Duckweed

A potential high protein feed source for domestic animals and fish

A report for the Rural Industries Research and Development Corporation

by Bio-Tech Waste Management Pty Ltd
December 1998

RIRDC Publication No 98/148
RIRDC Project No BWM-1A

ISBN 0 642 57887 7
ISSN 1440-6845

Duckweed – a potential high protein feed source for domestic animals and fish
Publication no 98/148
Project no. BWM-1A

Researcher Contact Details

Robert E. Bell
Bio-Tech Waste Management Pty. Ltd.
P.O. Box 870
ARMIDALE, NSW 2350

Phone: 02 6772 8791
Fax: 02 6771 3131
email: bellbtwm@northnet.com.au
Internet: http://www.duckweed.com.au

RIRDC Contact Details

Rural Industries Research and Development Corporation
Level 1, AMA House
42 Macquarie Street
BARTON ACT 2600
PO Box 4776
KINGSTON ACT 2604

Phone: 02 6272 4539
Fax: 02 6272 5877
Email: rirdc@netinfo.com.au
Website: http://www.rirdc.gov.au

Published in December 1998
Printed on environmentally friendly paper by the AFFA Copy Centre

Foreword

Duckweed species, botanical name *Lemnaceae*, have great potential to remove mineral contaminants from wastewaters emanating from sewage treatment works, intensive animal production industries, abattoirs, food processing plants and the ammonia from fish ponds.

When removing nitrogen, phosphorus, potassium and other minerals from wastewater, the duckweed, when harvested, becomes a high protein-rich biomass which can be used as a feed for animals and fish.

This publication considers some of the technologies available for drying large quantities of the harvested duckweed, which is 92 - 94% water, and converting the duckweed into a feed suitable for animals and fish.

Duckweed protein has higher concentrations of the essential amino acids, lysine and methionine, than most plant proteins. It also has high concentrations of trace minerals and pigments, and zanthophyll that makes duckweed meal an especially valuable feed supplement. Trials of feeding duckweed to laying hens, Pekin ducks, shedded sheep and abalone are covered in the publication.

This report, a new addition to RIRDC's diverse range of over 250 research publications, is part of the Corporation's New Plant Products R&D Program, which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

Peter Core
Managing Director
Rural Industries Research and Development Corporation

Acknowledgments

Bio-Tech Waste Management (BTWM) is greatful to the Rural Industries Research and Development Corporation for the financial support which made possible the research which was conducted and reported on in this Final Report.

BTWM would also like to acknowledge the invaluable contribution of the Senior Principal Investigator, Professor John V. Nolan, University of New England's Department of Animal Science.

Contents

Executive Summary

Characteristics of Duckweed

Duckweed is a small fast-growing, aquatic plant that floats on the surface of ponds. Interest in duckweed has increased recently along with the realisation that it can extract unwanted minerals such as nitrogen, phosphorus,potassium, aluminum, iron, magnesium and sodium from polluted water (sewage, factory effluent). Duckweeds need to be managed, protected from wind, maintained at an optimal density by judicious and regular harvesting and fertilised to balance nutrient concentrations in water to obtain optimal growth rates. When effectively managed in this way, duckweeds yield 10-30 tonne dry matter (DM/ha/year containing up to 43% crude protein (CP), 5% fat and a highly digestible dry matter.

Crop management is concerned with when to fertilise, harvest and buffer; how much to fertilise and to harvest and which nutrients to supply. Judicious management should be aimed at maintaining a complete and dense cover of duckweed. Any waste organic material can be used to supply duckweeds with nutrients. The most economical are wastewater effluent from homes, dairies, piggeries, beef cattle feedlots, abattoirs and food processing plants.

Research on using the duckweeds in the rations of domestic animals has been surprisingly scarce, perhaps because of the difficulities in growing sufficient quantities under experimental conditions.

Bio-Tech Waste Management's Duckweed Wastewater Treatment System has the potential to reduce the usually high loadings of mineral nutrients, particularly Nitrogen, Phosphorus, Potassium and Sodium at dairies, piggeries, beef cattle feedlots, abattoirs and poultry farms with processing plants, to levels that allow the effluent to be re-used on a continuous basis for either land applications or for cleaning sheds. The economic benefit to these industries would be enormous if they could produce re-usable wastewater plus have a crop which could be used as a high protein feed supplement.

Duckweed protein (35-45%) has higher concentrations of the essential amino acids, lysine and methionine than most plant proteins. It also has high concentrations of trace minerals and pigments, and zanthophyll that makes duckweed meal an especially valuable supplement for poultry, fish and other animal feeds.

Drying Program

With the plant being approximately 94% water, an economical method of drying the plant had to be found so that it could be turned into a feed for fish and animals. A number of technologies were evaluated, and the one most suited for drying duckweed was identified as a solar power drying chamber. When a heat exchanger is connectecd to the drying chamber, the efficiency increases substantially. When drying the duckweed in the winter months in a cold climate, it will probably be necessary to use gas heating to supplement the solar heat if drying large quantities of the plant. The quantity of duckweed being harvested in the colder months, for instance on the Northern Tablelands, will be substantially less than what is harvested during the warmer months.

The drying of duckweed will be required when the plant material is used in a pre-mix application, either as a dried plant material or incorporated into pellets. The drying trials emphasised the significant time and cost savings that could be realised if the harvested duckweed could be feed as a fresh plant. This is possible where the duckweed is cultivated on wastewater at a farm, such as a piggery, dairy, beef cattle feedlots or on aquaculture farms.

Feeding Trials

A total of 7 trials of feeding duckweed to animals or fish were conducted during the course of the project. The results are briefly discussed below.

Trial No. 1. Duckweed As A Protein Source In Diets For Layers

Egg production and egg characteristics were determined in two strains of layers (Tegel Hi-Sex and Tegel Super Brown) when given diets in which duckweed was included at 10, 30, 50, 80, 120 and 200 grams/kilo as feed. A total of 120 layers were used in the trial, 60 of both strain. The trial was conducted over a three month period August to November 1996.

The effects of performance, egg and egg shell characteristics and yolk colour were recorded in the laying hens.

Trial No. 1 demonstrated the potential of duckweed to provide a source of amino acids and minerals, and yolk pigments that impart an attractive golden colour to the egg youlks.

Trial No. 2 Use Of Dried Duckweed In Diets For Brown Egg Layers

In this trial, egg production of newer brown egg layers was recorded over a period of two months when given diets with and without duckweed.

Two strains of layers, initially about 55 weeks of age, (Tegel Super Brown and Tegel Hi-Sex, total 108 hens) were used in the trial. Feed intakes and egg production were recorded for 8 weeks. Egg weights, shell and internal characteristics were determined on eggs collected on 2 successive days at the end of the trial.

Feed intake and production was excellent and no clinical effects of the diets were evident. Birds ingested slightly more of the duckweed-containing diet, and produced slightly heavier eggs with attractive but darker yolks. The researchers concluded that duckweed is a useful protein, mineral and pigment source in diets for layers and it appears to contain no toxic or anti-nutritional factors.

Trial No. 3 Fresh And Dried Duckweed In Diets For Laying Pekin Ducks

Two trials were carried out with 100 Pekin ducks (70 weeks of age, after moulting) to investigate the use of fresh and dried duckweed or fresh azolla as components of diets.

In Trial No. 3, the ducks were maintained on a commercial diet for 3 weeks then allocated at random to 10 groups of 10 and given one of 5 diets formulated to duck-layer specifications. Feed intakes, duck-day egg and egg-mass production were determined.

Feed intake (corrected for spillage), egg production and Feed Conversion Ration did not differ between diets, indicating that duckweed can be used in diets, either fresh or dried, at inclusion levels of 5% or 10%, with no adverse effect on egg production or food conversion efficiency.

Trial No. 4 Fresh And Dried Duckweed And Azolla In Diets For Laying Pekin Ducks

In Trial No. 4, the ducks were maintained in the same groups and given commercial pellets. Feed intake and egg production were recorded. Ducks ingested, on average, 0.5 - 0.6 kilos of fresh duckweed or azolla per day. Egg production did not differ between diets. Indication are that fresh duckweed and fresh azolla are highly attractive and nutritious feeds for ducks.

In both Trials 3 and 4, egg production was excellent for second-lay ducks. Of the egg characteristics examined in both trials, only yolk colour differed between diets. Yolk colours were highly correlated with intake of duckweed, showing that duckweed has the ability to impart a rich golden colour to egg yolks.

Trial No. 5 Duckweed Feeding Trial On Merino Fine Wool Sheep

The primary objective was to determine if the sheep would eat duckweed, and if so, the benefit gained by the sheep. Bio-Tech Waste Management (BTWM) also wanted to determine the most appropriate method of feeding duckweed, in a dried or fresh state.

The sheep were assigned ot one of four dietary treatment groups using a random allocation technique. All of the sheep ate their feed and showed no adverse effects from their diets.

It would be incorrect to make any concrete conclusions regarding the duckweed feeding trials for Merino fine wool whethers other than they will eat both dried and fresh duckweed without any apparent ill effects.

The trial confirmed that duckweed represents alternative high protein supplement for sheep that are shedded and most probably for lot-fed lambs. In both instances, the diets of the animals is crucial in producing either extra fine wool or lean body weight.

Trial No.6 Duckweed Feeding Trial For Silver Perch

BTWM made arrangements with a silver perch fish farm in New South Wales to trial feeding dried duckweed as a high protein supplement in their feed for the silver perch.

Feeding trials started in October 1997 but were suspended shortly thereafter as the owners have decided to move the operations to Queensland. The trials did not last long enough to draw any conclusions other than the fish will eat the duckweed.

Trial No. 7 Duckweed Feeding Trials For Abalone

BTWM provided dried duckweed to a company manufacturing feed for the abalone industry. While the protein level of the duckweed is of importance in the abalone feed, the real interest is the level of beta carotene in the dried duckweed.

The duckweed is being incorporated into the standard abalone feed to try and give the abalone the orange/red colouring that they get when growing naturally in the ocean and eating a sufficient quantity of seaweed.

The trial is continuing and BTWM has been advised that the abalone are eating the feed containing the duckweed.

Recommendations

The results presented in this Report clearly show that there are very significant benefits to be achieved by installing the BTWM Duckweed Wastewater Treatment System, and using the harvested biomass as a feed for animals and fish.

BTWM believes that further research can be justified in duckweed feeding trials for pigs, dairy cows and in the aquaculture industry for prawns, silver perch, eels, abalone and baramundi.

1. Introduction

Duckweed species (botanical name Lemnaceae) have great potential to remove mineral contaminants from wastewaters emanating from Sewage Treatment Works (STW), intensive animal production industries, food processing factories, or from intensive irrigated crop production. Duckweed needs to be managed, protected from wind, maintained at an optimal density by judicious and regular harvesting and fertilised to balance nutrient concentrations in water to obtain optimal growth rates. When effectively managed in this way, duckweed yields 10-30 tonne dry matter (DM)/ha/year containing up to 43% crude protein (CP), 5% fat and a highly digestible dry matter. Crop management is concerned with when to fertilise, harvest and buffer, how much to fertilise and to harvest, and which nutrients to supply.

Judicious management should be aimed at maintaining a complete and dense cover of duckweed, low dissolved oxygen and a pH level of 6-7. A total crop suppresses algae growth, which minimises CO2 production from algal respiration and prevents its elevating effect on pH. Any waste organic material can be used to supply duckweed with nutrients. The most economical are wastewater effluent from homes, food processing plants, cattle feedlots, intensive pig and dairy production and abattoirs. Research on using the duckweed on the rations of domestic animals has been surprisingly scarce, perhaps because of the difficulities in growing sufficient quantities under experimental conditions. Duckweed has been fed to animals and fish to compliment diets, largely to provide a protein of high biological value.

Bio-Tech Waste Management's Duckweed Wastewater Treatment System has the protential to reduce the ususally high loadings of mineral nutrients, particularly Total Organic Nitrogen T(N), Total Phosphorus T(P), Potassium (K) and Sodium (Na) at beef feedlots, piggeries, dairies, poultry farms and abattoirs to levels that allow the effluent to be re-used on a continuous basis for either land applications or for cleaning sheds. The economic benefit to these industries would be enormous if they could produce re-usable wastewater plus have a crop which could be used as a high protein feed supplement. The social and environmental benefits would also be substantial.

Duckweed trials at municipal Sewage Treatment Works (STW) in NSW during 1993 and 1994, demonstrated duckweed can remove nutrients from municipal wastewater to New South Wales (NSW) Environment Protection Authority (EPA) sensitive water standards, and while doing so prevent algal growth.

2. Objectives

While removing N, P, K and other minerals in municipal wastewater, the duckweed becomes a high protein-rich biomass which when harvested, can be used as a feed for animals and fish. Using harvested duckweed primarily from a STW in northern New South Wales, the project analysed various drying technologies in an effort to find an economical method to dry large quantities of duckweed (the plant is 92-94% water) without destroying its high (35-45%) protein content.

While trialling drying technologies work took place on converting the dried duckweed into a feed for animals and fish. Duckweed protein has higher concentrations of the essential amino acids, lysine and methionine, than most plant proteins. It also has high concentrations of trace minerals and pigments, and zanthophyll that makes duckweed meal an especially valuable supplement for chickens, ducks and other animal feeds.

3. Methodology

The research project was divided into Three Stages which overlapped each other.

3.1 Stage 1

Drying Program 12-18 months in duration commencing in Month 1.

Evaluate and trial various drying and dewatering systems available which can dry the duckweed biomass economically. Remembering that the plant is approximately 93% water, the key to using it as a high protein feed supplement is finding a technique to remove the water as quickly and economically as possible without destroying the protein.

Bio-Tech Waste Management has dried duckweed in fan-forced ovens at 70 degrees Celsius (C) without destroying the protein level, however this is not an economical method.

Strategies and systems to be evaluated included partial dewatering on-site when harvesting. Trialling microwave drying ovens and solar powered drying systems were also evaluated. Wrapping the duckweed biomass in plastic similar to silage was also tried and evaluated.

Bio-Tech Waste Management (BTWM) has tried drying duckweed in the open air. This is not an appropriate technology as the protein levels are not protected.

Samples of dried duckweed were analysed for its protein levels, the concentrations of the essential amino acids, lysine and levels of methionine. The levels of trace minerals were also analysed.

3.2 Stage 2

Production of poultry and animal feeds. 18 Months duration

Before BTWM could start promoting duckweed as a high protein feed supplement for animals, it required the scientific data to prove that it was in fact an ideal feed and would not have a detrimental effect on the animals.

By Month 3 it was expected that there would be a limited quantity of duckweed biomass available which would allow work to commence on determining the levels, if any, of detrimental bacteria.

Research would also be conducted on the various ways of packaging the biomass as feed; ie., pellets, silage, powdered or possibly in a liquid form.

Methods of protecting the protein in ruminants would be evaluated.

3.3 Stage 3

Feeding Trials - 18 Months commencing in Month 7

Stage 3 was expected to produce the data that proves that the harvested duckweed from wastewater ponds can be successfully fed to poultry and other animals.

The poultry feeding trials were designed to commence first for a number of reasons, one of which was that large quantities of dried duckweed was not required. The trials for pigs and ruminants will require large amounts of dried duckweed and the ruminant trials will most likely require the duckweed to be protected.

Some of the key factors to be evaluated in Stage 3 are:

* determining the upper levels of inclusion of duckweed in the diet of poultry and pigs.

* for laying hens, the effect of duckweed on the shell thickness and yolk colouring.

* for ruminants, the emphasis would be to analyse the effects of feeding duckweed on the rumen function and whether the time required for the animals to aquire a taste for dried and ideally wet (fresh) duckweed.

4. Detailed Results

At the time of preparing the detailed proposal to RIRDC in February 1995, a review of all available literature indicated that the duckweed would have to be dried and then made into a feed for animals. A major effort in this Project has been to either locate existing technology that can economically dry the duckweed or develop a system that can dry the plant.

Convection Drying Ovens
These have been used at the University of New England (UNE) to dry the duckweed in quantities ranging from a few hundrew grams to 40 kilos for plant analyses and for trials in pelletising. These gas fired ovens are not an economical method of drying plant material which is 94% moisture. The oven does guarantee however,that any bacteria on the duckweed will be destroyed.

Solar Power Drying Chamber
BTWM visited an installation in northern Queensland that was using solar power to dry lemon grass. BTWM constructed a small version of the solar power drying chamber in Armidale to trial it for drying duckweed. The smaller version is not as efficient as the one visited in Queensland but it was still very effective in drying the duckweed-60 hours versus 150 hours in a plastic tunnel house. The operating cost of drying the duckweed in the solar power drying chamber was minimal.

While working with solar power as a source for drying the duckweed, BTWM was having discussions with firms that manufacture and install heat pumps and heat exchangers. It appears that the most promising method of drying duckweed would be to use the solar power drying chamber in conjunction with a heat exchanger. The concept is to recover the heat from the moist air in the drying chamber before it is expelled thereby making the solar chamber more efficient. It has also been suggested that the chamber use gas heating in the cooler seasons to keep the temperature in the chamber at a constant level.

Many of the municipal Sewage Treatment Works (STW) have bio-digesters and between 50% and 70% of the gas generated is flared. At the larger STW the idea will be to access the excess gas from the bio-digester and use it to heat the duckweed drying chamber in association with a heat exchanger. If this system is employed, then the cost of drying duckweed becomes very economical with the added benefit that it would be done on-site at the STW.

Plastic Tunnel House
While it was never intended to be a permanent method of drying the duckweed, the plastic tunnel house at UNE has been the method used to dry the majority of the duckweed used in the feeding trials. Generally BTWM dried approximately 800 kilos of duckweed at a time, and depending on the amount of sunlight and the minimum and maximum air temperatures, it took between 5 and 8 days to dry the duckweed. The plant was spread out on the floor of the tunnel house and was turned over twice a day. The only operating cost is the labour required to turn over the duckweed to ensure that it dries out evenly.

The biggest disadvantage of using the tunnel house is the inability to insulate the structure and retain the heat after the daylight hours.

Hydraulic Press
Several organisations contacted about drying technologies suitable for aquatic plants, suggested rolling or squeezing the duckweed to remove the water from the plant. BTWM tried rolling the duckweed but this proved to be a difficult process, certainly on a small scale and the plant tended to

cling to the rollers.

Bio-Tech Waste Management acquired a second hand hydraulic press as the initial results of squeezing or pressing the duckweed looked promising. The press is capable of pressing .25 cubic metres of duckweed and certainly removes a significant quantity of water from the plant, but in doing so the duckweed becomes
like a thick cake due to the light film of wax on its fronds (leaf). This makes the final drying process much more difficult and time consuming.

Microwave Technology
This has also been evaluated as a means of drying duckweed. The trials were conducted for BTWM by a firm specialising in microwave technology.

The first trial was not encouraging as microwaves are not efficient when the moisture content is over 50 percent. Given duckweed's 92% to 94% moisture content, using microwaves during the initial drying stages was not feasible. Subjecting the duckweed to microwaves equivalent to 80 degrees celsius for 10 minutes removed a very large quantity of water and did not destroy the plant cells but neither did it dry the plant. Various trials were conducted using microwaves with duckweed in a partially dried state. The use of microwaves to partially dry duckweed is not economically viable usless it is used in conjunction with another application such as treating the duckweed with enzymes. If the enzymes require heat to activate them, then microwaves can be used for this process while also finishing the drying of the duckweed.

Another possibility is to use the microwaves in applications where it is essential that all pathogens are killed without drying the plant or damaging the cells. The first trial referenced above would have killed all of the pathogens without destroying the plant cells.

5. Crude Protein Levels

The crude protein level of duckweed depends primarily on the level of Total Organic Nitrogen (Kjeldahl Nitrogen), expressed as T(N), in the wastewater. During the course of the feeding program, duckweed was harvested from a number of municipal STW which had duckweed growing on their evaporation ponds. Depending on the treatment system used at the STW, the crude protein level of the duckweed varied between 32% and 35%. The crude protein level of duckweed cultivated at a municipal STW is not likely to be higher than 35% as the level of T(N) rarely exceeds 20 mg/l.

Duckweed cultivated at a beef cattle feedlot reached 44% crude protein while piggery effluent, which had been heavily diluted, produced duckweed with a crude protein level of 39%.

Bio-Tech Waste Management had the duckweed cultivated on diluted piggery effluent analysed for crude protein, minerals and amino acid profile. Researching published data indicates that the analyses for the duckweed cultivated on diluted piggery effluent is very similar to the analyses of duckweed cultivated on municipal wastewater ponds and beef cattle feedlot effluent. It has been suggested that duckweed protein concentrate could be used as an effective protein supplement in diets low in lysine such as those based on corn or rice.

(*All Amounts Expressed As Per Cents)*

	Protein	**Ash**	**Fat**	**Fibre**
Duckweed cultivated on diluted piggery effluent	35.0	13.0	5.4	15.0

Amino Acid Profile

ASP	GLU	SER	GLY	HIS	ARG	THR	ALA	PRO	TYR	VAL	MET	CYS2
17.45	13.13	5.21	6.09	1.95	5.42	7.72	7.44	4.85	3.55	4.36	1.22	0.70

ILE	LEU	PHE	LYS
2.65	8.12	4.99	5.15

Mineral Content Of The Duckweed

P	S	K	CA	MG	NA	MN	FE	ZN	CU	AL	B	MO
%	%	%	%	%	%	ug/g	ug/g	ug/g	g/g	%	g/g	g/g
1.136	0.707	2.650	1.119	0.357	0.203	407.3	459.1	1732.9	11.7	0.024	236.6	2.4

Table 1

Essential Amino Acids in Protein Concentrate from Duckweed Cultivated on Diluted Piggery Effluent Compared to FAO Reference Pattern, Corn and Rice (g/100g of Protein).

Amino Acid	Duckweed	FAO	Corn	Rice
LYS	5.15	4.2	2.3	3.2
ILE	2.65	4.2	6.2	5.2
LEU	8.12	4.8	15.0	8.2
MET	1.22	2.2	3.1	3.4
PHE	4.99	2.8	5.1	5.0
THR	7.72	2.8	3.7	3.8
VAL	4.36	4.2	5.3	6.2
TRP	---	1.4	0.6	1.3

Source: *J. Agric. Food Chem* 1980, 28, 848-850
The duckweed data was analysed for Bio-Tech Waste Management.

6. Bacteria Levels

A common question asked at the start of the duckweed research was what effect would the pathogens have on the duckweed when it is cultivated on wastewater. It appears that pathogens and their effects on duckweed cultivated in wastewater has not been a topic that has been widely reported on.

When researching the subject, BTWM spoke with botanists and animal nutritionists. The general consensus was that the duckweed would not uptake the pathogenic bacteria and that if it had bacteria attached to it and was fed to animals, the digestive system of the animals would be able to handle the bacteria. If there was concern that the duckweed was being contaminated by the bacteria in the ponds, the duckweed should be rinsed before being fed wet (fresh) to the animals.

Escherichia Coli (E Coli) and Fecal Coliform were monitored twice monthly at the Inverell STW to compare the levels in the Control Pond with the levels in the Duckweed Pond. The coliform group of bacteria is the principal indicator of suitability of water for domestic, industrial or other uses.

When analysing the results for the Duckweed Pond, which had a very good cover of either duckweed or azolla throughout the trial, the fecal coliform readings at the point of discharge in the Duckweed Pond and the Control Pond were very similar and well within the NSW Environment Protection Authority limits for discharging into sensitive waters.

E coli is a member of the indigenous fecal flora of warm blooded animals. The occurrence of E coli is considered a specific indicator of fecal contamination and the possible presence of enteric pathogens. (Eaton et al. 1995). There were only two occasions when there were E coli readings for the Control Pond and the Duckweed Pond, and those readings occurred on the same day for both ponds. The other readings recorded a zero results.

Since practically all of the duckweed for the feeding trials was harvested from the Inverell STW, there was little chance that the duckweed would have been contaminated by pathogenic bacteria. The other STWs where BTWM was cultivating duckweed also record low levels of E coli and Fecal Coliforms.

The duckweed that was dried and analysed for bacteria content was found to be perfectly safe to be used as a feed for animals. The drying process in the tunnel house would have destroyed any bacteria.

7. Feeding Trials

7.1 Trial No.1

Duckweed As A Protein And Pigment Source In Diets For Layers.

Egg production and egg characteristics were determined in two strains of layers (Tegel Hi-Sex and Tegel Super Brown) when given diets in which duckweed was included at 10, 30, 50, 80, 120 and 200 g/kg as feed. A total of 120 layers were used in the trial, 60 of each strain. The birds were held in single-bird cages (banks of 6) distributed uniformly in an open shed. The trial was conducted over a three month period August to November 1996 in Shed C at the University of New England.

The study extended previous work which confirmed the value of duckweed as a protein-rich, pigment-rich ingredient when used in the diets of laying hens (Haustein et al., 1988; O'Neill et al., 1996). As before, duckweed was used as a partial replacement for soybean meal, but over a wider range of inclusion. The effects of performance, egg and egg shell characteristics and yolk colour were recorded in the Tegel Hi-Sex and Super Brown laying hens.

Duckweed (predominantly *Spirodela punctata*) was collected from the Sewage Treatment Works at Scone, NSW and dried in a tunnel house at the University. Six diets were formulated to contain 11.3 MJ ME and (g/kg as feed) 160 g crude protein, 40 Ca, 10P with increasing amounts of duckweed in lieu of soybean meal and wheat (Table 2).

Table 2.
Dietary formulations with increasing concentrations of duckweed (g/kg as feed).

Duckweed	10	30	50	80	120	200
Wheat	526	521	511	496	476	436
Soybean meal	120	105	95	80	60	20

Source: Department of Animal Science, University of New England, Armidale, NSW

Summary Of Trial No.1.

Trial No.1 clearly demonstrated the potential of duckweed to provide a source of amino acids and minerals, and yolk pigments that impart an attractive golden colour to the egg yolks.

The response to the experimental diets differed between strains. The Hi-Sex birds maintained a high intake and hen-day and egg-mass production, and a good feed conversion ratio on all diets. There was a small decline in production of Hi-Sex hens given diets with higher levels of duckweed whereas the Super Brown birds appeared to be more adversely affected as the level of inclusion of duckweed was increased and the amount of soybean and wheat were correspondingly decreased. The diets were not specifically balanced for individual amino acids and minerals as no analysis had been done on this material at the time of diet formulation.

All the birds in this trial consumed relatively large amounts of water and produced very wet droppings. A possible reason is the relatively high concentrations of total salts arising from the presence of duckweed in the diets. Individual minerals, such as boron and aluminium, may also have had detrimental effects, however there was no discernible trend in the wetness of droppings with level of inclusion of duckweed in the diet. (In a nearby trial, the same strains given a diet formulated using the same batch of soybean meal also produced wet droppings. The wetness declined after the birds were given a similar diet formulated with soybean meal from a different source, although there were other possible confounding factors). Thus it is not possible to conclude with certainty that duckweed was, or was not, the reason for the wet droppings. In view of the high concentrations of minerals that may accumulate in duckweed, it appears to be highly desirable to know the mineral content of duckweed when diets are being formulated so that levels of all minerals can be adjusted appropriately.

7.2 Trial No.2

Use Of Dried Duckweed In Diets For Brown Egg Layers

Trial No.2 commenced in December 1996 and was completed in February 1997. In this trial, egg production of newer brown egg layers was recorded over a period of two months when given diets with and without duckweed.

Two strains of layers, initially about 55 weeks of age, (Tegel Super Brown and Tegel Hi-Sex, total 108 hens) were held in single-bird cages (banks of 6) distributed uniformly in Shed C at the University of New England. All hens were given commercial crumbles for 4 weeks before being allocated to their dietary treatments, *viz Diet 1* Ridley's AgriProducts commercial layer crumble with 10% duckweed (Control 1); *Diet 2*, layer crumble (Control 2) and *Diet 3*, Duckweed layer crumble with 10% duckweed (DW). The three diets were formulated to have the same specifications, ie. 10.8 MJ/kg DM, 16.7% crude protein and 3.8% calcium.

Feed intakes and egg production were recorded for 8 weeks. Egg weights, shell and internal characteristics were determined on eggs collected on 2 successive days at the end of the trial. Some relevant results are given in Table 3.

Table 3
Summary Of The Duckweed Feeding Trial For Brown Egg Layers

	Strains		**Diets**			**Significance**	
	Hi-Sex Diet	Brown Diet	Ridley's Diet	Control Strain	DW Diet		
Intake gDM/d	142	140	137	139	147	NS	NS
Hen-day prod. %	88.4	86.0	90.7	84.9	85.9	NS	NS
Egg weight grams	67.4	62.4	63.6	62.8	66.2#	***	**
Shell thickness u	382	369	379	369	378	*	NS
Haugh units	74.0	73.4	72.6	73.9	74.6	NS	NS
Yolk colour Roche	12.6	12.7	12.2	12.5	13.2#	NS	***

** P<0.01; *** P<0.001 # indicates result is significantly higher than for other two diets.

There were significant strain and diet effects, but the findings were generally similar for both strains (no significant interactions). Feed intake and production was excellent and no clinical effects of the diets were evident. Birds ingested slightly more of the duckweed-containing diet, and produced slightly heavier eggs with attractive but darker yolks. The researchers concluded that duckweed is a useful protein, mineral and pigment source in diets for layers and it appears to contain no toxic or anti-nutritional factors.

7.3 Trial No.3

Fresh And Dried Duckweed In Diets For Laying Pekin Ducks

Two trials were carried out with 100 Pekin ducks (70 weeks of age, after moulting) to investigate the use of fresh and dried Duckweed (DW; 4.9 % DM, 35 % crude protein) or fresh Azolla (AZ; 3.9 % DM, 30 % CP) as components of diets.

The reason for conducting the trials was a 450 sow piggery in Tamworth, NSW is considering installing a BTWM Duckweed Wastewater Treatment System on its evaporation ponds. The surface area of the ponds is not large enough to cultivate a sufficient quantity of duckweed to incorporate into the pigs' diets, therefore the piggery owners are interested in identifying a use for the harvested duckweed.

Approximately 40 kilometres from the piggery there is a duck farmer with 14,000 Pekin ducks. He was very interested in determining if the duckweed and azolla could be used as a high protein feed supplement for the ducks' diet. If the duckweed proved to be a good protein substitute, then he would be interested in acquiring the duckweed from the piggery.

In Trial No.3, the ducks were maintained on a commercial diet for 3 weeks then allocated at random to 10 groups (n=10) and given one of 5 diets formulated to duck-layer specifications, *viz. Diet 1,* commercial pellets (Ridley Agri-Products); *Diet 2,* similar pellets with 10 % dried DW; *Diet 3,* same pellets as Diet 2 but with fresh DW (DM equivalent to Diet 2), *Diets 4 and 5* similar to Diets 2 and 3 but with 5 % DW. Feed intakes, duck-day egg and egg-mass production were determined (24/3 -13/4/97). Raw means are given in Table 4, with significance based on alalysis of covariance (covar).

Feed intake (corrected for spillage), egg production and FCR did not differ (P>0.05) between diets, indicating that DW can be used in diets, either fresh or dried, at inclusion levels of 5 % or 10 %, with no adverse effect on egg production or food conversion efficiency.

Table 4
Fresh And Dried Duckweed Feeding Trial For Laying Pekin Ducks

Measurement	Industry pellets	Dried DW 10 %DM	Fresh DW 10 %	Dried DW 5 %DM	Fresh DW 5 %	Signf. covar
Feed gDM/d	272	266	313	362	287	NS
Egg prod. %	89.7	76.5	73.5	89.1	83.0	NS
Egg-mass g/d	74.2	63.0	62.4	74.2	69.9	NS
FCR g/g	3.6	4.0	4.7	4.7	3.9	NS
Roche yolk colour	10.8	9.8	11.2	8.5	9.7	***

7.4 Trial No.4

Fresh And Dried Duckweed And Azolla In Diets For Laying Pekin Ducks

In Trial No.4, the ducks were maintained in the same groups and given commercial pellets (!4/4 - 18/5/97). On 19/5/97, 5 diets formulated to the same theoretical specifications as the Ridley's pellets, were then allocated at random to the 10 pens (2 replicates/diet), *viz. Diet 1,* commercial pellets; *Diet 2,* pellets with 10% dried DW; *Diet 3,* pellets with fresh DW equivalent to the duckweed DM in Diet 2; *Diet 4,* pellets similar to Diet 2 but with DW *ad libitum; Diet 5* pellets similar to Diet 2 but with AZ *ad libitum.* Results (19/5 - 2/6/97) are given in Table 5.

Table 5
Fresh And Dried Duckweed And Azolla Feeding Trial For Laying Pekin Ducks

Measurement	Industry pellets	Dried DW 10 % DM	Fresh DW 10 %	Fresh DW *ad libitum*	Fresh AZ *ad libitum*	Signf. covar
Egg prod. %	96.3	77.9	87.0	82.8	82.7	NS
Egg-mass g/d	80.4	64.9	71.4	70.9	70.2	NS
Roche colour	10.1	8.7	9.3	10.3	9.2	***

Feed intake and egg production were recorded in Trial No. 4. Ducks ingested, on average, 0.5 - 0.6 kg fresh DW or AZ per day. Again egg production did not differ (P>0.05) between diets. Indications are that fresh DW and fresh AZ are highly attractive and nutritious feeds for ducks.

Azolla was used in this feeding trial with the duckweed as the Inverell STW was cultivating a large quantity of azolla along with the duckweed on the trial evaporation pond. If azolla was proved to be as effective as the duckweed in removing minerals from the municipal wastewater, then it could represent another high protein feed supplement for animals.

In both Trials 3 and 4, egg production was excellent for second-lay ducks. Of the egg characteristics examined in both trials, egg weight (c. 84 g), shell weight, shell breaking strength and resistance to deformation, albumen height, Haugh units and yolk colour, only yolk colour differed ($P<0.001$) between diets. Yolk colours were highly correlated (r sq. = 0.61, Trial 2) with intake of DW, showing that DW has the ability to impart a rich golden colour to egg yolks.

7.5 Trial No.5

Duckweed Feeding Trial On Merino Fine Wool Sheep

When applying to RIRDC for research funding, BTWM thought that the feeding trials for ruminants would require the duckweed to be protected so that the protein would pass through the rumen to the small intestine. As the researchers progressed with their work on the various feeding trials and critically assessed the analyses and the duckweed plant, the more they questioned the need to protect the duckweed when feeding it to ruminants.

Phase 1 incorporated studies of digestion of fresh duckweed in the rumen of sheep using *in vitro* methods and a technique often referred to as the *in sacco* method. In the latter, the unknown feed (eg. duckweed) is suspended in porous bags and subjected to varying periods of microbial digestion in the rumen of sheep fitted with rumen cannulas. The sheep are referred to as *fistula sheep.*

Two trials using the *in vitro* method were conducted at the University on *fistula sheep* in October 1997, to determine the rate and extent of digestion of duckweed protein in the rumen. This affects whether or not the amino acids in duckweed become available to the ruminant host.

The results for the two trials were practically identical. After being exposed to rumen microbes for 3.5 hours, 70.8 % of the fresh duckweed remained. After being exposed to rumen microbes for 6.5 hours, 69.3 % of the fresh duckweed remained and after 8.5 hours in the rumen, 67.1 % of the fresh duckweed remained. After 24 hours exposed to rumen microbes 45 % of the fresh duckweed remained.

Research on ruminant digestion systems suggest that most feed would be digested within 8 hours of entering the rumen, therefore it would appear approximately 67% and 70 % of the fresh duckweed would pass through the rumen into the small intestine. With the crude protein level of the fresh duckweed at 35 %, the duckweed that passed through to the small intestine had approximately 24.5 % crude protein. This represents a very high level of protein for ruminants.

Bio-Tech Waste Management acquired 36 Merino fine wool wethers from the University of New England's "Kirby" property on 21 November 1997 and moved the sheep into Shed C animal house located across from the Department of Animal Science.

Building on the knowledge gained from the duckweed trials on the fistulated sheep, diets were formulated based on different inclusion levels of dried and fresh duckweed. The primary objective was to determine if the sheep would eat duckweed, and if so, the benefit gained by the sheep. BTWM also wanted to determine the most appropriate method of feeding duckweed, in a dried or fresh state.

When the sheep were brought into the shed on 21 November 1997, they had only been exposed to pasture grasses, so initially they were given lucerne chaff. It took approximately four days for all of the sheep to adjust to the lucerne chaff. They were then introduced to oats and chaff mixed with a small amount of lucerne. They were on this diet for three days before they were all eating their feed. They were then switched to a diet of 700 grams of oats and chaff, which had been calculated to be a maintenance diet. They all ate the straight oats and chaff within two days and were ready for the introduction of duckweed into their feed.

On 1 December 1997, all of the sheep were weighed. They were then introduced to a diet comprising 50 grams of dried duckweed mixed with 600 grams of oats and chaff. The protein of this diet equalled the protein level in the 700 grams of oats and chaff. By 4 December they were all eating the dried duckweed mixed with the oats and chaff. Most of the sheep would have even eaten the dried duckweed on its own without the oats and chaff. They all stayed on this diet until 21 January 1998 when fresh duckweed was introduced into their feed.

On 21 January, 200 grams of fresh duckweed was introduced into the sheeps' diet and the dried duckweed deleted. The fresh duckweed was mixed with the 600 grams of oats and chaff. The object was to ensure that they would all eat the dried and fresh duckweed before assigning them to one of the four dietary treatments. They stayed on the daily diet of 200 grams of fresh duckweed and 600 grams of oats and chaff until they were eating all of their feed. By 24 January the quantity of fresh duckweed in the sheeps' diet was increased to 300 grams per day, which they all ate.

On 26 January, the sheep were again weighed to determine if any experienced major weight loss. Since they were on a maintenance diet, we did not expect major weight gains, and indeed only five had very small gains in weight with the average being 0.9 kilos. Thirty-one of the sheep had weight losses with the average being 1.3 kilos. The researchers were not concerned with the weight loss as animals always experience a loss in weight when moved from an out of doors environment to confined space indoors.

The sheep were assigned to one of four dietary treatment groups using a random allocation technique. The four treatment groups were, 1. Control Group which received 700 grams of oats and chaff daily. 2. Group 2 received 50 grams of dried duckweed and 600 grams of oats and chaff daily. 3. Group 3 received 100 grams of dried duckweed and 570 grams if oats and chaff. 4. Group 4 received one kilo of fresh duckweed which was harvested from a municipal sewage treatment works in northern NSW and 630 grams of oats and chaff.

All of the sheep ate their feed and showed no adverse effects from their diets. On 17 February 1998, the sheep were weighed and only four experienced a weight loss, with an average of 350 grams. Thirty-two sheep gained weight with the average being 1.3 kilos. The weight gain equalled the earlier average weight loss, therefore the researchers were confident that the diets were very close to being reliable maintenance diets.

The feeding trials continued until 13 March 1998, when the sheep were again weighed and returned to UNE's "Kirby" property. An analysis of the sheep weights during the 24 day trial between 17 February and 13 March 1998, revealed that the sheep on Diet 1. Control Diet of 700 grams of oats and chaff, lost on average 830 grams. The sheep on Diet No.2, 50 grams of dried duckweed and 630 grams of oats and chaff, did not either gain or lose weight. The sheep on Diet No.3, 100 grams of dried duckweed and 570 grams of oats and chaff, lost on average 440 grams. The sheep on Diet No.4, one kilo of fresh duckweed and 630 grams of oats and chaff lost an average of 600 grams.

It would be incorrect to make any concrete conclusions regarding the duckweed feeding trials for Merino fine wool whethers other than they will eat both dried and fresh duckweed without any apparent ill effects. In most instances, they consumed all of their feed within a half-hour of being fed. During the 103 days that they were exposed to duckweed in their diet, the average weight of the sheep went from 39.2 kilos to 38.6 kilos, a decrease of 600 grams. Since the researchers were also trying to identify a maintenance diet for the sheep, a loss of 600 grams over 103 days was considered acceptable and close to a maintenance diet based on duckweed as a high protein supplement. Diet No.2 was very close to being the maintenance diet, as the sheep on average lost only 100 grams during the 103 day trial.

The trial confirmed that duckweed represents alternative high protein supplement for sheep that are shedded and most probably for lot-feed lambs. In both instances, the diets of the animals is crucial in producing either extra fine wool or lean body weight.

The researchers believe the results reported above justify further expenditures for research into the feeding of duckweed, both fresh and dried, to shedded sheep, lot-fed lambs and other ruminants like dairy cattle, where the very high protein of the duckweed would most probably result in the cow producing extra milk. If the cow is fed fresh duckweed containing 94% water, then the animals consumption of water would also be decreased.

7.6 Trial No.6

Duckweed Feeding Trial For Silver Perch

Bio-Tech Waste Management made arrangements in May 1997 with a silver perch fish farm in Condobolin, NSW to trial feeding dried duckweed as a high protein supplement in their feed for the silver perch. The fish farm was also very interested in combining the fresh duckweed with algae and crumbing it as a feed for the sliver perch.

BTWM has had continuing discussions with the fish farm regarding the trials and in August 1997 arranged to ship a large quantity of both dried and fresh duckweed to the fish farm. BTWM also discussed the concept of using duckweed to uptake ammonia from their fish ponds as the build-up of ammonia in fish ponds is a major problem and expense for fish farming industry.

Feeding trials started in October 1997 but were suspended shortly thereafter as the owners have decided to move the operations to Townsville, Queensland. The trials did not last long enough to draw any conclusions other than the fish will eat the duckweed. The owners would like to recommence the trials later in 1998 after they have relocated in Qld. if funding is available. The idea is to build a specific pond for the feeding and water cleansing trials.

In late 1996, BTWM supplied fresh duckweed from a duckweed trial at a municipal sewage treatment works, to a grazier with a fish pond. He was interested to see if his silver perch would eat the duckweed. He advised that not only did the fish consume the duckweed, it was very effective in cleansing the water in the pond and in doing so, grew very rapidly.

7.7 Trial No. 7

Duckweed Feeding Trials For Abalone

Bio-Tech Waste Management was contacted in early October 1997, by a company located in Woodside, South Australia, specialising in the manufacturing of feed for the abalone industry. Following a series of discussions, BTWM agreed to ship a quantity of dried duckweed to the company in South Australia. While the protein level of the duckweed is of importance in the abalone feed, the real interest is the level of beta carotene in the dried duckweed. BTWM had the duckweed analysed prior to shipping it to S.A. for the level of crude protein, ash, fibre, minerals and beta carotene. The beta carotene was analysed at 90 parts per million.

The duckweed is being incorporated into the standard abalone feed to try and give the abalone the orange/red colouring that they get when growing naturally in the ocean and eating a sufficient quantity of seaweed. When being farmed, they do not get the colouring required by the abalone customers. BTWM was advised that
initially the abalone were fed a large quantity of duckweed in the hope that the abalone would acquire the desired colouring over a very short period. Apparently the quantity of duckweed incorporated into the feed upset the mineral balance and the abalone did not respond well to the feed. The trial was abandoned in December 1997.

A new trial was started in February 1998 with small amounts of duckweed being incorporated into the abalone feed. The objective is to achieve the desired colouring over a six month period. At the preparation of this Final Report, the feed company is only able to advise that the abalone are eating the feed containing the duckweed.

8. Discussion of Results

The objectives of the project as contained in the Research Proposal: NPP-20 dated February 1995, were to develop and trial strategies of drying duckweed economically, converting the dried plant into a feed supplement and conducting preliminary feeding trials. If the trials proved successful, it should prove to be a strong financial incentive to intensive animal production industries, abattoirs, poultry farms and communities with wastewater ponding systems to use BTWM's Duckweed Wastewater Cleansing System to uptake mineral nutrients to either EPA sensitive water or continuous re-use standards, and either convert the harvested duckweed into a feed for animals or sell the biomass to feed companies.

BTWM has proved with a small scale protype, that a solar powered drying chamber is an economical method of drying duckweed. This method of drying can be speeded up if gas is used to supplement the solar power along with heat exchangers to capture the heat from the moist air before it is expelled from the drying chamber. If duckweed is cultivated at a sewage treatment works where there is a biodigester, the gas generated by the biodigester represents an inexpensive source of heat which can be used in the drying process.

Leaving the duckweed drain in a holding area out-of-doors for a day before beginning the drying process, also shortens the drying time by several days. BTWM is confident that it is viable to dry harvested duckweed and is continuing to explore alternative drying technologies.

Discussions were held with various feed manufacturers during the Project to determine their interest in producing feeds that includes dried duckweed for its high protein, mineral and beta carotene content. While there was genuine interest in the feeding results, the discussions broke down when they mentioned the very substantial quantities of dried duckweed that they would required on a continuous basis.

The quantity of dried duckweed produced by a municipal sewage treatment works serving a town or city of 20,000, would be sufficient to supply a poultry farm of 50,000 to 60,000 laying hens with a pellet containing 10% duckweed. The pellet would have the required amount of protein, minerals and beta carotene for the hen to produce a good quality egg with a nice yellow yolk.

The concept would be to identify specific users for the harvested duckweed from non-farm duckweed installations. These users would most likely require the duckweed to be in a dried state and probably pellitised or crumbed. The poultry and aquaculture industries are two that come to mind.

While evaluating various drying techniques, it became more apparent that if BTWM's Duckweed Wastewater Treatment System is going to be embraced by the intensive animal production industries, then trials will be required of feeding duckweed fresh to animals. This was not envisaged in the Proposal to RIDIC, but as reported above feeding fresh duckweed to animals became a significant part of the overall feeding trials.

The trials demonstrated that animals will in fact gladly eat fresh duckweed and benefit from it, possibly beyond the value of its level of protein. All of the information to date indicates that when fed to ruminants, the majority of the protein will pass through the rumen to the small intestine without any form of protection being required. More research and trials are required in this particular duckweed application, but it offers an exciting new area for duckweed systems at dairies, where the duckweed cleanses the wastewater from the milking sheds and is then fed back to the dairy cows as a fresh high protein feed supplement.

9. Implications

An Assessment Of How The Duckweed Trials Impact On Cities, Towns, Abattoirs, Food Processing Plants, Aquaculture And Intensive Animal Production Industries Producing Large Quantities Of Wastewater

Bio-Tech Waste Management has demonstrated at a number of sewage treatment works, a piggery and a beef cattle feedlot, that duckweed will uptake minerals from the wastewater to a level that it will allow the water to be either discharged into a river or be used continuously for irrigation. The BTWM system is substantially less expensive that other available technology and produces a valuable by-product, in the form of a high protein plant in the process.

With the State of NSW introducing Load Based Licensing (LBL) laws in July 1998, it becomes necessary for any business producing wastewater to cleanse the water to EPA standards. Indications are that the other states in Australia will adopt the NSW legislation within the next two years. Unless those sectors referenced above can find economical methods to cleanse their wastewater, there is a very real possibility that they will find that the LBL laws will either put them out of business or make them so marginal that funding for capital expenditures is no longer available.

As an example, the pork industry is experiencing very strong competition from overseas imports and very low prices for its products. The LBL laws will make a number of the small to medium piggeries nonviable if they cannot find a cost effective way to cleanse their wastewater. The BTWM System will not only cleanse the piggery effluent but will produce a fresh biomass which can be fed directly to the pigs. The piggeries will make substantial savings, as protein and minerals are two of the biggest expenses in the pigs' diet. The same situation applies to dairies and beef cattle feedlots.

The aquaculture industry is also facing a very difficult time due to new environmental laws, including the LBL laws coupled with escalating costs for fish and prawn feeds. While writing this report, BTWM has received two serious enquiries about installing the BTWM System at fish and prawn farms, not only to remove ammonia from the ponds but to generate substantial quantities of duckweed that can be used in conjunction with other feeds. The cost savings being estimated are very substantial.

Bio-Tech Waste Managaement's Duckweed Wastewater Treatment System, is expected to have a major impact on a number of industries in Australia. BTWM is not in a position to estimate the costs and benefits to the Australian economy at this time.

10. Recommendations

The results presented in this Report clearly show that there are very significant benefits to be achieved by installing the BTWM Duckweed Wastewater Treatment System, and using the harvested biomass as a feed for animals and fish.

BTWM believes that further research can be justified in duckweed feeding trials for pigs, dairy cows and in the aquaculture industry for prawns, silver perch, eels, abalone and baramundi. The suggestions for the aquaculture industry were provided by the NSW Fisheries Research Centre and the CRC Centre for Aquaculture.

BTWM also believes that further trials feeding fresh duckweed to shedded sheep to determine if the higher protein will result in finer wool is also justified. A number of graziers have also indicated an interest in feeding fresh duckweed cultivated on municipal wastewater ponds, to lot-fed lambs as a means of reducing substantially the feed costs. If fresh duckweed provides the protein and minerals required by the lambs, it could be mixed with an inexpensive feed supplement such as chaff. The financial benefits could be enormous for the lot-fed lamb producer. Feeding trials would be required to determine the optimum inclusion rate of fresh duckweed in the diet, while assessing the results on the lamb's body weight and fat content, just to mention two criteria.

The results of the duckweed feeding trials conducted to date have been disseminated to the appropriate industry groups and have been reported in various journals, newspapers, on radio programs and talk-back shows and featured in television programs. The two researchers on the Project have both been giving presentations and papers at seminars, symposiums and conferences. BTWM is preparing to include brief details and results from the feeding trials, in its home page on the internet.

11. Bibliography/References

1. Eaton, A.D., Clesceri, L.S., Greenberg, A. E. (1995)
   Standard Methods for the Examination of Water and Wastewater 19th Edition

2. Haustein, Gillman, A.T., Skilllicorn, R.H., Vergara, P.W., Guevara, V. and
   Gastanaduy, A. (1988)
   Duckweed, a useful strategy for feeding chicken: Performance of layers fed with
   sewage grown Lemanaceae species.
   Poultry Science. 69: 1835-1844

3. Journal of Agriculture Food Chemistry (1980) Vol. 28, 848-850

4. O'Neil, P.J., Nolan, J.V., Thomson, E. (1996)
   Proceedings Australian Poultry Science Symposium
   Vol. 8, 123-127

12. APPENDICES

DUCKWEED (*Spirodela punctata*) AS A PROTEIN AND PIGMENT SOURCE IN DIETS FOR LAYERS.

USE OF DRIED DUCKWEED IN DIETS FOR BROWN EGG LAYERS.

FRESH AND DRIED DUCKWEED AND AZOLLA IN DIETS FOR LAYING PEKIN DUCKS. TWO TRIALS.