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MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 3 F - 23

(1) Half Yearly Rpt 1/8/86 rec'd 7/29/88

(2) Final Rpt 11/27/87 rec'd 7/29/88

Attachments

PN-ABA-526

3.F.23

FINAL

SEWAGE GROWN LEMNA SPECIES AS A PROTEIN SOURCE FOR CHICKENS

LEMNA REPORT FROM
THE PERU DUCKWEED GROUP
TO USAID, LIMA.

NOVEMBER, 1987
LIMA-PERU

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SEWAGE GROWN LEMNA SPECIES AS A PROTEIN SOURCE FOR CHICKENS

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PROJECT SUMMARY

Sewage grown Lemna, with a protein content and amino acid profile similar to soy meal, can be successfully used to provide the protein component in poultry diets. Acceptable levels of Lemna in the diets of layers range between 5% to 25% of total feed. Lemna fed layers produce more eggs of the same or higher quality as controls birds fed standard diets. Levels of up to 15% Lemna produce equal growth rates in broilers and diets consisting of up to 15% Lemna species are suitable for feeding chicks under 3 weeks of age. We have also demonstrated that in commercial poultry farms 5% Lemna diets produce higher pigmentation and equal or better growth than do standard rations.

Given that corn and soy meal, the key ingredients in poultry feeds are imported, Peru stands to benefit from foreign exchange savings through the development of a sewage-Lemna-poultry industry. In many third world countries, where fish meal and/or soybean are unavailable, Lemna represents a potentially abundant and easily utilized source of high quality protein which can be produced locally using unexploited resources such as sewage or waste animal manures.

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I. INTRODUCTION

A. General description of Lemna

Duckweeds, (*Lemnaceae*), a family of small freshwater macrophytes, are the smallest and most simple of flowering plants, ranging in size from the *Spirodella polyrrhiza*, which reach 1.5 cm in diameter, to the pin-size *Wolffia arrhiza*. These floating plants which grow in dense blanket-like clusters on the surface of nutrient-laden, open fresh-water bodies have, over the years, received considerable attention from scientists studying basic plant physiology and biochemistry. The unique ability of Lemna species to reproduce by cloning, combined with their structural simplicity render them ideal experimental organisms in the study of plant cell function. (Hillman, 1961). In recent years, however, the focus of attention has shifted to Lemna species' potential as a biological agent in the treatment of waste-water, (Hillman and Culley, 1978; Culley and Epps, 1973; Harvey and Fox, 1973; Rusoff et al., 1977; Porath et al., 1979) and as a byproduct of that process, its utility as a feedstuff for fish (Shireman et al., 1978(a) and 1978(b); Van Dyke and Sutton, 1976) livestock (Rusoff et al., 1977, 1978, and 1980; Hillman and Culley, 1978) and poultry (Abdulayef, 1969; Muzafanou, 1968; Truax et al., 1972; Mutzar et al., 1976, 1977, 1978(a), 1978(b), 1978(c); Johri and Sharma, 1980).

There are 4 common genera of Lemna species (*Lemna, Spirodella, Wolffia* and *Wolffiella*) and more than 40 known species with a worldwide distribution (McClure, 1966). We found two species of duckweed, *Lemna gibba* and *Wolffia arrhiza*, growing naturally on the San Juan de Miraflores oxidation lagoons in the southern cone of Lima, Peru. *Lemna gibba*, a duckweed with a pea-like gibbous structure and *Wolffia arrhiza*, the smallest species (0.2-0.4 mm), were found growing in large stands in tertiary lagoon effluent and lagoon runoff. Growth blooms at San Juan de Miraflores are seldom coincident, resulting in pronounced swings in the percentage of the two species, although *Lemna gibba* was always dominant.

B. Project Rationale

The nutritive value of Lemna as poultry feed has long been recognized within the scientific community (Boyd, 1971). Among more recent studies, Truax et al., 1972 demonstrated that dehydrated Lemna, when substituted for dried alfalfa meal at up to 5% of mixed poultry feeds, produces superior weight gain in chicks of up to 3 weeks of age. They attributed this phenomenon to Lemna species' well balanced amino acid profile. Truax et al., 1972 also demonstrated in comparative feeding studies, that chickens fed diets consisting of up to 10% Lemna consistently weighed more than chickens fed diets containing similar percentages of alfalfa meal, as well as chickens fed exclusively on an "optimal" control diet.

Duckweed has also been proposed as an aquaculture mechanism for sewage treatment (Sutton and Ornes, 1977 and Oron, 1986) because of it's high growth rates and its ability to efficiently extract nutrients and trace elements from water. The melding of these two concepts suggests an efficient, low-cost sewage treatment process which produces a high quality poultry feed constituent as a byproduct.

We decided to first test the demand side of the equation before concentrating on the more expensive supply side (i.e., engineering efficient Lemna species based sewage treatment facilities). The Peru Duckweed project was therefore designed to test the utility of sewage grown Lemna species as poultry feed. Successful results would warrant subsequent research and development efforts to design efficient small and large-scale Lemna species based sewage treatment facilities.

C. Project Objectives

The overall project goal was to determine the safety and efficacy of sewage-grown Lemna species as feed for layers, broilers and chicks. Specific objectives included:

1. To determining the pathogenicity and toxicity of dry and wet harvested Lemna species.

2. To determine the relative contribution of Lemna species supplemented feeds to the prevalence of human enteric bacteria in poultry.

3. To determine the acceptability of fresh (wet) Lemna species as a feed supplement to commercial poultry feeds.

4. To determine the metabolizable energy of Lemna species meal as feed for poultry.

5. To determine the relative effect on egg laying performance of Lemna species substitution, for varying amounts of conventional protein constituents (soy and fish-meal), in controlled isocaloric and isonitrogenous feeding experiments.

6. To determine the relative effect on broiler performance of Lemna species substitution, for varying amounts of conventional protein constituents, in controlled isocaloric and isonitrogenous feeding experiments.

7. To determine the relative effect on chick performance of Lemna species substitution, for varying amounts of conventional protein constituents, in controlled isocaloric and isonitrogenous feeding experiments.

D. San Juan Sewage Lagoons

The San Juan de Miraflores lagoons, located in the southern cone of Lima Peru, consist of eighteen one to three hectare oxidation ponds configured in series to form three distinct sewage treatment facilities. The flow-through system is typical of an oxidation treatment facility designed for the tropics. Sewage flows successively through four ponds before finally being discharged to surrounding farm and grazing land. Lemna species was found growing naturally in all primary, secondary, tertiary lagoons. *Tilapia nilotica* fish, remnants of an experimental fish cultivation study conducted in the facility's quaternary lagoons, are thought to ingest all the Lemna species entering or growing in those lagoons. The best Lemna species growth was found to occur in a single 2.25 ha. tertiary lagoon which, had a stand of Lemna species throughout the year. Lemna species used in the study was harvested (weekly) from this lagoon as well as from a shallow pond formed from a lagoon runoff located West of the main lagoon batteries.

Lemna species growth varied considerably throughout the year. Several factors were observed to contribute to this phenomenon:

1. Wind and Water Level Fluctuations play a major role in depleting standing populations of Lemna species -- leaving it stranded on the shore. In order to prevent crowding of duckweed against the shore, as well as to effect a more even distribution of Lemna species throughout a lagoon, efforts were made to devise efficient and inexpensive floating baffles. The most successful effort involved stringing long strands of green carriso (a thin hollow bamboo-like cane) together and floating them across a lagoon. The carriso strands sprouted, sending up small leafy fronds which also helped buffer Lemna species from the wind. This is the method current being used to partially protect Lemna species from the strong southwest wind.

2. During the study two major die-offs were observed to occur in the main tertiary lagoon used by the study. Virtually the entire standing crop of Lemna species died. Die-offs were only witnessed in the one tertiary lagoon. Plant populations growing in other lagoons, while considerably smaller than that found in the main tertiary lagoon, remained stable. The die-offs occurred during the summer season (Feb and March) in both years of the study. The growth rate of tertiary lagoon Lemna species appeared to decline after the first die-off and never returned to pre-die-off levels. Interestingly, Lemna species growing in the runoff lagoons never exhibited any signs of disease or die-off. Growth rates remained stable allowing the runoff ponds to serve as reliable sources of Lemna species for the remainder of the study.

During May of the second year we found a large concentration of water fleas in our harvests; samples of which were dried and analyzed in the laboratory and found to contain approximately 30% protein -- close to that of Lemna species.

E. Harvesting and Drying

The prevalence of a brisk southwest breeze at the San Juan site ensures Lemna species crowding against the northeast shorelines of lagoons. Lemna species was manually harvested by lowering a tined leaf rake beneath the Lemna species from the perimeter of the lagoons and manually removing it into a wheelbarrow, bucket or plastic bag. The wet harvested Lemna species was then taken to the Food Processing Plant at The Universidad Nacional Agraria (UNA) in Lima. There, the two species were separated by washing them through several sieves. Lemna species was then sun dried to approximately 40% humidity on concrete aprons. Drying was completed to 10% total moisture using the Food Processing Plant's forced air oven for 15-30 minutes at 60°C. Complete sun drying was avoided to minimize pigment loss through ultraviolet exposure. This method ensured the retention of high xanthophyll levels (800 - 1000 ppm) in the finished Lemna meal. The dried Lemna was stored at room temperature in 200 liter black plastic feed sacks. This storage method minimized protein and pigment loss, with no spoilage occurring for the duration of the study.

F. Characteristics of Lemna species

Biochemical analyses of dried Lemna species samples were conducted at the IIN and Purina laboratories. Results generally confirmed expectations. Protein (N x 6.25) content in particular, which ranged from 25% to 39%, was well within the expected range. Values for protein, ash, carbohydrate and crude fiber of samples taken from several harvests are presented in Table 1.

The value of Lemna species as a feedstock is highly dependent on its amino acid profile. Amino acid analysis were performed at the laboratories of both the University of Maryland and Johns Hopkins University on samples harvested from primary, secondary and tertiary lagoons. The amino acid profile of Lemna species was found to be similar to that of soybean: high in lysine and somewhat deficient in methionine relative to most animal proteins. Initial results indicate considerable variation between samples. See Table 2.

The protein content of Lemna species is sensitive to levels of available nutrient in the aquatic environment (Humphrey, 1977). Consequently, Lemna found in relatively clear, low-nutrient waters grows slowly and contains high levels of crude fiber, ash and carbohydrates, but relatively little protein. In contrast, Lemna grown on sewage lagoons grows rapidly and has a high protein content. We also analyzed samples of Lemna species taken from an unmanaged stand in Lago Titicaca near Puno, Peru. Lemna species from this stand had a protein content of 18% and a relatively high fiber content. These results are characteristic of Lemna species which has not been harvested frequently.

Xanthophyll (pigment) is, from a commercial perspective, a very important component of Lemna species, and deserves specific mention. The xanthophyll content of wet Lemna species harvested from the San Juan lagoons was found to be extremely high - up to 1938 ppm. Exposure to direct ultraviolet radiation results in rapid degradation, however, with xanthophyll levels as low as 282 ppm recorded for Lemna species completely dried in the sun. See Table 1.

II. THE EXPERIMENTS

A. The Pathogenicity and Toxicity of Lemna species Meal

1. Heavy Metals

The uptake of toxic elements and compounds as represented by the ash component of Lemna species is highly dependant on the richness of the growth medium. In a high growth phase (i.e., suitable temperatures and high concentrations of nutrients in the growth medium) Lemna species plants grow extremely rapidly, continuously dividing and subdividing. Each plant, therefore, maintains a "young plant" profile: high protein, low fiber and low ash. Heavy metals, complex organics and inorganics represent only a minuscule portion of the entire plant biomass. In conditions where nutrients are constrained, however, Lemna species plants grow old - processing large amounts of water without subdividing or significantly increasing their biomass. Lemna species in this low nutrient-low growth phase acts like an organic vacuum cleaner; absorbing virtually anything in its effort to grow. It can directly employ complex organics as food, fixes nitrogen directly from the atmosphere, and removes trace amounts of phosphorus from the water. Having survived for some time in such a "hungry" condition (low nutrient) Lemna species plants have been shown to contain surprisingly high concentrations of toxic minerals and metals (Muztar et al., 1976).

We tested the heavy metal content of the Lemna species growing in primary, secondary and tertiary lagoons at the San Juan site. Dried samples were subjected to standard testing at the OSHA laboratory in Salt Lake City for the presence of toxic substances. Lemna species taken from the primary lagoon tested positive for Zinc (.0063%), Antimony (.0126%) and Copper (.0066%); Lemna species harvested from the secondary lagoon tested positive for Zinc (.0031%); and Lemna species taken from the tertiary lagoon also tested positive for Zinc (.0022%). All these values are well below toxicity standards applied to feedstuffs in the United States. We can conclude, therefore, that Lemna species harvested from the San Juan lagoons under conditions of normal growth and frequent harvesting are chemically safe for human or animal consumption.

2. Human Pathogens

In numerous replications (over 30 samples) Lemna species taken from all three San Juan lagoons (primary, secondary and tertiary) were washed in fresh water, and then comprehensively tested for the presence of enteric bacteria. In these studies when very selective conditions were used for isolation of Salmonellae we were able to isolate this bacteria from both the plants and the water. Other pathogens isolated from recently harvested wet Lemna species were Aeromonas and Vibrio species. Both, like salmonellae, are common environmental contaminants found in fresh water lagoons and on water plants under natural conditions. We were unable to detect the presence of these or any other human enteric bacteria on the dried Lemna species meal. We were able to conclude that dried Lemna species meal is bacteriologically safe as a feedstuff for animals.

Bacteriology of the San Juan Lagoons

The San Juan oxidation lagoons are used to process sewage in the Southern part of Lima. We examined bacteriologically at about biweekly intervals the water and the Lemna plants in a primary, secondary and tertiary treatment lagoon, from September 1985 to November 1986. The following assays were performed. We enumerated the fecal coliform and total aerobic plate counts. We also quantitatively determined the concentration of Aeromonas species in both the water and the Lemna plant using Ampicillin containing Blood Agar. We also used TCSB agar to isolate and quantify the number of Vibrio colonies. Salmonella species were isolated using double strength Selenite broth. The identity of all strains was confirmed using both API and other appropriate biochemical tests. Total plate counts and fecal coliform counts were performed using Standard Methods.

Results of Water and Lemna Bacteriology

We found that Lemna concentrated bacteria from the water. See table 3. This finding has been reported previously by Spira et. al., in Bangladesh (1981) and Gilman et. al., in India (1983).

Oxidation lagoons are able to produce considerable decreases in the concentration of fecal coliform bacteria and parasites such as *Giardia lamblia*. There was however, a paradoxical increase in the concentration of Aeromonas species and *Vibrio cholerae non-Ø1* in the secondary and tertiary lagoons in comparison to concentrations of these bacteria found in the primary lagoon. These two bacteria are not normally looked for in water studies. Yet, there is strong evidence that both may be enteropathogens responsible for human disease.

Vibrio cholerae was not isolated either from plants or water in any lagoon from the middle of July until the end of November in 1986. At all other times however *Vibrio cholerae Ø1* was isolated regularly from both water and plants of both plants. In the tertiary lagoon when *Vibrio cholerae-non Ø1* was isolated it was nearly always in concentrations above 3.Ø log1Ø CFU/ml.

Salmonella species were isolated regularly from the primary lagoon but was isolated only in 14% of the water samples taken from the tertiary lagoon. Fecal coliform counts decreased from 6.2 log1Ø CFU in the primary lagoon to 4.9 log1Ø CFU in the tertiary lagoon (p< .01). Lemna plants had a similar significant decline in concentration but with slightly higher concentrations of bacteria.

Based on these results feeding of wet Lemna to chickens or other poultry may be able to transmit enteropathogenic bacteria. Dried Lemna in contrast, carries little to no risk of transmission of enteropathogens.

3. Other Factors

In the tertiary lagoon, we observed portions of Lemna species that would turn white and eventually lose their buoyancy in a slowly spreading (outward) circle. This problem is particularly apparent during periods of slow growth resulted in significant reductions in plant populations in certain lagoons. During high growth phases the population growth far exceeds losses from the infection.

A common fungus, *(Oomycotes)* has been isolated from diseased Lemna species samples by the plant pathology department of CIP (Potato International Center) and is a possible Lemna species pathogen. Further studies will be required to determine whether or not this hypothesis is correct.

Crowded Lemna species populations (conditions for low growth) found in primary and secondary lagoons, were occationally infected with aphids. Parasites do not appear to significantly affect fast-growing populations.

4. Summary

Lemna species growing in the San Juan de Miraflores sewage lagoons represents an excellent potential source of protein for poultry feeds. It grows rapidly, and is easy to harvest. We were able to isolate Salmonellae, Aeromonas and Vibrio species from wet, recently harvested Lemna species samples but could isolate no human enteric bacteria from dried Lemna species samples. We concluded that dried Lemna species is bacteriologically safe as a feed for chickens. Similarly the Lemna species tested negative for all but a few toxic elements and compounds, and those that were detected were well below USA recommended safety levels for feedstuffs. We concluded that Lemna is both bacteriologically and chemically safe as a feedstuff for animals.

B. The Relative Contribution of Lemna to the Prevalence of Human Enteric Bacteria in Poultry

Infection of commercial poultry flocks by Campylobacter and Salmonella species is commonly reported throughout the world. Prevalence of Salmonella in US commercial poultry is high in some cases approaching 80%. Lemna would not be a safe ingredient if it caused an increase in the rate of enteropathogenic infection in chickens fed Lemna compared to chickens fed a standard diet. We also sought to determine whether Lemna feeds might contribute to infection of poultry flocks with other human enteropathogens.

We tested stool samples from live broiler chickens collected at random on five separate occasions from five different poultry wholesale centers in metropolitan Lima as well as free range chickens from Pueblos Jovenes. See Table 3a.

The results demonstrated conclusively that Lemna species feeds do not contribute to increased prevalence of human enteropathogens among layers (Table 3). The majority of samples taken from market chickens tested positive for Campylobacter species, while a significantly lower number of samples collected from experimental chickens were found to test positive. Neither market nor experimental chickens tested positive for Salmonella species.

While we did not test for the presence of common poultry diseases we were able to satisfactorily conclude that Lemna species meal does not significantly increase mortality risk for chickens. Only two study chickens died during the entire study period. This rate is significantly lower than that experienced by commercial poultry farms in the Lima vicinity.

C. Acceptability of fresh Lemna as a food supplement to commercial poultry feeds

Lemna, consisting of between 93% and 95% water requires drying, since it is generally used as a dry, stable food stuff which is then mixed with other dried feed components. Drying adds to the cost of production in any commercial Lemna production process. We decided to examine the potential for directly using wet, unprocessed Lemna as a feed supplement for broilers.

We successfully demonstrated that commercial feeds can be supplemented with 10% fresh Lemna species (on a dry weight basis). This effectively doubled the feed bulk, providing most of the liquid requirements of the chickens, as well as a 15% protein supplement over the existing commercial feed protein content. The increased bulk of this diet limits to 10% the amount of Lemna species that can be used wet. Under circumstances where transport is not a consideration this may be a suitable form of supplementation. Chickens accepted the 10% Lemna species supplemented feed with no apparent difficulty. Lemna species fed to chickens in a wet condition, potentially can transmit enteropathogenic bacteria.

D. Dried Lemna species: Determination of the Apparent Metabolizable Energy in Chicks

1. True Metabolizable Energy

Dried Lemna species samples (10% humidity) with approximately 35% protein were supplied to a student at the UNA for a True Metabolizable Energy evaluation (Sibbald, 1963(a),1963(b) and 1980). Her study, under the direct supervision of the University was performed on roosters. The animals were fed corn alone or Lemna species (15 gm, 20 gm, and 25 gm). Lemna species when fed at 3 different levels showed little variation in its true metabolizable energy valu The values obtained for the three Lemna species levels, (15 gm, 20 gm, and 25 gm.) were 2794, 2855 and 2706 Kcal/kg respectively.

The TME values for Lemna species are about the same as those obtained for toasted soybean (Hill, 1960). They are much higher than TME values obtained for other sewage products such as sludge and algae which have been fed to chickens. (Lipstein et. al., 1980(a), 1980(b), 1983 and 1984). The TME of Lemna (2700 Kcal/kg) is more than twice as high as the Apparent Metabolizable Energy (1200 Kcal/kg) determined in baby chicks.

2. Apparent Metabolizable Energy

We performed a study to measure the Apparent Metabolizable Energy of Lemna species (*Lemna gibba* and *Wolffia arrhiza*) on Baby chicks. Two methods were used for the determination of the Apparent Metabolizable Energy: a) the Total Collection Method, and b) the Indicator Method using Chromium Oxide.

The results obtained with the total collection method (1030 and 1146 kcal/kg for Lemna at 20 and 30% levels respectively) were slightly lower than those obtained by the indicator method (1382 and 978 kcal/kg for Lemna at 20 and 30% levels). However it was evident that the total collection method was a much more accurate technique. See table 4.

These studies were done under highly controlled conditions. From these results we think the ME should be specifically calculated for the age and possibly even race of bird to be used. It would appear that young birds do not digest Lemna species nearly as well as full grown roosters. Other studies also have shown differences in the ME with age (Petersen et al, 1976).

E. Layer Study - Determination of the relative effect on egg laying performance of Lemna species substitution for varying amounts of conventional protein constituents (soy and fish-meal) in controlled isocaloric and isonitrogenous experiments

Our studies were designed to examine the safety and efficacy of Lemna meal as a source of protein and pigment for laying hens, and to evaluate its impact on egg laying performance and egg quality. We performed the study on two lines of layers, Topaz and Whiteline Leghorn Hens.

1. METHODS

a Metabolizable Energy (ME)

The ME of Lemna species was determined, using the method described by Sibbald, 1963(a) and 1980) to be 2000 kcal/kg in mature roosters. This ME value was used in the formulation of the experimental diets in the first study (experiments 1a and 1b). The ME was later estimated using young broilers (0-21 days of age). The new ME value of 1200 kcal/kg was used in formulating diets for all succeeding studies. The latter value is closer to those obtained by other researchers using baby chicks.

b. Diet Formulation

All diets were formulated according to AEC requirements. Ingredients were purchased locally from the Ralston Purina Company. Dried *Lemna gibba* and *Wolffia arrhiza* were finely milled before preparation of the diet mixtures.

All diets were formulated to be isonitrogenous and isocaloric except when differences in energy between diets was the variable being tested (experiment 1b). Mixing of diets was performed in a small mill at the UNA Food Processing Plant. The pigmentation of the corn used in the diets was not able to be standardized because of the variability of the supply.

c. Measurement of Yolk and Skin Pigmentation

The Roche fan was used to measure pigmentation. Pigmentation in yolks or chicken skin was not able to be compared between different experiments because of the variability of the pigmentation of different lots of corn supplied to make each experimental diet. Thus, for example, comparison of pigment between two broiler studies done at different times and using different batches of corn is not possible. In any one experiment all chickens in the study were fed with the same lot of corn.

d. Pens

A temporary structure made of bamboo matting and fence posts was built to house the layer experiments. Individual pens measuring 2 x 3 meters were constructed within the "layer house" using wooden frames and heavy commercial grade fishing nets. Each unit was provided with a feeder, an automatic waterer, 5 nests and bedding consisting of wood shavings pre-treated for fleas.

e. Evaluation of Eggs - General Design of Operational Procedures

During a 14 day pre-experimental period in which egg production was carefully observed and hens reassigned in order to balance egg production between pens, all hens received a control diet (see table). Feed and water were supplied *ad libitum*. Feed consumption was measured weekly by subtracting residual feed from the total feed provided. Hens began receiving experimental diets on day 15. Each diet group consisted of 50 hens (5 units of 10 each). Feeding and watering and food consumption measurement protocols remained identical to those followed during the pre-experimental period. Eggs were collected, weighed and classified daily. During a monthly 'sampling week' (the fourth week of each 28 day interval during the experimental period), all eggs were weighed and classified individually, and randomly selected "standard size" (57 to 64 gm) eggs were used for external and internal quality measurements (ref). The parameters we used to measure the quality, were Haugh Units and yolk pigment. Haugh Unit was measured using a slide rule (Egg Quality Slide Rule, Designed by A.W. Brantx K.H. Norris, US Dept of Agriculture. The Kaw Co. Henniker, N.H. 03242 USA), and the pigment was measured using the Hoffman La ROCHE colorimetric fan.

f. Animals

Two different hen lines were utilized in the experiments. In the first experiments (1a and 1b) we used 41 week old TOPAZ layers, a heavy breed of hens producing brown eggs which were donated by Avicola Hannan S.A. (Pacific Breeders). In the second experiment we used a commercial line of White Highline Leghorn hens. These lighter, more delicate breed of hens which produce smaller eggs at a lower rate of production than do the TOPAZ variety, were obtained for the study at the age of 39 weeks.

g. Experiment #1a
(1) Experimental Design

Two hundred 43 week old Topaz layers were distributed into groups of 10 according to weight and placed in 21 pens following a pre-experimental period. Three diets were used in the study: a control diet with standard rations (no Lemna species), a diet with 15% Lemna, and a diet with 15% Wolffia. The diets were formulated to be isonitrogenous (17% crude protein) and isocaloric (ME of 2800 kcal/kg). See table 5. The experiment lasted 90 days including and adaptation period of 14 days during which no experimental data was collected.

Two hundred rectal swab samples (100 from control units and 100 from Lemna and Wolffia sp. fed units), were analysed for pathogens such as Vibrio and Aeromonas species, enterotoxigenic and enteropathogenic *E.coli* and Shigella and Salmonella species, using standard techniques.

Cleaning procedures of the units were carried out daily, and included change of water and bed turning. Layers were weighed individually at the end of every 28 day period.

h. Experiment #1b
(1) Experimental Design

One hundred TOPAZ layers from experiment 1a were used in this study. These layers were kept in their previous pen allocations (experiment 1a), while new diets were introduced. The control group was maintained on the same control diet and three new isoproteic diets were formulated. The new diets were the following: 25% Lemna (ME 2800 kcal/kg), 25% Lemna with a higher (ME 2900 kcal/kg) and 40% Lemna (ME 2800 kcal/kg). The study lasted 2 months. No pre-experimental or adaption periods were used since these layers had already been on diets similar to their new experimental diets.

(2) Operational Procedures

Four control units from experiment 1a were chosen at random, and remained as controls. In the same manner, six more units from the previous experimental diets were chosen and the new formulated diets were supplied. The study lasted two months.

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Feed, water, feed consumption, egg handling and pen procedures were the same as in experiment 1a. Layer weights were recorded at the beginning and at the end of the study.

i. Experiment #2

(1) Experimental Design

Two hundred, 41 week old, Leghorn White Highline hens were distributed in the pens groups of 10 according to weight. The diets used in this study were, a control (0% Lemna), 15% Lemna, and 25% Lemna with an ME of 2800 kcal/kg (see Table 5). Each diet group consisted of 40 hens (4 duplicates of 10 layers each). The study lasted 3 months including a 2 week adaption period during which no experimental data was collected.

(2) Operational Procedures

Egg production balancing procedures were identical to those in experiment 1a. Feed and water were supplied ad libitum, and feed consumption was recorded every 14 days. A the end of the pre-experimental period, the hens received the experimental diets. Sampling weeks, pen procedures and egg handling, also were identical to those in experiment 1a. Hens were weighed at the beginning and at the end of the study

(a) Biochemistry

In this experiment, protein measurements were performed on randomly selected standard size eggs collected on the last sampling week. Eggs were hard cooked to facilitate yolk and albumen separation, and stored at 4°C until assayed (Bair & Marion, 1978). Protein was measured using standard micro-Kjeldal techniques with bovine serum albumin as a standard. Shell calcium concentration was measured on standard size eggs collected on three sampling weeks in experiment 1a. Shells were digested with a HCl solution (68% v/v), and the calcium concentration was determined by Atomic Absorption (Tietz, 1980).

j. Data handling and statistics

All data was collected in forms, which were then transferred to Lotus 123 worksheets on an IBM PC and Bernoulli disks. Cleaning of the data was performed on the Lotus worksheets once all data was entered. Statistics were performed using Chi-Square, Fishers or Student t test.

k. Calculations

Data was calculated on a 28 day basis for each 10 hen unit, except for period one, where the first 2 weeks (adaption period) are not included. In addition since no significant difference was found in any parameter studied between hens receiving Lemna 25% at 2800 kcal/kg and 2900 kcal/kg the results from these two studies have been pooled.

Mean egg weights were calculated using the total number and weight of the eggs produced in each unit during each period. Conversions represent the ratio of the total egg weight produced, over the total food consumed over a week period (experiment 1a & 1b), or over a 2 week period (experiment 2).

2. RESULTS

All diets were isoproteic and isocaloric and formulated as shown in Table 5, except when the difference in energy between diets was the variable being tested (experiment 1b).

a. Experiment #1a and #1b

The inclusion of 15% Lemna and 15% wolffia in the diets, produced no significant differences in egg production, in ratio of feed conversion, or in mean egg weights when compared to the control group as seen in Table 6. In the third period (6-10 weeks), there was a slight but significant increase in the consumption of the Lemna 15% group ($p < 0.05$), when compared to the control. When comparing the initial and final periods (2 week vs 10 weeks), the control showed decreases in consumption $p < 0.001$ over time, while the Lemna 15% group, showed only a slight decrease ($p < 0.07$) in feed consumption, but a significant decline in egg production, $p < 0.009$.

In the second portion of this study (experiment 1b), we found no significant differences between the control group and the 25% Lemna fed hens in regard to any of the outcome variables shown in table 7. There was however, in the first period, a significant decrease in feed consumption of hens fed the 40% Lemna diet when compared to controls ($p < .005$). There was also a trend for decreased egg production in the first and second periods of the study between the control hens and those fed 40% Lemna species (see table 7). Feed conversion values were consistently but not significantly better when the the Lemna 15% group was compared to the other diet groups. No differences in egg weight were seen throughout the study.

The decline over time, for all groups, in rates of egg production and feed consumption seen in experiment 1a, continued in experiment 1b (Tables 6 & 7). This phenomenon was reported by Fletcher, (1982).

The effects of dietary Lemna species levels on yolk pigmentation and egg quality are presented in Table 8. In experiment 1a, there was a significant increase in pigmentation when 15% *Lemna gibba* or 15% *Wolffia arrhiza* were included in the diets compared to the control eggs ($p < 0.001$). Higher levels of Lemna species (25% and 40%) produced smaller incremental changes although still significantly different ($p < 0.005$) when eggs from hens fed 25% Lemna species were compared to those from the 40% group. The quality of the eggs (graded by Haugh units), was significantly different from the control, favoring Lemna species. No differences were found in the calcium concentration of the shells between the groups fed with 0, 15, and 25% of Lemna in their diets; values of calcium were 43%, 42.9% and 43% respectively (number per group =25).

Microbiological studies of Layers receiving either Wolffia or Lemna in study 1A had no significant increase in the percent of enteropathogens isolated compared to the layers fed a control diet. Interestingly, in Peru chickens are rarely colonized with Salmonella species, this was also the case in our experimental chickens. See table 9.

b. Experiment #2

There was a gradual but not significant increase in the rate of egg production of the Leghorn layers during the first two periods in the control group during t.e 2.5 months studied (Tabl 5). The Lemna 15% group also presented a gradual increase in production during the first two periods, with a subsequent but not significant decline during the last period. Inclusion of 25% Lemna in the diet, significantly decreased production during the last period (10 weeks), when compared to the control ($p < 0.025$).

There were no significant differences in consumption between the control and the 15% groups except during the first period when there was a significant decrease ($p < 0.01$) in the amount of feed consumed by the Lemna 15% group. Similarly, conversion values tended to be better in the 15% Lemna diet group throughout the study. See table 10a.

No changes in the mean egg weight were seen with increasing amounts of Lemna species. All groups showed a gradual significant increase in the mean egg weight over time.

The effects of dietary levels of Lemna species on pigmentation in this study were similar to the results obtained in experiment 1a (table 4). There was a marked increase in yolk pigmentation in eggs from hens fed 15% Lemna compared to the control group ($p < 0.001$). Eggs from hens fed 25% Lemna were significantly more pigmented than those from hens fed 15%. However the rate of increment in pigment was much less marked than was seen between the control and the 15% group.

The chemical values obtained for protein on eggs collected on the last period of this experiment are presented in table 10b. There was a significant increase in protein both in the albumen ($p < 0.001$) as well as in the yolk ($p < 0.001$) when 15% Lemna and 25% were used compared to the control.

Formal double blind taste tests of eggs from both hen lines, were performed at the Universidad Nacional Agraria (UNA), by well trained panelists. Pigmented yolks were preferred over the paler control yolks, and <u>when rated, the overall quality (flavour, smell, color, and appearance) of the 15% Lemna group had the highest rating</u> compared to eggs from the control, 25% and 40% diet groups. No unusual tastes were detected in any of the groups tested.

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3. SUMMARY: The effect of Lemna species on the performance of Layers

We have demonstrated in this study using the two different lines of hens that Lemna is an excellent nutrient source for layers. We used 15% Lemna in the diets which substituted a portion of the soybean and fishmeal normally included in the diet. Lemna not only maintained egg production levels and mean egg weight compared to the controls, but although not statistically significant, there was a trend for improved feed conversions as well. Also, augmenting the amount of Lemna in the diets of Leghorn hens increased the total protein of the eggs and desirability in the taste of the eggs.

Pigmentation is an important attribute that adds to the economic value of Lemna as dietary ingredients. High egg-yolk pigmentation is commercially desirable and correlates highly with dietary Lemna levels. We found that the addition of 15% Lemna in the diets resulted in high levels of pigmentation compared to controls. Higher levels of Lemna produced increased levels of pigmentation but at a less efficient rate.

Hens were able to feed on a diet containing 40% Lemna with only a 10% loss in productivity. In diets containing 40% Lemna, soybean is eliminated and fish meal severely restricted leaving corn as the major constituent. Hens fed this diet were able to maintain high rates of egg production although at somewhat reduced levels compared to controls. Although a diet of 40% Lemna is not recommended for commercial operations, it may be useful in household farms in the third world, especially in places where fish meal or soy bean are costly or not available.

F. Broilers - Determination of the relative effect on broiler performance of Lemna species substitution for varying amounts of conventional protein constituents in controlled isocaloric and isonitrogenous feeding experiments.

1. Study on Lemna in a Finisher Diet in Broilers

The purpose of these studies was to evaluate Lemna as a protein and energy source in broiler chickens (28-56 days of age), its effect on growth rate and to examine its influence on broiler skin pigmentation.

a. BROILERS I

(1) Experimental design and procedures

Three hundred and twenty TITAN male chicks were obtained locally from Avicola Hannan, as a donation. The birds were placed in 20 units provided with individual thermostats, and were fed a standard diet and water *ad libitum* during 4 weeks (28 days).

At the end of the 4 week starter period, the birds were weighed and randomized into 24 finisher units, discarding extreme weights, (6 treatments, 4 repetitions, 10 birds per unit). Six isocaloric and isonitrogenous experimental diets containing different Lemna species levels (0, 5, 10, 15, 20, and 25%) were formulated using an ME of 2000 Kcal/kg dried Lemna (calculated ME value). See table 11.

Birds were fed their experimental diet on day 28. On day 49, all animals were weighed. Feed consumption was recorded and calculated at the end of the study, as the total feed provided minus the residue on that day for each unit of 10 birds. Conversion values were calculated as the average feed consumed per unit over the mean weight gained.

Carcass measurements were performed on 8 birds from each group diet at the end of the study. This involved weighing of the head and neck, legs, viscera, abdominal fat, and carcass yield. Pigmentation of the skin was measured at the end of the seven weeks using the standard Roche fan.

(2) Results

The use of *Lemna gibba* (duckweed) in levels up to 15% in broiler finisher diets, resulted in a small but significant decrease in weight gain (up to 10%) compared to the control as shown in Table 12. Higher levels (20 and 25%), resulted in a 20% decrease in weight compared to controls ($p<.005$). However, as the food consumption of all groups declined with increments of Lemna species, it is interesting to note that the feed conversion increased by 14% with 25% Lemna species in the diet, a value significantly different from the controls. The carcass yield differed slightly but significantly compared to the controls ($p<.005$).

Pigment was found to increase significantly as the level of Lemna species was raised in the diets.

b. BROILERS II

(1) Experimental design and procedures

Two hundred and forty TITAN male birds were obtained from Avicola Hannan and placed in 20 battery units as in experiment 1. The birds were fed a standard diet until the age of 28 days. After this period, the animals were weighed and redistributed in cages in groups of 8. The 21 units received 8 different experimental diets until the age of 56 days. The experimental diets contained 0,10,15 and 25% Lemna at two different energy levels (2900 and 3100 kcal/kg) using an ME of 1200 kcal/kg for the Lemna. See Table 13.

Initial and final individual weights were measured on days 28 and 49 respectively. Again, feed and water were provided *ad libitum*, and consumption and conversion procedures were identical to those used in the previous experiments. Carcass measuring procedures were also identical to those in the previous studies. In this study, 6 birds per group diet were used for carcass measurements. Carcass composition was also performed in this study.

Birds were homogenized in a meat blender at the UNA Food Plant, and the total samples were then dried at 60-70 °C. Dried samples were then analyzed for total fat, protein and ash content.

(2) Results

In this study, chickens fed diets containing 15% or less Lemna species (formulated using an ME of 1200 Kcal/kg for Lemna) gained weight at the same rate as the controls. See Table 14. Chickens fed diets with 25% Lemna species gained weight at a rate 11% slower than did the controls. At a higher diet ME, weight gain in the 10 and 15% Lemna groups was approximately 8% lower than the controls and at 25% we observed a 12% loss in weight gain.

Feed conversion was lower with the higher ME, at all levels of Lemna species. See Graphs 1 and 2. Carcass yield was found to be higher at the lower ME for the control, 15 and 25% groups. However, at 10% Lemna, a higher ME resulted in a better, though not significant, carcass yield.

c. BROILERS III

(1) Experimental design and procedures

Two hundred male TITAN birds 35 days of age were randomized according to weight and placed into 20 units (10 birds per unit). Four experimental diets were formulated according to AEC requirements, containing different pigment sources: 2.5% Lemna, 0.3% marigold, 0.05 Carovet (a commercial pigment), and a control. Again, diets were isonitrogenous and isocaloric (3000 kcal/kg) using an ME of 2000 Kcal/kg dried Lemna. See Table 15. Initial and final weight measures were recorded at 35 and 56 days of age respectively. Feed consumption and final pigment measurement procedures were identical to the previous study.

(2) Results

Table 16 shows the pigment obtained on the skin of broilers (36-56 days of age), from different sources. The highest pigmentation was obtained with 0.3% marigold. See Graph 3. There was no statistical difference in the weight gained among the four groups, and the feed conversions did not differ either, except for a small increase in the carovet group.

2. Summary: Use of Lemna in Broiler diets

The decrease in weight gain in the chickens fed Lemna species was mainly due to the high ME value (2000 Kcal/kg) used to formulate the diet in this study. When the ME value of Lemna used to formulate the diet was 1200 Kcal/kg (Broilers 2) chickens fed diets containing up to 15% Lemna sp. did as well as chickens fed a standard diet.

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Higher levels (20 and 25%), resulted in a decrease in weight gain. Pigment was found to increase as the level of Lemna was raised in the diets. See Table 17.

G. Study on Lemna in a Finisher Diet in Broilers Grown on a Commercial Farm.

1. Experimental design and procedures

This study was performed on a commercial setup with the collaboration of AVICOLA HANNAN, who provided the animals, the housing, the feed and professional personnel. In this study, we used 800 one day old TITAN and 800 Arbor Acres birds (50% males and 50% females), were housed in a Commercial unit located in Huachipa.

The animals were separated into 6 male units (3 Titan and 3 A.A. units) and 6 female units. The level of Lemna species used in the diet was 5%. The chicks were fed a commercial starter diet (Nicolini) throughout the first 21 days. After this period, they were supplied the diets containing 5% Lemna, and the control diets for the finishing period until day 56.

Birds were fed a commercial diet and water *ad libitum*. On day 21, sample groups (approximately 60 per unit) were weighed. After weighing, the new experimental diets were supplied to each unit. The diets consisted of a control and a diet containing 5% Lemna, using an ME of 1200 kcal/kg for the calculation. Again, feed and water were supplied ad libitum. Birds were weighed on day 42 and 49. On these dates, pigment was also measured on every 5th bird using the Roche fan. Consumption and conversion was calculated at the end of the study (day 49) as in the previous studies.

2. Results

All groups fed 5% Lemna had increased weight gain compared to the groups fed a standard diet. See Table 18. The resulting final weights were always higher on birds fed Lemna species when compared to the controls for both lines, and in both females and males. Pigmentation of the birds, even at low levels of Lemna, was significantly higher in all groups when compared to their controls.

3. Summary: Commercial use of Lemna to feed broilers

The final study compared 260 broilers fed 5% Lemna with 130 control chickens fed a control diet in a commercial farm. Both groups did well but the group fed Lemna had a significant increase in weight and pigment compared to the controls.

Lemna may substitute for fish-meal in the diet of broiler chickens. In places like Peru fish-meal is often used in concentrations of up to 15%. Diets containing a high level of fish-meal may be associated with black vomit, a toxic effect in chickens, and gives the meat a fishy taste (Rojas et. al., 1976).

Lemna substitution will permit lower levels of fish meal to be used in broiler diets with the benefit of better appearance (golden yellow color) and better tasting meat.

H. Baby Chickens - Determination of the relative effect on chick performance of Lemna species substitution for varying amounts of conventional protein constituents in controlled isocaloric and isonitrogenous feeding experiments

This study was performed to evaluate Lemna species as a feedstuff for growing chicks. We had previously tested the effects of Lemna species on growing chicks using the ME of 2000 Kcal/kg determined on the rooster study. This ME was later demonstrated to be twice that found when baby chickens were tested.

In a preliminary study using a diet formulated with an ME of 2000 Kcal/kg for Lemna species, we found an incremental decline in the growth rate of baby chicks as the percent of Lemna species in their diet was increased. It was interesting however that when these low weight chicks were subsequently changed to a standard (control) diet, their rate of weight gain was greater than that of the control group.

1. Experimental design

In the current study we fed chicks from birth on Lemna species formulated at the empirically derived ME value of 1200 Kcal/kg.

a. Starter period

Two hundred, one day old Titan chicks obtained locally from Avicola Hannan were randomly distributed by weight in experimental units. Each unit held 11 birds and each level of diet was replicated three times. The units were maintained at 37 degrees C. We compared diets containing the following different levels of Lemna species: 10%, 15%, 20%, 25% and 30% Lemna with a standard diet not containing Lemna species. The diets were formulated using an ME value of 1200 Kcal/kg dried Lemna species. All diets and the water were supplied ad libitum. At the end of the experimental period (28 days) the birds were weighed. During the experimental period (28 days) feed consumption was recorded in each unit.

b. Catch up Growth

Prior studies had demonstrated that chicks fed Lemna species early in life and then fed a normal diet exhibited growth rates that were faster than chickens fed a normal diet. This phenomenon has been observed in children during recovery from illness and termed catch-up growth.

we also examined this phenomenon by examining the chicks fed Lemna species in the above studies and at day 33 putting half of them on a normal diet while the other half was maintained on their Lemna species diet at levels fed previously.

Birds from each triplicate group were on day 33 then separated into 2 groups of 10. One group was maintained on the corresponding Lemna species diet, and the other was changed to a control (0% Lemna) diet for a period of 2 weeks (recuperation period).

2. Results

Chicks (0-28 days of age) fed Lemna species at levels ranging from 5-20% had growth rates equal to or better than did chicks fed a control diet. At levels higher than 20% the rate of weight gain decreased significantly compared to controls ($p < .005$). See Graph 4. Chicks fed more than 10% Lemna species in the diet had decreased feed consumption.

Since weight gain was not decreased until chicks were fed diets containing more than 20% Lemna species, the feed conversion ratio in chicks fed diets with 20% or less Lemna species was lower than that seen in controls. See Table 19.

Chicks maintained on Lemna species from day 33 to day 46 however, had decreased weight gain compared to the control. The decrease in weight compared to controls was significantly lower when Lemna concentrations above 15% were exceeded. Feed consumption in these chicks decreased with decreasing amounts of Lemna species in the diet thus producing a net rise in feed conversion ratios. See Table 19 and Graphs 5 and 6.

Chicks previously fed Lemna species during the 0-33 day period and then switched to a standard starter diet had better weight gains than did the control group. In this group consumption was maintained at levels similar to the control group with slightly lower conversion rates compared to chicks fed the control diets from 0-46 days. Chicks fed Lemna species throughout the study had significantly lower weight gain and feed consumption associated with higher feed conversion ratios than did chicks fed the same level of Lemna species for up to 33 days and then switched to a standard diet (33-46 days). See table 19.

(3) Summary of Baby Chicken Studies

The use of diets containing up to 20% Lemna species appeared to be satisfactory for chickens in the first weeks of life (0-28 days) when an appropriate ME was used to formulate the diet.

Chickens fed Lemna species who were continued on this starter diet beyond the 4 week period had decreased weight gain compared to controls. This decrease was significant in chickens fed more than 15% of Lemna species in their diet.

However we observed that birds fed Lemna species for the first 28 days of life when switched to a standard diet improved their feed conversions and their weight gain compared to controls only fed a standard diet. These results suggest that the chicken may provide a model which simulates catch-up growth in children.

III. CONCLUSIONS

We would recommend that the optimal level of Lemna species in diets for chickens is about 15%. Although a diet of 40% Lemna is not recommended for layer commercial operations, it may be useful in household farms in the third world, especially in places where fish-meal or soybean are costly and not available.

IV. FUTURE NEEDS

Future studies will need to concentrate on optimizing growth rates of Lemna species in lagoons or canals.

Also integrated farm systems need to be further examined. For example, chicken feces could be used as nutrient for growing Lemna species. The Lemna after harvesting and drying could then be fed back to the chickens.

Systems for the treatment of water like the one in San Juan de Miraflores represent a potential source of nutrients for water plants such as Lemna species which could be used not only as a feedstuff for poultry, but for other small animals and fish as well.

Lake Titicaca has stands of Lemna species that at times of the year are a potential large source of Lemna species. Puno is very high, above the level where corn can be grown, and most animal feed needs to be brought in from areas lower down in the sierra. Lemna species could serve as an extremely useful source of animal feed in this poor region. Drying would be facilitated by the high altitude. Harvested Lemna species not only would serve as a source of food to poultry but could be used as a protein concentrate for cattle and pigs. This area has a high potential for developing a future large scale Lemna harvesting and drying project.

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V. PROJECT RECOGNITION

The Director of AID and the Rector of the Universidad de Agraria both gave speeches at the dedication ceremony of the two Chicken installations constructed with joint funds by the Project and the Universidad Nacional de Agraria. In addition to building two major experimental chicken houses, the project also provided thesis material for at least 5 students at the Universidad Nacional Agraria and one at the Universidad Ricardo Palma. The Lemna project was featured in a magazine article and covered on local Peruvian TV. UNICEF also made the use of Lemna in the diet the subject of a day long seminar on non-traditional uses of animal feed. In addition, a video was made demonstrating the project and shown to organizations such as UNDP, NYC. and foundations such as Ford, Pfizer and Rockfeller Foundation.

TABLE 1

CHEMICAL ANALYSIS OF LEMNA SP

SAMPLE	HUMIDITY %	PROTEIN %	FIBER %	ASH %	CALCIUM %	PHOSPH. %	FAT %	XANTHOPHYLS ppm	SALT %
Lemna gibba (wet)	95.93	33.71	14.72	11.13	0.9	0.5	3.69	469	
Lemna gibba (dried)	10	33.97	8.63	19.10	1.6	0.65	3.63	658	5.09
Lemna gibba (dried)	9.41	32.53	9.41	22.01	1.7	0.84	4.52	1063	5.65
Lemna gibba (dried)	9.23	32.65	9.13	23.61	2.65	0.9	3.42	868	5.12
Lemna minor (wet)	93.67	33.6	7.91	15	1	0.58	4.02	866	
Wolffia arrhiza (dried)	12.01	31.36	11.68	23.82	2.48	0.82	3.62	974	3.57

TABLE 2

AMINO ACID VALUES FOUND IN LEMNA GIBBA FROM THE SAN JUAN LAGOONS IN LIMA (*).

	%	+/-	SD	(N)
HUM	13.02	+/-	1.52	(5)
FAT	2.40	+/-	0.28	(5)
PROT	33.93	+/-	1.32	(5)
AMINO ACID	**% DRY MATTER**	**+/-**	**SD**	**(N)**
CYS	0.36	+/-	0.01	(5)
ASP	2.91	+/-	0.22	(5)
THR	1.25	+/-	0.07	(5)
SER	1.42	+/-	0.07	(5)
GLU	3.56	+/-	0.29	(5)
PRO	1.36	+/-	0.07	(5)
GLY	1.45	+/-	0.11	(5)
ALA	1.63	+/-	0.13	(5)
VAL	1.72	+/-	0.08	(5)
MET	0.52	+/-	0.05	(5)
ILE	1.27	+/-	0.07	(5)
LEU	2.14	+/-	0.21	(5)
TYR	0.94	+/-	0.05	(5)
PHE	1.30	+/-	0.17	(5)
HIS	0.61	+/-	[illegible]	(5)
LYS	1.64	+/-	[illegible]	(5)
NH3	0.71	+/-	0.24	(5)
ARG	1.65	+/-	0.16	(5)

(*) These analyses were performed by the Department of Poultry Science at the University of Maryland

T A B L E 3

ISOLATION OF ENTEROPATHOGENS AND INDICATOR BACTERIA FROM WATER AND LEMNA PLANTS IN THE PRIMARY, SECONDARY AND TERTIARY TREATMENT LAGOONS

BACTERIA INDICATOR AND ENTEROPATHOGENS	PRIMARY		SECONDARY		TERTIARY	
	Water n = 33	Lemna n = 33	Water n = 27	Lemna n = 19	Water n = 29	Lemna n = 29
Vibrio-Non 01						
No.+, (%)	6 (18%)	9 (36%)	9 (33%)	9 (47%)	10 (34%)	9 (31)
Mean Log10	1.36	2.1	1.36	2.6	1.4	1.65
+/- S.D	+/-2.0	+/-2.8	+/-2.0	+/-2.9	+/-2.0	+/-2.5
Aeromonas species						
No.+, (%)	31 (94%)	32 (97%)‡‡	26 (96%)	19 (100%)‡‡	28 (97%)	28 (97%)‡‡
Mean Log10	4.8	6.4	4.5	6.8	4.3	6.3
+/- S.D	+/-1.9	+/-1.5	+/-1.7	+/-0.9	+/-1.3	+/-1.6
Salmonella species						
No.+, (%)	22 (66%)	26 (79%)	2 (7%)++	12 (66%)‡‡	4 (14%)^^	8 (29%)^^
Fecal Coliform						
Mean Log10	6.2	6.5	5.7#	6.4##	4.9++	5.3^^
+/- S.D	+/-1.9	+/-1.1	+/-1.4	+/-0.8	+/-1.04	+/-0.91
Total Plate Count						
Mean Log10	7	8.1	6.7	7.8‡‡	6.4	7.6‡‡
+/- S.D	+/-7.9	+/-8	+/-0.97	+/-0.5	+/-1.09	+/-0.58

Significant difference between water specimens versus Lemna
‡ p < .05
‡‡ p < .01

Significant difference between primary and secondary
\+ p < .05
++ p < .01

Significant difference between primary and tertiary
^ p < .05
^^ p < .01

Significant difference between secondary and tertiary
\# p < .05
\## p < .01

TABLE 4

SUMMARY OF THE APPARENT METABOLIZABLE ENERGY STUDY, CORRECTED FOR NITROGEN (AMEn), ON TITAN EASY CHICKS USING TWO METHODS

TOTAL COLLECTION METHOD

AMEn OF LEMNA GIBBA AND WOLFFIA ARRHIZA (kcal/g dried matter)		
Inclusion level	Lemna gibba	Wolffia arrhiza
20%	1.030 +/- 0.170	1.050 +/- 0.020
30%	1.146 +/- 0.026	1.071 +/- 0.078

CHROMIUM OXIDE INDICATOR METHOD

AMEn OF LEMNA GIBBA AND WOLFFIA ARRHIZA (kcal/g dried matter)		
Inclusion level	Lemna gibba	Wolffia arrhiza
20%	1.302 +/- 0.052	1.257 +/- 0.177
30%	0.978 +/- 0.001	1.050 +/- 0.037

TABLE 5A

FORMULATION AND CALCULATED NUTRITIONAL VALUE OF DIETS FED TO TOPAZ LAYERS

INGREDIENTS	CONTROL (1)	LEMNA 15 (2)	WOLFFIA 15 (3)
CORN (%)	52	51	51
WHEAT SUB PRODUCT (%)	19	16	16
FISH MEAL (%)	8	8	8
SOY (%)	11		
LEMNA (%)		15	
WOLFFIA (%)			15
FISH FAT (%)	3	3	3
CALCIUM CARBONATE (%)	8	7	7
DICALCIUM PHOSPHATE (%)	0	0	0
IODATED SALT (%)	0		
DL METHIONINE (%)			
PRE MIX VITAMIN (%)	0	0	0
TOTAL	100	100	100
ME KCAL/KG	2800	2800	2800
TOTAL PROTEIN	17.1	16.86	17.5
LISINE %	0.94	0.96	0.94
METHIONINE %	0.35	0.35	0.35
MET. & CYST. %	0.62	0.65	0.62
CALCIUM %	3.3	3.3	[illegible]
AVAILABLE PHOSPH %	0.38	0.38	[illegible]
ASH (%)	11.26	13	[illegible].4
FIBER (%)	3.76	4.87	[illegible].75
FAT (%)	6.01	7	6.02

TABLE 5B

FORMULATION AND CALCULATED NUTRITIONAL VALUE OF DIETS FED TO WHITELINE LEGHORN LAYERS

INGREDIENTS (%)	CONTROL	15% LEMNA	25% LEMNA
LEMNA	0	15	25
WHEAT SUB-PRODUCT	3	2	0
YELLOW CORN	62	54	48
FISHMEAL	7	7	2
SOYBEAN	9	0	0
COTTON PASTE	5	5	5
BROWN SUGAR	5	5	5
GREASE	0	4	6
Ca(HPO4-)2	0	0	1
CALCIUM CARBONATE	5	4	4
SHELL	4	4	4
SALT	0	0	0
DL MET	0	0	0
PREMIX	0	0	0
TOTAL	100	100	100
EM, KCAL/KG	2806	2840	2840
PROTEIN %	16	16	16
LYSINE %	0.65	0.65	0.65
MET %	0.38	0.36	0.36
CALCIUM %	3.2	3.2	3.2
AVAIL. PHOSPH. %	0.35	0.35	0.35

TABLE 6

PERFORMANCE OF TOPAZ LAYERS FED DIETS CONTAINING 15% LEMNA SPECIES AS COMPARED TO CONTROLS.

DIET		WEEK 2	WEEKS 6	WEEKS 10
CONTROL	PRODUCTION (%)	92.23 +/- 4.44 (5)	91.50 +/- 5.10 (5)	88.79 +/- 5.71 (5)
	CONSUMPTION (Kg)	0.145 +/- 0.003 (5)	0.145 +/- 0.004 (5)#	0.133 +/- 0.003 (5)+
	CONVERSION	2.436 +/- 0.090 (5)	2.404 +/- 0.069 (5)	2.347 +/- 0.094 (5)
	MEAN EGG WT (Gm)	66.18 +/- 1.71 (5)	65.96 +/- 1.97 (5)	64.21 +/- 1.39 (5)
	MEAN WEIGHT GAIN (Gm)	163 +/- 75	43 +/- 68 #	-19 +/- 86 +
	NUMBER OF EGGS/HEN/WK	6.460	6.405	6.215
15% LEMNA	PRODUCTION (%)	93.71 +/- 0.95 (5)	92.71 +/- 1.67 (5)	90.07 +/- 2.12 (5)+
	CONSUMPTION (Kg)	0.147 +/- 0.004 (5)	0.146 +/- 0.005 (5)	0.140 +/- 0.006 (5)+
	CONVERSION	2.347 +/- 0.044 (5)	2.376 +/- 0.071 (5)	2.392 +/- 0.087 (5)
	MEAN EGG WT (Gm)	66.76 +/- 1.45 (5)	66.19 +/- 1.62 (5)	65.17 +/- 1.03 (5)*+
	MEAN WEIGHT GAIN (Gm)	117 +/- 124 +	60 +/- 106 #o	31 +/- 154
	NUMBER OF EGGS/HEN/WK	6.560	6.490	6.305
15% WOLFFIA	PRODUCTION	89.71 +/- 3.31 (5)	------------------	------------------
	CONSUMPTION	0.14[illegible] +/- 0.00[illegible] (5)	------------------	------------------
	CONVERSION	2.425 +/- 0.118 (5)	------------------	------------------
	MEAN EGG WT (Gm)	67.10 +/- 1.12 (5)	------------------	------------------
	MEAN WEIGHT GAIN (Gm)	131 +/- 115	------------------	------------------
	NUMBER OF EGGS/HEN/WK	6.2[illegible]	------------------	------------------

\# Represents a significant difference when comparing each diet group to the control
\+ Represents a significant difference between week 2 and week 10
o Represents a significant difference between week 2 and week 6
& Represents a significant difference between week 6 and week 10
* Represents a significant difference between Lemna 15% and Wolffia 15% groups
One Unit Cage contains 10 hens
Mean egg weights represent the total kg produced per period, divided by the total production number

T A B L E 7

PERFORMANCE OF TOPAZ LAYERS FED DIETS CONTAINING DIFFERENT VARYING PERCENTAGES OF LEMNA GIBBA AT DIFFERENT ENERGY LEVELS COMPARABLE TO A CONTROL DIET

DIET		WEEK 14		WEEK 16	
CONTROL	PRODUCTION (%)	66.66 +/- 3.67	(4)	64.46 +/- 4.60	(4)
	CONSUMPTION (Kg)	0.141 +/- 0.002	(4)	0.131 +/- 0.004	(4)+
	CONVERSION	2.434 /- 0.036	(4)	2.413 +/- 0.032	(4)
	MEAN EGG WT (Gm)	65.40 +/- 1.33	(4)	64.25 +/- 1.56	(4)
	MEAN WEIGHT GAIN (GM)			46	
	NUMBER OF EGGS/HEN/WK	6.081		5.913	
25% LEMNA ME: 2600 KCAL/KG	PRODUCTION (%)	66.56 +/- 1.36	(2)*	64.10 +/- 2.68	(2)
	CONSUMPTION (Kg)	0.132 +/- 0.009	(2)	0.131 +/- 0.004	(2)
	CONVERSION	2.504 +/- 0.112	(2)*	2.473 +/- 0.020	(2)+#
	MEAN EGG WT (Gm)	65.11 +/- 0.18	(2)	63.14 +/- 0.389	(2)
	MEAN WEIGHT GAIN (GM)			114	
	NUMBER OF EGGS/HEN/WK	6.033		5.888	
25% LEMNA ME:2800 KCAL/KG	PRODUCTION (%)	65.64 +/- 1.79	(2)*	67.32 +/- 0.54	(2)
	CONSUMPTION (Kg)	0.141 +/- 0.007	(2)	0.132 +/- 0.003	(2)
	CONVERSION	2.466 +/- 0.044	(2)*	2.382 +/- 0.013	(2)
	MEAN EGG WT (Gm)	63.96 +/- 0.76	(2)	63.61 +/- 0.91	(2)
	MEAN WEIGHT GAIN (GM)			134	
	NUMBER OF EGGS/HEN/WK	6.175		6.113	
40% LEMNA	PRODUCTION (%)	62.66 +/- 0.36	(2)	79.46 +/- 0.89	(2)+
	CONSUMPTION (Kg)	0.1[illegible] +/- 0.001	(2)*	0.125 +/- 0.001	(2)
	CONVERSION	2.[illegible] +/- 0.003	(2)	2.475 +/- 0.005	(2)+
	MEAN EGG WT (Gm)	6[illegible] +/- 1.05	(2)	63.57 +/- 0.15	(2)
	MEAN WEIGHT GAIN (GM)			-118	
	NUMBER OF EGGS/HEN/WK	5.[illegible]		5.563	

* Represents a significant difference between each groups vs the control

\+ Represents a significant difference between 14 weeks and 16 weeks.

\# Represents a significant difference between both 25% groups

Represents a significant difference between both 25% groups and the 40% group.

One Unit Case contains 10 hens

Mean egg weights, represent the total kg produced per period, divided by the total production number

T A B L E 8

PIGMENTATION OF THE YOLK IN EGGS FROM TWO DIFFERENT LINES OF LAYERS WITH DIFFERENT PERCENTAGES OF LEMNA SPECIES.

TORAZ LAYERS	CONTROL 1 2-10wks	WOLFFIA 15% 2wks	LEMNA 15% 2-10wks	CONTROL 2 11-18wks	LEMNA 25% 11-18wks	LEMNA 25% E 11-18wks	LEMNA 40% 11-18wks
Pigment							
mean	9.42 a	12.79 b	12.46 b	8.02 a	12.58 b	13.13 b	13.39 c
std	1.72	0.65	0.80	0.70	0.55	0.71	0.52
n	210	70	210	112	56	56	56

LEGHORN	CONTROL 1 2-10wks	LEMNA 15% 2-10wks	LEMNA 25% 2-10wks
Pigment			
mean	7.67 a	13.30 b	14.09 c
std	0.82	0.60	0.79
n	166	168	168

Yolk Pigmentation was measured with a ROCHE colorimetric fan
Different letters represent statistical differences between groups

T A B L E 9

Frequency of Campylobacter, Aeromona species and Pathogenic E. coli in layer hens fed control and duckweed diets.

Diet	Total Samples	Campylobacter	Aeromona hydrophila	Salmonella
Control	100	18 (18%)	1 (1%)	Ø
Duckweed	100	24 (24%)	4 (4%)	Ø

Frequency of Campylobacter, Aeromonas and Salmonella species and Pathogenic E.coli in Mark[illegible] Free Range Broiler Chickens in Lima.

Origin of Samples	Number of Samples	Campylobacter		Aeromonas species	ETEC	EPEC	Salmonella* species
Canto Grande*	50	25	50 (50%)	1 (2%)	8 (16%)	2 (4%)	Ø
Market	117	63	72^ (88%)	7 (6%)	1 (1%)	1 (1%)	Ø
University [illegible]*	30	7	11^ (64%)	Ø	4 (13%)	2 (7%)	Ø
TOTAL	197	95	133 (71%)	8 (4%)	13 (7%)	5 (3%)	Ø

* Free range

^ The number of chicken sampled for Campylobacter in the market and in the University were 72 and 11 respectively

ETEC- E coli which produce either or both ST and LT enterotoxin.

EPEC- E.coli which are enteropathogenic

ETEC and EPEC E.coli were detected using DNA probes.

Salmonella typhi was not isolated.

TABLE 10A

PERFORMANCE OF WHITELINE LEGHORN LAYERS FED DIETS CONTAINING VARYING PERCENTAGES OF LEMNA GIBBA IN THE DIETS AS COMPARED TO THE CONTROL.

DIET		WEEK 2	WEEK 6	WEEK 10
CONTROL	PRODUCTION (%)	76.96 +/- 9.43 (4)	73.46 +/- 8.65 (4)	82.23 +/- 6.73 (4)
	CONSUMPTION (Kg)	.120 +/- 0.005 (4)	.122 +/- 0.005 (4)	.120 +/- 0.001 (4)
	CONVERSION	2.682 +/- 0.360 (4)	2.500 +/- 0.251 (4)	2.308 +/- 0.154 (4)
	MEAN EGG WT (Gm) ‡	60.60 +/- 1.20 (4)	62.09 +/- 1.76 (4)	63.65 +/- 1.52 (4)+
	MEAN WEIGHT GAIN (GM)			149 +/- 195 (40)
	NUMBER OF EGGS/HEN/WK	5.388	5.500	5.756
15% LEMNA	PRODUCTION (%)	8[illegible] 14 +/- 4.79 (4)	83.75 +/- 4.27 (4)	75.38 +/- 5.73 (4)˚
	CONSUMPTION (Kg)	.113 +/- 0.002 (4)‡	.118 +/- 0.005 (4)	.118 +/- 0.005 (4)
	CONVERSION	2.293 +/- 0.079 (4)	2.311 +/- 0.050 (4)	2.334 +/- 0.225 (4)
	MEAN EGG WT (Gm)	60.22 +/- 1.53 (4)	60.84 +/- 0.75 (4)	62.85 +/- 0.73 (4)+
	MEAN WEIGHT GAIN (Gm)			122 +/- 214 (40)
	NUMBER OF EGGS/HEN/WK	5.750	5.663	5.556
25% LEMNA	PRODUCTION (%)	76.07 +/- 3.86 (4)	71.16 +/- 6.46 (4)˚	70.36 +/- 4.38 (4)‡
	CONSUMPTION (Kg)	.118 +/- 0.004 (4)˚	.119 +/- 0.004 (4)	.118 +/- 0.004 (4)
	CONVERSION	2.524 +/- 0.304 (4)	2.811 +/- 0.266 (4)˚	2.654 +/- 0.156 (4)‡
	MEAN EGG WT (Gm)	60.05 +/- 1.22 (4)	60.03 +/- 0.25 (4)	63.18 +/- 1.14 (4)+
	MEAN WEIGHT GAIN (GM)			74 +/- 234 (40)
	NUMBER OF EGGS/HEN/WK	5.325	4.981	4.925

‡ Represents a significant difference when compared to the control
\+ Represents a significant difference between week 2 and week 10 for the same diet
˚ Represents a significant difference between the Lemna 15% and the Lemna 25% groups

One Unit Cage contains 10 hens
Mean egg weights, represent the total kg produced per period, divided by the total production number

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TABLE 10B

PROTEIN CONTENT OF EGGS FROM LEGHORN HENS FED DIETS CONTAINING VARYING PERCENTAGES OF LEMNA SPECIES COMPARED TO AN ISONITROGENOUS AND ISOCALORIC CONTROL DIET. (*)

	TREATMENTS		
	CONTROL	15% LEMNA	25% LEMNA
PROTEIN (WHITE)	84.102 +/- 0.302 (40)	84.745 +/- 0.160 (40)***	86.055 +/- 0.576 (40)***
PROTEIN (YOLK)	15.642 +/- 0.232 (40)	16.293 +/- 0.125 (40)***	17.238 +/- 0.141 (40)***

(*) Values are presented as X +/- SD (n), on a dry matter basis.
*** = p < 0.001 when compared to the control.

TABLE 11

STUDY: BROILERS I (4-7 WKS)
DIETS: STANDARD (0-4 WKS), 6 EXPERIMENTAL (4-7 WKS)

Ingredients (%)	Standard 0-28days	Diet1 Control	Diet2 5%	Diet3 10%	Diet4 15%	Diet5 20%	Diet6 25%
Corn	58.38	57.28	57.72	58.64	61.88	63.22	66.53
Wheat subprod.	14	23.86	22.89	20.19	12.16	7.65	
Fishmeal	13	12.17	11.76	10.62	9.38	7.48	5.9
Soy	13.34	5.87	1.98				
Lemna			5	10	15	20	25
Fat							0.072
Calcium Carbonate	1.05	0.67	0.5	0.39	1.26	1.09	1.07
Dicalcium fosfate					0.16	0.41	1.26
DL-Met	0.073						0.034
Premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15
	100	100	100	99.99	99.99	100	100.02

BROILERS I
NUTRITIVE CALCULATED VALUE
OF THE EXPERIMENTAL DIETS

NUTRIENT	DIET1	DIET2	DIET3	DIET4	DIET5	DIET6
M.E. (Kcal/kg)	2900	2900	2900	2900	2900	2900
Total Protein (%)	19.57	19.05	18.69	18.46	18.3	18
Crude Fibre (%)	3.98	4.32	4.65	4.64	4.97	5.07
Ether extract (%)	4.27	4.4	4.45	4.34	4.29	4.26
Ash (%)	4.51	5.03	5.63	7.11	7.58	8.14
Lysine (%)	1.038	0.99	1.06	1.07	1.07	1.07
DL Methionine (%)	0.422	0.37	0.43	0.4	0.39	0.38
Met & Cys (%)	0.72	0.71	0.72	0.72	0.72	0.72
Available Phosphorous (%)	0.44	0.43	0.41	0.4	0.4	0.51
Calcium (%)	0.6	0.8	0.8	1.2	1.2	1.4
Sodium (%)	0.18	0.25	0.32	0.6	0.6	0.8

TABLE 12

STUDY No. 1:
PERFORMANCE CHARACTERISTICS OF TITAN BROILERS (28-49 DAYS) USING DIFFERENT PERCENTAGES OF LEMNA GIBBA IN THE DIETS.

% LEMNA IN THE DIET	0	5	10	15	20	25
METABOLIZABLE ENERGY	2900 kcal/kg	2900 kcal/kg	2900 kcal/kg	2900 kcal/kg	2900 kcal/kg	2900 kcal/kg
INITIAL WEIGHT (gm) +/- Std. (n)	1051.52 (4) +/-29.64	1052.93 (4) +/-10.04	1055.60 (4) +/- 9.57	1042.34 (4) +/-15.42	1053.38 (4) +/-17.52	1047.09 (4) +/-32.57
WEIGHT GAIN (gm)	1293.31 (4) +/-63.56	1250.63 (4) +/-45.99	1177.05 (4)* +/-45.62	1145.23 (4)* +/-50.45	1017.90 (4)*** +/-32.72	1018.23 (4)*** +/-45.63
CARCASS WEIGHT (gm)	1643.99 (8) +/-104.01	1651.83 (8) +/-144.44	1564.25 (8) +/-105.55	1573.53 (8) +/-115.31	1413.13 (8)*** +/-162.45	1420.00 (8)*** +/-126.45
CARCASS YIELD (%)	77.2 (8) +/- 0.71	77.07 (8) +/- 1.31	76.1 (8)*** +/- 0.45	76.02 (8)*** +/- 0.25	75.43 (8)*** +/- 0.53	75.04 (8)*** +/- 0.60
FEED CONSUMPTION (gm)	3014.60 (4) +/-67.44	3103.10 (4) +/-81.79	2958.23 (4) +/-105.02	2859.08 (4)* +/-80.61	2788.10 (4)* +/-70.11	2764.63 (4)* +/-68.90
FEED CONVERSION (kg feed cons./kg gained)	2.35 (4) +/- 0.06	2.40 (4) +/- 0.06	2.51 (4)*** +/- 0.03	2.49 (4)* +/- 0.07	2.74 (4)*** +/- 0.06	2.72 (4)*** +/- 0.09
PIGMENTATION (Roche fan)	10.00 (4) +/- 0.47	10.90 (4)* +/- 0.41	11.40 (4)*** +/- 0.32	11.45 (4)*** +/- 0.20	11.68 (4)*** +/- 0.18	11.93 (4)*** +/- 0.40

* p less than 0.05, *** p less than 0.005 when compared to the control.

TABLE 13

Study No. 2
Titan Broilers
Diet Formulas

INGREDIENTS	DIET 1	DIET 2	DIET 3	DIET 4	DIET 5	DIET 6	DIET 7	DIET 8
LEMNA	0	10	15	25	0	10	15	25
WHEAT BUB-PROD	20	19.73	7.54	0	18.32	10.46	3.31	0
FISHMEAL	13	11.93	11.03	7.66	14.38	14.26	12.72	9.33
CORN	59.04	56.43	64.62	62.85	55.93	61.53	65.99	58.97
SOYBEAN	7.23	0	0	0	7.66	0.3	0	0
FAT	0	1.5	1.5	3.89	2.76	2.8	2.8	6.3
DICALCIUM PHOSPHATE	0	0	0	0.45	0	0	0	0.25
CALCIUM CARBONATE	0.58	0.26	0.16	0	0.48	0.03	0.03	0
DL-METHIONINE	0	0	0	0	0.07	0	0	0
PREMIX	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
TOTAL	100	100	100	100	100	100	100	100

TABLE 14

STUDY No. 2

PERFORMANCE CHARACTERISTICS OF TITAN BROILERS USING DIFFERENT PERCENTAGES OF LEMNA GIBBA AT TWO ENERGY LEVELS IN THEIR DIETS.

% LEMNA IN THE DIET	0%	10%	15%	25%	0%	10%	15%	25%
METABOLIZABLE ENERGY (Kcal/kg)	2900	2900	2900	2900	3100	3100	3100	3100
INITIAL WEIGHT (gm)	905.92 (24) +/-74.33	935.42 (24) +/-73.25	942.67 (24) +/-74.67	943.38 (24) +/-56.94	907.50 (24) +/-67.27	939.17 (24) +/-68.38	931.25 (24) +/-74.22	925.63 (24) +/-66.33
WEIGHT GAIN (gm)	1400.75 (3) +/-134.70	1402.50 (3) +/- 53.13	1441.92 (3) +/- 43.84	1250.75 (3) +/- 56.67	1533.60 (3) +/- 85.03	1412.29 (3) +/- 56.22	1412.50 (3) +/- 54.85	1360.42 (3)* +/- 42.03
CARCASS WEIGHT (gm)	1705.00 (6) +/-31.36	1515.92 (6)*** +/-110.54	1702.50 (6) +/-55.58	1511.67 (6)*** +/-101.34	1659.83 (6) +/-145.50	1577.50 (6) +/-73.92	1625.93 (6) +/-113.37	1536.67 (6) +/-112.27
CARCASS YIELD (%)	65.6 (6) +/-0.638	64.31 (6)** +/-0.507	65.1 (6) +/-1.293	64.94 (6) +/-1.295	65.21 (6) +/-1.408	65.03 (6) +/-1.730	64.94 (6) +/-1.164	64.06 (6) +/-0.873
FEED CONSUMPTION (kg)	3.93 (3) +/- 0.07	3.95 (3) +/- 0.09	4.02 (3)a +/- 0.10	3.66 (3)** +/- 0.12	3.85 (3) +/- 0.10	3.80 (3) +/- 0.13	3.76 (3)b +/- 0.05	3.61 (3) +/- 0.04
FEED CONVERSION	2.87 (3)a +/- 0.24	2.82 (3) +/- 0.14	2.79 (3) +/- 0.03	2.92 (3) +/- 0.10	2.51 (3)b +/- 0.13	2.69 (3) +/- 0.06	2.66 (3) +/- 0.07	2.8 (3)*** +/- 0.03

* = p less than 0.05, ** = p less than 0.01, *** = p less than 0.005 when comp ared to the control.

Different letters represent a significant difference b tween groups with diets containing the same lemna levels, but different Energy levels

TABLE 15

Study No. 3
Diet Formulas
Pigmentation Study

INGREDIENTS (%)	EXPERIMENTAL DIETS 1	2	3
Corn	60	60	60
Wheat Subproduct	17.65	17.18	17.55
Fish meal	12	12	12
Soy	6	4.17	6
Fat	2.56	2.56	2.55
Calcium carbonate	1.15	1.15	1.15
Dicalcium phosphate	0.15	0.15	0.15
Salt	0.05	0.03	0.03
DL-Methionine	0.05	0.05	0.05
Premix	0.15	0.15	0.15
Lemna	--	2.5	--
Marigold	--	--	0.3
Carovet	--	--	--

CALCULATED NUTRITIONAL VALUE

Em.kcal/kg	3000
Protein,%	19.54
Lysine,%	1.03
Methionine,%	0.46
Met+Cyst,%	0.74
Treonine,%	0.71
Tryptophan,%	0.21
Calcium,%	1
Phosphates avail,%	0.45
Sodium,%	0.14
Fat,%	6.59
Fiber,%	3.56
Ash,%	5.42

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TABLE 16

STUDY No. 3
PERFORMANCE CHARACTERISTICS OF TITAN BROILERS USING DIFFERENT PIGMENT SOURCES

	PIGMENT SOURCE		
	CONTROL (0%)	2.5% LEMNA	0.5% MARIGOLD
METABOLIZABLE ENERGY OF DIET	3000 kcal/kg	3000 kcal/kg	3000 kcal/kg
INITIAL PIGMENT	5.14 +/- 1.34 (50)	5.54 +/- 1.51 (50)	5.42 +/- 1.3 (50)
FINAL PIGMENT	5.30 +/- 2.03 (50)	5.40 +/- 1.67 (50)	7.05 +/- 1.97 (50)
INITIAL WEIGHT	1155.70 +/- 171.87 (50)	1172.74 +/- 159.02 (50)	1151.60 +/- 192.43 (50)
FINAL WEIGHT (GRAMS)	2373.14 +/- 284.67 (43)	2342.81 +/- 250.56 (43)	2368.71 +/- 319.43 (43)
FEED CONSUMPTION	2.58 +/- 0.122 (5)	2.90 +/- 0.145 (5)	2.56 +/- 0.058 (5)
FEED CONVERSION kg feed cons /kg wt gained	2.49 +/- 0.156 (5)	2.43 +/- 0.145 (5)	2.45 +/- 0.149 (5)

TABLE 17

SUMMARY OF THREE STUDIES EXAMINING THE PERFORMANCE CHARACTERISTICS OF BROILERS FED DIETS CONTAINING VARYING PERCENTAGES OF LEMNA GIBBA EACH COMPARED TO THEIR RESPECTIVE CONTROL DIET (*)

PIGMENT (Roche Fan)

		LEMNA (%)						
	Days	0%	2.5%	5%	10%	15%	20%	25%
BROIL I	28-49	10.00	----	10.90	11.40	11.50	11.70	11.60
BROIL III	35-56	5.90	6.40					

WEIGHT GAIN (grams)

	Days	0%	2.5%	5%	10%	15%	20%	25%
BROIL I	28-49	1283.31	----	1290.83	1177.05	1149.23	1017.90	1018.23
BROIL II (2.9)	28-49	1400.75	----	----	1402.50	1441.52	----	1250.79
(3.1)		1533.80	----	----	1412.20	1412.50	----	1360.92
BROIL III	35-56	1210.00	1174.07	----	----	----	----	----

FEED CONVERSION (kg feed consumed/kg gained)

	Days	0%	2.5%	5%	10%	15%	20%	25%
BROIL I	28-49	2.36	----	2.40	2.51	2.52	2.73	2.73
BROIL II (2.9)	28-49	2.87	----	----	2.83	2.75	----	2.93
(3.1)		2.51	----	----	2.70	2.67	----	2.80
BROIL III	35-56	2.43	2.48	----	----	----	----	----

FEED CONSUMPTION (kg)

	Days	0%	2.5%	5%	10%	15%	20%	25%
BROIL I	28-49	3.027	----	3.103	2.958	2.659	2.788	2.764
BROIL II (2.9)	28-49	3.98	----	----	3.96	4.02	----	3.66
(3.1)		3.85	----	----	3.6	3.76	----	3.61
BROIL III	35-56	2.98	2.9	----	----	----	----	----

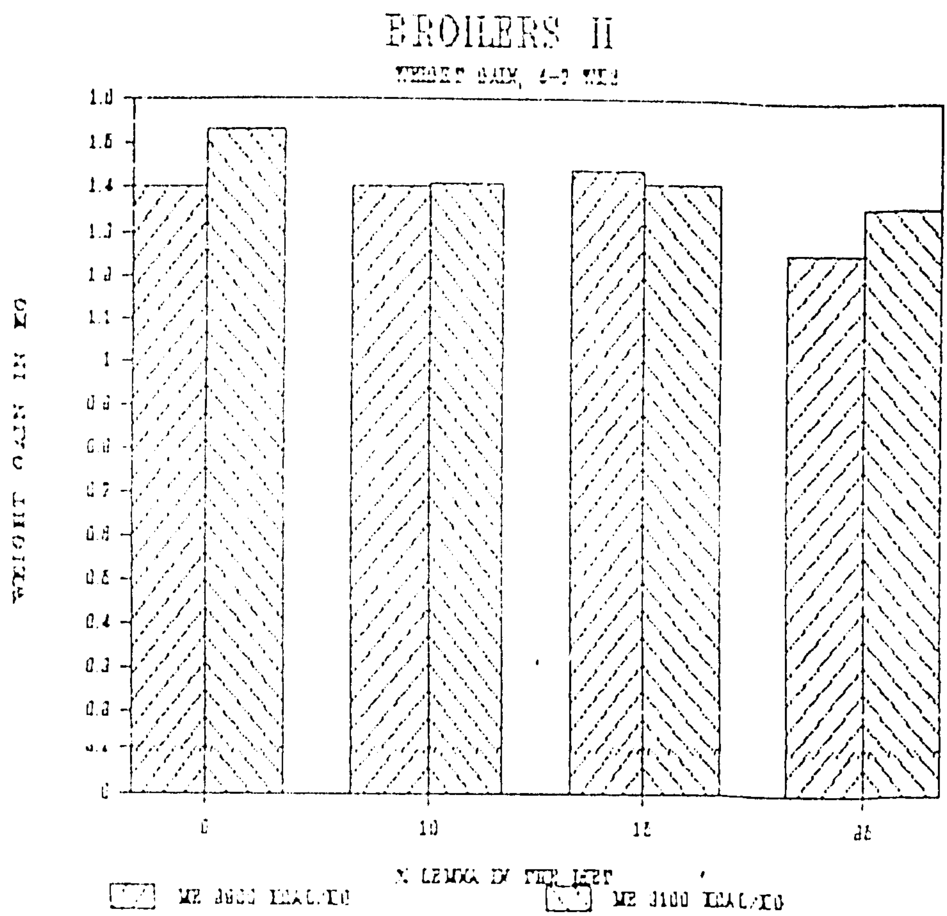
(*) The three broiler studies are not comparable due to the differences in diet formulation (different ME and pigment levels)

TABLE 12

STUDY No. 4
PERFORMANCE OF TWO LINES OF BROILERS FED A DIET CONTAINING 5% LEMNA SPECIES COMPARED TO A CONTROL DIET UNDER COMMERCIAL CONDITIONS.

BREED (Age (days)	DIET	SEX	INITIAL WT (GM) DAY 21	N DAY 21	FINAL WT (GM) DAY 45 +/- STD	N DAY 45	FINAL PIGMENT +/- STD	N
TITAN (21-45 days)	Control	Males	441.50	(60)	1774.57 +/- [illegible]	(125)	2.12 +/- 1.12	(25)
	Lemna 5%	Males	412.45	(145)	1804.[illegible] +/- 221.2[illegible]	(256)	3.43 +/- 1.73	(50)***
	Control	Females	415.10	(52)	1544.81 +/- 150.6	(125)	1.62 +/- 1.54	(25)
	Lemna 5%	Females	436.45	(118)	1639.13 +/- 160.91	(253)***	3.66 +/- 1.53	(53)***
ARBOR ACRES (21-45 days)	Control	Males	431.60	(64)	1680.62 +/- 256.67	(130)	1.73 +/- 1.05	(26)
	Lemna 5%	Males	411.40	(126)	1712.36 +/- 238.58	(263)	3.50 +/- 1.65	(52)***
	Control	Females	406.10	(57)	1441.69 +/- 112.27	(130)	1.50 +/- 0.56	(24)
	Lemna 5%	Females	399.25	(130)	1550.27 +/- 131/31	(259)***	4.02 +/- 1.66	(51)***

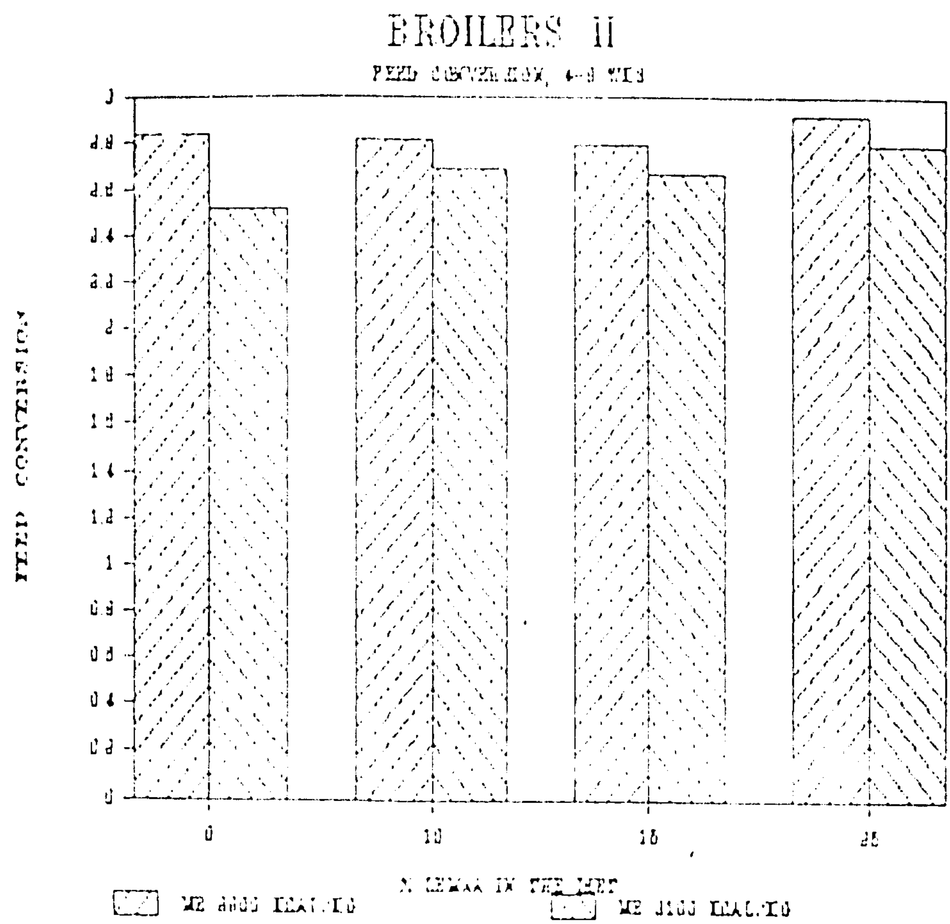
*** = p < 0.001



The effect on weight gain in Titan broilers fed varying percentages of Lemna gibba at two different energy levels.

Note: The diets were formulated using an ME value of 1200 kcal/kg for Lemna gibba.

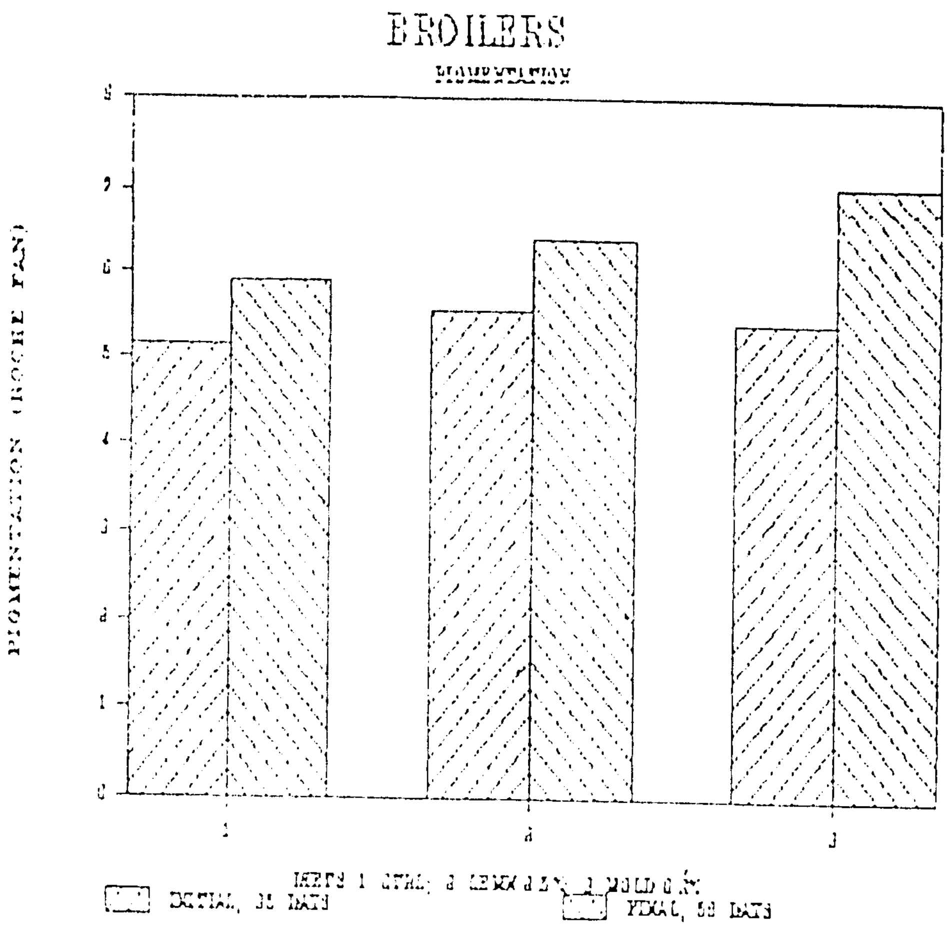
G R A P H 1



- The similarity of Feed Conversion ratios in Titan Broilers fed varying percentages of Lemna gibba at two different energy levels.

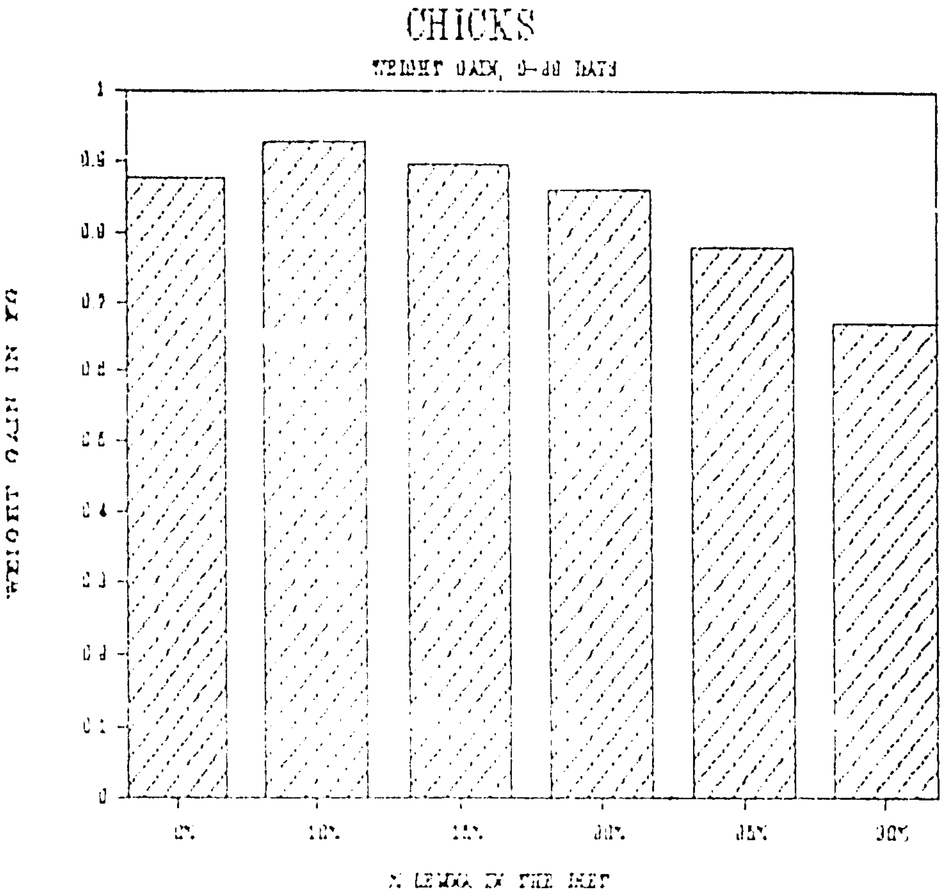
Note: The diets were formulated using an ME value of 1200 kcal/kg for Lemna gibba.

G R A P H 2



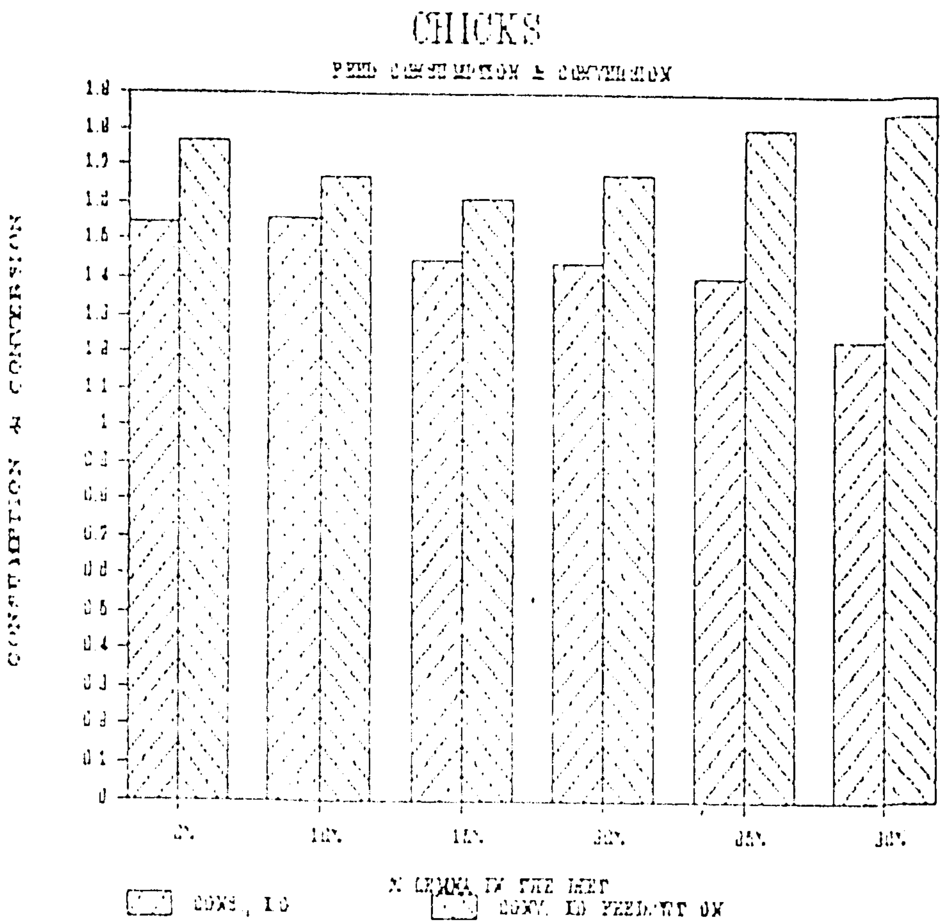
- The effect of different sources of pigment on broilers skin pigmentation.

GRAPH 3



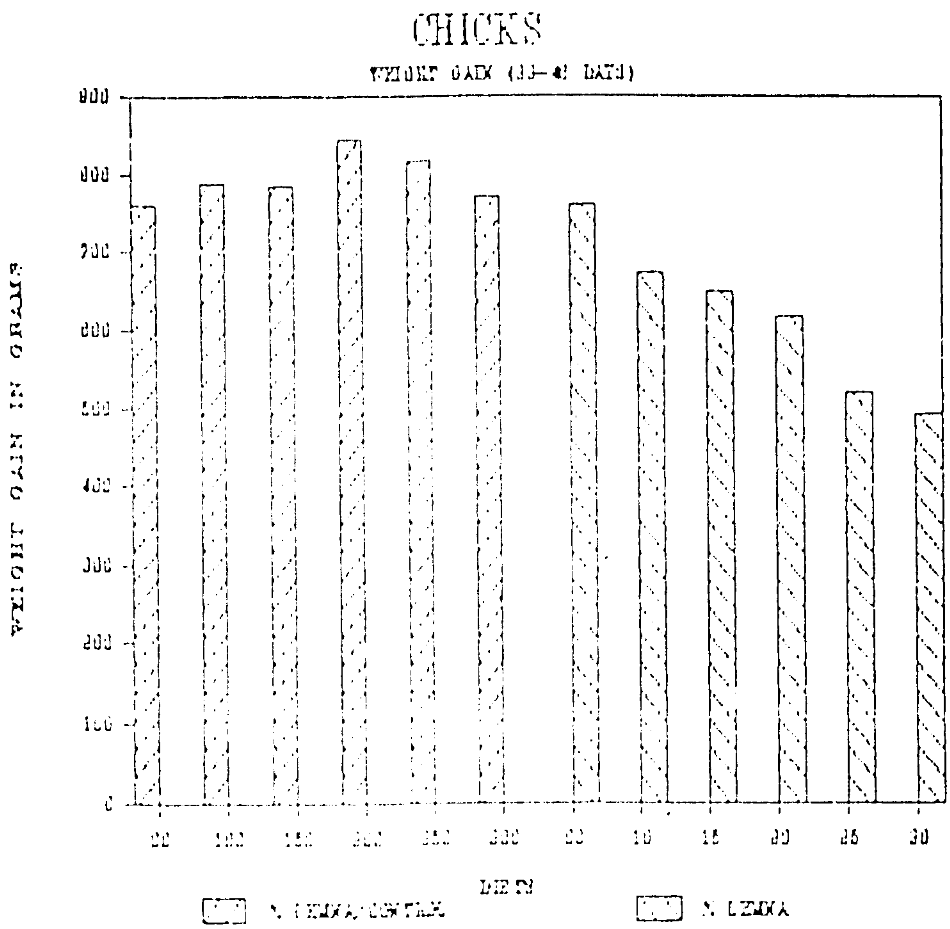
- The effect on weight gain in Titan baby chicks fed varying percentages of Lemna gibba in their diets.

GRAPH 4



The effect on Feed Consumption (kg) and Feed Conversion ratios (kg. of feed consumed / kg. weight gained), on baby chicks fed varying percentages of Lemna gibba in their diets.

G R A P H 5

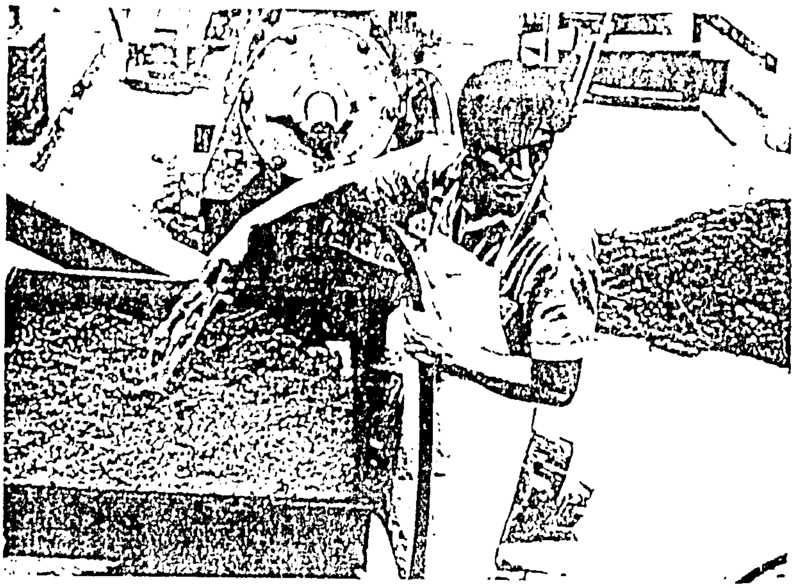


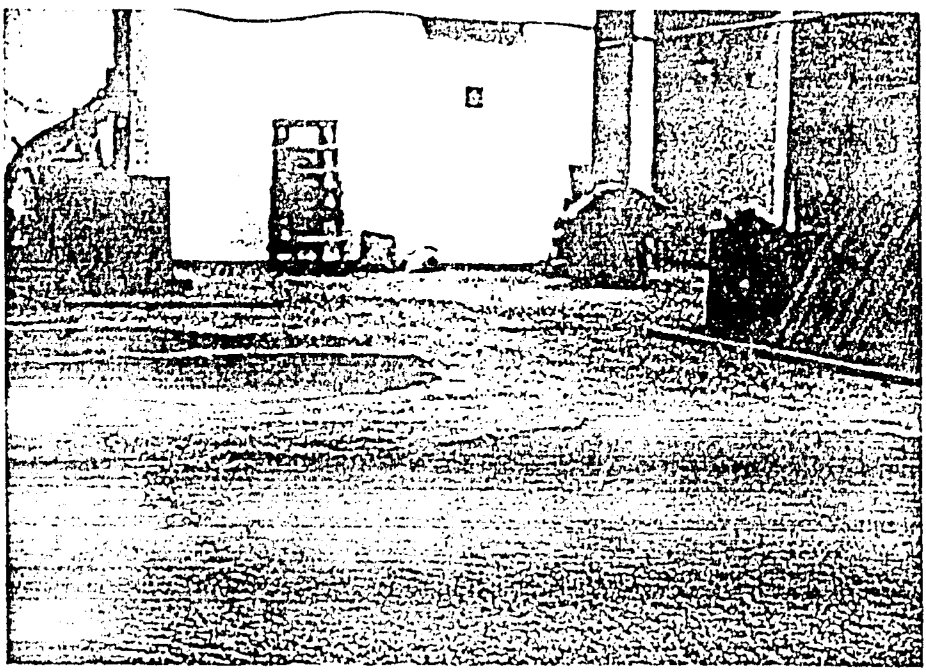
The effect of feeding a control diet versus continuing on a diet containing varying percentages of Lemna gibba during two weeks (33-48 days) in Titan baby chickens fed Lemna gibba for the first four weeks of life.

G R A P H 6

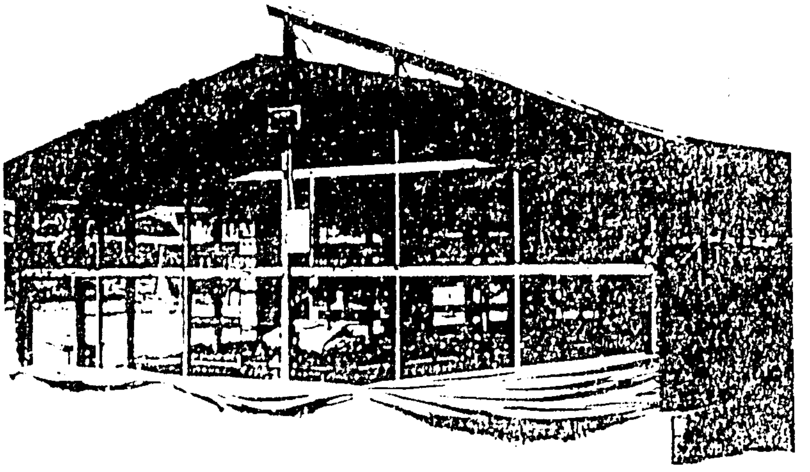
Picture #1: San Juan de Miraflores Sewage Lagoon. This photograph shows the baffles used to protect Lemna species from the strong wind. Harvesting is being performed by a worker.

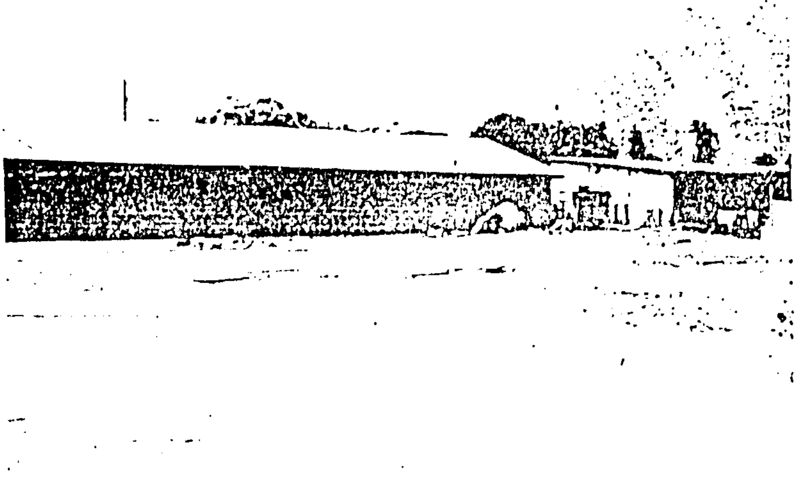
Picture #2. Lemna species transported wet to the Universidad Nacional Agraria where drying was performed.



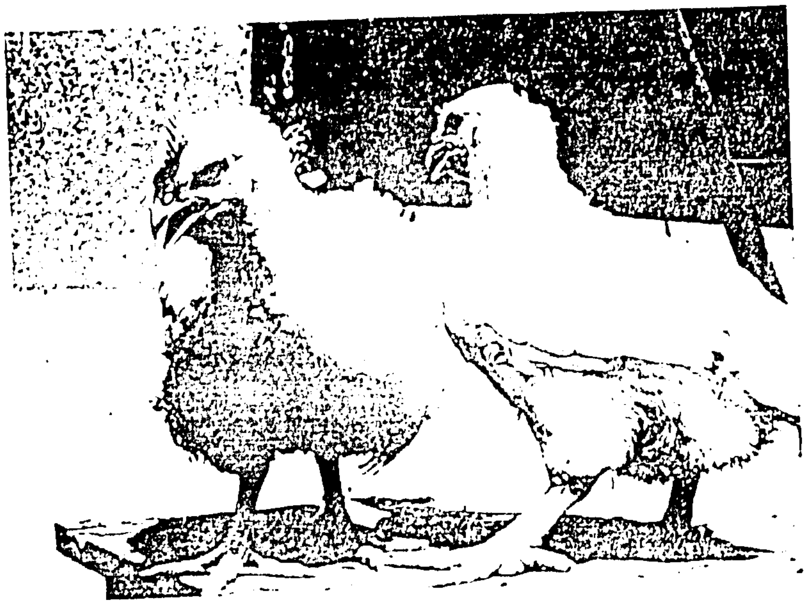


Pictures #[illegible] and #4. Washing and drying of lemna species at the Universidad Nacional Agraria. Drying was performed on concrete aprons first

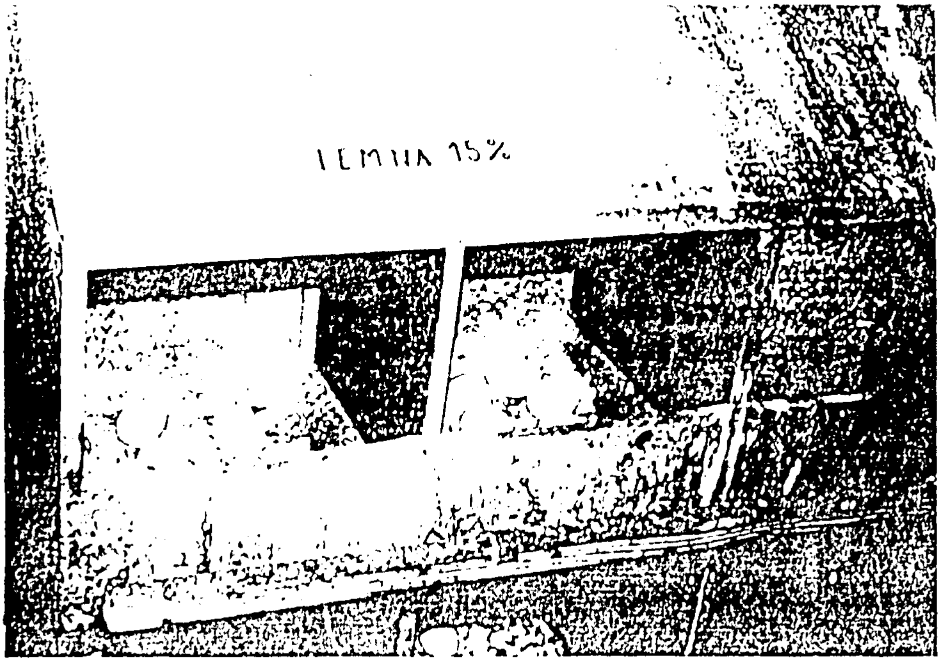


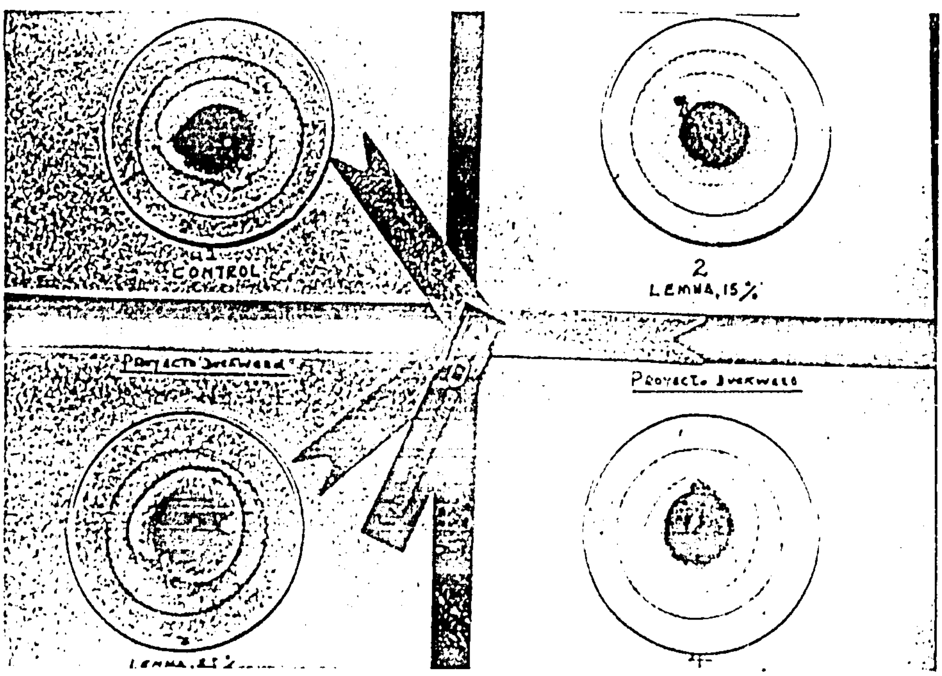


Pictures #5 and #6: Experimental Chicken House built by the Duckweed Project at the Universidad Nacional Agraria.



Picture #7: Titan Broiler Chickens: these chickens were fed a Control diet (left), and a diet containing Lemna (right) as can be seen by the pigmentation of the leggs.





Pictures #8 and #9 Egg production and pigmentation of the yolks; Picture 9 shows the yolk pigmentation of eggs from layer hens fed diets containing different levels of Lemna species (0%, 15%, 25% Lemna, and 15% Wolffia).