult house flies show a resistance developed to malathion. The effect of insecticides used seems to have been exhibited especially on house flies on the Java Island and in the urban area of Jakarta (Appendices 5 and 6).

Dr. Kuwatsuka majoring in soil science at the Nagoya University made a lecture on "The Present Situation of the Use of Agricultural Chemicals in Indonesia." He pointed out in his lecture that the crop of rice was 300 ∿ 400 kg per 10 ares when such chemicals were used and 100 ∿ 180 kg per 10 ares when no chemicals were used. In other words, the crop of rice is expected to increase by two to three times after these chemicals are applied. Of these chemicals, carbaryl, carbofuran, diazinon and fenitrothion have been recommended as insecticides chlorothainil and mancozeb as fungicides, and brodifacoum and coumarin as rodenticides.

Although DDT is prohibited to use for farming, it can be purchased from a hospital when it is asserted to be applied to the control of malaria.

By the way, the annual crop of rice was 23,160,000 metric tons (M/T) in Indonesia in 1982.

According to the Japan International Cooperation Agency (JICA) (Country Report 1983: Group Training in Pesticide Utilization for Plant Protection which deals with current problems on human hazard, environmental and food pollution, and the development of pesticides in fungi and insects), the crop of rice was 1.5 ∿ 2.0 M/T per hectare before the introduction of a new technique, but has increased to 4 ∿ 5 M/T per hectare after the application of this technique.

Data reveal that a total of 370 formulations of pesticides are known in Indonesia, and that of them, 115 formulations have been licensed temporarily and the remaining 255 permanently (Appendix 7).

After obtaining the information mentioned above, items for analysis were decided and the primary analysis was carried out.

### 1.3 Primary analysis on animal diet and raw materials

On the basis of the information supplied by Mr. Sasaki, of P.T. Otsuka Indonesia, a guideline was established for analysis on animal diet and raw materials produced in Indonesia. Then Mr. Sasaki kindly sent samples of these diet and raw materials to Japan.

These samples reached Japan at the end of July, 1984. Some of them were found to be insect-eaten and destroyed by burning or treated by smoking in the plant quarantine.

1) Purpose of primary analysis

A team of feed experts is sent out to make investigation and give advice regarding diet for laboratory animals raised and bred at the national laboratory mentioned above (NQCL). To provide the team with preliminary knowledge, preparatory analysis is conducted on diet and raw materials in Indonesia.

Items considered to be necessary for the preparatory analysis were selected on the basis of data on raising and breeding of rabbits given by Mr. Sasaki, of P.T. Otsuka Indonesia, and information on diet and raw materials offered by Mr. Takagi, of P.T. Bina Satwa.

These items consisted mostly of those concerning known interfering contaminants in the GLP. Furthermore, mycotoxins and microbial contamination were examined, as mentioned in section 1.2.

2) Materials and methods of primary analysis

Seven samples supplied by P.T. Otsuka Indonesia were used as materials of analysis. They consisted of two samples of commercial diet and five samples of raw materials. The two samples were those of two products, Concentrate BU and TU, of the EKA Poultry Industrial Enterprise used for breeding and raising of rabbits. Each product contained five main components, corn, fish meal, rice bran, skim milk and soybean meal.

A total of 46 items were selected for analysis. They included the five components mentioned above, contaminative chemical substances, mycotoxins, microorganisms, estrogen, nitrosamine, alkali neutralization number and peroxide value (Appendix 8).

The analytical method used was that mentioned in the GLP standard and used by every manufacturer in Japan as a standard method. For its details, refer to a technical book published.

In the U.S.A., the Standard Methods of the Association of Official Analytical Chemists (AOAC) in 1975 are referred to for such analytical method. They are cited in EPA Report No. 600/1-81-040 (Appendix 9).

Since the standards for known interfering contaminants are high, it is necessary to take the limit of detection in consideration.

3) Results of primary analysis

The primary analysis was performed as a preparatory analysis to provide a team of diet experts to be sent out to Indonesia with data required. Therefore, it was conducted only on the supplied samples as they were. In

other words, many points remained unknown about these samples, such as the condition of sampling, the type of product, the date of production, and the process of production of animal diet from raw materials.

There were problems on numerical values in the results of experiments concerning moulds, the coliform group, acid value and peroxide value. It seemed necessary to carry out a continuing survey in the future to verify those values (Appendix 8).

4) Discussion and conclusions of primary analysis

If was quite sure that the philosophy of the manufacturer on the five components of the product, as well as the raising condition of laboratory animals and the species of these animals raised in Indonesia, may have been incorporated in the products of the EKA Poultry Industrial Enterprise. Therefore it was not appropriate to express the opinion on these products at that stage.

Of the raw materials used for the production of diet, soybean meal seemed to be a by-product of oil manufacture from soybeans by pressing. Corn was poor in protein and high in moisture probably because of the effect of had conditions of transportation. Rice bran was also scanty in protein. No other components met the Japanese Standard for the Composition of Animal Diet or the "Values of Diet Analysis in Indonesia" supplied by the Nippon Formula Feed Manufacturing Co., Ltd. either (Appendix 10). Fish meal seemed to have been imported, judging from its composition. There was no problem on skim milk. It was common to all the five components of diet that moisture was rather high, probably because water might have been absorbed during storage or in the course of transportation.

Since chemical contaminants are threatening for diet for laboratory animals, data on diet analysis were compared between Indonesia and other countries in the world. These data were collected from EPA Report No. EPA 600/1-81-040, NCTR, Greenman's "Commercial Laboratory Animal Diets, Toxicant and Nutrient Variability," J.K. Eva's "Quality Control in Diets - the Attainable, and the Draft of Steps to Meet the GLP drawn by the Japanese Association of Laboratory Animal Diets." In this manner the actual condition of diet in Indonesia was compared with that in Japan, the U.K. and the U.S.A. (Appendices 11 to 14).

There was no large difference in the results of heavy-metal analysis between the data collected in Indonesia and those collected in other

countries in 1974 ∿ 1982. From the review of literature it had been presumed that there might be a problem on residual agricultural chemicals. All the samples, however, were found free from the agricultural chemicals on which analysis had been performed. Nevertheless, it is too hasty to rest assured. We must keep sharp watch and carry out analysis continually for a long time.

Of the mycotoxins, aflatoxin, deoxy-nivalenol, nivalenol (trichothecenes) and ochratoxin have attracted attention as toxic substances to be contained in diet components. None of them, however, were detected from any sample.

In the microbiological examination, the total viable microbial count was negligible in all the components, except rice bran. Moulds were contained abundantly in every components, most probably because of high moisture. The MPN of the coliform group was large. Since the production and storage of diet are conducted under very filthy conditions in Indonesia, there are many problems to be solved from a hygenic point of view.

As no nitrosamine was detected from any component, the samples seemed to be safe in this regard. Something doubtful was found about estrogen, for which examination must be continued in future.

Rather large acid values and peroxide values were detected. If a quality control is performed thoroughly, there will be an improvement in both values.

Mentioned above are the results of analysis and some discussion on them. It is concluded that there are important problems on diet from a hygenic point of view. There are many items of analysis which were found normal in the present survey, but which may possibly be sources of trouble in future. It is desirable to continue a regular survey on these items and make a collective judgment on the items some other day, on the basis of cumulative data collected in Indonesia.

## Chapter II Fact-Finding Survey in Indonesia by a Team of Diet Experts

### 2.1 Purpose and steps of survey by a team of diet experts

A team of diet experts was organized in Japan in July, 1984. The duty to be shared was assigned to each member of the team by the departure to Indonesia in September of the same year. All the members were prepared for their own duty.

1) Purpose of survey

It was the purpose of the survey by the team to study conditions required for the production of animal diet for raising and breeding of such laboratory animals as guinea pigs, mice, rabbits, and rats to be used in the project of pharmaceutical quality control in Indonesia within the territory of this country.

To accomplish this purpose, information was collected beforehand by the review of literature, and preparatory analysis performed on raw materials for diet.

Every effort was made especially to carry out what is mentioned in items (i) to (vi) in section 1.1.

2) Members and schedule of team

Four Japanese members were appointed for the team. They are as follows: Yoshitaka AIDA; technical officer, Division of Toxicology, Biological Safety Research Center, National Institute of Hygienic Sciences, Ministry of Health and Welfare; expert in laboratory animals. Yoji ANDO; chief, Section of Business Administration, Tokyo Branch Office, Japan Clea Co., Ltd.; expert in formula diet and design. Yoneo YOSHIDA; associate counselor, Division of New Market Development, Nippon Formula Feed Manufacturing Co., Ltd.; expert in quality control. Susumu KUSAKA; junior engineer, Section of Raw Material Production, Chita Plant, Nippon Formula Feed Manufacturing Co., Ltd.; expert in diet production.

When they arrived at Indonesia, Dr. Jiro KAWAMURA joined the team as leader and Dr. Pudjoprajitno as counterpart personnel from Indonesia.

The schedule of the team was made for fifteen days, from 2nd to 16th September, 1984. In it, thirteen facilities were inspected. The detailed schedule is as follows.

| | | | |
|---|---|---|---|
| September | 2nd (Sun.) | Narita ∿ Jakarta | Meet Dr. Kawamura, team leader, at the hotel to adjust the schedule |
| | 3rd (Mon.) | Visit to NQCL (P.P.O.M. in Indonesian) for inspection and conference; visit to C.B.R. | |
| | 4th (Tues.) | Jakarta ∿ Ciawi | Visit to B.T.P. |
| | 5th (Wed.) | Ciawai ∿ Bandung<br>Bandung ∿ Bogor | Visit to Hipki "Mekar"<br>Visit to Bio Farma |
| | 6th (Thur.) | Bogor ∿ Jakarta | Meeting of the investigation group |

| | | |
|---|---|---|
| 7th (Fri.) | Bogor ∿ Jakarta | Visit to L.P.P.H, P.T. Hirema, and P.T. Bina Satwa |
| 8th (Sat.) | Jakarta | Meeting of the investigation group; disposition of business |
| 9th (Sun.) | Jakarta ∿ Surabaya ∿ Tretes | |
| 10th (Mon.) | Tretes ∿ Malang<br>Malang ∿ Surabaya | Visit to P.T. Otsuka Indonesia |
| 11th (Tues.) | Surabaya ∿ Semarang<br>Semarang ∿ Surakarta | Visit to EKA Poultry Industrial Enterprise |
| 12th (Wed.) | Visit to P.T. Air Mancur.<br>Surakarta ∿ Yogyakarta ∿ Jakarta | |
| 13th (Thur.) | Jakarta ∿ Bogor | Arrange matters with P.T. Bina Satwa; Visit to P.T. Pfizer Indonesia, P.T. Vaksindo Satwa Utama Raya, and P.T. Kapo Trading Co., Ltd. |
| 14th (Fri.) | Sampling of diet and raw materials at P.T. Bina Satwa; prepare a report of inspection by the Investigation Group | |
| 15th (Sat.) | Meeting to report the results of inspection at NQCL (P.P.O.M.) | |
| 16th (Sun.) | Jakarta ∿ Hong Kong ∿ Narita | |

2.2 Results of survey

The results of inspection and observation at the 13 facilities are as follows.

i) Biomedical Research Center (CBR)

The center was equipped with the following instruments for the production of diet for laboratory animals.

- A grinding machine (Nara Machine Co., Japan) 3 h.p.
- A pellet mill, CPM 2 h.p.
- A ribbon mixer (Miyasaka Precision Instrument Co., Japan) (about 300 kg)
- A desiccator

These instruments were used for the production of diet for laboratory animals. Since no steam was introduced when pellets were formed by the pellet mill, the resulting pellets were soft. When they were actually

given to animals, some portions of them remained to be powder and were scattered around noticeably.

Of the laboratory animals kept at the center, mice belonged to the Swiss breed and rats to the Wistar breed. All of them were bred by cross mating. Rabbits were rather large in size, originated from the western part of the Java Island, and allowed to multiply at the center for self-sufficiency. Sawdust and wild grass were used as litter in cages.

Two kinds of diet were produced in the form of pellets of ø 3/16 inch. One of them was for mice and rats and the other for rabbits.

ii) Project of Animal Research and Development (B.P.T.)

B.P.T. is facilities constructed by the cooperation of the Netherlands. Laboratories and instruments for chemical analysis were of the latest type and sufficient. Diet analysis was conducted on the five components, heavy metals, mycotoxin (aflatoxin), hormones, agricultural chemicals, PCB and others. It is desirable that B.P.T. will extend cooperation to NQCL in performing diet analysis in future.

B.P.T. was equipped with three pellet mills of horizontal type made in the U.K. for molding. Nevertheless, it had no desiccators. Pellets were allowed to stand on the concrete floor of a diet preparation room for drying.

iii) Hipki "Mekar"

This is a breeder who produces rabbits for meat and fur. It organized an association with twelve breeders in the neighborhood. According to them, a male or female breeding rabbit 4 months old costs 45,000 Rp and rabbit meat 3,000 Rp per kilogram. The rabbit cage in use was satisfactory, or 1.5 times as large as that used for breeding a rabbit in Japan.

Rabbits of some pure breeds had been introduced for the production of meat and fur rabbits, but none of these breeds had been preserved. It is regrettable that those rabbits were used all for cross mating. The technique of breeding, however, has been established on this farm. If technical advice is given to this breeder, it will be possible to entrust the production of rabbits to it.

Rice bran and elephant grass were used mainly for feeding. In addition, wild grass was fed (Appendix 15).

iv) Bio Farma

As laboratory animals, hamsters, mice and rabbits were raised. Recently, monkeys began to be kept on this farm. All the animals, except monkeys, have been bred on the farm and supplied, upon request, to universities and other institutions.

There were golden hamsters, Swiss mice, Wistar rats and Rhesus monkeys on the farm.

Plastic cages sufficient in size were used. They were arranged at four levels of seven rows each in a rack. Eighteen racks were placed in a room.

Formerly, diet had been produced on the farm. At present, it is purchased from Charoen Phoc Phand.

Mice and rats were fed artificial sow's milk for piglets of late growing stage (ø 3/16 inch x 5 mm), rabbits mash for adult chickens or artificial sow's milk for piglets of late growing stage and elephant grass, and monkeys diet for swine.

Rice hulls were used as litter for mice and rats.

Bio Farma has raised 400 ∿ 700 guinea pigs, 14,000 ∿ 18,000 mice, 7,000 ∿ 10,000 weaned mice, 20 ∿ 100 rabbits, and 30 ∿ 100 monkeys on the average per month. It was an institution where laboratory animals were raised in the best condition in Indonesia.

v) Institute of Animal Disease Research (L.P.P.H.)

This institute was accomplished by the aid of Australia. In it, guinea pigs, mice, rabbits and rats have been raised. All of them, except rabbits, have been bred experimentally. Rabbits were purchased from a breeder in the neighborhood. They had been suffered most frequently from coccidiosis and psoroptic mange.

The laboratories of the institute were well equipped to perform pathological and microbiological examinations.

The number of laboratory animals raised was relatively small. Two kinds of diet have been produced in the institute. One of them was for mice and rats (ø 6 mm x 15 mm) and the other for guinea pigs and rabbits. When the team visited the institute, no diet for rabbits was seen and rabbits were fed Setaria grass.

Various raw materials for diet stored were inspected by the team. Fish meal was spoiled remarkably with bad smell. Coconut meal was moldy and solidified. Any other material was also in a similar condition. According to a staff member who explained these materials, the institute purchased all the materials from a pet food shop.

vi) P.T. Hirema

This animal diet plant is operated by a company which is only one of its kind that has been established independently by its own capital. It began to work in 1977 to produce diet for layer hens principally. To meet the recent trend of demand, it was going to be equipped with a pellet mill for the production of diet for broilers. Its producing capacity was 58 tons per day (about 7 working hours). At present, about 600 tons of diet could be sold per month. The whole facilities of production were on a very small scale. They were of American perpendicular type. Cumulative measuring gauges were set under the tanks of raw materials and finished products. A one-ton mixer was installed in an underground room below the room where the tanks were located.

It was hardly understandable that there was no system of tubes for steam in the pellet mill for molding (40 h.p.).

vii) P.T. Bina Satwa

In P.T. Bina Satwa a conference was held to discuss the availability of raw materials produced in Indonesia.

It was decided that raw materials which seemed to be available for the production of diet for laboratory animals were borne to Japan for analysis. Samples collected for analysis are as follows:

Corn, milo, rice bran, wheat pollard, soybean meal (Brazil), fish meal (Peru and domestic), meat and bone meal (New Zealand), coconut meal, Daun petai cina (meal and leaves), sesami meal (China), papaya leaves, cassava leaves, elephant grass, green bean (L.P.P.H.), ground nut meal (L.P.P.H.), Setaria grass, lumuput lapangan, cocoa residue

In addition to these twenty, three samples were collected from diet actually used in C.B.R. and L.P.P.H. and on Bio Farma, respectively. In short, 23 samples were to be analyzed in Japan.

viii) P.T. Otsuka Indonesia

Since this company provided the team with data on rabbits raised in it before the survey was started, the team could inspect the company smoothly. The rabbits raised in the company were much smaller than meat rabbits reared in Lembang in the western part of the Java Island. In Malang, where the company is located, in the eastern part of this island, adult rabbits weigh 1.5 ∿ 2.0 kg and are used for the pyrogen test in this company. A rabbit weighing about 2 kg can be purchased at a price of 1,800 Rp. Since rabbits in Malang are too small, they are now bred by cross mating. As a result, cross-bred rabbits can be used when they reach 3 months of age.

Concentrate TU (for rabbit raising) produced by the EKA Poultry Industrial Enterprise, Semarang, which was to be visited later by the team, was used for growing rabbits and diet RC-4 of the Oriental Yeast Co., Ltd. for breeding rabbits.

A total of 400 breeding rabbits were kept in the company and 70 ∿ 80 rabbits produced every month. The litter size ranged from five to eight. The weaning rate was about 70 ∿ 80%.

Facilities and raising condition were generally good. They will be advanced further, if the latest reproductive technique is introduced in this company.

ix) EKA Poultry Industrial Enterprise

In this company discussion was made on diet for rabbits on the basis of data obtained from the preparatory analysis. In it a main topic was the crude fiber contents of the diet. Alfalfa is commonly used in Japan, but it is too expensive for rabbits to be fed in Indonesia. Therefore, petai cina and papaya leaves are used as substitutes for alfalfa. The limit of mixing rate is about 7% for petai cina. Rabbits fed petai cina excessively will suffer from alopecia by the action of mimosine contained in this grass. It was explained that papaya leaves could be fed to rabbits to a mixing rate of about 18% (Appendix 16).

Chief raw materials used by this company for the production of diet for rabbits were corn, rice bran, soybean meal, meat and bone meal, fish meal and ground nut meal (= peanut meal). Strenuous effort has been made to prevent these materials from molding. The use of antimycotic agents

was stressed in the company. The plant of the company was small in scale with equipments manufactured by the Lister Company in the U.K. Its main product was chicken diet. Its daily output was about 6 tons, 70% and 30% of which were diet for layers and broilers, respectively.

x) P.T. Air Mancur

This is a pharmaceutical company with galenicals as main products. The team inspected its library, analytical and research laboratories. There was a field where herbs were grown for experimental purposes.

As laboratory animals, hamsters, guinea pigs, mice and rabbits were raised. Hamsters and mice were fed pellets (ø 2 cm) produced in the company itself, and rabbits Kang kung.

Animals were used only for a simple toxicity test of pharmaceutical products to confirm their safety.

xi) P.T. Pfizer Indonesia

This is an important selling base of the company in Southeast Asia. It was explained to be playing a central role in quality control and santation in that region. The team inspected its plant.

It is hot and humid in Indonesia. The storehouse of raw materials and products is air-conditioned. A low-temperature storehouse is used for articles which must be preserved particularly at low temperature. Arrangement and labelling of stored things were carried out in such manner in the storehouse that the principle "First taken in, first taken out" might be observed strictly.

The plant of the company was partitioned into small rooms for pharmaceutical production. In other words, it seemed to be composed of private rooms. One or two persons worked in each room. The plant was designed so thoughtfully and efficiently with a satisfactory equipment of air conditioning that there was much about the plant to be kept for future reference.

In addition, each worker put on protective clothes fit for the type of work to do. A notice was posted on the door of every room to indicate the type of work in operation, the name of the worker and the working hours. It was very impressive.

Every laboratory was large enough and sufficiently equipped. As laboratory animals, rabbits were raised for the pyrogen test and fed Kang kung.

xii) P.T. Vaksindo Satwa Utama Raya

This institution began to be constructed in 1980 and was accomplished in 1982. It was given technical advice and assistance by the Nippon Institute for Biological Science, Tokyo, Japan. It started the production of four vaccines for animal use in January, 1984. Water is the most important thing in vaccine production. It was supplied from a well about 180 meters deep. There was also a large cleaning bed which was used efficiently.

As laboratory animals, small numbers of chickens, dogs, goats, mice and rabbits were raised in this institution. Mice were given diet produced by the C.B.R.

xiii) P.T. Kapo Trading Co., Ltd.

This company produces Pre-mix. It acts as a general agency of importation for the Roche, Ciba-Geigy and Abbott Companies.

An additive will be produced by order if it amounts to 1 kg at least, containing a minimum unit of vitamin or mineral.

The team inspected the plant of the company, the equipments of which seemed to be most likely to be contaminated. The price list of the company is presented elsewhere (Appendix 17).

## 2.3 Conclusions of survey by the team of diet experts

When the survey was finished in Indonesia, the following conclusions are drawn. If small rabbits in the eastern part of the Java Island are bred and raised in a closed colony, they will be good laboratory animals. To do this, various data are required. It is also necessary to establish a long-term plan, under which microbiological, genetical, and environmental control must be carried out.

As is anticipated from the preparatory analysis, raw materials of diet for laboratory animals must be examined for decay, rottenness, and molding from a hygenic point of view. Petai cina was expected to be available as a source of fibers, but it can hardly be used as such, since it contains mimosine. In the present project, however, this grass is to be treated by heat. So that, mimosine contained in it will be destroyed.

Anyhow, sufficient care and technique must be taken for the storage and quality control of raw materials and products, taking the climate condition in Indonesia into consideration.

Final conclusions will be drawn after results are obtained from the analysis of samples collected.

The equipments and techniques possessed by animal diet manufacturers operated on a small and moderate scale are hardly available for the present project. Those possessed by P.T. Bina Satwa and other manufacturers operated on a large scale can be relied upon in performing this project.

It seems to be efficient to carry out diet analysis by utilizing the equipments and techniques of the Project of Animal Research and Development (B.P.T. standing for Balai Penelitian Ternak). In this case it is necessary to perform a cross check of the results of analysis.

Samples of grass for hay making were collected from petai cina, elephant grass, Setaria grass, papaya leaves, cassava leaves, and lumuput lapangan (wild grass). They were brought to Japan for analysis.

Whether pre-mix should be produced by the NQCL (P.P.O.M.) itself or by any other plant by a contract is to be decided after discussion is made again in Indonesia. Whether NQCL should produce the whole amount of required pre-mix or only a part of it, will be determined after its capacity of production is confirmed.

# Chapter III Secondary Analysis and Formulation of Animal Diet for the National Drug and Food Quality Control Laboratory Project in Indonesia

## 3.1 Purpose and conclusions of secondary analysis

### 1) Purpose

It was the purpose of the secondary analysis to carry out an analysis of minimum requirements for the production of diet for various laboratory animals in Indonesia. It was expected from this analysis that what types of raw materials and what combination of these materials would be suitable for such diet.

2) Materials and methods

To attain the purpose mentioned in the preceding paragraph 1), a total of 23 samples were analyzed. They consisted of 18 samples collected at P.T. Bina Satwa from 16 raw materials which were available in Indonesia and which included wild grasses expected to serve as substitutes of alfalfa, two samples collected at L.P.P.H. from two raw materials and three samples from three products of diet. They are mentioned in detail as follows.

i) The 18 samples collected from 16 raw materials, including wild grasses, at P.T. Bina Satwa are as follows:

Maize, milo, rice bran, wheat pollard, soybean meal (Brazil), coconut meal, fish meal (Peru and domestic), meat and bone meal (New Zealand), sesame meal (China), cocoa residue

Samples were collected from the following sources of fibers:

Daun petai cina (meal and leaves), papaya leaves, cassava leaves, elephant grass, Setaria grass, lumuput lapangan

ii) The two samples collected from two raw materials at the Institute of Animal Disease Research (L.P.P.H.) are as follows:
Green bean, groundnut meal (peanut meal)

iii) The three samples collected from three products of diet are as follows:

Commercial pig starter of Charoen Pokphand (Bio Farma), diet for rabbits (L.P.P.H.), diet for mice and rats (C.B.R.)

The samples mentioned above were subjected to the secondary analysis, which was carried out in the same manner as the primary analysis mentioned in paragraph 2) of section 1.3.

3) Results and discussion

The results of material analysis on 20 samples collected from 18 raw materials (Appendix 18) were compared with the analytical data published by the Nippon Formula Feed MFG Co., Ltd. and the 1983 Feedstuffs Analysis Table printed in the Feedstuffs (February 14, 1983 issue) (Appendices 10 and 19).

There was no problem on maize or milo. In rice bran, fat and ash were a little larger in value, but smaller than those obtained from the primary analysis. This difference may have been derived from that in the process of production.

Wheat pollard was assumed to be similar to wheat shorts. Soybean meal (Brazil) was rich in protein and resembled soybean extract produced in Japan.

Both Peruvian and domestic fish meal presented better AV and POV than those in the primary analysis. The team was satisfied with the results of the secondary survey. Peruvian fish meal seemed to be too rich in fat.

There was no problem on meat and bone meal or coconut meal. Sesame meal produced in China was considered to be a little poor in protein and fat.

Of the raw materials serving as sources of fibers, Daun petai cina was assumed to be hardly available as a component of diet, as it contains mimosine which is toxic enough to cause alopecia in animals. It has been reported that mimosine can be destroyed by heat treatment. For reference, the following paper is introduced here, since it deals with a method of determination of mimosine: "The determination of mimosine and 3,4-dihydroxypyridine in biological material" Aust. J. Agric. Res., 1964, 15, 168-179 (Appendix 20).

Judging from the inspection in the present survey, elephant grass and Setaria grass are only two materials that can be used as components of diet. A further survey, however, is required on both grasses before they are actually used as materials for diet production. Any other raw material from a source of fibers may serve as a substitute of elephant or Setaria grass.

Of the three products of diet analyzed, the commercial pig starter was high in AV and contained no antibacterials, contrary to expectation. The diet for rabbits of L.P.P.H. was poor in protein and very scanty in fibers. It did not seem to be complete pelleted diet. It was high in AV, formula molds and coliform group, showing an insufficient Ca/P ratio. The diet for mice and rats of C.B.R. was considered to be satisfactory in all the points, except AV and molding condition.

Anyhow, the results of the secondary analysis could be anticipated from the quality of the raw materials collected.

Besides, analysis of volatile basic nitrogen (VBN) was performed on fish meal and meat and bone meal to obtain satisfactory results. Analyses of AV, POV and NaCl were conducted with results shown in Appendix 18.

Mentioned above are the results of analysis on the raw materials and products of diet collected.

4) Conclusions

Judging from the meteorological condition, distribution of raw materials, technical condition of storage and technique of diet production in Indonesia, it is considered that rather satisfactory results were obtained from these analyses. The diet which is going to be produced for the present project will be a factor of great reproducibility for animal experiments to be performed in future. It is as important as laboratory animals themselves.

Raw materials of diet must be inspected sufficiently. A table of raw material inspection should be prepared to inspect materials whenever they are taken in the storehouse. It is necessary to accept materials of the same quality at all times for the production of diet. Especially, it is expected that the sensory test will be carried out on these materials.

Bactericidal effect can be expected from the use of steam in the process of production. In the process of drying it seems possible to lower the moisture of diet to such level as tolerable for animals. After that, it is essential to store the finished product with a care not to allow micro-organisms to recontaminate the product.

3.2 Tentative formulation of diet materials for the project in Indonesia

1) Basic principles of formulation

Basic principles of tentative formulation for the production of diet for laboratory animals used in the project are as follows: (1) The diet contains so few known interfering contaminants that it may meet the requirements mentioned in the GLP. (2) The composition of a given diet once established must not be altered to keep the reproducibility of the animal experiment. (3) Care must be taken not to use too many types of raw materials for the production of diet in order to prevent the analytical value of the finished diet from fluctuating noticeably.

Efforts should be made to be faithful to these principles of formulation, taking economic conditions into consideration.

2) Formulation for mice and rats (draft)

In the present survey, materials for analysis were collected to make the first formulation. After they were used for analysis, their remaining portions were mixed up tentatively to prepare 1 kg of diet. Then, a simple palatability test of this diet was carried out. As a result, this diet was

a little poor in palatability. Therefore, the second formulation was prepared.

The remaining portions of the materials collected were too little to be mixed into pellet. Accordingly, mash was prepared and used for the palatability test. The form of diet may have some effect on the palatability. The first formulation, therefore, was not abolished, but is still pending.

[ First Formulation ]

| | |
|---|---|
| Maize and milo | 30% |
| Wheat pollard | 20% |
| Soybean meal | 15% |
| Fish meal (Peru) | 10% |
| Coconut meal | 10% |
| Sesame meal | 5% |
| Cassava meal | 9% |
| Vitamin and mineral mix | 1% |
| Total | 100% |

The analytical values of these components are shown in Appendix 18. The five components presented the following values: Crude protein, 23.3%; crude fat, 5.1%; crude fiber, 4.9%; crude ash, 5.3%; moisture, 11.2%. From these values nitrogen free extracts (NFE) was calculated to be 50.2%.

The analytical values of vitamins and minerals were used to calculate the value of Pre-mix.

Pre-mixes are not added in the first formulation.

[ Second Formulation ]

| | |
|---|---|
| Maize | 18.0% |
| Milo | 13.0% |
| Wheat pollard | 20.0% |
| Soybean meal (Brazil) | 15.0% |
| Fish meal (Peru) | 13.0% |
| Coconut meal | 5.0% |
| Sesame meal | 5.0% |
| Cassava meal | 8.0% |
| Coconut oil | 0.5% |

| | |
|---|---|
| Molasses | 0.5% |
| Salt | 0.5% |
| $CaCO_3$ | 0.8% |
| *Pre-mixes | 0.7% |
| Total | 100.0% |

*Pre-mixes are prepared to consist of the following amounts of the following components per 100 g of diet.

| | | |
|---|---|---|
| Vitamin A | 1,000 | iu |
| Vitamin $D_3$ | 200 | iu |
| Vitamin E | 10 | mg |
| Vitamin $B_1$ | 1.5 | mg |
| Vitamin $B_2$ | 1.5 | mg |
| Vitamin $B_6$ | 1.5 | mg |
| Vitamin $B_{12}$ | 7 | µg |

3) Formulation for guinea pigs and rabbits (draft)

The first formulation was set up. The remaining portions of raw materials after analysis were used tentatively for the production of diet under this formulation. A palatability test was carried out on this diet in the same manner as mentioned in paragraph 2) of section 3.2. As a result, this diet was poor in palatability, as well as diet for mice and rats. Therefore, the second formulation was prepared.

[ First Formulation ]

| | |
|---|---|
| Maize and milo | 20% |
| Wheat pollard | 20% |
| Soybean meal (Brazil) | 10% |
| Fish meal (Peru) | 5% |
| Coconut meal | 4% |
| Elephant grass and Setaria grass | 40% |
| Vitamin and mineral mix | 1% |
| Total | 100% |

The analytical values of these components are shown in Appendix 18. The five components presented the following values: Crude protein, 17.8%; crude fat, 3.7%; crude fiber, 13.7%; crude ash, 8.1%; moisture, 9.0%. NFE was 47.7%. As the value of crude fiber is remarkably different from that obtained by calculation, it must be re-examined for confirmation.

Pre-mixes are not added in this diet, as well as that for mice and rats.

[ Second Formulation ]

| | |
|---|---|
| Maize | 8.0% |
| Milo | 6.3% |
| Wheat pollard | 20.0% |
| Soybean meal (Brazil) | 15.0% |
| Fish meal (Peru) | 5.0% |
| Coconut meal | 2.0% |
| Coconut oil | 1.0% |
| Molasses | 0.5% |
| Cassava meal | 10.0% |
| Elephant grass | 15.0% |
| Setaria grass | 15.0% |
| Salt | 0.7% |
| $CaCO_3$ | 0.8% |
| Vitamin C | 150 mg/100 g |
| **Pre-mixes | 0.7% |
| Total | 100.0% |

** It is planned to prepare pre-mixes last of all, so that they may be used for this diet, as well as for that for mice and rats.

3.3. System of production and economic character of diet produced by the formulation of raw materials

1) System of production

There are two systems to settle the problem of production of diet. They are complete and half self-production.

In the half self-production, 3 ∿ 5 half-finished products (mash) are purchased as materials and mixed finally into diet at the NQCL.

By using this system, it is possible to prevent workers engaged in diet production to the largest possible extent from committing errors in formulation to use the space for storage of these materials efficiently, and to carry out a thorough management of what is in storage.

By the way, it is necessary for the NQCL to be equipped with an ultra cutting mill to grind hay for the production of diet for guinea pigs and rabbits.

A final decision should be made on the manufacturing system to be adopted when a training in diet production takes place in April, 1985.

2) Economic character

No economic character of a product can be judged without taking the objective or characteristics of the product into consideration. At present, the price of the diet produced by the tentative formulation is calculated within an extent of information available.

The price of the raw materials mixed in the first formulation to produce diet for mice and rats was Rp 224 per kilogram, and that to produce diet for guinea pigs and rabbits Rp 158 per kilogram. The price of the raw materials mixed in the second formulation to produce diet for mice and rats and diet for guinea pigs and rabbits may have been Rp 230 and Rp 160 per kilogram, respectively. The price of pre-mixes is not included in either diet.

Then, the price of diet on sale in Indonesia was examined. Concentrate TU and SU of the EKA Poultry Industrial Enterprise cost Rp 325 and Rp 350 per kilogram, respectively. Charon Phoc Phand's pig starter used on the Bio Farma costs Rp 362 per kilogram.

The raw materials used at the NQCL, however, are for laboratory animals. If importance is attached to the reproducibility of the test, raw materials will be selected seriously and a rise in cost unavoidable.

# Chapter IV Recommendation on Raising and Breeding of Laboratory Animals at the National Drug and Food Quality Control Laboratory in Indonesia

## 4.1 Raising and breeding of mice and rats

It is recommended for raising and breeding of mice and rats to prepare a manual completely for management of animals by closed colonies and to collect basic data on these animals. These data include growth curve, drinking water intake, food consumption, amount of urine voided, hematological data, rate of pregnancy, litter size and weaning rate. For details refer to technical books.

For microbial control it seems necessary to collect basic information on diseases of laboratory animals in Indonesia. Especially, it is recommended to start a survey on diseases related to SPF conditions. It is essential to pay attention to zoonoses.

In brief, it is indispensable for raising and breeding of laboratory animals to carry out microbial, environmental, genetical and dietetic controls. Mice and rats must always be subjected to these controls.

### 4.2 Raising and breeding of guinea pigs and rabbits

It is desirable to use guinea pigs and rabbits produced in Indonesia.

Since breeding is particularly difficult in guinea pigs, these animals should be raised by floor feeding in the same manner as in the L.P.P.H. Otherwise, a large and broad cage should be used for them. Not only pelleted diet but also fresh vegetable is required for breeding guinea pigs.

It is the best step for rabbit breeding to begin with the formation of a closed colony of rabbits small in body size belonging to a breed raised in the eastern part of the Java Island.

### 4.3 Closing words

It is the purpose of this report to present a formulation of raw materials for the production of diet for laboratory animals which are raised and bred at the National Drug and Food Quality Control Laboratory in Indonesia. To attain this purpose, a team of diet experts was sent from Japan to Indonesia to conduct a survey. Before and after the survey analysis was performed on raw materials and finished products of diet. As a result, the present report could be prepared.

In preparing the report a helping hand was extended by the following persons: Dr. Akira Takanaka, Head of Pharmacological Division, National Institute of Hygienic Science; Mr. Tanaka, section chief of the same institute; Mr. Honma, research scientist of Nippon Pellet Feed Co., Ltd.; Mr. Ishibashi, chief of Division of Test and Assay; Mr. Dobashi, section chief, and Mr. Kato, unit chief of the Tokyo Microscopical Research Institute, Inc. The team wish to express their deep appreciation to those who supported their activity.

# A P P E N D I C E S

Appendix 1 - (1)

PT. OTSUKA INDONESIA
JL. SUMBER WARAS 25
LAWANG - INDONESIA.

# LOCAL MADE RABBIT FOOD IN INDONESIA

## PREFACE

Since 1975 PT. Otsuka Indonesia has been using rabbits for pyrogen analysis. Rabbits used are, between 1,5 - 2,7 Kg of weight , originated from Batu area, around 25 Km from Malang.

Due to the difficulties in obtaining suitable qualified rabbit food from Japan ( Type RC-4 , Oriental Yeast Co. Ltd. ), investigations have been conducted for ,

1. rabbit breeding
2. suitable local made rabbit food .

## RESULTS OF INVESTIGATIONS MADE

1. Fresh vegetables consisting of "kangkung" ( Ipomoea sp. ) and carrot ( Daucus carrota ).
   Result :-well accepted by rabbits
   - dirty cages
   - less healthy rabbits , eg. too slow increase of bddy weight .

2. Self made rabbit food (pellet-form) consisting of :
   - corn powder 30 %
   - soy bean 20 %
   - coconut powder 15 %
   - finely ground rice 10 %
   - green pea 10 %
   - fish powder 15 %

   Result : - well accepted by rabbits
   - less healthy rabbits, eg. too slow increase of weight
   - not practical to produce because of facility and capacity .

   Data : refer to no. 5.

Appendix 1 - (2)

3. Rabbit food Konsentrat TU (pellet-form) , product of EKA Poultry Industrial Enterprise ,

- protein 16 %
- coarse fibre 10 %
- fat 2 %
- vitamine
- mineral

Result : - well accepted by rabbits
- increase of body weight similar to RC-4 type
- healthy rabbits
- price Rp. 325 ,- per Kg ( RC-4 : Rp. 1870 ,- per Kg )

Data : refer to no. 5

4. For the purpose of breeding :

from pregnancy until child birth and breast feeding given rabbit food type Konsentrat TU.

Result : - less healthy mother and child , increase of body - weight and abnormal of body-hair growth .
( It is about 10 - 15 % )

Correction by giving the Konsentrat SU type or RC-4 type could normalize the condition .

Further investigations are being conducted ti give the Konsen - trat BU type especially for expectant females , and the Konsen - trat SU type for breast feeding .

Note : - SU type well accepted by rabbit
- price : Rp. 350 ,- per Kg

5. Data :

5.1. Data of 5 rabbits given self made rabbit food (see no.2)

| body weight (g) after tho...day | No.1 | No.2 | No.3 | No.4 | No.5 |
|---|---|---|---|---|---|
| 0 | 1040 | 1290 | 1320 | 1200 | 1320 |
| 1 | 1060 | 1320 | 1300 | 1200 | 1320 |
| 2 | 1120 | 1290 | 1300 | 1190 | 1320 |
| 3 | 1170 | 1350 | 1310 | 1240 | 1310 |
| 4 | 1100 | 1310 | 1310 | 1210 | 1330 |
| 5 | 1100 | 1240 | 1320 | 1220 | 1350 |
| 7 | 1100 | 1250 | 1330 | 1210 | 1340 |
| 8 | 1120 | 1300 | 1360 | 1260 | 1380 |
| 9 | 1170 | 1300 | 1360 | 1260 | 1410 |
| 10 | 1170 | 1360 | 1340 | 1260 | 1410 |

Appendix 1 - (3)

| body weight (g) after the...day | No.1 | No.2 | No.3 | No.4 | No.5 |
|---|---|---|---|---|---|
| 11 | 1190 | 1390 | 1380 | 1220 | 1460 |
| 12 | 1230 | 1340 | 1370 | 1220 | 1400 |
| 14 | 1220 | 1430 | 1370 | 1300 | 1430 |
| total | 180 g | 140 g | 50 g | 100 g | 110 g |

5.2. Data of 6 rabbits given Konsentrat TU (see no.3)

| body weight (g) after the...day | No.1 | No.2 | No.3 | No.4 | No.5 | No.6 |
|---|---|---|---|---|---|---|
| 0 | 1200 | 1120 | 1000 | 1210 | 990 | 890 |
| 1 | 1220 | 1000 | 940 | 1210 | 990 | 920 |
| 2 | 1280 | 1180 | 1060 | 1210 | 920 | 940 |
| 3 | 1320 | 1190 | 1070 | 1260 | 1010 | 960 |
| 4 | 1300 | 1220 | 1100 | 1240 | 1070 | 990 |
| 5 | 1340 | 1250 | 1120 | 1240 | 1070 | 1010 |
| 7 | 1380 | 1290 | 1150 | 1210 | 960 | 1070 |
| 8 | 1380 | 1320 | 1140 | 1240 | 1030 | 1100 |
| 9 | 1420 | 1350 | 1170 | 1160 | 1040 | 1150 |
| 10 | 1450 | 1350 | 1200 | 1240 | 1030 | 1120 |
| 11 | 1460 | 1360 | 1200 | 1280 | 1070 | 1150 |
| 12 | 1520 | 1390 | 1250 | 1320 | 1080 | 1180 |
| 14 | 1540 | 1400 | 1270 | 1320 | 1120 | 1240 |
| total | 340 g | 280 g | 330 g | 110 g | 130 g | 350 g |

5.3. Data of 5 rabbits given RC-4 .

| body weight (g) after the...day | No.1 | No.2 | No.3 | No.4 | No.5 |
|---|---|---|---|---|---|
| 0 | 1380 | 1340 | 1320 | 1280 | 1400 |
| 1 | 1430 | 1360 | 1300 | 1380 | 1450 |
| 2 | 1440 | 1320 | 1280 | 1290 | 1470 |
| 3 | 1460 | 1510 | 1300 | 1300 | 1470 |
| 4 | 1430 | 1370 | 1310 | 1280 | 1440 |
| 5 | 1400 | 1350 | 1400 | 1390 | 1510 |
| 7 | 1450 | 1380 | 1370 | 1320 | 1560 |
| 8 | 1450 | 1440 | 1380 | 1350 | 1540 |
| 9 | 1500 | 1400 | 1410 | 1370 | 1490 |
| 10 | 1200 | 1400 | 1420 | 1360 | 1590 |

Appendix 1 - (4)

| body weight (g) after the...day | No.1 | No.2 | No.3 | No.4 | No.5 |
|---|---|---|---|---|---|
| 11 | 1520 | 1520 | 1460 | 1410 | 1600 |
| 12 | 1520 | 1490 | 1500 | 1380 | 1600 |
| 14 | 1560 | 1550 | 1460 | 1430 | 1640 |
| total | 130 g | 310 g | 260 g | 150 g | 240 g |

5.4. Summary data of increasing body weight after 14 days .

| No. | Konsentrat TU | Self made | RC - 4 |
|---|---|---|---|
| 1 | 340 mg | 180 mg | 180 mg |
| 2 | 280 mg | 140 mg | 310 mg |
| 3 | 330 mg | 50 mg | 260 mg |
| 4 | 110 mg | 100 mg | 150 mg |
| 5 | 130 mg | 110 mg | 240 mg |
| 6 | 350 mg | | |
| average | 256,7 g | 116 g | 228 g |

*CONCLUSION*

1. Eventhough further investigations are still needed, there is a bright future to replace the rabbit food imported from Japan, type RC-4 Oriental Yeast Co. Ltd. by the local made products rabbit food type Konsentrat TU , BU , SU from EKA Poultry Industrial Enterprise, Jl. Imam Bonjol 138 B Semarang.
2. Breeding is made possible especially because of the low cost rabbit food .

---oOo---

NOTE :

* Now we are doing the experimentation of Konsentrat SU for breeding.

Lawang , 10.05.1984

Drs. Hendro Santoso

manager

Appendix 2

§58.90 Animal Care.

(a) There shall be standard operating procedures for the housing, feeding, handling, and care of animals.

(b) All newly received animals from outside sources shall be placed in quarantine until their health status has been evaluated. This evaluation shall be in accordance with acceptable veterinary medical practice.

(c) At the initiation of a nonclinical laboratory study, animals shall be free of any disease or condition that might interfere with the purpose or conduct of the study. If, during the course of the study, the animals contract such a disease or condition, the diseased animals shall be isolated. If necessary, these animals may be treated for disease or signs of disease provided that such treatment does not interfere with the study. The diagnosis, authorizations of treatment, description of treatment and each date of treatment shall be documented and shall be retained.

(d) Warm-blooded animals, excluding suckling rodents, used in laboratory procedures that require manipulations and observations over an extended period of time or in studies that require the animals to be removed from and returned to their home cages for any reason (e.g., cage cleaning, treatment, etc.), shall receive appropriate identification (e.g., tattoo, toe clip, color code, ear tag, ear punch, etc.). All information needed to specifically identify each animal within an animal-housing unit shall appear on the outside of that unit.

(e) Animals of different species shall be housed in separate rooms when necessary. Animals of the same species, but used in different studies, should not ordinarily be housed in the same room when inadvertent exposure to control or test articles or animal mixup could affect the outcome of either study. If such mixed housing is necessary, adequate differentiation by space and identification shall be made.

(f) Animal cages, racks and accessory equipment shall be cleaned and sanitized at appropriate intervals.

(g) Feed and water used for the animals shall be analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed or water are not present at levels above those specified in the protocol. Documentation of such analyses shall be maintained as raw data.

(h) Bedding used in animal cages or pens shall not interfere with the purpose or conduct of the study and shall be changed as often as necessary to keep the animals dry and clean.

(i) If any pest control materials are used, the use shall be documented. Cleaning and pest control materials that interfere with the study shall not be used.

Appendix 3

## Maximum Allowable Concentrations of Toxic Feed Contaminants

| CONTAMINANT | UNIT | USEPA | NCTR | MIN. REQ. |
|---|---|---|---|---|
| Cadmium | mcg/g | 0.16 | 0.05 | - |
| Lead | mcg/g | 1.5 | 1.00 | - |
| Arsenic | mcg/g | 1.0 | 0.25 | - |
| Mercury | mcg/g | 0.1 | 0.05 | - |
| Selenium | mcg/g | 0.6 | 0.5 | 0.1 |
| PCBs | ng/g | 50 | 50 | - |
| DDTs | ng/g | 100 | 50 | - |
| Malathion | mcg/g | 2.5 | 0.5 | - |
| Lindane | ng/g | 20 | 10 | - |
| Dieldrin | ng/g | 20 | 10 | - |
| Aflatoxins (Total) | ng/g | 5 | 1.0 | - |
| Estrogenic Activity (DES Equiv.) | ng/g | 1 | 2 | - |

Appendix 4

# Present State of Agricultural Chemicals in Indonesia

(a) List of agricultural chemicals for farming authorized by the Indonesian Ministry of Commerce for importation as of May 8, 1970

List No.: Directorate Impor 2306/DIN/70

| Organic chlorine preparation | Organic phosphorus preparation | Miscellaneous |
|---|---|---|
| Dieldrin | Malathion | Walfatox |
| Aldrin | Parathion (E) | Copper Sulphate |
| DDT | Parathion (M) | Zinc Phosphate |
| BHC | Sumithion | |
| | Dipterex | |
| | Diazinon | |
| | Dimecron | |

(b) Stock of agricultural chemicals of the Public Corporation of Agricultural Material Distribution in 1970

List No.: Stc. No. 182/v/92/70

| Name of agricultural chemical | Amount of stock kℓ or ton | |
|---|---|---|
| | Jan., 1970 | May, 1970 |
| Endrin (19.5% EC) | 872. kℓ | 810. kℓ |
| BHC (6% G) | 1,175. ton | 1,082. ton |
| Diazion (60 EC) | 668. kℓ | 770. kℓ |
| Sumithion (50 EC) | 123. kℓ | 112. kℓ |
| Malathion (50 EC) | 30. kℓ | 29. kℓ |
| Thiodan (50 EC) | 353. kℓ | 353. kℓ |
| Dipterex (85 WS) | 36. kℓ | 36. kℓ |
| (E) Parathion (50 EC) | 8. kℓ | 7. kℓ |
| Zinc Phosphate | 163. ton | 158. ton |

(c) Example of agricultural investment in the Bimas Project (agricultural chemicals in rice farming)

Western Java Province in the dry season of 1969

| Item | Amount required /Ha | Unit cost Rp/kg or lt. | Expense required* Rp/Ha |
|---|---|---|---|
| Urea | 100 kg | 35 | 3,500 |
| T. S. P. | 50 kg | 30 | 1,500 |
| Sumithion | 4.2 ℓ | 610 | 2,560 |
| Zinc Phosphate | 0.1 kg | 500 | 50 |
| Sprayer (hand) | One machine /15 Ha | - | 245 |
| Seed (PB-5) | 30 kg | 40 | 1,200 |
| Extension fee | - | - | 600 |
| Total | | | 9,657/Ha |

* Projected increase in yield: 3.5 tons by wet padi. Price of rice at the time of increase in yield by about 1.0 ton: 12∿15 Rp ≒ Yen/kg wet padi.
Remarks: Rp ≒ Yen.

(d) Example of agricultural investment of cabbage-rearing farmer (agricultural chemicals in field crop rearing)

Northern Sumatra Province in the rainy season of 1970

| Item | Expense required Rp/Ha |
|---|---|
| Seed (From Takii) | 7,200 |
| Fertilizer (Urea Tsp) | 30,000 |
| Insecticide (12 times/season) | 37,000 |
| Fungicide (5 times/season) | 18,500 |
| Labour charge (Sprayers) | 62,500 |
| Total | 155,200 Rp/Ha |

Remarks: Export price of cabbage 7 Rp/kg
Production 25 ton/Ha/season
Cost: ca. 6.2 Rp/kg
7 - 6.2 = 0.8 Rp/kg
ca. 20,000 Rp/Ha/season ≒ 4 month.

## Appendix 5

$LC_{50}$ values of 5 insecticides to 16 colonies of housefly larvae in Indonesia (ppm).

| Colony name | Diazinon | Sumithion | Malathion | DDT(tech.) | γ-BHC |
|---|---|---|---|---|---|
| No. 1. Java | 30.12 | 13.77 | 23.92 | 95.24 | 23.36 |
| No. 2. Java | 137.74 | 44.25 | 36.22 | 233.64 | 18.59 |
| No. 3. Java | 34.48 | 30.67 | 29.41 | 92.93 | 23.55 |
| No. 4. Java | 194.55 | 176.68 | 61.50 | 239.23 | 22.83 |
| No. 5. Java | 12.82 | 9.29 | 12.85 | 38.76 | 24.51 |
| No. 6. Sumatra | 26.74 | 10.92 | 11.18 | 53.53 | 6.44 |
| No. 7. Sumatra | 29.41 | 6.74 | 16.18 | 52.36 | 97.47 |
| No. 8. Sumatra | 18.11 | 5.47 | 14.41 | 274.73 | 244.41 |
| No. 9. Celebes | 60.09 | 154.32 | 25.64 | 190.11 | 12.25 |
| No. 10. Celebes | 26.18 | 7.39 | 17.73 | 480.76 | 190.66 |
| No. 11. Ambon | 17.30 | 3.78 | 10.20 | 369.71 | 46.73 |
| No. 12. Celebes | 21.09 | 5.35 | 14.41 | 190.11 | 3.88 |
| No. 13. Flores | 161.81 | 0.37 | 90.91 | 92.94 | 6.01 |
| No. 14. Bali | 44.64 | 8.68 | 19.01 | 165.56 | 19.01 |
| No. 15. Timor | 0.14 | 0.30 | 2.83 | 378.78 | 38.61 |
| No. 16. Lombok | 3.79 | 0.67 | 9.52 | 322.58 | 22.83 |
| Takatsuki | 1.15 | 1.59 | 4.76 | 362.31 | 82.91 |

Source: Insect Control Science, Japan, Vol. 39, IV

## Appendix 6

$LD_{50}$ values for 9 insecticides of the adult female house flies in Indonesia (μg/Insect).

| Colony name (Collection site) | Allethrin | Pyrethrins | Sumithion | Diazinon | Malathion | DDVP | Baytex | γ-BHC | DDT |
|---|---|---|---|---|---|---|---|---|---|
| No. 1. Java (Bogor) | 0.423 | 0.110 | 0.224 | 0.171 | 0.710 | 0.060 | 0.133 | 0.312 | 1.397 |
| No. 2. Java (Jakarta) | 0.307 | 0.146 | 0.407 | 0.330 | 0.787 | 0.143 | 0.216 | 0.515 | 1.062 |
| No. 3. Java (Jakarta) | 0.285 | 0.182 | 0.560 | 0.259 | 0.220 | 0.126 | 0.151 | 0.327 | 1.542 |
| No. 4. Java (Jakarta) | 0.198 | 0.103 | 0.615 | 0.273 | 1.292 | 0.094 | 0.185 | 0.358 | 0.603 |
| No. 5. Java (Cirebon) | 0.497 | 0.213 | 0.146 | 0.175 | 0.467 | 0.033 | 0.110 | 1.611 | 3.752 |
| No. 6. Sumatra (Medan) | 0.154 | 0.188 | 0.072 | 0.126 | 0.318 | 0.021 | 0.043 | 0.330 | 1.449 |
| No. 7. Sumatra (Berastagi) | 0.369 | 0.150 | 0.178 | 0.204 | 0.393 | 0.039 | 0.089 | 0.509 | 4.249 |
| No. 8. Sumatra (Kabanjahe) | 0.543 | 0.191 | 0.435 | 0.309 | 0.698 | 0.066 | 0.174 | 0.906 | 2.932 |
| No. 9. Celebes (Ujung Pandang) | 0.534 | 0.188 | 0.069 | 0.075 | 1.854 | 0.032 | 0.051 | 0.394 | 0.703 |
| No. 10. Celebes (Ujung Pandang) | 0.487 | 0.269 | 0.246 | 0.235 | 0.582 | 0.076 | 0.128 | 1.404 | 7.251 |
| No. 11. Ambon (Ambon) | 0.070 | 0.280 | 0.054 | 0.089 | 0.217 | 0.011 | 0.034 | 0.153 | 2.683 |
| No. 12. Celebes (Manado) | 0.142 | 0.423 | 0.076 | 0.171 | 0.249 | 0.011 | 0.025 | 0.033 | 1.567 |
| No. 13. Flores (Maumere) | 0.160 | 0.071 | 0.024 | 0.019 | 0.269 | 0.007 | 0.018 | 0.006 | 0.433 |
| No. 14. Bali (Denpasar) | 0.247 | 0.095 | 0.058 | 0.128 | 0.222 | 0.029 | 0.041 | 0.116 | 0.406 |
| No. 15. Timor (Kupang) | 0.326 | 0.153 | 0.013 | 0.015 | 0.097 | 0.029 | 0.016 | 0.179 | 0.830 |
| No. 16. Lombok (Mataram) | 0.087 | 0.044 | 0.049 | 0.084 | 0.097 | 0.013 | 0.027 | 0.105 | 0.908 |
| Takatsuki | 0.481 | 0.387 | 0.088 | 0.293 | 0.454 | 0.076 | 0.135 | 4.547 | 46.900 |

Source: Insect Control Science, Japan, Vol. 39, III

Appendix 7 - (1)

## CURRENT PROBLEM ON HUMAN HAZARD, ENVIRONMENT & FOOD POLLUTION AND THE DEVELOPMENT OF PESTICIDES IN FUNGI & INSECTS

Indonesia is developing its agricultural sector in order to increase agricultural production and job opportunities, ensuring increase of the per capita income of the farmers.

Food production must be increased to 3.8 - 4 percent annually in order to be able to fill the need of food for the people, which is increasing at the rate of about 2.34 percent annually.

Foreign exchange earning from the plantation sub sector must be increased step by step, to replace petroleum export, to support industry and to increase societies income. All of these make us use new technology. For example: rice product. Before using a new technology the agricultural production was about 1.5 - 2 ton rice per hectare. Now by using new technology agricultural production reaches about 4 - 5 ton rice per hectare. This high production can be reached by energy input: rice seedling, fertilizer, mechanization, pesticides (insecticide, herbicide, fungicide), and frequency of planting (3 times annual, 5 x 2 annual).

The use of pesticides for Bimas (Massive Guidance) was about 1.26 l/kg/ha in 1979 and 1.34 l/kg/ha in 1980.

Pests

Pests include insects, pathogens and weeds which are injurious to plants. Pests multiply very fast, so may be harmful to man and cause great economic

loss by damaging or destroying agricultural crops and other valuable plants.

In Indonesia, Nilavarvata lugens is known to exist and multiply since 1972 until now.

In 1975 - 1979 losses to the rice reached 300.000 ton of the annual agricultural production.

Pest also can damage 19 - 24. percent of the annual agricultural production.

Pesticide

Pesticide is used for controlling various kind of pests. Pesticide has been used since the past century. People first used organic matter from plant such as pyrethrum, nicotine. Then, sulfur and copper sulfur mixed to quick lime.

The synthetic pesticide has been used since the second world war with the discovery of DDT [(C14 H9 C19) di chloro diphenyl trichloro ethane]. Since that time plenty of synthetic pesticides were found and hundred of formulations of pesticides were flooding the world markets.

Recently, there are 370 formulations of pesticides which have been licensed in Indonesia, 115 formulations by provisional license and 255 formulations by permanent license. These sixteen formulations are in just restricted use and need the permit from the Agriculture Minister or Chairman of Pesticide Committee if we use these pesticides.

Appendix 7 - (3)

To know the risk of using pesticide, the way of quality model of pesticide application is given below:

1. Pesticide sprayed in air will soon mix with air and will be affected by the sun.
2. Pesticide can decompose in air.
3. Pesticide can undergo percolation, carried by air current.
4. Post of the pesticide falls on plant.
5. Pesticide can stick and spread and cover surface of plant.
6. Pesticide can enter plant through mouth of leaf.
7. Pesticide can have a phytotoxic effect on plant.
8. Pesticide can change in body of plant.
9. Insect pests infesting the plant will be killed if they come in contact with the pesticide (contact poisons). If they bite the plant, they will be killed (stomach poisons).
10. Predators and parasites will be killed by pesticide too.
11. Insects with pollinizing activity, frog, snake and so on will be killed by pesticide too.
12. Pesticide will fall on soil and water. Here pesticide will have influence on water insect, soil arthropod, microbia and so on.
13. Pesticide degrades in water and soil.
14. Persistent pesticide (DDT, Aldrin, Dieldrin) will not undergo degradation in soil, but will communicate in soil.
15. In water, pesticide can result in the increase in size biologically (particularly persistent pesticide).
16. If granular formulation is used, they will enter the body of plant and evaporate around the plant, if they have systemic action. Granular is not destroy predator and parasite, but they will have negative influence to fauna and water.

Appendix 8 - (1)

# CERTIFICATE

Analitical result of feedstuffs and products
in Indonesia

5 Oct. '84
CLEA JAPAN INC.
YOJI ANDO D.V.M.

| Name | Concentrate BU | Concentrate TU | Realizable limit | Method |
|---|---|---|---|---|
| Subject | | | | |
| Moisture | 10.7 % | 11.6 % | | 135 ± 2° C 2hr |
| Crude Protein | 16.4 % | 16.8 % | | Kjeldahl |
| Crude Fat | 7.7 % | 8.7 % | | Soxhlet s extractor |
| Crude Ash | 12.2 % | 12.1 % | | 550° C 2hr |
| Crude Fiber | 3.8 % | 4.8 % | | AOAC |
| Total Mercury | non detect | non detect | 0.01 ppm | FL-AASP |
| Cadmium | 0.08 ppm | 0.11 ppm | | AASP |
| Lead | 0.5 ppm | 0.8 ppm | | AASP |
| Arsenic | 0.32 ppm | 0.40 ppm | | Colorimetry |
| Nitrite | non detect | non detect | 0.5 ppm | Colorimetry |
| D D T | non detect | non detect | 0.005 ppm | ECD-GC |
| B H C | non detect | non detect | 0.005 ppm | ECD-GC |
| Dildrin | non detect | non detect | 0.005 ppm | ECD-GC |
| Heptachlor | non detect | non detect | 0.005 ppm | ECD-GC |
| Malathion | non detect | non detect | 0.005 ppm | FPD-GC |
| Selenium | non detect | non detect | 0.1 ppm | Fluorescence |
| P C B | non detect | non detect | 0.005 ppm | ECD-GC |
| Aflatoxin | negative | negative | 5 ppb | TLC |
| Aldrin | non detect | non detect | 0.005 ppm | ECD-GC |
| Paration | non detect | non detect | 0.005 ppm | FPD-GC |
| Chromium | 3.8 ppm | 2.9 ppm | | AASP |
| Deoxynivalenol | non detect | non detect | 0.05 ppm | HPLC |
| Nivalenol | non detect | non detect | 0.05 ppm | HPLC |
| Ochratoxin | non detect | non detect | 0.05 ppm | HPLC |

ECD-GC: Electron Capture Detector - Gas Chromatography
FPD-GC: Flame Photometric Detector - Gas Chromatography
TEA-GC: Thermal Energy Analyzer - Gas Chromatography
HPLC: High Perfomance Liquid Chromatography
AASP: Atomic Absorption Spectrophotometer
FL-AASP: Flame Less - Atomic Absorption Spectro Photometer
TLC: Thin Layer Chromatography
RIA: Radio Immuno Assay
AOAC: Association of Official Analytical Chemists

| Name | Concentrate BU | Concentrate TU | Realizable limit | Method |
|---|---|---|---|---|
| Subject | | | | |
| Viable total count | $1.2 \times 10^4$ /g | $1.1 \times 10^4$ /g | | |
| Mould | 100 /g | 50 /g | | |
| Staphylococcus | negative | negative | 0.01 /g | Modified |
| Enterococcus | negative | negative | 0.01 /g | The Sanitaion |
| Pseudomonas aeruginosa | negative | negative | negative /3g | Low in Japan |
| Salmonella | negative | negative | negative /20g | |
| Coliform group | 230 MPN/100g | negative | negative /0.333g | |
| Antibacterials | negative | negative | negative /50g | Bio Assay |
| N-nitrosopyrrolidine | non detect | non detect | 1 ppb | TEA-GC |
| N-nitrosomorpholine | non detect | non detect | 1 ppb | TEA-GC |
| N-nitrosodimetlyamine | non detect | non detect | 1 ppb | TEA-GC |
| Estron | 1.0 ppb | 1.8 ppb | | RIA |
| Estriol | 0.9 ppb | 0.7 ppb | | RIA |
| Estradiol | 0.5 ppb | 0.9 ppb | | RIA |
| Acid Value | 144 | 148 | | Titrate |
| Peroxid Value | 93 meq/kg | 78 meq/kg | | Titrate |
| Retinol | 0.25 mg%(830IU/100g) | 0.22 mg%(730IU/100g) | | Colorimetry |
| Vitamin $B_1$ | 0.79 mg% | 0.86 mg% | | Fluorescence |
| Vitamin C | non detect | non detect | 3 mg% | Colorimetry |
| Calcium | 2533 mg% | 2544 mg% | | AASP |
| Phosphorus | 1172 mg% | 1340 mg% | | Colorimetry |
| Benzo(a)pyrene | non detect | non detect | 5 ppb | TLC |

| Name | Soybean meal | Corn | Rice bran | Realizable limit | Method |
|---|---|---|---|---|---|
| Subject | | | | | |
| Moisture | 10.7 % | 15.6 % | 13.6 % | | 135 ± 2° C 2hr |
| Crude Protein | 39.5 % | 7.9 % | 11.5 % | | Kjeldahl |
| Crude Fat | 2.4 % | 4.3 % | 17.2 % | | Soxhlet s extractor |
| Crude Ash | 7.8 % | 1.3 % | 9.2 % | | 550° C 2hr |
| Crude Fiber | 6.3 % | 1.4 % | 7.9 % | | AOAC |
| Total Mercury | non detect | non detect | non detect | 0.01 ppm | FL-AASP |
| Cadmium | 0.14 ppm | 0.15 ppm | 0.18 ppm | | AASP |
| Lead | 1.4 ppm | 0.7 ppm | 1.1 ppm | | AASP |
| Arsenic | 0.19 ppm | 0.08 ppm | 0.14 ppm | | Colorimetry |
| Nitrite | non detect | non detect | non detect | 0.5 ppm | Colorimetry |
| D D T | non detect | non detect | non detect | 0.005 ppm | ECD-GC |
| B H C | non detect | non detect | non detect | 0.005 ppm | ECD-GC |
| Dildrin | non detect | non detect | non detect | 0.005 ppm | ECD-GC |
| Heptachlor | non detect | non detect | non detect | 0.005 ppm | ECD-GC |
| Malathion | non detect | non detect | non detect | 0.005 ppm | FPD-GC |
| Selenium | non detect | non detect | non detect | 0.1 ppm | Fluorescence |
| P C B | non detect | non detect | non detect | 0.005 ppm | ECD-GC |
| Aflatoxin | negative | negative | negative | 5 ppb | TLC |
| Aldrin | non detect | non detect | non detect | 0.005 ppm | ECD-GC |
| Paration | non detect | non detect | non detect | 0.005 ppm | FPD-GC |
| Chromium | 0.8 ppm | 0.1 ppm | 1.0 ppm | | AASP |
| Deoxynivalenol | non detect | non detect | non detect | 0.05 ppm | HPLC |
| Nivalenol | non detect | non detect | non detect | 0.05 ppm | HPLC |
| Ochratoxin | non detect | non detect | non detect | 0.05 ppm | HPLC |
| Viable total count | $2.7 \times 10^4$/g | $8.3 \times 10^5$/g | $2.8 \times 10^6$/g | | ┐ |
| Mould | 720 /g | 36000 /g | 16000 /g | | │ |
| Staphylococcus | negative | negative | negative | 0.01 /g | │ Modified |
| Enterococcus | negative | negative | negative | 0.01 /g | │ The Sanitaion |
| Pseudomonas aeruginosa | negative | negative | negative | negative /3g | │ Low in Japan |
| Salmonella | negative | negative | negative | negative /20g | │ |
| Coliform group | 230 MPN/100g | 110000 MPN/100g | 110000 MPN/100g | | ┘ |
| Antibacterials | negative | negative | negative | negative /50g | Bio Assay |
| Acid Value | - | - | 142 | | Titrate |
| Peroxid Value | - | - | 60 meq/kg | | Titrate |

| Name | Fish meal | Skim milk | Realizable limit | Method |
|---|---|---|---|---|
| Subject | | | | |
| Moisture | 10.2 % | 11.1 % | | 135 ± 2° C 2hr |
| Crude Protein | 62.1 % | 34.0 % | | Kjeldahl |
| Crude Fat | 7.2 % | 0.2 % | | Soxhlet's extractor |
| Crude Ash | 16.9 % | 7.9 % | | 550° C 2hr |
| Crude Fiber | non detect | non detect | 0.1 % | AOAC |
| Vieble total count | $2.2 \times 10^3$/g | - | | |
| Mould | 150 /g | - | | Modified |
| Staphylococcus | negative | - | 0.01 /g | The Sanitaion |
| Enterococcus | negative | - | 0.01 /g | Low in Japan |
| Pseudomonas aeruginosa | negative | - | negative /3g | |
| Salmonella | negative | - | negative /20g | |
| Coliform group | 430 MPN/100g | - | | |
| Antibacterials | negative | - | negative /50g | Bio Assay |
| Calcium | 4502 mg% | - | | AASP |
| Phosphorus | 2960 mg% | - | | Colorimetry |
| Acid Value | 52 | - | | Titrate |
| Peroxid Value | 135 meq/kg | - | | Titrate |
| Total Mercury | non detect | - | 0.01 ppm | FL-AASP |
| Cadmium | 0.93 ppm | - | | AASP |
| Lead | 1.3 ppm | - | | AASP |

Appendix 9

Table II

| CONTAMINANT | AUTHOR, OR REFERENCE | LIMIT OF DETECTION |
|---|---|---|
| Arsenic | AOAC 1975 | .05 mcg/g |
| Cadmium | AOAC 1975 | .05mcg/g |
| Manganese | AOAC 1975 | .11 mcg/g |
| Lead | AOAC 1975 | .05 mcg/g |
| Selenium | Gutteman and Lisk 1961 | .05 mcg/g |
| Mercury | Hatch and Ott 1968 | .05 mcg/g |
| All Others by ICP | | |
| Aflatoxin ($B_1$,$B_2$,$G_1$,$G_2$) | Pons et al., 1975 | 2.00 ng/g |
| Organophosphorus & Chlorinated Insecticides | AOAC 1975 | 5.00 ng/g |
| Polychlorinated Biphenyls | AOAC 1975 | 10.00 ng/g |
| Chlortetracycline | AOAC 1975 | .55 mcg/g |
| Oxytetracycline | AOAC 1975 | 1.21 mcg/g |
| Penicillin | AOAC 1975 | 2.20 mcg/g |
| Streptomycin | AOAC 1975 | 2.80 mcg/g |
| Estrogenic Activity (DES Equiv.) | NCTR 1973 | 4.00 ng/g |

## Analytical Values of Feed in Indonesia

| Raw materials of animal diet in Indonesia (average value) | | | | | | Japanese standard animal diet components (1975 edition) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Component / Raw material | % Moist | % C.Pro | % C.Fat | % C.Fib | % C.Ash | Component / Raw material | % Moist | % C.Pro | % C.Fat | % C.Fib | % C.Ash |
| Maize | 12.0 | 9.4 | 4.0 | 2.3 | 1.4 | Maize | 13.5 | 9.0 | 4.0 | 2.0 | 1.4 |
| Milo | 13.9 | 9.1 | 2.8 | 2.9 | 1.6 | Milo | 12.9 | 9.5 | 3.1 | 2.0 | 1.7 |
| Coconut meal | 9.9 | 19.7 | 10.1 | 10.5 | 6.4 | Coconut meal (pressed) | 10.8 | 20.9 | 8.5 | 9.7 | 6.9 |
| Soybean meal (Brazil) | 11.3 | 43.6 | 1.5 | 5.8 | 6.4 | Soybean meal (extracted) | 11.9 | 46.3 | 1.3 | 5.0 | 6.0 |
| Soybean meal (US) | 12.4 | 49.7 | 0.8 | 4.2 | 5.9 | | | | | | |
| Soybean meal (domestic) | 10.6 | 34.9 | 16.2 | 7.1 | 7.1 | | | | | | |
| Rice bran | 12.2 | 13.3 | 12.9 | 8.5 | 10.3 | Rice bran (pressed) | 12.8 | 15.0 | 17.1 | 7.2 | 8.5 |
| Fish meal (A) | 13.5 | 47.8 | 4.7 | | 32.1 | | | | | | |
| Fish meal (B), domestic | 10.4 | 52.4 | 14.5 | | 18.6 | Fish meal (crude) | 8.7 | 50.5 | 12.0 | | 25.1 |
| Fish meal (C), imported | 10.3 | 59.8 | 3.7 | | 23.1 | Fish meal (Peru) | 9.2 | 64.3 | 7.6 | | 17.4 |
| Hay (papaya) | 13.8 | 22.3 | 4.5 | 8.4 | 15.2 | Alfalfa (Dehai) | 8.9 | 18.5 | 3.4 | 20.8 | 10.3 |
| Hay (peti cina) | 10.2 | 29.3 | 5.5 | 12.9 | 3.7 | | | | | | |

Appendix 11

(a) PARAMETRIC STATISTICS - TOXIC ELEMENTS

| ELEMENT (mcg/g) | | CAT | MONKEY | G. PIG | RODENT | RABBIT | DOG |
|---|---|---|---|---|---|---|---|
| PB | N | 41 | 38 | 35 | 40 | 17 | 11 |
| | X | 1.12 | .37 | .55 | .58 | .60 | 1.11 |
| | Median | .79 | .19 | .48 | .48 | .52 | .90 |
| | SD | .92 | .44 | .24 | .40 | .24 | .42 |
| | CV% | 82 | 118 | 42 | 69 | 40.7 | 37.9 |
| | Range | .2-5.8 | .06-2.47 | .26-1.34 | .26-2.59 | .33-1.4 | .6-1.87 |
| Cd | N | 41 | 38 | 35 | 40 | 17 | 11 |
| | X | .19 | .15 | .17 | .15 | .14 | .19 |
| | Median | .15 | .12 | .17 | .15 | .13 | .07 |
| | SD | .09 | .14 | .04 | .05 | .04 | .4 |
| | CV% | 47.9 | 94.4 | 21.0 | 30.5 | 29.7 | 200 |
| | Range | .09-.48 | .07-.96 | .10-25 | .09-.26 | .1-.29 | 0-1.3 |
| AS | N | 41 | 38 | 35 | 40 | 17 | 11 |
| | X | .36 | .09 | .18 | .39 | .13 | .05 |
| | Median | .33 | .10 | .17 | .38 | .15 | 0 |
| | SD | .15 | .08 | .08 | .09 | .11 | .07 |
| | CV% | 40.2 | 92.4 | 41.7 | 22.9 | 82.0 | 140.4 |
| | Range | .11-.72 | 0.0-.23 | 0.0-.40 | .17-.58 | 0-.39 | 0-1.17 |
| Se | N | 41 | 38 | 35 | 40 | 17 | 11 |
| | X | .38 | .21 | .35 | .42 | .43 | .37 |
| | Median | .31 | .12 | .26 | .36 | .34 | .18 |
| | SD | .18 | .23 | .18 | .16 | .22 | .32 |
| | CV% | 47.6 | 110.9 | 55.6 | 39.1 | 50.6 | 86.5 |
| | Range | .15-.96 | .04-1.05 | .11-.90 | .24-1.04 | .14-.85 | .05-.94 |

(b) PARAMETRIC STATISTICS - TOXIC MOLECULAR CONTAMINANTS

| TOXICANT (Unit) | | CAT | MONKEY | G. PIG | RODENT | RABBIT | DOG |
|---|---|---|---|---|---|---|---|
| Malathion (mcg/g) | | | | | | | |
| | N | 41 | 38 | 35 | 40 | 17 | 11 |
| | X | .08 | .09 | .17 | .11 | .09 | .05 |
| | Median | 0 | .04 | .1 | .11 | .06 | .01 |
| | SD | .16 | .10 | .23 | .07 | .77 | .06 |
| | CV% | 199.4 | 115.2 | 129.4 | 62.6 | 83 | 124.4 |
| | Range | 0-.96 | 0-.400 | 0-1.20 | 0-.36 | 0-.28 | 0-.18 |
| PCBs (ng/g) | | | | | | | |
| | N | 40 | 37 | 33 | 39 | 17 | 11 |
| | X | 6.75 | 2.51 | 4.48 | 19.61 | 2.23 | 0 |
| | Median | 0 | 0 | 0 | 0 | 0 | 0 |
| | SD | 14.73 | 7.31 | 8.19 | 33.59 | 5.06 | 0 |
| | CV% | 218.2 | 290.7 | 182.6 | 171.2 | 226.1 | - |
| | Range | 0-57 | 0-36 | 0-31 | 0-185 | 0-15 | 0-0 |
| DDTs (ng/g) | | | | | | | |
| | N | 40 | 37 | 34 | 39 | 17 | 11 |
| | X | 3.1 | .22 | 2.73 | 13.74 | .35 | 0 |
| | Median | 0 | 0 | 0 | 0 | 0 | 0 |
| | SD | 7.57 | 1.31 | 9.2 | 21.8 | 1.46 | 0 |
| | CV% | 244 | 608 | 336 | 158 | 412 | - |
| | Range | 0-26 | 0-8 | 0-47 | 0-84 | 0-6 | 0-0 |

## Results of Analyses of Commercial Diets

| Analyte | Annual average | | | | | Accumulated 5-Yr value | | |
|---|---|---|---|---|---|---|---|---|
| | 1974 (n=14) | 1975 (n=15) | 1976 (n=54) | 1977 (n=34) | 1978 (n=31) | Mean (n=148) | Maximum value | Minimum value |
| Lindane (ppm) | 0.002 | 0.006 | 0.002 | 0.001 | <0.001 | 0.002 | 0.04 | <0.001 |
| Heptachlor (ppm) | 0.003 | 0.004 | 0.001 | <0.001 | <0.001 | 0.001 | 0.012 | <0.001 |
| Malathion (ppm) | 0.11 | 0.38 | 0.62 | 0.1 | 0.1 | 0.33 | 2.4 | 0.005 |
| DDT (ppm) | 0.014 | 0.152 | 0.139 | 0.018 | 0.010 | 0.028 | 0.309 | <0.001 |
| PCB (ppm) | <0.001 | <0.001 | <0.001 | 0.02 | 0.017 | 0.009 | 0.055 | <0.001 |
| Dieldrin (ppm) | 0.005 | 0.012 | 0.001 | 0.001 | <0.001 | 0.002 | 0.026 | <0.001 |
| Cd (ppm) | 0.079 | 0.085 | 0.086 | 0.093 | 0.087 | 0.087 | 0.168 | 0.005 |
| As (ppm) | 0.038 | 0.01 | 0.38 | 0.32 | 0.01 | 0.25 | 0.92 | 0.01 |
| Pb (ppm) | 0.39 | 0.89 | 0.42 | 0.34 | 0.55 | 0.47 | 1.92 | 0.02 |
| Hg (ppm) | 0.036 | 0.037 | 0.023 | 0.019 | 0.02 | 0.024 | 0.16 | 0.007 |
| Vitamin A (IU/100g) | 3695 | 2768 | 4908 | 4031 | 3873 | 4160 | 43900 | 1150 |
| Vitamin $B_1$ (mg/100g) | 7.36 | 8.91 | 9.03 | 9.45 | 9.61 | 9.1 | 13.1 | 5.7 |
| Protein (%) | — | — | 24.4 | 23.8 | 24.5 | 24.2 | 44.4 | 21.7 |
| Fat (%) | — | — | 5.3 | 5.7 | 5.5 | 5.5 | 6.6 | 4.0 |

Note) Greenman, D. L. et al. NCTR. 1980 : Commercial Laboratory Animal Diets, Toxicant and Nutrient Variability: J. Toxicol. Environ. Health 6 : 235-246

Appendix 13 - (1)

TABLE 2 Found Contamination in Raw Materials - U.K. 1979

| Contaminant | | Cereals | Cereal Byproduct | Vegetable Proteins | Animal Proteins | Fish Meals | Grass Meals |
|---|---|---|---|---|---|---|---|
| $NaNO_2$ | ppm | ND-4 | ND-3 | ND-3 | ND-100 | ND-7 | ND-10 |
| $NaNO_3$ | " | ND-23 | ND-29 | ND-9 | ND-200 | 2-33 | ND-4000 |
| Pb | " | ND-1.00 | ND-1.00 | ND-4.00 | 2.0-30.0 | 1.0-10.0 | ND-10.0 |
| As | " | ND | ND | ND | ND-5.0 | 2.0-12.0 | ND-5.0 |
| Cd | " | ND | ND-0.2 | ND-0.3 | ND-4.0 | ND-1.2 | ND-1.0 |
| Hg | " | ND-0.08 | ND-0.06 | 0.01-0.03 | ND-0.02 | 0.10-0.25 | ND-0.05 |
| Se | " | 0.03-0.16 | 0.04-0.90 | 0.09-0.26 | 0.04-0.10 | 1.00-1.30 | ND-0.05 |
| Dieldrin | ppb | ND | ND-8 | ND-6 | | ND-23 | |
| DDT's | " | ND-94 | ND-70 | ND-50 | | ND-80 | |
| Lindane | " | ND-13 | 3-48 | 1-10 | | 2-10 | |
| Heptachlor | " | ND-30 | ND-10 | ND-4 | | ND-5 | |
| P.C.B's | " | ND | ND | ND | | ND | |
| Malathion | ppm | ND-36.0 | ND-1.0 | ND-0.01 | | ND | |
| Aflatoxins | ppb | ND-8.0 | ND | ND-60 | | ND | ND |
| T.V.C. | /g | $10^3$-$10^7$ | $10^3$-$10^6$ | $10^5$-$10^7$ | $10^4$-$10^6$ | $10^3$-$10^5$ | $10^4$-$10^7$ |
| Meso.Spores | " | $10^2$-$10^5$ | $10^2$-$10^4$ | $10^4$-$10^6$ | $10^3$-$10^5$ | $10^2$-$10^4$ | $10^4$-$10^6$ |
| Coliforms | " | Rare | Rare | Rare | Frequent | Frequent | Frequent |
| E.Coli 1 | " | " | " | " | " | " | " |
| Salmonellae | " | ND | ND | ND | Rare | Rare | Rare |
| Fung.Spores | " | $10^2$-$10^4$ | $10^2$-$10^4$ | $10^3$-$10^4$ | ND-$10^4$ | ND-$10^4$ | $10^2$-$10^3$ |
| Antibiotics | " | ND | ND | ND | ND | ND | ND |

notes:- Rare - found in not more than 10% of samples tested.
Frequent - found in more than 20% of samples tested.
E.Coli 1 - confirmed in about 10% of samples in which Coliforms were found.

*The majority of micro-contaminants found in diets directly reflect the* levels of those contaminants in the raw materials. In considering attainable quality in diets it is necessary to have some idea of desirable quality and Table 3 illustrates various published values - there is considerable divergence of opinion.

Appendix 13 - (2)

TABLE 4 Found Contamination in Diets

| Contaminant | | | Mean | Range |
|---|---|---|---|---|
| Pb | (+Meat & Bone Meal) | ppm | 2.9 | 0.5 - 6.5 |
| " | (- " " " " ) | " | 1.3 | 0.5 - 4.0 |
| Cd | (+ " " " " ) | " | 0.65 | 0.2 - 1.1 |
| " | (- " " " " ) | " | 0.35 | 0.05 - 0.9 |
| $NaNO_3$ | (+Grass Meal) | " | 440.0 | 1.0 -900.0 |
| " | (- " " ) | " | 15.8 | 1.0 - 70.0 |
| $NaNO_2$ | | " | 1.8 | 1.0 - 7.0 |
| F | (+Meat & Bone + Phosphate) | " | 43.4 | 11.0 - 70.0 |
| " | (+Phosphate only) | " | 18.3 | 10.0 - 35.0 |
| " | (Low Phosphate only) | " | 5.1 | 1.0 - 11.0 |
| Hg | | " | 0.02 | 0.01 - 0.12 |
| As | | " | 0.3 | 0.20 - 1.10 |
| Se | | " | 0.19 | 0.02 - 0.66 |
| Lindane | | 1978 | " 0.017 | 0.001- 0.170 |
| (overall Mean 0.013 ppm) | | 1979 | " 0.023 | 0.001- 0.300 |
| | | 1980 | " 0.006 | 0.001- 0.035 |
| | | 1981 | " 0.004 | 0.001- 0.011 |
| Heptachlor | | 1978 | " 0.001 | 0.001- 0.011 |
| (overall Mean 0.004 ppm) | | 1979 | " 0.008 | 0.001- 0.065 |
| | | 1980 | " 0.003 | 0.001- 0.010 |
| | | 1981 | " 0.001 | 0.001- 0.002 |
| Dieldrin | | 1978 | " 0.003 | 0.001- 0.120 |
| (overall Mean 0.003 ppm) | | 1979 | " 0.006 | 0.001- 0.035 |
| | | 1980 | " 0.002 | 0.001- 0.014 |
| | | 1981 | " 0.001 | 0.001- 0.002 |
| DDT's | | 1978 | " 0.033 | 0.001- 0.250 |
| (overall Mean 0.023 ppm) | | 1979 | " 0.039 | 0.001- 0.165 |
| | | 1980 | " 0.011 | 0.001- 0.070 |
| | | 1981 | " 0.008 | 0.001- 0.018 |
| Malathion | | 1978 | " 0.190 | 0.02 - 1.00 |
| (overall Mean 0.148 ppm) | | 1979 | " 0.330 | 0.02 - 4.80 |
| | | 1980 | " 0.030 | 0.02 - 0.09 |
| | | 1981 | " 0.025 | 0.02 - 0.06 |
| Total Viable Organisms | | | /g 7300 | 1000 - 75000 |
| Mesophilic Spores | | | " 3400 | 100 -100000 |
| Fungal Spores | | | " 83 | ND - 1100 |

Cadmium and Lead are quite specifically related to formulation, significantly higher levels being found in diets containing Meat and Bone Meal. Lead levels are 3x higher and Cadmium levels are 2x higher than those levels found in diets not containing this ingredient. With respect to certain published permitted maxima, there is no way in which diets containing Meat and Bone Meals could be made in the U.K. to comply regularly with a Lead limit of 1.5 ppm, and no diet at all would regularly comply with a Cadmium limit of 0.25 ppm.

Certain other contaminants also show specific raw material relationships - Nitrate levels are very high in diets containing Grass Meal and Fluoride is related both to Meat and Bone Meal and to Mineral Phosphates. For the majority of these contaminants there are no real problems in achieving levels similar to, or rather less than, published maxima.

Appendix 14 - (1)

# Plan of the Laboratory Animal Feed Association for Steps to Be Taken to Meet the GLP

Kenichi Nakagawa

Chairman of
Japan Animal Feed Association

Table 1. Values of Contaminants in Various Areas

| | | | E P A | Purina | Wayne | Agway | Feed association (draft) |
|---|---|---|---|---|---|---|---|
| Aflatoxin | | (ppb) | 5 | 10 | 10 | 5 | 10 |
| Extrogen activity | | (ppb) | 1 | | | | |
| Cadmium | | (ppm) | 0.16 | 0.5 | 0.5 | 0.2 | 0.5 |
| Arsenic | | (ppm) | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Lead | | (ppm) | 1.5 | 1.5 | 1.5 | 1.5 | 1.5* |
| Mercury | | (ppm) | 0.1 | 0.2 | 0.2 | 0.1 | 0.2 |
| Selenium | | (ppm) | 0.1∿0.6 | | | | |
| P C B | | (ppm) | 0.05 | 0.15 | 0.15 | 0.05 | 0.15 |
| Nitrosamine | | (ppb) | 10 | | | | |
| Organic chlorine group | B H C | (ppm) | 0.02 | 0.05 | 0.05 | 0.05 | 0.05 |
| | Heptachlorine | (ppm) | 0.02 | 0.05 | 0.03 | 0.03 | 0.05 |
| | Heptachlorine epoxide | (ppm) | | 0.05 | 0.03 | 0.03 | |
| | DDT (including related substances | (ppm) | 0.1 | 0.15 | 0.15 | 0.1 | 0.15 |
| | Dieldrin | (ppm) | 0.02 | 0.05 | 0.03 | 0.03 | 0.05 |
| | Aldrin | (ppm) | | 0.05 | 0.03 | 0.03 | 0.05 |
| | Endrin | (ppm) | | 0.05 | 0.03 | 0.03 | |
| | Chlordane | (ppm) | | 0.05 | 0.05 | 0.05 | |
| | Toxaphane | (ppm) | | | | 0.2 | |
| Organic phosphorus group | Malathion | (ppm) | 2.5 | 0.5 | 0.5 | 0.1 | 2.5 |
| | Timet | (ppm) | | 0.5 | 0.5 | 0.2 | |
| | Diazinon | (ppm) | | 0.5 | 0.5 | 0.3 | |
| | Desulfaton | (ppm) | | 0.5 | 0.5 | 0.3 | |
| | Methyl parathion | (ppm) | | 0.5 | 0.5 | 0.3 | |
| | Parathion | (ppm) | | 0.5 | 0.5 | 0.3 | 0.5 |
| | Thiodan | (ppm) | | 0.5 | 0.5 | 0.3 | |
| | Ethion | (ppm) | | 0.5 | 0.5 | 0.3 | |
| | Trithion | (ppm) | | 0.5 | 0.5 | 0.3 | |

* 3.0 ppm for dog use.

Reference: Trial proposal quality control of experimental animal feed.

Table 2. Maximum Values of Contaminants in Products of Various Companies in the Past 3 Years

| | Limit of detection (ppm) | Diet for mice and rats | | Diet for guinea pigs and rabbits | | Diet for dogs | |
|---|---|---|---|---|---|---|---|
| | | No. of samples analyzed | Maximum value (ppm) | No. of samples analyzed | Maximum value (ppm) | No. of samples analyzed | Maximum value (ppm) |
| Aflatoxin | 0.005 | 144 | ND | 62 | ND | 32 | ND |
| Cadmium | 0.01 | 252 | 1.18 | 200 | 0.46 | 72 | 0.24 |
| Arsenic | 0.1 | 251 | 0.7 | 204 | 0.5 | 72 | 0.6 |
| Lead | 0.05 | 254 | 1.6 | 202 | 1.5 | 68 | 2.0 |
| Mercury | 0.01 | 251 | 0.05 | 197 | 0.07 | 70 | 0.03 |
| P C B | 0.01 | 143 | 0.04 | 89 | 0.03 | 36 | ND |
| B H C | 0.05 | 143 | ND | 62 | ND | 36 | ND |
| Heptachlorine | 0.01 | 140 | ND | 62 | ND | 36 | ND |
| DDT (including related substances) | 0.1 | 143 | ND | 62 | ND | 36 | ND |
| Dieldrin | 0.01 | 143 | ND | 62 | ND | 36 | ND |
| Aldrin | 0.01 | 128 | ND | 48 | ND | 28 | ND |
| Malathion | 0.05 | 125 | 1.61 | 59 | 2.10 | 32 | 0.77 |
| Parathion | 0.05 | 128 | ND | 48 | ND | 28 | ND |

ND: Not detected.

Table 3. Cumulative Results of Analytical Values of Contaminants in Products of Various Companies

| Item | Kind of diet | No. of samples analyzed | No. of samples with contaminants not detected | No. of samples used for statistics | Average value (ppm) | Maximum value (ppm) | Minimum value (ppm) |
|---|---|---|---|---|---|---|---|
| Lead | For mice and rats | 254 | 0 | 254 | 0.55 | 1.6 | 0.03 |
| | For guinea pigs and rabbits | 202 | 25 | 177 | 0.65 | 1.5 | 0.02 |
| | For dogs | 68 | 0 | 68 | 0.75 | 2.0 | 0.25 |
| Cadmium | For mice and rats | 252 | 0 | 252 | 0.12 | 1.18 | 0.01 |
| | For guinea pigs and rabbits | 200 | 21 | 179 | 0.14 | 0.46 | 0.02 |
| | For dogs | 72 | 0 | 72 | 0.11 | 0.24 | 0.01 |
| Chromium | For mice and rats | 147 | 4 | 143 | 1.13 | 5 | 0.09 |
| | For guinea pigs and rabbits | 57 | 5 | 52 | 2.04 | 8.54 | 0.11 |
| | For dogs | 29 | 0 | 29 | 1.70 | 4 | 0.55 |
| Mercury | For mice and rats | 251 | 99 | 152 | 0.011 | 0.05 | 0.005 |
| | For guinea pigs and rabbits | 197 | 101 | 96 | 0.013 | 0.07 | 0.005 |
| | For dogs | 70 | 47 | 23 | 0.012 | 0.03 | 0.005 |
| Arsenic | For mice and rats | 251 | 13 | 238 | 0.23 | 0.7 | 0.03 |
| | For guinea pigs and rabbits | 204 | 47 | 157 | 0.11 | 0.5 | 0.004 |
| | For dogs | 72 | 1 | 71 | 0.14 | 0.6 | 0.01 |
| Silver nitrite | For mice and rats | 115 | 34 | 81 | 0.61 | 1.3 | 0.3 |
| | For guinea pigs and rabbits | 41 | 18 | 23 | 0.54 | 1.0 | 0.3 |
| | For dogs | 19 | 9 | 10 | 0.61 | 1.5 | 0.3 |
| Mala-thion | For mice and rats | 125 | 13 | 112 | 0.55 | 1.61 | 0.05 |
| | For guinea pigs and rabbits | 59 | 9 | 50 | 0.66 | 2.10 | 0.03 |
| | For dogs | 32 | 6 | 26 | 0.20 | 0.77 | 0.04 |

Appendix 15

5 Sep. '84

HIPKI " MEKAR "
TERNAK PEMBIBITAN KELINCI
JENIS UNGGUL
JLN.DR.SETYA BUDHI KM.12,7
LEMBANG - BANDUNG.

AYUM SUWITA
Pimpinan

## CARA-CARA BETERNAK KELINCI.

1. UKURAN KANDANG.
   a. Kandang induk dan anak-anaknya.
      a. Panjang kandang : 1 meter.
      b. Lebar kandang : 75 centi meter.
      c. Tinggi kandang : 60 centi meter.
   b. Kandang pejantan.
      a. Panjang kandang : 75 centi meter.
      b. Lebar kandang : 75 centi meter.
      c. Tinggi Kandang : 60 centi meter.
   c. Tnggi tiang dari permukaan tanah (kolongnya) 1 meter.

2. JENIS KELINCI.
   a. Jenis Belanda : Plamceres.
   b. Jenis Australia : Blouwinder.
   c. Jenis Jerman : Jerman.
   d. Jenis Jepang : Yamamoto (Anggora).
   e. Jenis Lokal : Kelinci pribumi.

3. BIBIT KELINCI YANG BAIK.
   a. Telinganya panjang dan lebar, berbadan besar, ekor hampir lurus dengan punggung.
   b. Sehat dan tidak berpenyakit.

4. CARA MENGAWINKAN.
   a. Bilamana sudah berumur 6 atau 7 bulan, sudah cukup waktunya untuk dikawinkan.
   b. Cara mengawinkannya sang betina (induk), dibawa ke kandang pejantan selama 20 menit (3 kali puas). Kalau ada waktu perkawinannya dilihat supaya jelas.
   c. Tanggal perkawinannya dicatat supaya tidak lupa.
   d. Lama hamil (mengandung) hanya 1 bulan, jadi bila kawin tanggal 1 April, tanggal 1 Mei melahirkan.

5. WAKTU MEMISAHKAN ANAK DARI INDUKNYA.
   a. Anaknya mencapai umur 50 hari, harus dipisahkan (dipindahkan) dari induknya.
   b. Induknya istirahat selama 10 hari, untuk menunggu perkawinan yang ke dua kali (selanjutnya).
   c. Tepat umur anaknya 2 bulan, induknya dikawinkan lagi. Jadi kelinci dalam 1 tahun bisa beranak 4 kali.

6. WAKTU MEMBERI MAKAN.
   a. Pagi hari kira-kira jam 8.$^{00}$, diberi dedak (huut) yang sudah dilarutkan (tidak terlalu lembek), sedikat diberi garam.
   b. Siang hari kira-kira jam 12.$^{00}$, diberi makan rumput seperun makan sore (malam).
   c. Sore hari kira-kira jam 17.$^{00}$, diberi makan rumput secukupnya.

7. WAKTU MEMISAHKAN JANTAN DAN BETINA.
   a. Umur anak kelinci mencapai 3 bulan, harus sudah dipisahkan antara jantan dan betina.
   b. Maksud pemisahan ini supaya tidak kawin muda.

8. PERAWATAN DAN KEBERSIHAN KANDANG.
   a. Tiap hari kandang harus selalu bersih.
   b. Bila perlu kandang harus di pel (digosok)
   c. Kolongnya jangan sampai becek, tempat pembuangan kotorannya sebaiknya jauh dari kandangnya.

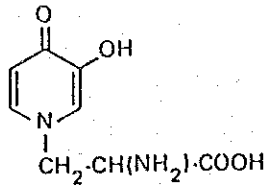
---

Appendix 16

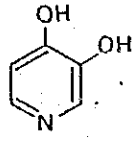
## Mimosine

When diets contain less than 30 percent leucaena (dry weight), cattle thrive for prolonged periods. But when leucaena makes up more than half the diet, and feeding is continued for more than 6 months, the result may be general ill-health with loss of tail and rump hairs, excessive salivation (drooling), and poor growth. The cause has recently been traced to the underproduction of thyroxine by the animal's thyroid gland, which results in goiter.† Swollen thyroids (goiters) are common among cattle feeding on leucaena. The cause is 3,4-dihydroxypyridine (DHP) 2* created in the animals' rumens by bacteria that produce it by chemically transforming the amino acid mimosine 1. Mimosine comprises 3–5 percent (dry weight basis) of the protein of the now-available leucaena types. In single-stomached animals (horses, pigs, rabbits, etc.), mimosine causes hair to fall out. In cattle, the rumen microorganisms transform it to DHP so quickly that even when animals are fed on a diet rich in leucaena their blood, meat, and milk is quite free of mimosine.

Fear of mimosine's effects have for years been a barrier to leucaena's wider use as forage. Today, with better understanding of its pharmacology, much of this fear is being dispelled.




1 Mimosine




2 3,4-dihydroxypyridine

Under field conditions, cattle with goiter don't die; the effects are reversible and can be seen early enough that the animals can be removed from the leucaena pasture to recover. Leucaena contains little or no cyanide, selenium, or bloat-causing agents that *do* kill cattle feeding on pastures such as white clover or alfalfa.‡ Mimosine has no known effect on the meat or milk of ruminants that can be detrimental to humans.

Nonetheless, mimosine is a concern, and searches are being made for low-mimosine varieties. Most leucaena strains have about equal mimosine levels, but some from Colombia and other species such as *Leucaena pulverulenta* (from northern Mexico and southern United States) have much less. Pioneering researchers in Hawaii and Australia have crossed leucaena (i.e., *L. leucocephala*) with *L. pulverulenta* to obtain hybrids with less than half leucaena's mimosine content (Figure 24). The research in Australia has reached an advanced stage, and low-mimosine leucaena lines should be available for grazing trials in 2 years. Goat-feeding trials have shown that, compared with strains available today, the new hybrids markedly reduce adverse effects caused by mimosine.*

When fresh moist leucaena leaves are heated, their mimosine content decreases, causing their feed value to increase. The reduction is greatest at temperatures above 70°C (158°F). Adding ferrous sulfate to rations containing unheated leucaena leaf meal also reduces mimosine toxicity.†

*Information supplied by E. M. Hutton and R. J. Jones.
†Matsumoto et al. 1951. See Selected Readings.

Appendix · 17 - (1)

DAFTAR HARGA OBAT-OBATAN HEWAN

| Product : | Packing : | Price p.Unit : |
|---|---|---|
| A G R I B O N | 10 g/zak | Rp. 400,-- |
| | 100 g/zak | Rp. 3.300,-- |
| | 100 g/tin | Rp. 4.050,-- |
| | 1 kg/tin | Rp. 29.400,-- |
| | 5 kg/tin | Rp. 139.150,-- |
| C O R Y L SP | 10 g/zak | Rp. 500,-- |
| | 100 g/zak | Rp. 4.500,-- |
| | 1 kg/tin | Rp. 41.000,-- |
| | 5 kg/tin | Rp. 190.000,-- |
| D O D E C A L | 5 g/zak | Rp. 150,-- |
| | 10 g/zak | Rp. 300,-- |
| | 100 g/zak | Rp. 2.550,-- |
| | 1 kg/tin | Rp. 23.000,-- |
| | 5 kg/tin | Rp. 105.000,-- |
| D I M E T O C H I N A | 1 Lt/btl | Rp. 21.500,-- |
| L I Q U A C Y C L I N E | 100 cc/btl | Rp. 5.000,-- |
| R O V I M I X 428 | 20 kg/box | Rp. 440.000,-- |
| | 5 kg/alfoil | Rp. 112.500,-- |
| R O V I M I X 430 | 20 kg/box | Rp. 465.000,-- |
| R O V I M I X AB2D3E | 1 kg/tin | Rp. 24.000,-- |
| | 5 kg/tin | Rp. 110.000,-- |
| R O V I M I X AD3 500/100 | 5 kg/tin | Rp. 160.000,-- |
| R O V I S O L AD3EC ORAL | 100 cc/btl | Rp. 2.300,-- |
| | 1 Lt/btl | Rp. 17.500,-- |
| | 6 Lt/btl | Rp. 90.500,-- |
| I N J A C O M ADE INJECTABLE | 100 cc/btl | Rp. 9.550,-- |
| GALLIMYCIN (ERYTHROMYCIN ABBOTT) | 25 gr/zak | Rp. 600,-- |
| | 0,5 Lb/zak | Rp. 4.850,-- |
| | 1 kg/tin | Rp. 18.300,-- |
| | 25 kg/tin | Rp. 447.850,-- |
| GALLIMYCIN - 50 PREMIX | 50 Lb/drm | Rp. 9.200,--/Lbs |
| S P E C T A M W.S. | 200 g/btl | Rp. 48.000,-- |
| S P E C T A M INJECTABLE | 500 cc/btl | Rp. 42.000,-- |
| I O D O P H A X 12,5 | 5 Lt/drm | Rp. 22.750,-- |
| ALBAC ZINC BACITRACIN 10% | 25 kg/zak | Rp. 2.800,--/kg |
| Z O O S O L KAPO | 1 Lt/btl | Rp. 21.000,-- |
| I V A L B O N INJECTABLE | 100 ml/btl | Rp. 6.500,-- |

Catatan: Harga-harga tersebut diatas tidak terikat dan dapat berubah setiap waktu tanpa pemberitahuan terlebih dahulu.

SOLE DISTRIBUTOR/IMPORTIR : PT. KAPO TRADING CO, LTD.

Head Office : Jl. K.S. Tubun IIC/30

Telp. : 540622,540885,540343, JAKARTA-BARAT

Telex : 45369 CIGYFC IA

Jakarta, 1 Juli 1984

Appendix 17 - (2)

DAFTAR HARGA OBAT2AN UNTUK HEWAN

EX CIBA - GEIGY

| PRODUCT : | PACKING : | PRICE P. UNIT : |
|---|---|---|
| ACTOPHOR | 4 x 5 ltr | Rp. 11.000,--/ltr |
| ACEDIST | 1 x 100 tablet | Rp. 31.500,--/tin |
| BRADOPHEN | 25 kg/ Drum | Rp. 32.500,--/kg |
| COSUMIX PLUS | 500 gr/tin | Rp. 25.000,--/tin |
| | 50 gr/zak | Rp. 2.650,--/zak |
| ESB3 | 25 kg/ Drum | Rp. 51.575,--/kg |
| | 30 gr/zak | Rp. 1.850,--/zak |
| ERTILEN | 100 ml/btl | Rp. 8.530,--/btl |
| ERTILEN CO | 100 ml/btl | Rp. 9.840,--/btl |
| IOSAN CCT | 4 x 5 ltr | Rp. 6.400,--/ltr |
| IOSAN CCT | 100 ml/btl | Rp. 725,--/btl |
| ILCOCILLINE DRY COW | 4 x 10 ml (box) | Rp. 9.450,--/box |
| ILCOCILLINE P.S. | 100 ml/btl | Rp. 12.500,--/btl |
| LOPATOL | 1 x 12 Tab/Box | Rp. 16.650,--/box |
| NEOCIDOL 40 WP | 1 kg/zak | Rp. 16.275,--/kg |
| | 20 gr/zak | Rp. 425,--/zak |
| OSTRILAN | 100 ml/btl | Rp. 6.150,--/btl |
| OPTICORTENOL S. | 20 ml/btl | Rp. 8.660,--/btl |
| OXYTOXIN INJEC.SOLUTION | 50 ml/btl | Rp. 6.770,--/btl |
| OXYSENTINA | 100 ml/btl | Rp. 8.670,--/btl |
| SOCATYL PASTE | 200 gr/tube | Rp. 7.875,--/tube |
| UTOCYL FORTE | 10 pess/box | Rp. 12.700,--/box |
| VEBONOL 2,5% INJEC.SOL | 10 ml/btl | Rp. 18.700,--/btl |
| VETIBENZAMINE | 100 ml/btl | Rp. 8.270,--/btl |
| VECORTENOL - VIOFORM OINT. | 10 gr/Tube | Rp. 5.050,--/tube |
| VETIDREX 5% INJEC. SOLUTION | 10 ml/btl | Rp. 4.460,--/btl |
| VIOFORM AEROSAL 0,5% SPRAY | 70 gr/btl | Rp. 3.800,--/btl |
| VESULONG 20% INJEC. SOLUTION | 100 ml/btl | Rp. 4.600,--/btl |
| BELORAN 500 | 5 lt/ Drum | Rp. 8.400,--/ltr |

Catatan : Harga2 tersebut diatas tidak mengikat dan dapat berubah setiap waktu tanpa pemberitahuan terlebih dahulu.

SOLE DISTRIBUTOR/IMPORTIR : PT. KAPO TRADING COY LTD.
Jl. Aipda K.S. Tubun .IIC/30
JAKARTA
Phone : 540622 - 540885 - 540343
Telex : 45369 CIGYFC IA

Jakarta, 1 Agustus 1984

Appendix 18-(1)

The Laboratory of Food Hygiene,
Tokyo Kenbikyo-in
(The Institution authorized by
the Ministry of Health & Welfare of Japan)
8-32, Kudan-Minami 4-chome
Chiyoda-ku, TOKYO
102 JAPAN
Telephone: 03-265-6606

CERTIFICATE No.E-0575-51~55

Applicant: CLEA JAPAN, INC.

Sample: As per attached sheet

Subject: As per attached sheet

As a result of tests carried out on the sample submitted under the above-mentioned name on October 19, 1984, we are enclosing here with as attachment.

Fumio Miyazawa, D.V.M., Ph.D.
Director
The Laboratory of Food Hygiene,
Tokyo Kenbikyo-in

Trans., December 19, 1984

財団法人 東京顕微鏡院

Appendix 18 - (2)

E-0575-51

| Name | | 1.Maize | 2.Milo | 3.Rice Bran | 4.Wheat Pollard | 5.Soy Bean Meal (Brazil) |
|---|---|---|---|---|---|---|
| Subject | | | | | | |
| C.Pro | % | 8.7 | 8.6 | 11.9 | 15.7 | 47.3 |
| C.Fat | % | 3.9 | 2.9 | 14.2 | 4.3 | 0.4 |
| C.Fib | % | 2.1 | 2.6 | 12.7 | 6.3 | 4.0 |
| C.Ash | % | 1.0 | 1.5 | 11.7 | 3.7 | 5.8 |
| Moist | % | 13.1 | 14.1 | 12.4 | 12.3 | 12.7 |
| Ca | % | 0.01 | 0.02 | 0.07 | 0.09 | 0.28 |
| P | % | 0.19 | 0.23 | 1.36 | 0.71 | 0.61 |
| AV | | - | - | 148.5 | - | - |
| POV | meq/kg | - | - | 17.4 | - | - |
| Retinol | | non detect | non detect | non detect | non detect | non detect |

E-0575-52

| Name | | 6.Fish Meal (Peru) | 7.Fish Meal (Domestic) | 8.Meat & Bone Meal | 9.Coconut Meal | 10.Daun Petai Cina (Meal) |
|---|---|---|---|---|---|---|
| Subject | | | | | | |
| C.Pro | % | 62.9 | 55.6 | 51.7 | 19.5 | 14.7 |
| C.Fat | % | 12.4 | 14.4 | 10.4 | 11.0 | 7.8 |
| C.Fib | % | - | - | - | 15.5 | 8.1 |
| C.Ash | % | 14.0 | 18.8 | 31.3 | 5.6 | 13.0 |
| Moist | % | 8.9 | 9.4 | 6.4 | 11.4 | 11.8 |
| Ca | % | 5.16 | 6.21 | 10.92 | 0.16 | 2.70 |
| P | % | 2.41 | 3.24 | 5.22 | 0.54 | 0.19 |
| AV | | 15.0 | 37.6 | 6.6 | - | - |
| POV | meq/kg | 4.4 | 0.6 | 34.9 | - | - |
| VBN | mg% | 0.01 | 0.13 | 0.01 | - | - |
| NaCl | % | 1.42 | 1.04 | - | - | - |
| Retinol | | 0.08mg%(2701U/100g) | non detect | non detect | non detect | non detect |

E-0575-53

| Name | | 11.Sesame Meal | 12.Papaya Leave | 13.Cassava Leave | 14.Daun Petai Cina | 15.Napier or Elephant Grass |
|---|---|---|---|---|---|---|
| Subject | | | | | | |
| C.Pro | % | 41.9 | 17.8 | 17.7 | 24.2 | 6.7 |
| C.Fat | % | 1.5 | 10.0 | 5.7 | 4.5 | 0.7 |
| C.Fib | % | 7.5 | 15.3 | 23.3 | 13.3 | 16.3 |
| C.Ash | % | 13.9 | 11.2 | 8.2 | 7.4 | 5.5 |
| Moist | % | 10.6 | 12.7 | 11.5 | 10.3 | 53.5 |
| Ca | % | 2.01 | 2.19 | 1.25 | 1.79 | 0.15 |
| P | % | 1.43 | 0.27 | 0.24 | 0.16 | 0.08 |
| Retinol | | non detect | non detect | non detect | non detect | non detect |

Appendix 18 - (3)

E-0575-54

| Name | | 16.Bio Farma Bandung | 17.Green Bean (LPPH) | 18.Groundnut Meal (LPPH) | 19.Setaria Grass | 20.Feed for Rabbit (LPPH) |
|---|---|---|---|---|---|---|
| Subject | | | | | | |
| C.Pro | % | 19.2 | 24.1 | 14.6 | 8.4 | 11.9 |
| C.Fat | % | 5.6 | 0.7 | 4.8 | 1.2 | 5.3 |
| C.Fib | % | 4.0 | 4.5 | 13.8 | 26.4 | 7.0 |
| C.Ash | % | 6.7 | 3.3 | 12.1 | 9.2 | 17.0 |
| Moist | % | 11.5 | 15.1 | 10.4 | 43.0 | 9.4 |
| Antibacterials | | negative | - | - | - | negative |
| Ca | % | 1.24 | 0.13 | 0.70 | 0.21 | 4.77 |
| P | % | 0.82 | 0.39 | 1.16 | 0.15 | 0.63 |
| AV | | 112 | - | - | - | 159 |
| POV | meq/kg | 15 | - | - | - | 12 |
| Mould | /g | 20 | - | - | - | 700 |
| Coliform group | | negative | - | - | - | 2400 MPN/100g |
| Retinol | | - | non detect | non detect | non detect | - |

E-0575-55

| Name | | 21.Lumuput Lapangan | 22.Cocoa Residu | 23.CBR |
|---|---|---|---|---|
| Subject | | | | |
| C.Pro | % | 7.4 | 17.0 | 21.1 |
| C.Fat | % | 1.4 | 8.0 | 7.6 |
| C.Fib | % | 26.8 | 17.4 | - |
| C.Ash | % | 7.9 | 4.4 | 13.7 |
| Moist | % | 11.7 | 13.3 | 7.9 |
| Antibacterials | | - | - | negative |
| Ca | % | 0.51 | 0.29 | 1.88 |
| P | % | 0.19 | 0.43 | 1.17 |
| AV | | - | - | 135 |
| POV | meq/kg | - | - | 18 |
| Mould | /g | - | - | 30 |
| Coliform group | | - | - | negative |
| Retinol | | non detect | non detect | - |

Appendix 18 - (4)

E-0619-41

| Name | | Feed for Project | | Limit of detection | Method |
|---|---|---|---|---|---|
| | | 1. House·Rat | Rabbit 2. Guinea pig | | |
| Subject | | | | | |
| Crude Protein | % | 23.3 | 17.8 | | AOAC method |
| Crude Fat | % | 5.1 | 3.7 | | AOAC method |
| Crude Fiber | % | 4.9 | 13.7 | | AOAC method |
| Crude Ash | % | 5.3 | 8.1 | | AOAC method |
| Moisture | % | 11.2 | 9.0 | | AOAC method |
| Calcium | mg% | 580 | 325 | | Atomic Absorption Analysis |
| Phosphorus | mg% | 720 | 493 | | Colorimetric |
| Retinol | | non detect | non detect | 0.03 mg% | Absorptiometry |
| Magnesium | mg% | 249 | 228 | | Atomic Absorption Analysis |
| Potassium | mg% | 1021 | 1336 | | Flame Photometric Analysis |
| Sodium | mg% | 82 | 102 | | Flame Photometric Analysis |
| Manganese | mg% | 8.17 | 7.37 | | Atomic Absorption Analysis |
| Copper | mg% | 0.87 | 0.86 | | Atomic Absorption Analysis |
| Zinc | mg% | 5.92 | 4.60 | | Atomic Absorption Analysis |
| Iron | mg% | 20.7 | 47.8 | | Colorimetric |
| Cobalt | mg% | 0.02 | 0.02 | | Atomic Absorption Analysis |
| Iodine | mg% | non detect | non detect | 0.5 mg% | Titration |
| Linoleic acid | % | 1.25 | 0.99 | | Gas Chromatography |

E-0619-42

| Name | | Feed for Project | | Limit of detection | Method |
|---|---|---|---|---|---|
| | | 1. House·Rat | Rabbit 2. Guinea pig | | |
| Subject | | | | | |
| Vitamin $B_1$ | mg% | 0.51 | 0.43 | | Fluorescence analysis |
| Vitamin $B_2$ | mg% | 0.33 | 0.45 | | Fluorescence analysis |
| Vitamin $B_6$ | mg% | 0.55 | 0.46 | | Bioassay |
| Vitamin $B_{12}$ | μg% | 2.8 | 1.3 | | Bioassay |
| Vitamin C | | non detect | non detect | 3 mg% | Absorptiometry |
| Vitamin $D_3$ | IU/100g | 50 | 110 | | High Perfomance Liquid Chromatography |
| Vitamin E | mg% | 5.5 | 2.2 | | Gas Chromatography |
| Pantothenic acid | mg% | 1.20 | 1.22 | | Bioassay |
| Folic acid | mg% | 0.14 | 0.13 | | Bioassay |
| Niacin | mg% | 6.07 | 6.61 | | Bioassay |
| Choline | mg% | 180 | 110 | | Bioassay |
| Biotin | μg% | 28.4 | 28.2 | | Bioassay |
| Inositol | mg% | 176 | 81 | | Bioassay |

Appendix 18 - (5)

E-0619-43

| Name | Feed for Project | | Limit of detection | Method |
|---|---|---|---|---|
| | 1. Mouse·Rat | Rabbit 2. Guinea pig | | |
| Subject | | | | |
| Fatty acid composition | | | | Gas Chromatography |
| $C_{10}$ | 1.1 % | 0.4 % | | |
| $C_{12}$ | 11.3 % | 6.8 % | | |
| $C_{14}$ | 5.9 % | 3.9 % | | |
| $C_{15}$ | 0.1 % | 0.6 % | | |
| $C_{16}$ | 16.5 % | 17.5 % | | |
| $C_{16:1}$ | 1.9 % | 1.5 % | | |
| $C_{17}$ | traces | traces | | |
| $C_{17:1}$ | 0.3 % | 0.2 % | | |
| $C_{18}$ | 2.8 % | 2.7 % | | |
| $C_{18:1}$ | 17.7 % | 17.2 % | | |
| $C_{18:2}$ | 27.6 % | 33.0 % | | |
| $C_{18:3}$ | 2.8 % | 4.9 % | | |
| $C_{18:4}$ | 0.9 % | 0.7 % | | |
| $C_{20}$ | 0.3 % | 0.6 % | | |
| $C_{20:1}$ | 0.6 % | 0.7 % | | |
| $C_{20:4\omega3}$ | 0.5 % | 0.2 % | | |
| $C_{20:4\omega6}$ | 0.2 % | 0.2 % | | |
| $C_{20:5}$ | 3.7 % | 2.4 % | | |
| $C_{22:1}$ | 0.4 % | 0.3 % | | |
| $C_{22:5}$ | 0.5 % | 0.3 % | | |
| $C_{22:6}$ | 3.6 % | 3.0 % | | |
| $C_{24:1}$ | 0.6 % | 0.3 % | | |
| non identification | 0.7 % | 2.6 % | | |

E-0619-44

| Name | Feed for Project | | Limit of detection | Method |
|---|---|---|---|---|
| | 1. Mouse·Rat | Rabbit 2. Guinea pig | | |
| Subject | | | | |
| Amino acid composition | | | | Amino acid Automatic Analysis |
| Arginine | 1.70 % | 1.10 % | | |
| Lysine | 1.30 % | 0.96 % | | |
| Histidine | 0.62 % | 0.45 % | | |
| Phenylalanine | 1.12 % | 0.87 % | | |
| Tyrosine | 0.70 % | 0.44 % | | |
| Leucine | 1.89 % | 1.40 % | | |
| iso-Leucine | 0.97 % | 0.75 % | | |
| Methionine | 0.44 % | 0.28 % | | |
| Valine | 1.25 % | 0.96 % | | |
| Alanine | 1.32 % | 0.98 % | | |
| Glycine | 1.16 % | 0.87 % | | |
| Proline | 1.23 % | 0.90 % | | |
| Glutamic acid | 3.94 % | 2.70 % | | |
| Serine | 1.14 % | 0.78 % | | |
| Threonine | 0.92 % | 0.69 % | | |
| Aspartic acid | 2.17 % | 1.60 % | | |
| Tryptophan | 0.32 % | 0.25 % | | |
| Cystine | 0.40 % | 0.28 % | | |

# 1983 FEEDSTUFFS ANALYSIS TABLE



This chart published to assist feed manufacturers and students of nutrition in formulating feeds. The values are not average, but carry a "margin of safety" so that the feed should contain at least as much protein, fat, minerals and vitamins, and not as much fiber as the final calculations show.

| Protein % | Fat % | Fiber % | Calcium % | Phosphorus % Total | Phosphorus % Available | Ash % | FEEDSTUFF | Dry Matter % | Swine Metabolizable energy | Poultry Productive energy Cal./lb. | Poultry Metabolizable energy Cal./lb. | True Metabolizable energy Cal./lb. | Milligrams Per Lb. Riboflavin | Niacin | Pantothenic acid | Thiamine | Choline | Biotin | Folic Acid | Pyridoxine | Carotene | Xanthophyll | Alpha tocopherol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 3.5 | 21.0 | 1.45 | 0.27 | 0.22 | 10.5 | Alfalfa leaf meal (dehy.) | 93 | 1,100 | 385 | 740 | 755 | 7.0 | 21.0 | 14.9 | 1.8 | 730 | 0.15 | 1.2 | 3.6 | 72 | 128 | 60 |
| 17 | 3.0 | 25.0 | 1.4 | 0.23 | 0.18 | 9.0 | Alfalfa meal (dehydrated) | 93 | 1,050 | 300 | 720 | 640 | 5.5 | 20.0 | 13.5 | 1.5 | 680 | 0.15 | 0.95 | 3.0 | 54 | 85 | 50 |
| 15 | 2.0 | 27.0 | 1.2 | 0.22 | 0.17 | 8.5 | Alfalfa meal (dehydrated) | 93 | 580 | 240 | 680 | 510 | 4.8 | 19.0 | 9.0 | 1.3 | 600 | 0.11 | 0.7 | 2.8 | 33 | 75 | 40 |
| 13 | 1.5 | 33.0 | 1.20 | 0.20 | 0.16 | 9.0 | Alfalfa meal (either type) | 89 | 820 | 200 | 250 | — | 4.0 | 9.0 | 8.0 | — | 300 | — | — | — | — | — | 18 |
| 11.5 | 1.8 | 6.5 | 0.06 | 0.36 | 0.12 | 2.5 | Barley | 89 | 1,320 | 800 | 1,140 | 1,375 | 0.70 | (24.0) | 2.8 | 2.3 | 500 | 0.05 | 0.14 | 1.8 | — | — | 10.0 |
| 9.5 | 1.8 | 6.5 | 0.05 | 0.33 | 0.11 | 2.5 | Barley (Pacific Coast) | 88 | 1,335 | 780 | 1,180 | 1,380 | 0.50 | (20.0) | 3.3 | 0.4 | 450 | 0.04 | 0.13 | 1.3 | — | — | 6.1 |
| 22 | 1.0 | 4.5 | 0.10 | 0.40 | 0.13 | 6.0 | Beans (navy cull) | 90 | 1,540 | 450 | 1,060 | — | 0.9 | 11.0 | 0.9 | 2.8 | 800 | 0.05 | 0.59 | 0.13 | — | — | — |
| 8 | 0.5 | 18.0 | 0.60 | 0.10 | 0.03 | 5.6 | Beet pulp, dried | 91 | 1,020 | 200 | 290 | — | 0.30 | 7.0 | 0.7 | 0.2 | 370 | — | — | — | — | — | — |
| 9 | 0.6 | 15.5 | 0.5 | 0.05 | 0.02 | 8.0 | Beet pulp & molasses | 92 | 1,000 | 200 | 300 | 955 | 0.3 | 7.4 | 0.7 | — | 370 | — | — | — | — | — | — |
| 35 | 1.0 | 1.0 | 0.28 | 0.22 | 0.22 | 4.5 | Blood meal, flash dried* | 91 | 1,050 | 1,010 | 1,250 | 1,435 | 0.65 | 14.0 | 0.5 | 0.2 | 340 | 0.04 | 0.04 | 2.0 | — | — | — |
| 27 | 6.6 | 13.5 | 0.25 | 0.6 | 0.15 | 6.6 | Brewers dried grains** | 93 | 1,040 | 1,000 | 1,140 | 1,400 | 0.6 | (20.0) | 3.9 | 0.3 | 950 | 0.03 | 0.1 | 0.77 | — | — | 11.0 |
| 45 | 1.0 | 3.0 | 0.10 | 1.40 | 1.4 | 6.5 | Brewers dried yeast | 93 | 1,400 | 570 | 920 | 1,335 | 15.0 | 200.0 | 50.0 | 45.0 | 1,750 | 0.34 | 5.0 | 19.7 | — | — | 0.9 |
| 11 | 2.5 | 11.0 | 0.06 | 0.30 | 0.1 | 2.1 | Buckwheat | 88 | 1,300 | 820 | 1,185 | 1,230 | 0.6 | (8.0) | 5.5 | 1.5 | 200 | — | — | 1.2 | — | — | — |
| 32 | 5.0 | 0.4 | 1.30 | 0.9 | 0.9 | 9.6 | Buttermilk, dried | 93 | 1,380 | 780 | 1,250 | — | 12.0 | 3.8 | 13.0 | 1.3 | 830 | 0.13 | 0.18 | 1.0 | — | — | — |
| 6 | 3.4 | 13.5 | 2.00 | 0.10 | 0.03 | 6.3 | Citrus pulp, dried | 90 | 1,100 | 420 | 600 | — | 1.00 | 9.0 | 5.5 | 0.6 | 350 | — | — | — | — | — | — |
| 15 | 3.5 | 28.0 | 0.5 | 0.2 | 0.15 | 6.0 | Coastal bermudagrass (dehy.) | 90 | — | — | — | — | 4.0 | 28.0 | 6.0 | — | 540 | — | — | — | 40 | — | 33 |
| 21 | 6.0 | 13.0 | 0.30 | 0.55 | 0.18 | 6.5 | Coconut oil meal (copra) | 92 | 1,520 | 600 | 680 | — | 1.4 | 11.0 | 2.8 | 0.5 | 420 | — | 0.5 | 2.0 | — | — | — |
| 8.5 | 3.8 | 2.5 | 0.01 | 0.25 | 0.08 | 1.5 | Corn (yellow) | 86 | 1,500 | 1,100 | 1,530 | 1,610 | 0.50 | (9.0) | 1.7 | 1.5 | 230 | 0.03 | 0.13 | 2.1 | 0.9 | 7 | 7.7 |
| 10.1 | 4.0 | 3.7 | 0.02 | 0.20 | 0.07 | 1.8 | Corn, high lysine | 90 | 1,560 | 1,130 | 1,530 | — | 0.5 | (9.0) | 1.7 | 1.5 | 230 | 0.03 | 0.13 | 2.1 | 1.0 | 7 | 7.7 |
| 7.5 | 3.0 | 8.2 | 0.04 | 0.20 | 0.07 | 1.5 | Corn & cob meal (yellow) | 86 | 1,300 | 900 | 1,290 | — | 0.40 | (7.2) | 1.7 | 1.0 | 160 | 0.02 | 0.09 | 0.8 | 0.8 | 5.6 | 4 |
| 2.3 | 0.4 | 32.5 | 0.11 | 0.04 | 0.01 | 1.5 | Corn cobs | 90 | 140 | — | 240 | — | 0.5 | (3.9) | 1.7 | — | — | 0.01 | — | 1.1 | — | — | — |
| 20.0 | 1.0 | 12.0 | 0.30 | 0.50 | 0.17 | 4.0 | Corn germ meal (wet milled) | 93 | 1,360 | 520 | 770 | — | 1.7 | (19.0) | 2.0 | 2.8 | 800 | 0.1 | 0.09 | 2.7 | 0.9 | — | 39 |
| 22 | 2.5 | 9.0 | 0.30 | 0.75 | 0.27 | 7.3 | Corn gluten feed | 90 | 1,120 | 510 | 785 | 1,075 | 1.0 | (30.0) | 7.5 | 0.9 | 690 | 0.08 | 0.09 | 6.8 | 1.0 | 10 | 6.7 |
| 42 | 2.0 | 5.0 | 0.1 | 0.4 | 0.13 | 4.5 | Corn gluten meal 41% | 91 | 1,425 | 895 | 1,300 | 1,440 | 0.70 | (20.0) | 4.5 | 0.5 | 200 | 0.08 | 0.1 | 3.6 | 10 | 60 | 9.2 |
| 60 | 2.0 | 2.5 | 0.02 | 0.5 | 0.2 | 1.8 | Corn gluten meal 60% | 90 | 1,600 | 1,240 | 1,820 | 1,810 | 0.7 | (37.0) | 1.6 | 0.1 | 150 | 0.08 | 0.1 | 2.8 | 20 | 120 | 11.7 |
| 23 | 0.0 | 0.0 | 0.06 | 1.8 | 1.5 | 7.8 | Corn ferm. extractives, cond. | 52 | 920 | — | 707 | 545 | 2.7 | (40.0) | 6.8 | 1.3 | 1,270 | 0.15 | — | 4.0 | — | — | — |
| 50 | 1.0 | 8.5 | 0.15 | 1.2 | 0.5 | 6.0 | Cottonseed meal (solv.) | 91 | 1,150 | 750 | 1,140 | — | 2.00 | 20.0 | 4.5 | 2.3 | 1,370 | 0.24 | 1.0 | 1.8 | — | — | 4.0 |
| 41 | 3.7 | 14.0 | 0.15 | 0.9 | 0.3 | 6.5 | Cottonseed meal (expeller) | 91 | 1,245 | 690 | 900 | — | 1.9 | 17.0 | 3.5 | 2.3 | 1,270 | 0.25 | 1.24 | 2.2 | — | — | 6.0 |
| 41 | 0.5 | 13.0 | 0.15 | 0.95 | 0.32 | 6.5 | Cottonseed meal (solv.) | 90 | 1,130 | 580 | 970 | — | 1.8 | 18.0 | 3.2 | 3.5 | 1,300 | 0.25 | 1.21 | 1.8 | — | — | 4.0 |
| 27 | 9.0 | 13.0 | 0.05 | 0.35 | 0.35 | 2.1 | Dist. dr. grains (light) (corn) | 92 | 930 | 800 | 900 | — | 1.5 | 17.0 | 2.5 | 0.9 | 400 | 0.4 | 0.4 | — | — | — | 2.2 |
| 27 | 8.0 | 8.5 | 0.14 | 0.9 | 0.9 | 4.6 | Dist. dr. gr., with sol. (corn) | 91 | 1,180 | 890 | 1,100 | 1,335 | 4.1 | 33.0 | 5.0 | 1.5 | 1,400 | 0.3 | 0.4 | 3.0 | 1.0 | 1 | 13 |
| 28 | 9.0 | 4.0 | 0.33 | 1.4 | 1.4 | 7.2 | Distillers dr. solubles (corn) | 95 | 1,600 | 1,020 | 1,350 | 1,415 | 7.6 | 52.0 | 9.0 | 3.1 | 2,200 | 0.5 | 0.5 | 5.9 | 1.0 | 0.8 | 25 |
| 9.5 | 1.5 | 1.5 | 0.06 | 0.24 | 0.1 | 4.0 | Dried bakery product | 91 | 1,650 | 1,315 | 1,750 | — | 0.7 | 8.6 | 6.6 | 0.6 | 560 | 0.3 | 0.07 | 14.0 | — | — | 11 |
| 85 | 2.5 | 1.5 | 0.20 | 0.75 | 0.75 | 3.8 | Feathers (hydrolyzed poultry) | 95 | 995 | 770 | 1,365 | 1,825 | 0.8 | 7.6 | 3.5 | 0.5 | 400 | 0.02 | 0.1 | 2.0 | — | — | — |
| 63.5 | 10.0 | 1.0 | 3.6 | 2.4 | 2.4 | 16.5 | Fishmeal, anchovy | 92 | 1,600 | 860 | 1,490 | 1,350 | 4.3 | 42.5 | 4.4 | 0.3 | 1,700 | 0.09 | 1.0 | 1.4 | — | — | — |
| 72 | 8.4 | 1.0 | 2.0 | 1.5 | 1.5 | 10.5 | Fish meal, herring | 93 | 1,265 | 950 | 1,380 | 1,740 | 4.00 | 40.0 | 5.0 | 0.3 | 1,600 | 0.09 | 0.15 | 2.0 | — | — | 6 |
| 61 | 9.4 | 1.0 | 5.2 | 2.9 | 2.9 | 19.0 | Fish meal, menhaden | 92 | 1,200 | 940 | 1,300 | 1,530 | 2.1 | 25.0 | 3.8 | 0.3 | 1,300 | 0.09 | 0.1 | 1.2 | — | — | 3 |
| 57 | 8.0 | 1.0 | 6.4 | 3.4 | 4.0 | 25.0 | Fish meal, red fish | 94 | 1,175 | 866 | 1,465 | 1,080 | 3.0 | 18.5 | 3.5 | 0.1 | 1,400 | 0.09 | 0.09 | — | — | — | — |
| 52 | 7.0 | 1.0 | 7.7 | 4.0 | 4.0 | 25.0 | Fish meal, tuna | 93 | 1,060 | 900 | 1,300 | — | 3.0 | 30.0 | 4.0 | — | 1,400 | 0.04 | — | — | — | — | — |
| 60 | 4.0 | 1.0 | 7.0 | 3.50 | 3.5 | 22.0 | Fish meal, white | 91 | 1,200 | 825 | 1,330 | 1,520 | 4.00 | 30.0 | 3.0 | 0.7 | 700 | 0.03 | — | 2.7 | — | — | 2.5 |
| 31 | 5.5 | 0.5 | 0.2 | 0.59 | 0.59 | 8.6 | Fish solubles (cond.) | 50 | 730 | 470 | 800 | — | 6.00 | 75.0 | 16.0 | 2.5 | 1,500 | 0.06 | 0.17 | 1.2 | — | — | — |
| 10 | 6.0 | 5.0 | 0.05 | 0.50 | 0.17 | 3.0 | Hominy feed (yellow) | 91 | 1,525 | 850 | 1,310 | — | 0.9 | (20.0) | 3.4 | 0.6 | 430 | 0.05 | 0.13 | 5.0 | 0.6 | 1.6 | — |
| 10 | 2.5 | 2.5 | 0.02 | 0.3 | 0.1 | 1.5 | Kafir corn | 89 | 1,480 | 1,080 | 1,590 | 1,560 | 0.6 | (16.0) | 5.0 | 1.4 | 200 | 0.04 | 0.09 | — | — | — | — |
| 32 | 4.0 | 9.0 | 0.4 | 0.8 | 0.27 | 6.0 | Linseed meal (expeller) | 91 | 1,250 | 510 | 690 | — | 1.6 | 16.0 | 8.0 | 2.3 | 810 | 0.15 | 1.3 | 2.5 | — | — | 3.5 |
| 33 | 0.5 | 9.5 | 0.4 | 0.8 | 0.27 | 6.0 | Linseed meal (solvent) | 91 | 1,145 | 450 | 640 | 1,210 | 1.30 | 20.0 | 6.4 | 4.2 | 635 | 0.16 | 0.8 | 2.5 | — | — | 2.6 |
| 25 | 1.2 | 15.0 | 0.20 | 0.70 | 0.2 | 6.5 | Malt sprouts, dried | 92 | 1,000 | 540 | 670 | — | 1.4 | 23.0 | 4.2 | 3.8 | 770 | — | 0.09 | — | — | — | 1.9 |
| 45 | 8.6 | 2.1 | 10.00 | 5.1 | 5.1 | 38.0 | Meat and bone meal | 94 | 790 | 660 | 840 | — | 0.9 | 16.0 | 2.1 | 0.2 | 600 | — | — | — | — | — | — |
| 50 | 10.0 | 2.8 | 8.1 | 4.1 | 4.1 | 32.7 | Meat and bone meal | 94 | 1,000 | 760 | 900 | 1,320 | 1.7 | 19.0 | 2.5 | 0.2 | 870 | 0.02 | 0.2 | 0.7 | — | — | 0.3 |
| 54 | 7.1 | 2.5 | 7.4 | 3.8 | 3.8 | 29.0 | Meat meal | 94 | 1,200 | 760 | 910 | — | 2.3 | 26.0 | 2.2 | 0.2 | 900 | 0.02 | 0.2 | 0.7 | — | — | 0.3 |
| 9 | 2.5 | 2.7 | 0.02 | 0.27 | 0.09 | 1.8 | Milo maize | 89 | 1,450 | 1,100 | 1,500 | 1,560 | 0.5 | (18.0) | 5.0 | 1.8 | 300 | 0.08 | 0.1 | 1.7 | — | — | 4.5 |
| 6 | 0.0 | 0.0 | 0.10 | 0.02 | 0.01 | 9.0 | Molasses, beet | 77 | 1,060 | 710 | 875 | — | 1.00 | 18.0 | 2.0 | — | 400 | — | — | — | — | — | 2 |
| 3 | 0.0 | 0.0 | 0.8 | 0.08 | 0.04 | 8.5 | Molasses, cane | 75 | 1,000 | 715 | 890 | — | 1.3 | 15.0 | 17.0 | 0.4 | 390 | 0.3 | — | — | — | — | 3 |
| 11.5 | 4.0 | 12.0 | 0.08 | 0.33 | 0.11 | 3.5 | Oats | 89 | 1,215 | 800 | 1,130 | 1,470 | 0.5 | (6.0) | 5.8 | 1.1 | 420 | 0.05 | 0.15 | 0.9 | — | — | 13 |
| 9.0 | 4.5 | 12.0 | 0.08 | 0.30 | 0.1 | 4.0 | Oats (Pacific Coast) | 91 | 1,190 | 800 | 1,210 | 1,575 | 0.50 | (6.5) | 5.5 | 1.1 | 400 | 0.05 | 0.15 | 0.9 | — | — | 9.0 |
| 16.0 | 5.5 | 3.0 | 0.07 | 0.4 | 0.13 | 2.5 | Oats, feed rolled, oat groats | 91 | 1,590 | 1,150 | 1,450 | 1,730 | 0.7 | (3.7) | 5.9 | 2.9 | 500 | 0.10 | 0.16 | 0.5 | — | — | 6.8 |
| 2 | 0.5 | 36.5 | 0.09 | 0.10 | 0.03 | 6.5 | Oat hulls | 93 | 395 | 0.0 | 140 | — | 0.50 | (3.5) | 1.0 | — | 85 | — | — | — | — | — | — |
| 3.5 | 1.0 | 22.0 | 0.25 | 0.20 | 0.07 | 6.0 | Oat mill byproduct | 92 | 505 | 100 | 170 | — | 0.72 | (4.0) | 1.5 | 0.5 | 145 | — | — | — | — | — | — |
| 43.0 | 6.0 | 13.0 | 0.15 | 0.55 | 0.18 | 5.8 | Peanut meal and hulls (exp.) | 92 | 1,315 | 850 | 1,100 | — | 2.1 | 75.0 | 23.0 | 2.7 | 900 | 0.17 | 0.3 | 2.4 | — | — | 1.3 |
| 45 | 1.0 | 15.6 | 0.1 | 0.60 | 0.2 | 4.8 | Peanut meal and hulls (solv.) | 92 | 1,110 | 850 | 1,000 | — | 2.1 | 75.0 | 23.0 | 2.6 | 900 | 0.17 | 0.3 | 2.5 | — | — | 1.3 |
| 58 | 13.9 | 2.3 | 3.2 | 1.7 | 1.7 | 14.0 | Poultry byproduct meal | 94 | 1,330 | 900 | 1,320 | 1,900 | 5.0 | 21.0 | 8.5 | 0.1 | 2,720 | 0.04 | 0.23 | 2.0 | — | — | 2.0 |
| 37 | 1.7 | 11.5 | 0.6 | 1.1 | 0.45 | 8.0 | Rapeseed Meal (solv.)(Canola) | 92 | 1,227 | 500 | 860 | 1,040 | 1.6 | 72 | 4.3 | 2.3 | 3,045 | 0.40 | 1.0 | 3.2 | — | — | — |
| 12.5 | 13.0 | 13.0 | 0.06 | 1.6 | 0.23 | 12.5 | Rice bran | 91 | 1,430 | 770 | 950 | — | 1.1 | (125.0) | 9.5 | 10.0 | 570 | 0.19 | 1.0 | 13.0 | — | — | 27 |
| 12.0 | 11.5 | 4.0 | 0.04 | 1.2 | 0.3 | 8.0 | Rice polishings | 92 | 1,560 | 950 | 1,377 | — | 0.8 | (185.0) | 26.0 | 9.0 | 600 | 0.28 | — | 12.0 | — | — | 41 |
| 7.3 | 1.7 | 9.0 | 0.04 | 0.26 | 0.09 | 5.2 | Rice (rough) | 89 | 1,230 | 811 | 1,215 | — | 0.4 | (13.0) | 4.0 | 1.2 | 450 | 0.03 | 0.08 | 3.0 | — | — | 4 |
| 22 | 1.0 | 31.0 | 0.25 | 0.50 | 0.18 | 6.0 | Safflower seed meal, solvent. | 91 | 905 | 460 | 540 | — | 1.00 | 5.0 | 15.0 | 1.0 | 700 | 0.6 | 0.22 | — | — | — | — |
| 42 | 1.3 | 15.1 | 0.40 | 1.25 | 0.42 | 7.8 | Safflower seed meal, solvent. | 93 | 1,320 | 540 | 875 | — | 1.1 | 10.0 | 18.0 | 2.0 | 1,480 | 0.7 | 0.7 | 5.0 | — | — | — |
| ~~44~~ | ~~5.0~~ | ~~6.1~~ | ~~2.00~~ | ~~1.5~~ | ~~0.5~~ | ~~11.5~~ | ~~Sesame meal~~ | 93 | ~~1,340~~ | ~~780~~ | ~~1,000~~ | — | ~~1.50~~ | ~~6.0~~ | ~~2.5~~ | ~~1.2~~ | ~~680~~ | — | — | ~~5.7~~ | — | — | — |
| 46 | 3.0 | 12.0 | 7.00 | 1.50 | 1.5 | 26.0 | Shrimp meal | 90 | 760 | 400 | 760 | — | 1.80 | — | — | — | 2,000 | — | — | — | — | — | — |
| 33 | 1.0 | 0.3 | 1.25 | 1.00 | 1.0 | 8.0 | Skim milk, dried | 94 | 1,500 | 760 | 1,140 | 965 | 9.00 | 5.0 | 15.0 | 1.6 | 640 | 0.15 | 0.28 | 1.8 | — | — | 4.0 |
| 21.0 | 4.0 | 8.0 | 0.15 | 0.6 | 0.2 | 7.5 | Sorghum gluten feed | 91 | 1,400 | 500 | 980 | — | 1.1 | (44.0) | 8.0 | 2.6 | 790 | — | — | — | — | — | — |
| 42.0 | 4.3 | 3.5 | 0.04 | 0.4 | 0.13 | 3.0 | Sorghum gluten meal | 91 | 1,440 | 850 | 1,240 | — | 0.70 | (15.0) | 4.0 | 0.5 | 300 | — | — | — | — | — | — |
| 37.0 | 17.5 | 5.5 | 0.25 | 0.58 | 0.2 | 5.0 | Soybeans (whole cooked) | 90 | 1,770 | 1,025 | 1,500 | — | 1.20 | 10.0 | 5.0 | 2.1 | 1,300 | 0.17 | 1.0 | 1.8 | — | — | 15 |
| 12.0 | 1.5 | 33.0 | 0.35 | 0.15 | 0.05 | 7.0 | Soybean millfeed | 89 | — | 200 | 350 | — | 1.6 | 11.0 | 6.0 | 1.0 | 290 | 0.10 | 0.09 | 1.0 | — | — | — |
| 42 | 3.5 | 6.5 | 0.20 | 0.60 | 0.2 | 6.0 | Soybean meal (expeller) | 90 | 1,360 | 780 | 1,100 | — | 1.40 | 12.0 | 6.0 | 1.8 | 1,100 | 0.15 | 0.3 | — | — | — | 3 |
| 44 | 0.5 | 6.5 | 0.25 | 0.60 | 0.2 | 6.0 | Soybean meal (solvent) | 90 | 1,280 | 760 | 1,020 | 1,205 | 1.30 | 12.0 | 6.0 | 2.1 | 1,200 | 0.15 | 0.3 | 3.0 | — | — | 1.3 |
| 48.5 | 0.5 | 3.5 | 0.20 | 0.65 | 0.22 | 6.0 | Soybean meal (solv.)(dehulled) | 90 | 1,430 | 750 | 1,140 | 1,320 | 1.2 | 9.5 | 6.0 | 1.1 | 1,250 | 0.15 | 0.3 | 3.2 | — | — | 1.3 |
| 28 | 1.0 | 24.0 | 0.35 | 0.9 | 0.3 | 7.7 | Sunflower meal with hulls | 90 | 820 | — | 700 | — | 1.0 | 90.0 | 14 | 11 | 1,300 | — | — | 5.0 | — | — | 5.0 |
| 60 | 9.0 | 2.0 | 4.6 | 2.5 | 2.5 | 21.2 | Tankage, meat meal | 92 | 980 | 800 | 1,200 | 1,350 | 1.1 | 17.0 | 1.1 | 0.2 | 1,000 | — | 0.7 | — | — | — | — |
| 15 | 1.1 | 5.5 | 0.05 | 0.3 | 0.1 | 4.0 | Triticale | 90 | 1,400 | 1,000 | 1,425 | — | 0.2 | — | — | — | 210 | — | — | — | — | — | — |
| 12.5 | 1.5 | 2.4 | 0.05 | 0.35 | 0.12 | 1.7 | Wheat, hard | 86 | 1,475 | 940 | 1,450 | 1,580 | 0.5 | (22.0) | 4.1 | 2.0 | 490 | 0.04 | 0.19 | 1.0 | — | — | 6.5 |
| 10 | 1.5 | 2.2 | 0.05 | 0.28 | 0.09 | 1.9 | Wheat, soft western | 90 | 1,500 | 950 | 1,450 | 1,550 | 0.5 | (21.0) | 5.0 | 1.9 | 350 | 0.05 | 0.1 | 0.7 | — | — | 7.0 |
| 14.5 | 3.0 | 11.0 | 0.08 | 1.15 | 0.4 | 7.0 | Wheat bran | 88 | 1,000 | 440 | 560 | 775 | 1.4 | (95.0) | 13.0 | 3.0 | 960 | 0.05 | 0.54 | 4.2 | — | — | 12 |
| 16 | 4.5 | 6.5 | 0.05 | 0.4 | 0.13 | 2.5 | Wheat shorts (red dog) | 88 | 1,300 | 720 | 1,140 | — | 2.0 | (48.0) | 11.0 | 8.9 | 910 | — | 0.36 | 2.13 | — | — | 14 |
| 24 | 8.0 | 3.5 | 0.04 | 0.8 | 0.3 | 4.3 | Wheat germ meal | 88 | 1,530 | 735 | 1,225 | — | 2.5 | (31.0) | 10.0 | 10.0 | 1,360 | 0.1 | 1.0 | 4.76 | — | — | 69 |
| 15 | 4.0 | 8.5 | 0.05 | 0.90 | 0.3 | 5.5 | Wheat millrun | 88 | 1,160 | 570 | 760 | — | 2.0 | (43.0) | 12.0 | 5.7 | 930 | — | 0.59 | 3.49 | — | — | 17 |
| 15.5 | 4.0 | 7.5 | 0.05 | 0.80 | 0.27 | 5.5 | Wheat middlings, standard | 88 | 1,335 | 700 | 960 | — | 2.1 | (78.0) | 12.0 | 7.0 | 930 | 0.17 | 0.68 | 4.2 | — | — | 20 |
| 14 | 2.0 | 1.5 | 0.05 | 0.30 | 0.1 | 0.5 | Wheat feed flour | 89 | 1,540 | 885 | 1,340 | — | 0.4 | (18.0) | 6.0 | 0.6 | 450 | — | — | — | — | — | — |
| 12 | 0.5 | 0.0 | 0.90 | 0.70 | 0.7 | 10.0 | Whey, dried | 94 | 1,415 | 700 | 755 | — | 9.0 | 4.3 | 40.0 | 2.0 | 460 | 0.18 | 0.4 | 1.1 | — | — | — |
| 16 | 1.0 | 0.0 | 1.5 | 1.2 | 1.2 | 16.0 | Whey product, dried | 94 | 1,240 | 700 | 935 | — | 23.0 | 8.0 | 31.0 | 2.4 | 800 | 0.11 | 0.4 | 1.8 | — | — | — |

( ) Niacin in parentheses may be wholly unavailable. * Conventionally dried blood meal contains 80% protein and 5.9% lysine. ** Brewers dried grains from users of liquid adjuncts will have less protein.

Appendix 20 - (1)

# THE DETERMINATION OF MIMOSINE AND 3,4-DIHYDROXYPYRIDINE IN BIOLOGICAL MATERIAL

By M. P. Hegarty,* R. D. Court,* and Peggy M. Thorne†

[*Manuscript received March* 13, 1963]

*Summary*

A simple, specific method is described for the determination of mimosine and 3,4-dihydroxypyridine (DHP) in extracts of leaves and seeds of *Leucaena glauca* Benth. and in urine.

Plant material is extracted with cold 0·1N hydrochloric acid, and interfering substances are removed by chromatography on a cation-exchange resin. Organic cations are displaced from the resin with 2N ammonium hydroxide and the concentrated eluate chromatographed in one direction with the use of mesityl oxide : formic acid : water (41 : 7 : 6 by volume). The spots of mimosine and DHP, revealed with a ferric chloride spray, are cut out and the colour fully developed and measured. Urines are analysed in the same way except for a preliminary hydrolysis of the conjugated DHP. The method is satisfactory for estimating amounts of mimosine and DHP in the range 10–160 μg applied to the paper. Recoveries of these substances added to various extracts and to urine have varied between 98–102%.

Appreciable destruction of mimosine, with the formation of some DHP, occurred when fresh *L. glauca* leaves were dried even under mild conditions. In fresh material no loss of mimosine occurred when it was placed immediately in 0·1N hydrochloric acid.

The ready hydrolysis of mimosine to DHP by boiling 0·1N hydrochloric acid has been demonstrated for the first time.

## I. Introduction

Mimosine,‡ β-[N-(3-hydroxypyridone-4)]-α-aminopropionic acid, occurs in the shoots and stems of *Mimosa pudica* L. and in large amounts in the leaves, stems, and seeds of *Leucaena glauca* Benth., a tropical shrub legume. Wibaut (1953) and Klingsberg (1960) have reviewed the detailed chemical investigations, which showed that the compounds isolated from *M. pudica* and *L. glauca* were identical (Kleipool and Wibaut 1950). The correct structural formula was determined from degradation studies and confirmed by synthesis (Wibaut 1946; Adams and Johnson 1949).

Owen (1958) has summarized the extensive literature on the depilatory and other toxic effects caused by feeding *L. glauca*, or its active principle mimosine, to rats, mice, pigs, horses, and ruminants. The depilatory effects of mimosine have recently been studied in greater detail. Crounse, Maxwell, and Blank (1962) have shown that mimosine, an analogue of tyrosine, caused inhibition of growth of anagen (growing) hairs in mice, and suggest that mimosine may interfere with tyrosine

* Division of Tropical Pastures, CSIRO, Cunningham Laboratory, St. Lucia, Qld.

† Division of Tropical Pastures, CSIRO, Cunningham Laboratory, St. Lucia, Qld.; present address: Department of Agriculture, Stock and Fisheries, Port Moresby, Territory of Papua and New Guinea.

‡ The *Chemical Abstracts* nomenclature for mimosine is 3-hydroxy-4-oxo-1(4H)-pyridine-alanine, but because of widespread usage the older nomenclature is used here.

*Aust. J. Agric. Res.*, 1964, 15, 168–79

metabolism. Hegarty, Schinckel, and Court (1964) have shown that administration of mimosine to sheep caused inhibition of mitotic activity in the wool follicle bulb, resulting in shedding of the fleece. These workers have also shown that mimosine is readily detoxicated in the rumen of sheep to 3,4-dihydroxypyridine (DHP), which is subsequently excreted in the urine, partly in conjugated form.

A sensitive and specific method for the estimation of mimosine and DHP in biological extracts was needed for investigations into the metabolism of these compounds in plants and animals. Existing methods for the determination of mimosine (Yoshida 1944; Matsumoto and Sherman 1951) are based on the extraction of mimosine from plant material with boiling 0·1N hydrochloric acid, clarification of the extract, and colorimetric estimation of mimosine as its purple ferric chloride complex at 535 mμ. Under these conditions of extraction some mimosine is hydrolysed to DHP, which has a ferric complex of similar colour. Thus the Matsumoto and Sherman method, while sensitive, lacks specificity. Paper chromatographic studies showed that even mild conditions of drying fresh *L. glauca* leaves caused destruction of mimosine and production of some DHP. DHP had not been reported previously in extracts of *L. glauca*.

Carañgal and Catindig (1955) and Hegarty (1957) examined the free amino acids of *L. glauca* by use of two-directional chromatography. The former authors suggested that mimosine could be estimated as its ferric complex on paper chromatograms, but gave no details of technique or results.

This paper describes methods for the quantitative extraction of mimosine and DHP from leaves and seeds of *L. glauca* and from urines. Specific chromatographic techniques are described for the analysis of these extracts. The method requires only simple apparatus and has been used for the analysis of large numbers of samples.

## II. Experimental

### (a) *Materials*

*Hydrochloric acid*: 0·1N, 0·2N, 6N, 10N.

*Cation-exchange resin*: Dowex 50-X4 (100–200 wet mesh) ($H^+$ form), from Bio-Rad Laboratories, Richmond, California, U.S.A.

*Ethanol*: 80%.

*Paper*: Whatman No. 1 filter paper sheets ("for chromatography"), 55 by 40 cm, were used for all experiments. The paper was not pretreated in any way.

*Solvent mixture*: Mesityl oxide : formic acid : water (41 : 7 : 6 by volume) was used at 10 ± 1°C. These formed a single phase mixture stable down to 0°C. Commercial grade mesityl oxide was distilled under an efficient fractionating column (75 cm of glass helices) and the fraction boiling at 127–128°C was collected and stored at −20°C. Distillation of the solvent without fractionation gave a solvent mixture in which mimosine and DHP streaked badly. Formic acid (A.R. 98%) was used.

*Ferric chloride*: 0·05% in 0·02N hydrochloric acid.

*Mimosine*: Crude mimosine was isolated from seeds of *L. glauca* by the method of Bickel and Wibaut (1946) and purified as described by Yoshida (1944). It was finally recrystallized several times from hot water.

*DHP*: Chromatographically pure DHP was prepared by vacuum pyrolysis of mimosine (Adams, Jones, and Johnson 1947) but a more convenient method with use of boiling 0·1N hydrochloric acid is described later in this paper.

### (*b*) *Methods*

(i) *Extraction of Mimosine and DHP and Separation from Interfering Substances*

(1) *Procedures for Plant Material.*—Small samples (1–5 g) of fresh leaf material are preserved by placing immediately in sufficient 0·2N hydrochloric acid (HCl) to give a final concentration of approximately 0·1N after making allowance for the moisture content of the sample. The leaves are later ground in a glass pestle and mortar, and the insoluble material is filtered or centrifuged off, and washed with 0·1N HCl until the washings give a negative test with ferric chloride. The washings and extracts are combined and made up to a known volume with distilled water. A volume of 50–100 ml/2 g fresh weight is convenient. Larger quantities of leaves are similarly preserved in 0·1N HCl, extracted in a macerator, filtered, washed, and made up to volume.

Dry leaf material and seed are ground in a micro-hammer mill to pass a 1·0 mm sieve and stored in screw-capped bottles for analysis. Leaf material (0·5 g) and seed (0·2–0·5 g) are thoroughly ground with 0·1N HCl (20–30 ml) in a glass pestle and mortar and allowed to stand overnight. The contents of the mortar are transferred to a 50 ml centrifuge tube with 0·1N HCl (5 ml) and centrifuged. The residue is washed with 0·1N acid (5 ml) and recentrifuged. The residue is transferred with 20–30 ml of 0·1N HCl to a 50 ml conical flask which is stoppered and shaken for 5 hr on a mechanical shaker. The suspension is centrifuged and the residue washed with 2×5 ml of 0·1N HCl. All extracts and washings are combined and made up to 200 ml with distilled water—which should make the solution about 0·05N with respect to hydrochloric acid.

(2) *Ion-exchange Resin Chromatography.*—The plant extract (20–40 ml containing 6–8 mg of mimosine) is purified on a column (80 by 7 mm) of Dowex 50-X4 cation-exchange resin ($H^+$ form). The extract is allowed to pass through the column under gravity and the column is then washed with distilled water (18 ml). Ethanol (80%, 18 ml) is then put through the column to desorb polyphenolic substances (Thompson, Morris, and Gering 1959). The ethanol is eluted with distilled water (15 ml). Air pressure (3 in. of mercury) applied to the top of the column has been used to speed up the ethanol and water washes. Organic cations are displaced from the column with 2N ammonium hydroxide ($NH_4OH$) (15 ml), the effluent being

collected in a tapered 15 ml tube which has been accurately calibrated in the region of 1·5 ml. Collection of eluate is commenced when the ammonia front is within 1 cm of the bottom of the resin bed. The eluate is concentrated in the tube at 40°C under reduced pressure to a volume of approximately 1 ml. One drop of 2N $NH_4OH$ is added and the tube carefully tilted and rotated to dissolve material on the walls. The solution is finally made up to the calibration mark, transferred to a small stoppered sample tube, and stored at 5°C for analysis.

(3) *Procedures for Urines.*—Urines are stored at −20°C. Samples for analysis are allowed to come to room temperature, thoroughly mixed, and centrifuged. In the analysis for mimosine and free DHP an aliquot (5 ml) of the supernatant in a centrifuge tube is acidified to pH 3 with 2N HCl, and an equal volume of absolute ethanol is added. The mixture is kept at 5°C overnight and then centrifuged. The supernatant is removed and the residue washed with 2×2 ml of 50% ethanol. The combined supernatant and washings are concentrated to less than half their original volume at 40°C under reduced pressure. Urine concentrates are diluted to three times their volume and absorbed on columns (130 by 7 mm) of Dowex 50-X4. The columns are washed with water and 80% ethanol, and cations are displaced with ammonium hydroxide as described in subsection (2) above.

Total DHP in urines is estimated after acid hydrolysis. Suspended material is removed, and the urine (2 ml) is made 6N with respect to hydrochloric acid and is hydrolysed in a sealed tube at 110°C for 4 hr. The sample is then centrifuged and the precipitate washed with 2×1 ml of 6N HCl. Supernatant and washings are combined and concentrated to dryness *in vacuo* at room temperature. Water (3 ml) is added and the solution treated with absolute ethanol as described for mimosine and free DHP analyses.

(ii) *Paper Chromatography and Quantitative Estimation*

Aliquots (10–50 μl) of the prepared samples are applied in duplicate on sheets of Whatman No. 1 paper together with aliquots (10–80 μl) of standard solutions of mimosine (1·98 mg/ml) and DHP (1·11 mg/ml). Papers are placed in tanks for descending chromatography, and allowed to equilibrate with the solvent mixture at 10 ± 1°C for 5 hr. The sheets are developed with the solvent mixture (30 ml per sheet) at 10°C for approximately 15 hr. They are withdrawn from the tanks and dried in a fume hood at room temperature.

The paper is lightly sprayed with ferric chloride reagent to reveal the ferric-positive substances. Tracings of typical chromatograms are shown in Figure 1. Mimosine and DHP, which appear as orange-red spots, are cut out in ⅜ in. squares and placed in 3 by 1 in. flat-bottomed sample tubes. Blank areas of similar dimensions and positions on the paper are treated in the same way. Ferric chloride reagent (5 ml) is added to each tube, completely covering the pieces of filter paper, and the tubes are stored in the dark for 15 min, and are gently agitated twice during this period. The absorbance of the purple solution is read against a distilled water blank at 535 mμ in a 2 cm cuvette, after allowing 30 sec for detached paper fibres to settle.

*(c) Tests of Methods*

(i) *Recoveries*

The methods have been tested on fresh leaves of *L. glauca*, on leaves dried at different temperatures, on seeds, and on sheep urines containing mimosine and DHP

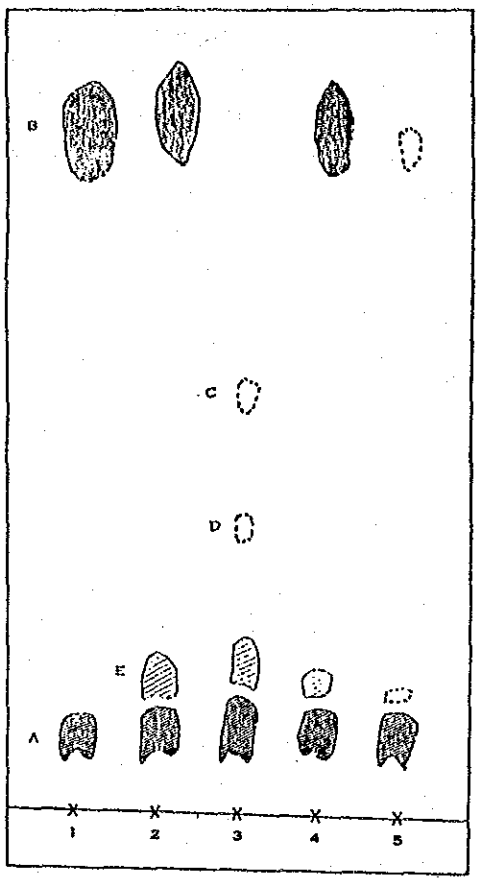



Fig. 1.—Typical chromatograms of extracts of *L. glauca* and sheep urine. Mesityl oxide : formic acid : water (41 : 7 : 6 by vol.) was used as the solvent. Colour was developed with ferric chloride solution. *1*, standards; *2*, urine from sheep fed on mimosine via the rumen; *3*, urine from sheep fed on mimosine intravenously; *4*, *L. glauca* dried at 60°C; *5*, fresh *L. glauca*. *A* is mimosine; *B* is DHP; *C*, *D*, and *E* are unidentified compounds. Hatching indicates approximately the intensity of the coloured spots.

(Table 1). Recoveries of mimosine added to extracts of the samples mentioned above have been within the range 98–102%. The recoveries of DHP added to various plant extracts and urines have also been satisfactory (Table 1).

Appendix 20 - (6)

DETERMINATION OF MIMOSINE AND 3,4-DIHYDROXYPYRIDINE

TABLE 1

ESTIMATIONS AND RECOVERIES OF MIMOSINE AND DHP

| Sample | Mimosine (mg) | | Recovery (%) |
|---|---|---|---|
| | Added | Found | |
| Fresh *L. glauca* leaf extract: | | | |
| 5 ml | | 8·35±0·07* | |
| 2 ml (3·34 mg mimosine) | 4 | 7·32±0·10* | 100±3* |
| *L. glauca* leaf air-dried: | | | |
| 0·5 g | | 32·70±0·57† | |
| 0·5 g | 20 | 53·08±0·42* | 102±1* |
| *L. glauca* leaf dried at 45°C: | | | |
| 0·5 g | | 29·05±0·26† | |
| *L. glauca* leaf dried at 60°C: | | | |
| 0·5 g | | 21·74±0·13* | |
| 0·5 g | 20 | 41·30±0·18* | 98±1* |
| *L. glauca* seed ground: | | | |
| 0·5 g | | 42·7±0·75† | |
| 0·028 g (2·46 mg mimosine) | 4 | 6·44±0·10† | 99±3† |
| Fresh white clover leaf extract: | | | |
| 5 ml | 4 | 3·89±0·04 | 97±1 |
| Sheep urine: | | | |
| 5 ml | | 5·7±0·09* | |
| 5 ml | 10 | 15·7±0·15* | 100±2* |
| Sheep urine: | | | |
| 5 ml | | 0·07±0·01* | |
| 5 ml | 2 | 2·10±0·06* | 102±3* |
| | DHP (mg) | | |
| | Added | Found | |
| *Sheep urine:* | | | |
| 5 ml | | 0·129±0·01* | |
| 5 ml | 1·387 | 1·525±0·03* | 101±2* |
| *L. glauca* leaf dried at 60°C: | | | |
| 0·5 g | | 3·59±0·05* | |
| 0·5 g | 2 | 5·54±0·08* | 100±2* |

* Standard deviation of determinations on three separate aliquots of plant extract or urine sample.

† Standard deviation of determinations on three separate samples.

(ii) *Specificity of Chromatographic Techniques*

Since there are no other published methods specific for the estimation of mimosine and DHP in biological extracts we have checked the specificity of our chromatographic techniques in the following ways.

Various purified extracts were chromatographed in two directions in phenol : water and butanol : acetic acid : water (Smith 1960) and in one direction in the proposed solvent mixture. No additional ferric-positive spots were detected on the two-directional chromatograms. Other solvent systems were investigated. Phenol : water, butanol : ammonia : water, and lutidine : water (Smith 1960) were unsuitable because of poor separations, excessive streaking, or high paper blank. Butanol : acetic acid : water (120 : 30 : 50 by volume) at room temperature (Smith 1960) gave separations qualitatively similar to those obtained with the mesityl oxide solvent mixture. Quantitative measurements of mimosine in various extracts were carried out in the two solvent mixtures.

There was good agreement in the mimosine content of extracts of dried leaf in the two solvents. However, in fresh leaf extracts the butanol solvent gave higher mimosine values (6·16 mg/2 ml) than the proposed solvent (5·67 mg/2 ml). This suggested that either the separation of mimosine from other ferric-reactive substances was inferior in the butanol solvent or that losses of mimosine occurred in the proposed solvent. The quantitative recoveries obtained in the mesityl oxide solvent and variable recoveries in the butanol solvent showed that only the former was suitable for quantitative analysis.

(iii) *Completeness of Extraction*

Samples of dried leaf and seed (0·5 g) were extracted as previously described, but the extracts from the two stages were kept separate. The residue was shaken with 0·1N HCl (20 ml) for a further 12 hr. All three extracts were then analysed. The three extractions removed 96·2, 3·7, and 0·1% respectively of the total extractable mimosine of dried leaf. Similar results were obtained for seed and for DHP in dried leaf. The third extraction was therefore considered unnecessary.

(iv) *Stability of Colour*

The development and stability of the final colour were found to depend on the pH of the ferric chloride solution, in agreement with the results of Matsumoto and Sherman (1951). Below pH 1·5, colour development was low; above pH 2·8 the typical purple colour was not produced. The ratio of ferric chloride to mimosine or DHP was found not to be critical. Standard solutions of mimosine and DHP developed with ferric chloride solutions containing 0·25 to 0·75 g per litre gave the same absorbance.

Light was found to cause rapid fading of the purple ferric chloride complex in the test-tube, a fact not previously reported. The absorbance of solutions decreased by up to 50% on standing in diffused daylight for 30 min, but no change in absorbance occurred in solutions kept in the dark for 4 days. The colour developed on the lightly sprayed paper was stable. No decrease in absorbance of the eluted colour was observed in chromatograms which had been kept in diffused daylight for 5 hr after spraying. Sprayed chromatograms kept for 7 days in the dark also showed no loss of colour.

(v) *Losses during Storage of Samples*

Analyses were usually completed within 3 weeks of collection of the sample. Under these conditions mimosine and DHP added at the initial extraction were

recovered quantitatively (Table 1). The following observations were made on changes during storage in the mimosine content of the original samples, extracts, and final solutions.

The mimosine content of a 0·1N HCl extract of fresh leaf material decreased from 8·7 to 8·1% on storage at 4°C for 6 months. The mimosine content of the dried samples also decreased on storage at room temperature in dark, screw-capped bottles. Leaf material dried at 60°C decreased from 5·0 to 4·6% mimosine over 6 months and to 4·2% after a further 16 months. No changes in mimosine content occurred in urines stored at −20°C for 12 months, but a slight increase in free DHP was noticed, presumably due to slow hydrolysis of the conjugated form(s). The greatest losses on storage have been observed in the final solutions for paper chromatography. Losses of mimosine of up to 25% in 9 months' storage at 4°C have been observed in the final solutions from urines, while similar solutions from plant material have shown a loss of 40% in 5 months.

(vi) *Stability of Mimosine in Plant Material*

Matsumoto, Smith, and Sherman (1951) showed that considerable decomposition of mimosine occurred when fresh leaves of *L. glauca* were subjected to conditions of moist heat. These authors were attempting to produce maximum decomposition of mimosine so that the material could be used as a poultry ration. One object of the present study was to devise conditions for the drying and preserving of plant materials such that decomposition of mimosine would be minimized.

A large quantity (1500 g) of fresh *L. glauca* leaves (including petioles) was collected. The collection was limited to the unopened leaf and the fully opened two top leaves of the plant. These were cut in two and the whole thoroughly mixed before subsampling. Four subsamples, each 250 g, were treated as follows. One (sample 1, Table 2) was placed immediately in sufficient 0·2N HCl to give a final concentration of 0·1N. Samples 2, 3, and 4 were dried at room temperature in a current of air, in a forced draught at 45°C for 10 hr, and at 60°C for 3 hr respectively. The samples were then analysed. The results, expressed on an oven-dry basis, are shown in Table 2.

The substance of high $R_F$ value on chromatograms of extracts of samples in Table 2 (see Fig. 1) was shown to be identical with DHP by cutting out the spot, eluting the compound, and co-chromatographing it with authentic DHP. The chromatography was carried out in two directions with four different solvent mixtures.

(*d*) *Conversion of Mimosine to DHP by Boiling* 0·1N *Hydrochloric Acid*

Previous investigators (Yoshida 1944; Matsumoto and Sherman 1951) extracted mimosine from plant material by boiling with 0·1N HCl for 1–2 hr. Adams *et al.* (1945) showed that mimosine was unaffected by long refluxing with constant boiling hydrobromic or hydriodic acids. Bickel and Wibaut (1946) recovered one of the nitrogen atoms of mimosine as ammonia, but only after 28 days at 170°C in 38% hydrochloric acid. However when mimosine was refluxed with 0·1N HCl for short periods (1–3 hr) and the reaction mixture analysed by the paper chromatographic

method, a new substance chromatographically identical with DHP was detected. This substance was isolated and identified in the following experiment.

Mimosine (2·8 g) was refluxed with 0·1N HCl (200 ml) for 3 hr and the solution concentrated to dryness in a rotary evaporator. The crystalline residue was dissolved in water and placed on a column (15 by 2 cm) of Dowex 50-X4 ($H^+$ form) (100–200 mesh), and the column was washed with water (300 ml), after which 0·1N HCl (900 ml) was used to elute the DHP, leaving unhydrolysed mimosine on the column. The acid eluate was concentrated to dryness to give a yellow crystalline hydrochloride (1·8 g) which was converted to the free base (1·3 g) by the method of Hirs, Moore, and Stein (1954). This was purified by recrystallization from absolute ethanol, high vacuum sublimation, and finally by recrystallization from ethanol. The product was obtained as white needle-like crystals, m.p. 243–244°C (after sintering at 235°C), undepressed on admixture with an authentic sample of DHP of the same melting point. (Found: C, 53·9; H, 4·6; N, 12·6%. Calc. for $C_5H_5O_2N$: C, 54·0; H, 4·5; N, 12·6%.) The infrared spectrum* was identical with that of an authentic sample of 3,4-dihydroxypyridine.

TABLE 2

EFFECT OF DIFFERENT TREATMENTS ON THE MIMOSINE AND DHP CONTENT OF FRESH L. GLAUCA LEAF

Results expressed on oven-dry basis

| Sample | Treatment | Mimosine (%) | DHP (%) |
|---|---|---|---|
| 1 | Fresh leaf in 0·1N hydrochloric acid | 8·7 | Tr.* |
| 2 | Air-dried at room temperature | 6·4 | Tr. |
| 3 | Dried at 45°C for 10 hr in forced draught | 6·3 | 0·2 |
| 4 | Dried at 60°C for 3 hr in forced draught | 5·0 | 0·7 |

* Tr., trace.

The hydrolysis of mimosine by boiling dilute acid was further investigated by refluxing mimosine (39·6 mg, 0·2 mmole) with 0·1N HCl (20 ml) and withdrawing aliquots at hourly intervals. After removal of the acid, these were analysed for mimosine and DHP. The results are presented in Figure 2.

After 0·5 hr of refluxing, 16% conversion of mimosine to DHP had occurred. Boiling 0·1N acid should therefore not be used to extract mimosine from biological material. Mimosine is stable in 0·1N HCl at room temperature for several weeks. When mimosine was refluxed with concentrated hydrochloric acid no appreciable conversion to DHP occurred, which confirmed the results of Adams *et al.* (1945). Mimosine added to urine and hydrolysed with 6N HCl at 110°C for 4 hr was recovered quantitatively.

* The infrared spectra were measured in Nujol and hexachlorobutadiene by Mr. H. J. Schnitzerling, CSIRO, Veterinary Parasitology Laboratory, Yeerongpilly, on a Perkin-Elmer model 21 spectrophotometer.

## III. Discussion

The low solubility of mimosine in aqueous solvents and almost complete insolubility in organic solvents have been a major problem in developing quantitative methods. The unusual lability of mimosine in hot, dilute acid and its insolubility in cold 80% ethanol necessitated the two-stage, cold acid extraction. Concentrated eluates from the resin columns may contain up to 15 mg of mimosine, and the technique of adjusting to final volume was devised to overcome the difficulty of redissolving the mimosine crystallized on drying. The insolubility of mimosine has been one of the limiting factors in the choice of chromatographic solvents (see Section II(c)(ii)). The two-phase solvent mixture of Bryant and Overell (1951) was modified to a suitable single-phase mixture by the technique of Smith (1960). In it mimosine and DHP were separated from all interfering substances. Brown pigmented material present in solutions for chromatography remained at the point of application.

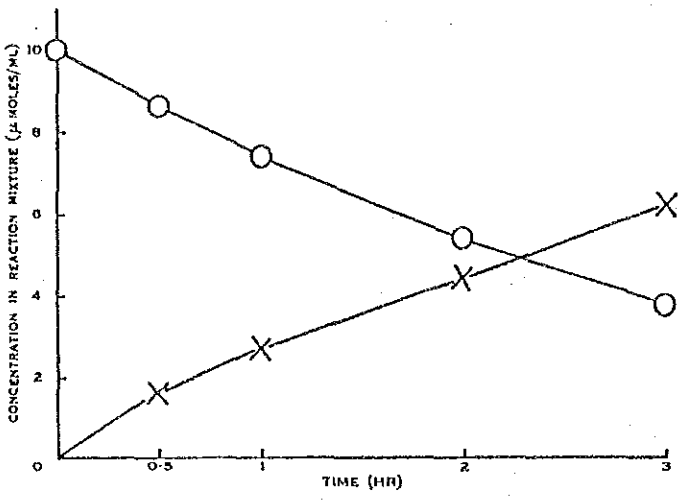



Fig. 2.—Conversion of mimosine to DHP in boiling 0·1N HCl.
°O Mimosine; × DHP.

Other compounds giving ferric chloride colours similar to that of mimosine and DHP have been detected on some of our chromatograms (see Fig. 1) but usually in amounts too small for quantitative analysis. As little as 2 $\mu$g of mimosine can be detected visually on paper, but about 10 $\mu$g is needed for accurate measurement of absorbance under the conditions of the method. These other compounds have not been identified, but as they are well separated on the chromatograms it is likely that once identified, they could be estimated by the proposed method.

The ease with which DHP was separated from mimosine on a Dowex 50 column by elution with 0·1N hydrochloric acid made it necessary to control the pH and volume of the acid extract placed on the column. No loss of DHP was observed with volumes of extract up to 40 ml (approx. 0·05N) containing up to 2·2 mg of

DHP. In early work 0·5N hydrochloric acid was used as an extracting agent, but the two-stage extraction was found necessary for quantitative results, and the 0·5N acid extract could not be applied directly to the columns without loss of DHP.

It can be seen (Table 2) that even mild conditions of drying caused appreciable losses of mimosine. The most convenient method of preserving fresh plant material with the minimum loss of mimosine has been to place it immediately in 0·2N hydrochloric acid. Samples 1 and 2 contained only traces of DHP. In other experiments, sap from *L. glauca* leaves was expressed on to a filter paper sheet, dried rapidly in a stream of cool air, and chromatographed. Trace amounts of DHP were found. It is not known whether the DHP was present in the intact leaf or was an artefact of the method of extraction. Drying fresh *L. glauca* leaves at higher temperatures resulted in increased formation of DHP, but approximately 25% of the mimosine originally present in the fresh leaf (sample 1) could not be accounted for as mimosine plus DHP in samples 2, 3, and 4. No increase in mimosine content of sample 4 resulted after hydrolysis for 4 hr at 110°C in 6N hydrochloric acid, which showed that no mimosine was present in combined form. The fate of the mimosine which could not be accounted for is not known. The results shown in Table 2 indicated the need for careful control of preparative techniques in studying the variation in mimosine content among strains of *L. glauca*. An investigation of this variation is at present being made by means of the described analytical method.

The conversion of mimosine to DHP (which probably exists as 3-hydroxy-4-pyridone) by boiling 0·1N hydrochloric acid is of interest because of the stability of mimosine to hot, concentrated acids. The dissociation constants of mimosine are: $pK_1$, 2·1 (COOH); $pK_2$, 7·2 ($NH_3^+$); $pK_3$, 9·2 (OH) (Spenser and Notation 1962), and in acid solution it will exist as a mixture of uncharged and variously charged forms. Those forms relevant to the present discussion are thought to be the uncharged molecule (I), the form (II) in which the α-amino group is protonated, the form (III) in which the ring nitrogen is protonated, and the form (IV) in which both nitrogen atoms are protonated. In 0·1N hydrochloric acid, II will predominate, but there seems to be no mechanism by which this form can undergo N-alkyl cleavage. Cleavage of IV would be expected to be an extremely slow reaction (Hine 1956). The equilibria II ⇌ I ⇌ III are likely to be immeasurably fast, and it is suggested that mimosine is converted to DHP in boiling 0·1N acid by an attack on the primary side-chain carbon atom of III by the neighbouring α-amino group (Hine 1956), and resultant N-alkyl cleavage. It is also suggested that in concentrated acids the stable form IV predominates to the almost complete exclusion of other forms.