MINISTRY OF EDUCATION AND TRAINING
CAN THO UNIVERSITY

School year: 2010-2012

DANG THI MY TU

MANIPULATION OF THE NUTRITIVE VALUE OF DUCKWEED (*LEMNA MINOR)* AS A FEED RESOURCE FOR LOCAL MUSCOVY DUCKS

MASTER OF SCIENCE THESIS IN AGRICULTURAL SCIENCES
ANIMAL HUSBANDRY

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Scientific supervisors:

1-Associate professor Dr. Nguyen Thi Kim Dong

2-Professor Dr. Thomas Reg Preston

AN APPROVAL OF THE SCIENTIFIC EVALUATION COMMITTEE

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COMMITMENT

I assure that this thesis is a scientific work which was implemented by myself. All the figures and results presented in the thesis are true and not published in any previous theses.

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CONTENTS

ABSTRACT 10
Paper 1: 10
Paper 2: 10
ABBREVIATIONS 12
Introduction 13
Hypothesis 13
Review of literature 14
Duck production in the Mekong delta 14
Duck production systems 15
Scavenging systems 15
Intergrated duck production systems 15
Intensive confinement systems 16
Local Muscovy ducks 16
Feeds for ducks 16
Rice bran 17
Soybean meal 17
Duckweed (Lemna spp.) 17
Biodigester effluent as fertilizer for the growing of duckweed 18
Traffering nitrogen of duckweed to starch 18
Digestibility 19
Excreta digestibility 19
Gut microflora in poultry 19
Conclusions 20
References 20
Effect oncomposition of duckweed (*Lemna minor)* of different levels of biodigester effluent in the growth medium and of transferring nutrient-rich duckweed to nutrient-free water 25
Abstract 25
Introduction 26
Materials and methods 27
Treatment and experimental design 27
Biodigester effluent 28
Measurements and chemical analyses 29
Statistical analysis 29
Results and discussion 29
Chemical composition of duckweed 29
Biomass yield and root length of duckweed 30
Effect of the "shock" treatment on the composition of the duckweed 32
Conclusions 34
Acknowledgments 34
References 34
Effect on growth, apparent digestibility coefficients and carcass quality of local Muscovy ducks of feeding high or low protein duckweed (*Lemna minor)* as replacement for soybean meal in a rice bran basal diet 36
Abstract 36
Introduction 37

Objectives 38
Materials and Methods 38
Location and climate of the study area 38
Birds, experimental design and treatments 38
Housing and management 40
Diets and feeding 40
Sampling procedure for excreta 41
Measurements and data collection 41
Feed and nutrient intakes 41
Growth parameters 41
Carcass parameters 41
Economic analysis 41
Chemical analysis 41
Statistical analysis 41
Results and discussion 42
Growth performance 42
Chemical composition of dietary ingredients 42
Feed intakes 42
Daily gain and feed conversion ratio 43
Carcass evaluation 44
Economic analysis and health status 45
Apparent digestibility coefficients 46
Conclusions 47
Acknowledgements 47
References 47

ABSTRACT

A series of experiments was carried out to determine: (i) the effects of fertilization with biodigester effluent on yield and composition of duckweed; (ii) changes in composition of duckweed when transferred from nutrient rich water to nutrient-free water; and (iii) the effects of source of duckweed (low and high protein) on apparent digestibility, feed intake and growth rate of local Muscovy ducks fed diets based on rice bran.

Paper 1:

Two experiments were conducted on a private farm in Binh Thuy District, Cantho City to study: (i) the yield and composition of duckweed cultivated with different levels of biodigester effluent; and (ii) the effect on duckweed composition of a "shock" treatment of transferring high quality duckweed to plain water containing no nutrients. In experiment 1, the treatments were 6 levels (0, 4, 8, 12, 16 and 20%) of biodigester effluent added to fresh water in plastic containers containing duckweed. The surface of water in each container was 0.4 m^2 with 20 cm depth giving a volume of 60 liters. Duckweed was inoculated at a rate of 400 g/m^2. The yield of duckweed was measured over a period of 14 days by removing and weighing one third of the biomass every 48 h.

There was a curvilinear response in yield, and in crude protein content of the duckweed, to level of effluent with maximum values for both at a ratio of 12% effluent and 88% water (72 mg N/ liter). Duckweed from this treatment was then transferred to fresh water and the composition studied over 5 days. The content of starch increased and that of crude protein decreased with increasing time in the fresh water.

It was concluded that yield and crude protein content of duckweed was optimized when the culture medium contained 12% biodigester effluent and 88% water. The "shock" treatment of transferring the best quality duckweed to nutrient-free fresh water led to decreases in crude protein and corresponding increases in starch. However, the effects were relatively small and unlikely to have significance from the points of view of duckweed as a feed for ducks or as a substrate for ethanol production.

Key words: *ash, NDF, fertilizer, protein, shock treatment, starch*

Paper 2:

The experiment was done with 72 local Muscovy ducks fed the experimental diets over a period of 84 days. Measurement of coefficients of apparent digestibility was carried out over the period 70 to 77 days; growth performance was measured over the whole period of 56 days (8 weeks). The ducks were bought at 1-day of age from smallholder breeding flocks in Cantho city and fed a commercial diet from 1 to 28 days of age. The treatments were: CTL, a basal control diet including rice bran with soybean meal; HPDW, rice bran with high protein duckweed; LPDW, rice bran with low protein duckweed. The three dietary treatments contained the same level of crude protein of 15% in DM. A premix (vitamins and minerals) was supplied at 2% of the CTL diet. In treatments HPDW and LPDW it was assumed that the duckweed would provide the necessary vitamins and minerals. The HPDW was cultivated in ponds supplied with biodigester effluent according to the optimum treatment in the earlier experiment (paper 1). For LPDW, the duckweed was collected from natural ponds of local farmers.

Final live weight and daily live weight gain of Muskovy ducks fed a rice bran basal diet were highest when the supplementary protein was from high protein duckweed and lowest when low protein duckweed was the supplement. The growth rate of Muscovy ducks fed rice bran supplemented with soybean meal was lower than that of Muscovy ducks fed rice bran supplemented with high protein duckweed. There were few differences in carcass traits when the ducks were slaughtered, except for a more attractive skin color for the ducks fed duckweed. The heavier gizzard in ducks fed duckweed probably reflected the higher fiber content of these diets. N retention was highest on the high protein duckweed diets and lowest for the low protein duckweed diets. The higher digestibility of the NDF fraction on the duckweed diets probably reflected the differences in the nature of the NDF fraction between rice bran and duckweed, as rice bran represented a lower proportion of the DM in the duckweed diets. The better economic results on the high duckweed diet resulted from lower feed costs and higher weight at slaughter.

Key words: *ash, NDF, fertilizer, protein, shock treatment, starch, Muscovy ducks, duckweed, digestibility*

ABBREVIATIONS

ADF	Acid detergent fiber
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
Ca	Calcium
CTL	Control based diet
CF	Crude fiber
CP	Crude protein
DM	Dry matter
FCR	Feed conversion ratio
HPDW	High protein duckweed
LPDW	Low protein duckweed
LW	Live weight
Mekarn	Mekong basin animal research network
N	Nitrogen
NDF	Neutral detergent fiber
OM	Organic matter
Prob/P	Probability
SBM	Soybean meal
SEM	Standard error of the mean

Introduction

Duckweed is easily identified by the presence of only one root per small green frond, and is common throughout the Delta. It is an aquatic plant which often forms dense floating mats in atrophic ditches and ponds (Driever et al 2005). It is a tiny green plant that grows on pond surfaces, grows well in different climates, and is a fast growing, high protein plant that can efficiently absorb nitrogen and phosphorus as well as heavy metals (Logsdon 1989). Duckweed has high nutrient uptakes rates, is cold tolerant and less sensitive than other aquatic plants to high nutrient stress, droughts, pests and disease. It grows best in tropical and temperate zones and give high biomass yields of 10-30 tonnes DM/ha/year, containing high levels of crude protein with a good amino acid balance (Leng et al 1995).

Growth rates of duckweed colonies are reduced by a variety of stresses: such as nutrient scarcity or imbalance; toxins; extremes of pH and temperature; crowding by overgrowth of the colony and competition from other plants for light and nutrients. However, when conditions are good, duckweed contains considerable amounts of protein, fat, starch and minerals which appear to be mobilized for biomass growth when nutrient concentrations fall below critical levels for growth (Leng et al 1995). According to research of Cheng and Stomp (2009), growing duckweed on hog wastewater can produce more starch. The protein content of the duckweed responds quickly to the availability of nutrients in the aquatic environment. It is considered that the use of effluent from biodigesters for growing duckweeds could be a way of increasing feed availability for animals and at the same time reducing problems of pollution to the environment.

Poultry production in Vietnam is based on agricultural production, and accounts for 19% of the total livestock production, being second only to pig production (Vang et al 2000). Most chickens are kept by small farmer households, who typically raise 10 - 12 birds by scavenging, supplemented by agricultural by-products.

The use of duckweed as poultry feed has been recognized by many authors (Haustein et al, 1987, 1990; Islam et al 1997; Rodriguez et al 1997, Leng, 1999; Samnang, 1999). Duckweed has high crude protein content and a well-balanced amino acid profile and is also a good source of vitamins and minerals for livestock (Landolt et al 1987; Men et al 2001). Duckweed can play important role in poultry feeding.

Hypothesis

The hypotheses to be tested were:

Paper I: When duckweed is grown on high nutrient density substrate (eg: with biodigester effluent) and then is suddenly transferred to zero-nutrient water, the metabolism changes to convert some of the protein and other nutrients into starch.

Paper II: Duckweed grown in ponds fertilized with biodigester effluent will support better growth rate of ducks than duckweed collected from natural water surfaces that are not fertilized.

Review of literature

Duck production in the Mekong delta

The Mekong Delta is situated in the South of Vietnam, and has a population of 22 million and an area of 39,551 km^2. It is the major agricultural region of Vietnam, and is also considered to be the most important rice granary for the country, accounting for about 48% of the total rice production. The delta is a four million hectare flat lowland plain of alluvial, acid and saline soils watered by the Mekong River and its canal networks. The ambient temperature fluctuates between 22 and 25 ^{0}C in the coolest months (December – January) and 32-33 ^{0}C in the warmest months (April – May). Annual rainfall is 1400 – 2400 mm, and average humidity varies from 76 to 80%. Most of the arable land is used intensively for rice cultivation or other crops throughout the year, and yields are generally high. Therefore there are considerable quantities of agricultural by-product available throughout the year, many of which are potentially valuable as feed resources for livestock.

Duck production plays an important role in the Mekong Delta, providing meat and eggs in the diets of people and income from local sales in the markets of Ho Chi Minh city and abroad. The population of ducks has increased in recent years in Vietnam, and was estimated at 132 million in 2009 (FAOSTAT data 2009). Although ducks are raised throughout the country, production is concentrated to the Mekong Delta, which has more than 50% of the total population, made up of 80% common ducks (*Anasplatyrhynchos*) and 20% Muscovy ducks (*Cairinamoschata*) (Quac 1990). In the Mekong Delta more than 95% of the total production is from smallholder farmers, who use different traditional systems, such as rearing around gardens, along canals, on the seashore and in the rice fields post-harvest. In these systems ducks forage for themselves and consume locally available feeds, and are normally only supplemented small amounts of rice or not at all. Because of the low or non – existent inputs these systems are therefore quite profitable. However, duck producers have a problem of limited scavenging, due to the introduction of intensive, high-yielding rice varieties. Also, in recent years some local governments in the Mekong Delta have campaigned to prohibit raising ducks in scavenging systems in order to protect irrigation systems and prevent water pollution and disease transmission. Therefore confinement systems have been developed, especially for resource-poor, peri-urban producers, who raise ducks on a small scale for income generation. However, they usually feed their birds concentrates or provide supplements of conventional protein feeds, such as fish meal or soybean meal, which are currently expensive. The producers have also been vulnerable to changes in the price of feed, and lose money when the price of the conventional feeds increases, and therefore have started to utilize alternative protein resources to reduce production costs.

Many cities and towns in the Mekong Delta have rapidly increasing human populations and consequently increasing demands for duck meat and eggs. Thus, to meet the increasing consumer demand, and increase profit margins, there is an increasing interest in confinement of improved breeds reared on cheap, locally available materials and unconventional feed.

Duck production systems

Scavenging systems

Duck farming based on scavenging is a particularly important activity in many countries in South – East Asia. Most local duck breeds are suitable for rearing in this way. Various systems have evolved in Vietnam, where duck production occurs either in full scavenging system or under semi-scavenging conditions. Ducks might be kept in partial confinement or be enclosed during the night only. The scavenging systems are characterized by low feed inputs and low standards of management and housing, and although resulting in low outputs can be quite profitable. Scavenging ducks in the Mekong Delta can be found in several different environments. Often small group of ducks (common and Muscovy) are kept in backyards, eating natural feeds as well as kitchen waste and purchased feed. These systems are common in peri–urban areas. The delta area is cluttered with rivers and canals and it is beneficial to keep ducks along the canal banks although in some provinces problems with erosion have led to this practice being banned. Moreover, in the coastal areas shellfish are abundant, and are a good source of protein and minerals, and thus scavenging ducks kept for the production of table eggs are mainly concentrated to the seashores (Becerra 1994). However, a major disadvantage of this system is the difficulty in controlling health problems. Especially from early 2004 until now Avian Influenza outbreaks have occurred in Vietnam and other Asian countries, and there have been widespread H5N1 infection in ducks and chickens throughout the country, and in particular in the Mekong Delta. Considerable evidence indicates that most ducks in scavenging systems can carry the H5N1 virus without showing symptoms, and asymptomatic ducks can rapidly spread the virus to other flocks and humans (Recombinomics 2005).

Integrated duck production systems

Many farming systems in Vietnam involve the integration of several components, including livestock, aquaculture, horticulture and rice cultivation. In common with producers in other Asian countries with experience of raising ducks (Kang et al1995; Ketaren 1998), farmers in Vietnam integrate ducks in many combinations such as: pig-ducks-chickens-vegetables-fruit-aquaculture, pig-ducks-goat-rice-vegetables-aquaculture and pig-ducks-cattle- vegetables-aquaculture (Devendra 1997). In the Mekong Delta these systems are common and are collectively called the VAC system, where the abbreviation stands for the Vietnamese word for the garden, fishpond and animals (Ogle and Phuc 1997). A VAC system can for example comprise ducks, fish, water plants and fruit trees, where the feces from the ducks become feed for fish, and fertilizer for plants and trees, while the ducks can utilize part of the plants and fish as feed. The system implies an efficient utilization and circulation of nutrients and is better for the environment. Other systems based on rice fields are integrated duck – rice cultivation, which has been shown to reduce or eliminate insects and weeds (Men et al 2001a), and to increase rice yields (Villamora et al 2000). The fish-duck system is particularly beneficial as it is environmentally friendly, and results in improved feather quality for the ducks (Edwards 1986).

Intensive confinement systems

In these systems ducks are kept in total confinement and all facilities are provided *i.e.* water and feed, in a sheltered area or pen. A well balanced ration is needed. Water pens should be designed such that the ducks cannot sprinkle the water. This system has mainly developed in peri-urban areas, due partly to limited possibilities to keep ducks in scavenging systems. In Vietnam this system is popular for raising exotic breeding ducks and for fattening growing meat – type ducks. High productivity can be achieved with intensive confinement systems, since the production, including feeding and management, is under controlled conditions, which optimize out put. In this system ducklings are usually reared up to eight or nine weeks age (Nho et al 1995; Quoc et al 1995), which is the market age preferred by consumers. Only a relatively small number of improved breeds (Cherry Valley and Super – Meat ducks) are produced in confinement using commercial feeds, because the systems requires high inputs, such as labour and capital investments for feed and housing (Scott and Dean 1991). Intensive confinement systems for ducks have become more common in certain areas of Vietnam, for example around Ho Chi Minh City, but in the Mekong Delta the small scale full or semi-confinement systems are still predominant. Especially in urban and peri-urban areas growing ducks are raised in these systems and fed unconventional feeds, such as locally available agro – industrial by – products, kitchen wastes and market wastes and thus should be more profitable, as a result of low feed costs. The meat from birds grown semi – intensively in unpolluted areas has a better taste and food value than from those grown intensively (Sandhu and Dean 2005).

Local Muscovy ducks

Several different breeds are found in the Mekong Delta, including local and exotic Muscovies and crosses. Local Muscovy ducks have been raised on a small scale for a long time. They are commonly allowed to scavenge around the backyards and garden or confined in simple shelters in small flocks of 10 – 50 head, and have low performance due to their small body size. Their products, including a carcass with red meat, are mainly consumed within the family. French Muscovy ducks were imported into Vietnam and acquired by Cantho University in 1993. They have black and white coloration and bright red caruncles on their face and over the base of their bill. Crosses of French and local Muscovy ducks have been produced and raised on farms, and have a bigger body size and better growth rate than local Muscovy ducks (Phuoc et al 1994). Subsequently they were introduced to local producers who were advised to feed them with good quality concentrate in the urban and peri-urban areas and on local feed resources in the villages (Dong and Ogle 1995). Average performance was found to be 2.13 kg for market weight (Dong 1996). The Muscovy is characterized by the production of a less fatty carcass (Parkhurt and Mountney 1998), large pectoral muscles, and sexual dimorphism which is in favour of the male. Optimum carcass weight quality requires that the female should be slaughtered at 10 weeks and the male at 11 – 12 weeks (Larbier and Leclercq 1994).

Feeds for ducks

Global production of duck meat shows a continuous and rapid increase (FAOSTAT 2009). The growth and protein accretion potential of ducks have been improved by selective breeding in recent decades with respect to change in body composition and improvement in feed conversion ratio (Timmler and Jeroch 1999). Studies on the requirements of the modern breeds for essential amino acids (EAA), however, are few, but do exist (Elkin 1987). Ducks can grow well, whether by scavenging or consuming a complete ration. However, the feed offered should contain enough nutrients and the ratio should be balanced with regards to the requirements for growth, maintenance and reproduction.

Rice bran

The traditional diets for monogastric livestock, especially chickens and ducks, in the Mekong Delta are based on rice, either paddy rice or rice by-products, such as broken rice and rice bran. As reported by Lung and Man (1999), broken rice and rice bran are widely used, and provide up to 80-90% of the energy in diets for growing ducks, and rice bran commonly accounts for 20% of the energy for both growing and breeding ducks. With the recent expansion of animal production, the demand, and consequently the price, for these feeds have increased. Since the price of rice also fluctuates widely, the profitability of duck production varies (Becerra 1994). Some producers use commercial concentrates for feeding in intensive confined systems, which can give good performance results, but low profits.

Soybean meal

Soybean meal (SBM) is an important source of dietary protein and energy for poultry throughout the world. However, not so much soybeans meal are grown in Vietnam, so the price is generally too high to use it in animal feeds. The raw soybean seeds contain a number of natural anti-nutritional factors for poultry, the most problematic being trypsin (protease) inhibitors. Thus, to increase the protein nutritive value (Balloun 1980), these anti-nutritional factors must be destroyed. Trypsin inhibitors disrupt protein digestion, which results in decreased release of free amino acids, and their presence is characterized by compensatory hypertrophy of the pancreas due to stimulation of pancreatic secretions. Fortunately, the heat treatment done during processing is usually enough to destroy trypsin inhibitors and other toxins such as lectins (haemagglutinin) (Gohl 1998). The growth depressing effect of lectins is belived to be due primarily to their damaging impact on intestinal enterocytes (Pustzai et al 1979) and to appetite depression (Liener 1986).

Duckweed (Lemna spp.)

Duckweed is a monocotyledon species of the family Lemnaceae adapted to grow in water at temperatures between 6 and 33 ^{0}C (Leng et al 1995). It is a small floating aquatic plant that grows very well on stagnant ponds and is commonly found throughout tropical countries (Leng et al 1995). Crude protein yields of between 6 and 10 tonnes/ha/year have been recorded when the N content in the water is in the range of 10 to 30 mg/liter (Nguyen Duc Anh 1997b). Not only the yield but also the crude protein of duckweed responds to the nutrient content of the water, increasing from 15% in DM with 10 mg N/liter to 40% crude protein in DM with 60 mg N/litre (Rodriguez and Preston 1996). Many trials have been carried out using duckweed as the major feed to raise fish, pig, chicken and also ducks. The use of duckweed as poultry feed has been recognized by many authors (Haustein et al 1987, 1990; Islam et al 1997; Rodriguez et al 1997, Leng 1999; Samnang 1999). Duckweed has high crude protein content and a well-balanced amino acid profile and is also a good source of vitamins and minerals for livestock (Landolt et al 1987; Men et al 2001). Even though the moisture content of duckweed can be the first limiting factor for chickens, duckweed can play important role in poultry feeding.

Biodigester effluent as fertilizer for the growing of duckweed

The protein content of duckweed responds quickly to the availability of nutrients in aquatic environment. It is considered that the use of effluent from biodigester for growing duckweeds could be a way of increasing feed availability for animals and at the same time reducing problems of pollution to the environment.

The effluents from biodigesters, suitably diluted are very effective media for growing duckweed. These can be extremely simple systems, easily incorporated into small farming areas based on home-biodigesters, constructed from plastic. In all cases the excrement plus washings from animals held under penned conditions are collected and put into an enclosed container which allows anaerobic microbes to grow and convert the residual carbohydrates to carbon dioxide and methane. Biodigester effluent has been shown to be an excellent fertilizer for duckweed ponds (Rodriguez and Preston 1996).

Transferring protein to starch

Duckweed has traditionally been studied because of its inherently high protein content at 30% to 35% on a dry-weight basis. It grows best in tropical and temperate zones and give high biomass yields of 10-30 tonnes DM/ha/year, containing high levels of crude protein with a good amino acid balance (Leng et al 1995).

Since the late 1960s, scientists have studied duckweed for animal and human consumption because of its high protein content. Researchers are now tapping into the plant's innate environmental benefits, from desalinating waste water to exploring its potential as a viable starch-based feedstock for ethanol production (Bryan Sims, No date)

According to Cheng and Stomp (2009) duckweed can be used to clean up animal waste at industrial hog farms and could be used to make ethanol. They have determined that duckweed grown on swine wastewater can produce five to six times more starch than corn.

It appears as it there are possibilities to modify the composition of certain strains of duckweed (Cheng and Stomp 2009). According to these authors, a starch content of 45% was achieved in *Spiridelapolyrrhiza* through simple transfer of the fresh duckweed fronds from a nutrient-rich solution to tap water for 5 days. The mechanism of the formation of starch was described by Armstrong (2003). He identified the lateral dark bodies at the base of the mother plant of *Lemna turionifera* called turions and indicated that they formed when the plant was subjected to nutrient or environment stress (eg: low temperature). Because the specific gravity of starch is about 1.5, the turions sink to the bottom of the pond or container in which the duckweed is growing. Under environment stress, the turions produce bubbles of carbon dioxide and rise to the surface. This is the reason of Lemna response to the shock treatment. It is not known if other species of Lemna (eg: *Lemna minor)* respond in a similar manner when subjected to environmental stress.

Digestibility

Measurements of digestibility are essential in order to define the efficiency of utilization of nutrients within foods, to classify the nutritional quality of food items, and to formulate diets for captive birds. Digestibility may be expressed in terms of apparent or true digestibility. Apparent digestibility is the relationship between the amount of nutrients consumed in the diets and the amount that disappears from the gastrointestinal tract: (nutrient intake – nutrient in feces) / nutrient intake. Apparent digestibility indicates that the measurement is biased by the amount of a nutrient that was absorbed but then excreted back into the digestive tract, as well as by endogenous nutrient losses, such as those from the shedding of the intestinal epithelia and mucous secretions. True digestibility corrects for those components of the excreted nutrients that were not originally in the food. It corrects for the portion of nutrient in the feces that is of endogenous origin. Thus the values obtained for true digestibility are always greater than those for apparent digestibility. The separation of endogenous losses arising from the digestive tract from the metabolic losses excreted in urine is difficult in birds, due to the simultaneous voiding of faeces and urine. In practical condition in Vietnam measurements that include endogenous losses for poultry are lacking. Therefore apparent digestibility of nutrients has been applied commonly for monogastric animals in Vietnam, especially chicken and ducks.

Excreta digestibility

This was first used by Kuiken and Lyman (1948), who measured the difference between amino acids consumed in the feed and in the corresponding feces. In birds, because of the mixing of feces and urine (excreta), excreta digestibility (metabolizability) is employed unless birds are surgically modified. In digestibility studies with intact birds based on this technique most of the available published data have been derived from excreta analysis (Ravindran et al 1999; Svihus and Hetland 2001; Jamroz et al 2002). Determination of apparent digestibility through analysis of excreta samples has been criticized because this approach fails to distinguish amino acids voided which are not of direct dietary origin (endogenous excretory losses) (Short et al 1999). Also the major criticism of both the fecal and excreta digestibility methods of amino acid assessment is that microbial activity in the lower intestine, particularly in the caeca, may affect amino acid digestibility by deaminating undigested amino acid residues. If this occurs, the digestibility values will be higher than in birds in which microbial action has been prevented.

Gut microflora in poultry

There is evidence that microbial activity in the digestive tract of broilers is mainly affected by the diet in relation to nutrient digestion. In poultry, fermentation occurs mainly in the caeca. Relative to other parts of the intestinal tract of poultry, the caeca provide a stable environment for micro-organisms and, as a result, contain the largest and most complex ecosystem. Raharjo and Farrell (1984a) and Ravidran et al (1999) reported that amino acid metabolism by hindgut microflora in chickens may be substantial, and that digestibilities measured in the terminal ileum are more accurate measures of amino acid availability than those measured in excreta. The digestion and absorption of nutrients by an animal depend on the rate of hydrolysis by the animal's enzymes and activity of the gastrointestinal tract of poultry. Bacteria can play an important role in metabolism in the intestinal tract (Savage 1986; Fuller and Cole 1988). In the small intestine, the bacteria population appears to be established within approximately 2 weeks (Smith 1965). However it takes much longer for the cecal ecosystem to develop (Bames et al 1972; Mead and Adams 1975).

The development of the microflora is also affected by the digestibility of the diet. Lee (1985) reported that dietary factors such as nutrient digestibility can influence the ecosystem of the intestinal tract, notably in the caeca and large intestine. It has been suggested that the microflora compete with the host animal for dietary nutrients. For a highly digestible diet this competition is usually in favor of the host. However, if birds receive poorly digestible diets rich in non-digestible carbohydrates, more substrate moves to the lower part of the intestine tract, thus favoring the microflora. The digestive capacity of young animals is still not fully developed (Nitsan et al 1991; Nir et al 1993). This leaves more substrate for microbial fermentation. Several studies indicate that part of the reduction in N digestibility can be explained by the fact that the micro-organisms can incorporate dietary amino acids into microbial protein (Salter and Coates 1974). Other evidence is that an increase in microbial activity stimulates proliferation of mucosal cells (Sakata 1987; Goodlad et al 1989), probably associated with increased losses of epithelial cells, which increases endogenous losses. This contributes to a greater feacal N output and, therefore, to a decrease in the apparent digestibily of N.

In poultry, much of the microbial fermentation takes place in the caeca (McNab 1979), and because the caeca take the form of two blind sacs, it is possible for much of the feed residue to bypass them. Salter (1973) tabulated the possible effects of the microflora on nitrogen excretion and protein utilization and concluded that the microflora can deaminate (Buraczewka and Buraczewski 1985) and synthesize (Deguchi et al 1978) amino acids and even alter the rates of mucosal cell proliferation and shedding (Khoury et al 1969). Jamroz et al (2001) found considerable amino acid synthesis by microbes in the caeca-colon of chicken, ducks and especially geese, and these amino acids are not absorbed and utilized in the body but excreted in the feces. Consequently, many assays for bioavailable amino acids involve procedures to reduce interference by microbial fermentation, particularly in the hindgut.

Conclusions

- Vietnam is an agricultural country with huge annual rice production, so the by-product (rice bran from mills) is abundant. This is a cheap and available feed resource for duck production.
- Duckweed grows widely on natural water surfaces in the Mekong Delta. It has high nutritive value, especially the protein content, and is a low-cost supplement suitable for complementing the protein-deficient rice bran in diets for ducks.

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Effect on composition of duckweed (*Lemna minor*) of different levels of biodigester effluent in the growth medium and of transferring nutrient-rich duckweed to nutrient-free water

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Abstract

Two experiments were conducted on a private farm in Binh Thuy District, Cantho City to study: (i) the yield and composition of duckweed cultivated with different levels of biodigester effluent; and (ii) the effect on duckweed composition of a "shock" treatment of transferring high quality duckweed to plain water containing no nutrients. In experiment 1, the treatments were 6 levels (0, 4, 8, 12, 16 and 20%) of biodigester effluent added to fresh water in plastic containers containing duckweed. The surface of water in each container was 0.4 m^2 with 20 cm depth giving a volume of 60 liters. Duckweed was inoculated at a rate of 400 g/m^2. The yield of duckweed was measured over a period of 14 days by removing and weighing one third of the biomass every 48 h.

There was a curvilinear response in yield, and in crude protein content of the duckweed, to level of effluent with maximum values for both at a ratio of 12% effluent and 88% water (72 mg N/liter). Duckweed from this treatment was then transferred to fresh water and the composition studied over 5 days. The content of starch increased and that of crude protein decreased with increasing time in the fresh water.

It was concluded that yield and crude protein content of duckweed was optimized when the culture medium contained 12% biodigester effluent and 88% water. The "shock" treatment of transferring the best quality duckweed to nutrient-free fresh water led to decreases in crude protein and corresponding increases in starch. However, the effects were relatively small and unlikely to have significance from the points of view of duckweed as a feed for ducks or as a substrate for ethanol production.

Key words: *ash, NDF, fertilizer, protein, shock treatment, starch*

Introduction

Duckweed (*Lemna minor*) is a simple tiny water plant that grows very well on pond surfaces. It can tolerate high nutrient stress and appears to be more resistant to pests and diseases than other aquatic plants. Moreover, it has high protein and carotene contents (Bui Xuan Men et al 1995). The protein content of duckweed responds quickly to the availability of nutrients in a water environment (Leng et al 1995). Duckweed has been used as a main protein supplement for pigs (Bui Hong Van et al 1997) and ducks (Bui Xuan Men et al 1995; Nguyen Duc Anh et al 1997b). Duckweed has received research attention because of its high nutritive value, especially the high protein content and also because of its capacity to grow rapidly on nutrient-rich waste water and produce biomass rich in protein (Leng et al 1995).

The use of tubular plastic biodigesters for anaerobic digestion to convert organic matter to biogas and effluent (Botero and Preston 1995) is a very simple and practical system that is flexible and uses low-cost materials (Preston and Rodríguez 2002; Mette 1998; Bui Xuan An et al 1997) when compared to other types of biodigester (Mikkle et al 1996; Timothy and Gohl 1996). The effluent has been shown to be a good fertilizer for duckweed (Rodríguez and Preston 1996; Le Ha Chau 1998; Lampheuy 2003). Biomass yield increased with level of organic fertilizer and was higher for the effluent from a biodigester than for the raw manure (Lampheuy 2003). This agrees with the findings of Le Ha Chau (1998) who also compared biodigester effluent with manure but at a fixed N level of 150 kg/ha. There was no interaction level*fertilizer for biomass production.

The results from these studies showed that the growth of duckweeds is similar to that of any other plant. Under experimental conditions the annual production reached 183 tonnes/ha of DM, however, under practical conditions a yield of up to 30 tonnes of DM/ha is more feasible (Leng et al 1995). Moderate conditions of temperature and light and liquid medium with the necessary nutrients are essential for good growth. Also, duckweeds adapt well to a wide range of conditions and are easy to grow (Cross 2001). It is considered that the use of effluent from biodigester for growing duckweeds could be a way of increasing feed availability for animals and at the same time reducing problems of pollution to the environment.

It appears there are possibilities to modify the composition of certain strains of duckweed (Cheng and Stomp 2009). According to these authors, a starch content of 45% was achieved in *Spiridela polyrrhiza* through simple transfer of the fresh duckweed fronds from a nutrient-rich solution to tap water for 5 days. The mechanism of the formation of starch was described by Armstrong (2003). He identified the lateral dark bodies at the base of the mother plant of *Lemna turionifera* called turions and indicated that they formed when the plant was subjected to nutrient or environment stress (eg: low temperature). Because the specific gravity of starch is about 1.5, the turions sink to the bottom of the pond or container in which the duckweed is growing. It is not known if other species of Lemna (eg: *Lemna minor)* respond in a similar manner when subjected to environmental stress.

Hypothesis

The hypotheses to be tested were:

- When duckweed is grown on high nutrient density substrate (eg: with biodigester effluent) and then is suddenly transferred to zero-nutrient water, the metabolism changes to convert some of the protein and other nutrients into starch.
- Duckweed exposed to environment “shock” (by transfer from nutrient-rich pond to plain water pond) will have a higher content of starch and thus have a higher nutritive value for growing ducks.

Materials and methods

Location and duration

The experiment was conducted on a farm in Binh Thuy District, Cantho City andin the laboratory of the Department of Agriculture and Applied Biology, Cantho University, Vietnam, from April to June 2011.

Treatment and experimental design

The experiment was a completely randomized design with 6 treatments and three replications. The treatments were 6 levels of biodigester effluent (BE) added to duckweeds growing in plastic containers, used as experimental ponds. The percentage of biodigester effluent was 0, 4, 8, 12, 16 and 20% (Table 1). For different concentrations the quantities of biodigester effluent and water were adjusted accordingly.

Table 1. The levels of biodigester effluent used in the treatments.

Treatment	**0BE**	**4BE**	**8BE**	**12BE**	**16BE**	**20BE**
Effluent,%	0	4	8	12	16	20
Fresh water,%	100	96	92	88	84	80

The surface of water in each basket was 0.4 m^2 with 20 cm depth giving a volume of 60 liters. Duckweed fronds obtained from natural ponds around the University were inoculated at a rate of 400 g/ m^2 (160 g/ basket) in each treatment. The different proportions of biodigester effluent and water were added to plastic containers to produce different concentrations of N (Table 2). The biodigester effluent was stored in a container (160 liters) and a sample analyzed at the beginning of the experiment.

Photo 1. Experimental layout.

Table 2. The measured N content of the pond water after application of biodigester effluent.

Treatment	0BE	4BE	8BE	12BE	16BE	20BE
N, %	0	0.0024	0.0048	0.0072	0.0096	0.012
N, mg/liter	0	24	48	72	96	120
N, kg/ha	0	36	72	108	144	180

The "shock" treatment was carried out by selecting the best treatment (based on biomass yield and N content of the duckweed) to measure the duckweed response to the transfer from nutrient-rich to plain water. After the transfer, samples of the duckweed were harvested daily for 6 days and analyzed for DM, N, starch, NDF and ash.

Water

Water used in experiment were taken from only source water at the farm and applied for all plastic containers.

Biodigester effluent

The biodigester effluent was obtained from a plug-flow tubular plastic biodigester charged with cattle manure (Photo 2).

Photo 2. The tubular plastic biodigester

Measurements and chemical analyses

The duckweed was allowed to grow for a period of 14 days at which time all the biomass was harvested and weighed and samples taken for analysis of DM, CF, Ash, NDF, N and starch. The root length of the duckweed was measured with a graduated ruler (Rodriguez and Preston 1996). The contents of DM, CF, Ash, NDF, N and starch were determined by procedures of AOAC (1990).

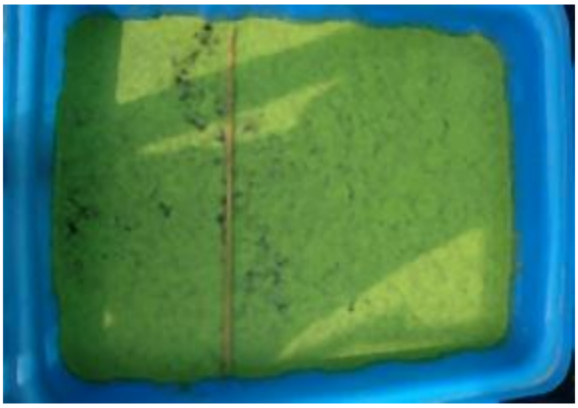

Photo 3. Harvesting of duckweed (one third of the surface is harvested every 48h)

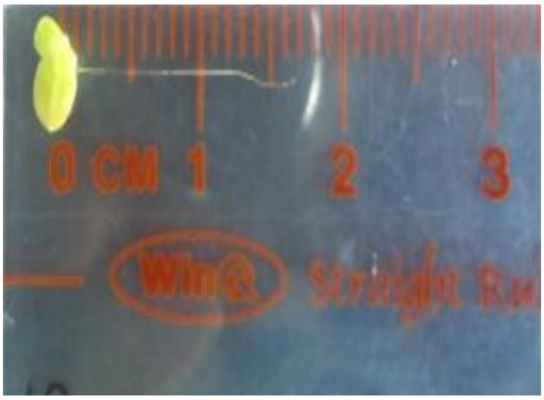

Photo 4. Measurement of the root length of duckweed

Statistical analysis

The data were analyzed by the General Linear Model procedure of the ANOVA program in the Minitab (2000) software. Sources of variation were: Biodigester effluent level and error. When the F test showed significant differences at $P<0.05$, Tukey's test for treatment comparisons was used (Minitab 2000).

Results and discussion

Chemical composition of duckweed

The DM content of the duckweed did not vary with level of effluent that was applied (Table 3). The values (4.99 to 5.04%) were similar to those (4.99 to 5.85%) reported in the study of Lampheuy (2003). The content of ash tended to increase with level of effluent.

Table 3. Chemical composition of duckweed in the different treatments

	0BE	**4BE**	**8BE**	**12BE**	**16BE**	**20BE**	SEM	P
DM, %	5.00	4.99	5.04	5.01	5.02	5.02	0.017	0.335
	------------------------------------	As % of DM	--------------------------					
OM	88.4^{a}	87.2a^{b}	85.3^{ab}	85.4^{ab}	85.7^{ab}	84.1^{b}	0.227	0.028
N	3.74^{a}	3.75^{a}	4.47^{ab}	4.89^{b}	4.14^{ab}	4.22^{ab}	1.42	0.028
CP	23.4^{a}	23.4^{a}	28.0^{ab}	30.6^{b}	25.9^{ab}	26.4^{ab}	0.661	0.019
CF	10.9^{a}	9.15^{ab}	8.76^{b}	7.41^{bc}	7.34^{bc}	5.66^{c}	0.734	0.016
NDF	19.8^{a}	18.3^{ab}	18.1^{ab}	16.0^{ab}	14.0^{ab}	13.1^{b}	0.457	<0.001
Ash	21.7^{a}	22.6^{ab}	23.9^{ab}	24.6^{ab}	23.8^{ab}	25.5^{b}	1.28	0.019

abc Mean values without common letter differ at P<0.05

There was a close negative relationship between the NDF content of the duckweed and the level of biodigester effluent that was applied (Figure 2). However, the relationship between crude protein and biodigester effluent was curvilinear with the maximum protein content at the 12% level of effluent (Figure 1), equivalent to 108 kg N/ha. By contrast, Lampheuy (2003) reported linear increases in crude protein content of duckweed (from 16.7 to 34.5% in DM) with levels of biodigester effluent N up to 200 kg/ha. The maximum level of crude protein reached in the present experiment (30.6% in DM with 108 kg N/ha) was similar to the level reported by Lampheuy (2003) for the 100 kg/ha level of N.

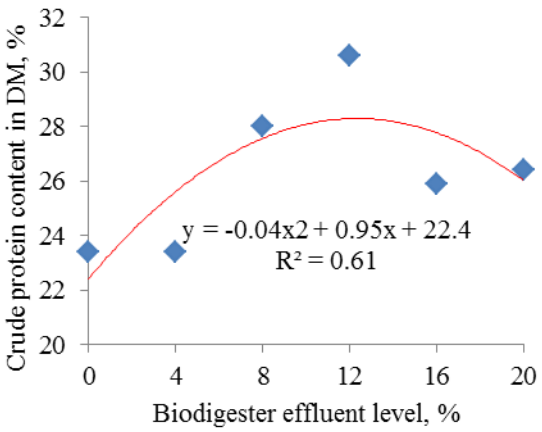

Figure 1. Relationship between effluent concentration and crude protein content of duckweed

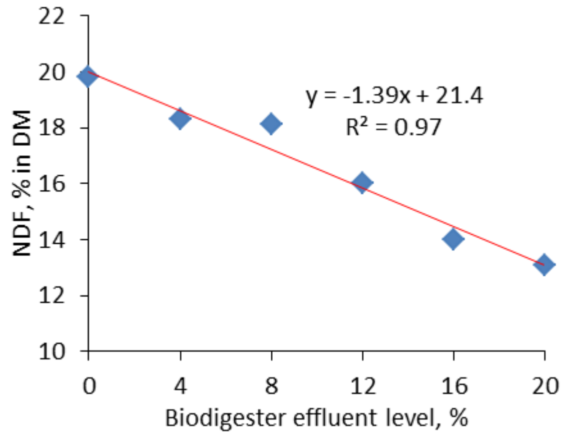

Figure 2. Relationship between effluent concentration and NDF content of duckweed

Biomass yield and root length of duckweed

The relationship between biomass yield and effluent level showed a similar curvilinear relationship as was observed for the crude protein content (Table 4, Figure 3), with maximum yield being obtained with the 12% level, equivalent to 108 kg N/ha. In terms of annual yield of DM, this was equivalent to 19 tonnes/ha. This agrees with the findings of Leng et al (1998) who reported yields of duckweed from 10 to 20 tonnes of DM/ha/year.

Table 4. Mean values for biomass yield of duckweed according to levels of biodigester effluent

Biomass yield	**0BE**	**4BE**	**8BE**	**12BE**	**16BE**	**20BE**	SEM	P
Fresh, g/0.4m^2	382^b	440^{ab}	523^{ab}	587^a	571^a	501^{ab}	37.9	0.018
DM, g/0.4m^2	19.1^b	21.9^b	26.4^{ab}	29.4^a	28.7^a	25.1^{ab}	1.9	0.018
Fresh, g/m^2	955^b	1100^b	1308^{ab}	1468^a	1428^a	1253^{ab}	94.9	0.018
DM, g/m^2	47.8^b	54.8^b	66.0^{ab}	73.5^a	71.6^a	62.8^{ab}	4.7	0.018
Tonnes DM/ha/yr	12.5	14.3	17.2	19.2	18.7	16.4		
Tonnes CP/ha/yr	2.92	3.34	4.82	5.86	4.83	4.32		

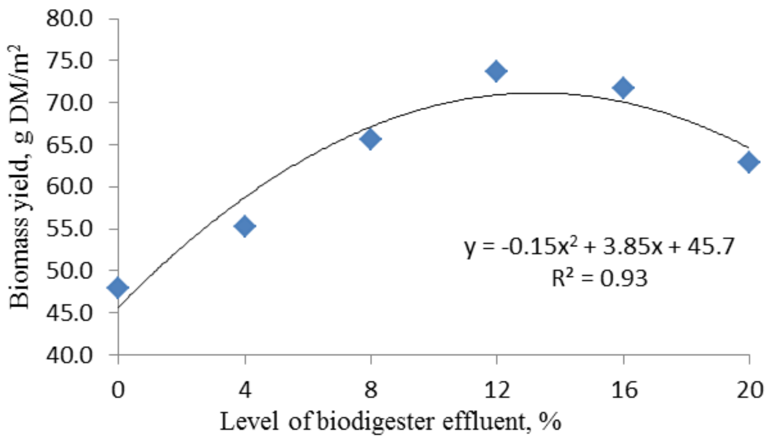

Figure 3. Relationship between effluent concentration and biomass DM yield of duckweed

The root length of the duckweed was negatively related with crude protein content (Table 5; Figure 4), although the relationship (R^2=0.32) was not as strong as was reported by Rodriguez and Preston (1996a) (R^2=0.86) and Lampheuy et al (2003) (R^2=0.82). There were closer relationships between root length and biomass yield (Figure 5) and between the N content of the water in the ponds and root length (Figure 6). There are many experimental observations (Nguyen Duc Anh et al 1997; Le Ha Chau 1998; Rodriguez and Preston 1996) that have shown that over short growth periods there is a close negative relationship between root length and the N content of the pond water.

Table 5. Mean values for root length of duckweed according to levels of biodigester effluent in the ponds

	0BE	4BE	8BE	12BE	16BE	20BE	SEM	P
Root length, cm	2.45	1.48	1.21	0.78	0.53	0.37	0.05	<0.001

Therefore the root length of duckweed is a good indicator of the crude protein of the duckweed, biomass yield and the N content of the pond water.

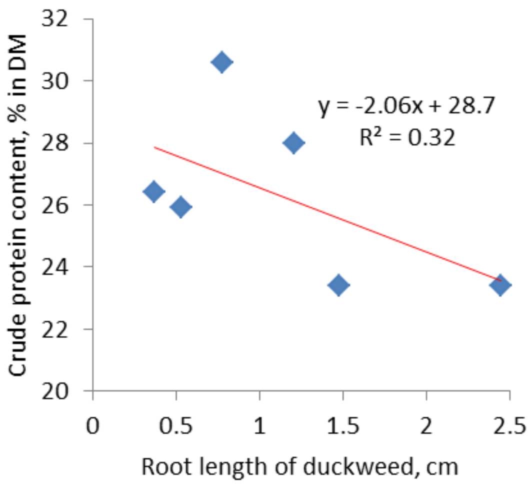

Figure 4. Relationship between root length and crude protein content of duckweed

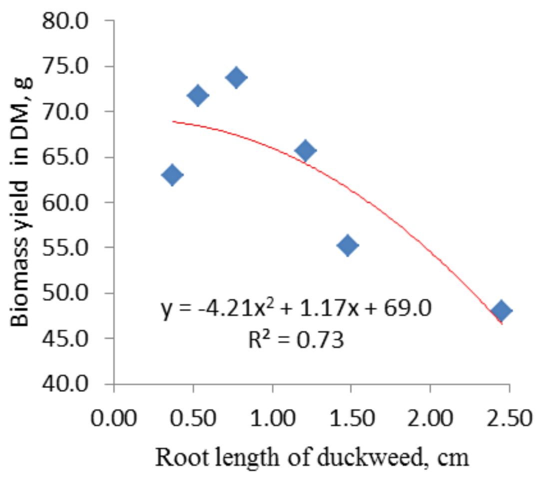

Figure 5. Relationship between root length and biomass yield of duckweed

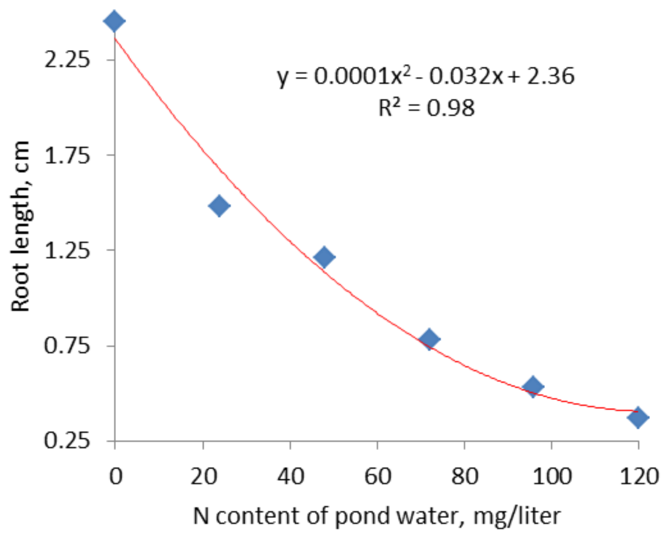

Figure 6. Relationship between N content of the pond water and the root length of the duckweed

Effect of the "shock" treatment on the composition of the duckweed

There were changes in the composition of the duckweed after transfer from nutrient-rich to plain water (Table 6). The crude protein and the ash content decreased and the starch content increased with increasing exposure to the nutrient-free water (Figures 7-9).

Table 6. Effect of the "shock" treatment on composition of the duckweed during the 5 days after transfer from nutrient-rich to plain water

	Days after transfer							
	0	**1**	**2**	**3**	**4**	**5**	SEM	P
DM, %	4.84	4.64	4.43	4.15	4.07	4.67	0.177	0.054
% in DM								
OM	82.4[a]	84.6a[b]	85.7[ab]	86.7[b]	86.5[b]	86.9[b]	0.806	0.014
CP	29.8[a]	25.9[ab]	25.0[ab]	24.5a[b]	24.1[b]	22.1[b]	1.175	0.013
NDF	22.4	20.4	18.3	20.8	19.7	19.0	0.889	0.077
Ash	24.9[a]	24.2[ab]	21.3[ab]	20.7[b]	20.8[b]	20.7[b]	0.775	0.005
Starch	2.05	2.16	2.22	2.36	2.53	2.63	0.130	0.056

abc Mean values without common letter differ at P<0.05

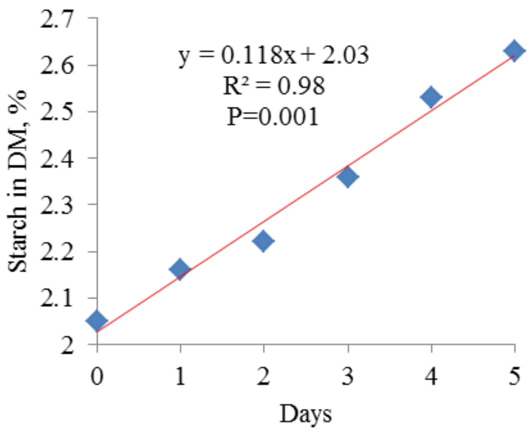

Figure 7. Relationship between time exposed to nutrient-free water and starch content of duckweed

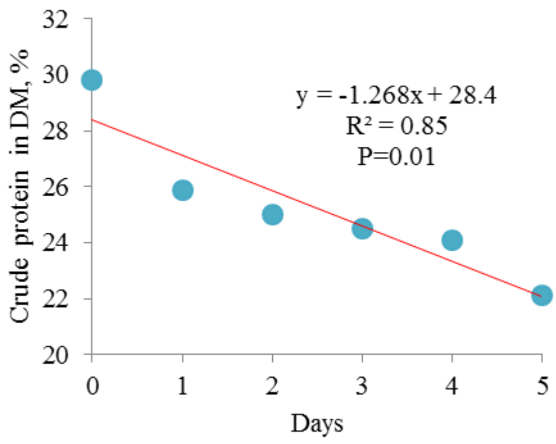

Figure 8. Relationship between time exposed to nutrient-free water and crude protein content of duckweed

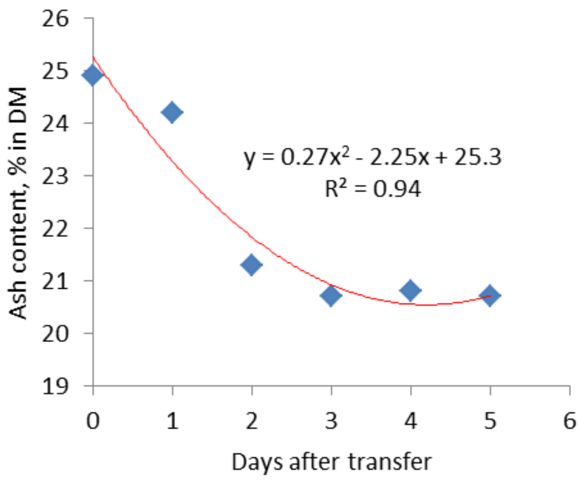

Figure 9. Relationship between time exposed to nutrient-free water and Ash content of duckweed

The above trends agree with the findings of Cheng and Stomp (2009) who reported that the starch content of duckweed increased after transfer for 5 days from nutrient-rich to plain water. However, the degree of change of starch concentration in our experiment (from 2.05 to 2.63%) was much less than that in the report of Cheng and Stomp (2009). According to these authors, a starch content of 45% was achieved in *Spiridela polyrrhiza* through a simple transfer of the fresh duckweed fronds from a nutrient-rich solution to tap water for 5 days. The reason for this difference is perhaps because of the different kind of duckweed used (*Lemna minor* in my experiment compared with *Spiridela polyrrhiza* in the report of Cheng and Stomp 2009) (Photos 5 and 6).

Photo 5. *Lemna minor (Wikipedia No date)*

Photo 6. *Spiridelap olyrrhiza (Wikipedia, No date*

Conclusions

- Yield and crude protein content of duckweed was optimized when the culture medium contained 12% biodigester effluent and 88% water, equivalent to 72 mg N/liter
- The "shock" treatment of transferring the best quality duckweed to nutrient-free fresh water led to decreases in crude protein and corresponding increases in starch; however, the effects were relatively small and unlikely to have significance from the points of view of duckweed as a feed for ducks or as a substrate for ethanol production.

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Effect on growth, apparent digestibility coefficients and carcass quality of local Muscovy ducks of feeding high or low protein duckweed (*Lemna minor)* as replacement for soybean meal in a rice bran basal diet

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Abstract

The experiment was done with 72 local Muscovy ducks fed the experimental diets over a period of 84 days. Measurement of coefficients of apparent digestibility was carried out over the period 70 to 75 days; growth performance was measured over the whole period of 56 days (8 weeks). The ducks were bought at 1-day of age from smallholder breeding flocks in Cantho city and fed a commercial diet from 1 to 28 days of age. The treatments were: CTL, a basal control diet including rice bran with soybean meal; HPDW, rice bran with high protein duckweed; LPDW, rice bran with low protein duckweed. The three dietary treatments contained the same level of crude protein of 15% in DM. A premix (vitamins and minerals) was supplied at 2% of the CTL diet. In treatments HPDW and LPDW it was assumed that the duckweed would provide the necessary vitamins and minerals. The HPDW was cultivated in ponds supplied with biodigester effluent according to the optimum treatment in the earlier experiment (paper 1). For LPDW, the duckweed was collected from natural ponds of local farmers.

Final live weight and daily live weight gain of local Muscovy ducks fed a rice bran basal diet were highest when the supplementary protein was from high protein duckweed and lowest when low protein duckweed was the supplement. The growth rate of Muscovy ducks fed rice bran supplemented with soybean meal was lower than that of Muscovy ducks fed rice bran supplemented with high protein duckweed. There were few differences in carcass traits when the ducks were slaughtered, except for a more attractive skin color for the ducks fed duckweed. The heavier gizzard in ducks fed duckweed probably reflected the higher fiber content of these diets. N retention was highest on the high protein duckweed diets and lowest for the low protein duckweed diets. The higher digestibility of the NDF fraction on the duckweed diets probably reflected the differences in the nature of the NDF fraction between rice bran and duckweed, as rice bran represented a lower proportion of the DM in the duckweed diet. The better economic results on the high duckweed diet resulted from lower feed costs and higher weight at slaughter.

Key words: *Biodigester effluent, fiber, NDF, N retention*

Introduction

Poultry production is a common activity in Southeast Asia, and is a major source of livelihood for over a million people in the rural areas. In the last two decades, Asian duck production has become more important, making up 87% of the world's duck population, and duck meat and egg production has increased more than four times (Chein Tai and Jui-Jane Liu Tai, 2001). This expansion has mainly come from the preservation of local breeds and strains, such as the local Muscovy duck and several Vietnamese breeds such as the Co and Tau duck (Duong Thanh Liem 2001), and imports of exotic and improved breeds.

Duck production is one component of integrated farming systems which are regarded as being part of a sustainable development in agriculture. Ducks *(Anasplatyrhynchos)* can be integrated with rice, orchards, cash crops, livestock and fish. Thus, the stakeholders not only can develop their livelihoods without accumulating debts, but also can get extra income through off-farm and non-farm activities (Le Thanh Phong et al 2007). The Mekong Delta, located in the South of Vietnam, is considered as the country's granary, accounting for 48% of the national rice production (followed by the Red River Delta). Mekong Delta has a warm ambient temperature and high annual rainfall that is suitable for duck production. Natural resources, including paddy rice fields, canal networks, and plant and grasses, for instance, are advantageous for ducks to increase in number. Ducks can effectively utilize low quality feed (agricultural residues, by-products and insects) and can produce highly nutritional foods for humans (Bui Xuan Men et al 1998).

Duck production is diversified into several raising systems according to economic criteria, for example, industrial integrated, medium to large commercial, medium to small commercial and mixed farming systems (integration of rice-ducks, ducks-fish or rice-fish-ducks) or spatial criteria, such as scavenging, semi-confined and confined systems (Edan et al 2006). The large scale system has developed only recently in some areas of the delta. It is generally agreed that better breeds, together with improvements in management of stock health and using local feed resources, as well as other appropriate technologies should enhance sustainable small-scale duck production.

However, the free-raising of ducks in the rice fields or canals (scavenging system) without strict management of outbreaks of diseases is a risk for community health and also duck production. In order to deal with this important issue and create a sustainable duck production, semi-confined and confined systems are being introduced and widely extended, with the aim of limiting the spread of infectious diseases such as Duck Plague and Avian influenza.

Annually, rice mills produce large quantities of grain for export, as well as the by-products (rice husk, rice bran and broken rice). The broken rice is not as valuable as rice grain but it also can be exported or used locally for human consumption. Rice bran is the outer layer of the brown rice kernel (after separating the husk) which is removed while milling brown rice to white. Rice bran is a rich source of nutrients and a pharmacologically active compound and is currently used as livestock feed and for oil production (Tahira et al 2007). According to Houston (1972), rice bran often occupies 5-8 percent of paddy rice (whole grain). Commonly, in Vietnam, the rice mills have produced three kinds of rice bran: the initial bran (mixed with rice husk fragments) and two types of bran produced in the polishing process which are very fine and have higher nutritive value than the initial bran. In the Mekong Delta, rice bran is cheaper than paddy rice and broken rice so it is the most widely available feed resource for duck production.

Objectives

- To evaluate the use of duckweed as a replacement for soybean meal in a basal diet of rice bran on the growth performance and carcass traits of growing Muscovy ducks.
- To determine apparent nutrient digestibility and nitrogen retention of Muscovy ducks fed experimental diets.

Materials and Methods

Location and climate of the study area

The experiment was carried out in the experimental farm of Cantho Univeristy in Binh Thuy District, Cantho City and the laboratory of the Department of Agriculture and Applied Biology, Cantho University, Vietnam, from July to October 2011. The climate is divided into two seasons: the rainy season (from May to November) and the dry season (from December to April). The ambient temperature fluctuates between 22^0C and 25^0C in the coolest months (December-January) and $32\text{-}33^0$C in the warmest months (April-May). Annual rainfall is 1400-2400 mm, and the average humidity varies from 76 to 80%.

Birds, experimental design and treatments

The experiment was done with 72 local Muscovy ducks fed the experimental diets over a period of 84 days. Measurement of coefficients of apparent digestibility we carried out over the period 70 to 75 days; and (ii) growth performance was measured over the whole period of 56 days. The ducks were bought at 1-day of age from smallholder breeding flocks in Cantho city. They were fed a commercial diet from 1 to 28 days of age. The birds were identified and then individually weighed (average initial live weights were around 950 g). All the birds were vaccinated with Duck Plague vaccine and Pasteurellosis vaccine at three and four weeks.

Photo 1. Ducklings in breeding period.

The experiment was arranged as a completely randomized design with 3 diets (Table 1) and 4 replications, and 6 birds per replicate (3 males and 3 females). The treatments were: CTL, a basal control diet including rice bran with soybean meal; HPDW, rice bran with high protein duckweed; LPDW, rice bran with low protein duckweed. The three dietary treatments contained the same level of crude protein of 15% in DM. A premix (vitamins and minerals) was supplied at 2% of the CTL diet. In treatments HPDW and LPDW it was assumed that the duckweed would provide the necessary vitamins and minerals.

Photo 2. Duckweed and rice bran prepared before feeding

The HPDW was cultivated in ponds supplied with biodigester effluent according to the optimum treatment in the earlier experiment (paper 1). For LPDW, the duckweed was collected from natural ponds of local farmers.

The feeding trial started when the ducks were 28 days old and lasted to 84 days of age. Apparent total tract digestibility was measured from day 70 to 75 using 2 ducks from each of the treatments.

Table 1. Feed ingredient composition of the diets % (DM basis)

	Diet		
Feed ingredient	**CTL**	**HPDW**	**LPDW**
Rice bran	84	80	70
Soybean meal	14	-	-
HP duckweed	-	20	-
LP duckweed	-	-	30
Premix-vitamins-minerals	2	-	-

Table 2. Composition of the premix used in the control diet

Premix of vitamins and minerals	Per kg
Vitamin A	2,500,000 IU
Vitamin D_3	500,000 IU
Vitamin E	1,500 IU
Niacinamide (Vitamin B_3)	5,000 mg
Calcium Pantothenate	3,000 mg
Vitamin C	3,300 mg
Riboflavin (Vitamin B_2)	1,200 mg
Vitamin K_3	1,000 mg
Thiamine (Vitamin B_1)	1,000 mg
Pyridoxine (Vitamin B_6)	550 mg
Folic acid	440 mg
Biotin (Vitamin B_7)	33,000mcg
Vitamin B_{12}	5,500 mcg
Premix of minerals	
Fe, Cu, Mn, Zn, I_2, Co, organic Se	121,200 mg
Biotin	18 mg
Dicalcium phosphate (DCP)	1,000 mg

Housing and management

The ducks were confined in pens constructed from bamboo, with thatched roofs, wire floors and surroundings with nylon nets (Photo 1). The average density was 3 birds per m^2. Natural light was used in the day and electric bulbs at night to allow eating as well as to deter mice. Feeders and drinkers were put in each pen. The housing, feeders and drinkers were cleaned and duck manure removed daily in the morning.


Photo 3. Experimental housing

Photo 4. Ducks growing in pens with different dietary.

Diets and feeding

In the preliminary period (from day 1 to day 28 after hatching) the ducklings were fed a commercial starter diet ad libitum, which contained 12.2 ME MJ/kgDM and 21% CP. The Muscovy ducks were kept in groups of 6 from 28 to 84 days. The high protein duckweed was cultivated on ponds enriched with nutrients by providing biodigester effluent at the level of 12 % (which gave the highest biomass yield and CP content in Paper 1). The low protein duckweed was collected from natural ponds which received no fertilizer. Both sources of duckweeds were harvested daily in the early morning. After collection they were put in large plastic baskets and cleaned by a strong water jet and then left for one hour to drain the excess water.

The rice bran and soybean meal were bought on one occasion from a feed store in the city and used during the whole experiment. For treatments HPDW and LPDW, the duckweeds were mixed with the rice bran in proportions to ensure a CP content of 15% in DM. The ducks were given fresh feed ad libitum 4 times a day at 07:30, 11:00, 14:00 and 19:00 h. Water was freely available for the ducks during day and night. The refusals and spillages were collected and weighed daily in the morning to calculate the feed intake. Samples were taken two times per week for analysis of chemical composition. The rice bran, soybean meal and duckweed were analyzed at the start of the experiment. The duckweed was analyzed two times per week.

Sampling procedure for excreta

During the 5-day collection period, samples were taken of the diets. Excreta were quantitatively collected three times daily at 7:00, 13:00 and 18:00 h, then frozen at - 20^0C. Care was taken to avoid contamination from feathers, scales and debris. Before analysis, excreta was thawed, then pooled within each diet and replicate and dried for 24 h at 55-60^0C. The dried excreta was weighed, homogenized, and ground to pass through a 0.5mm sieve, and representative samples were taken and stored in airtight plastic containers at -4^0C for analysis (Ravindran et al 1999).

Measurements and data collection

Feed and nutrient intakes

Daily feed intakes were calculated according to the total feed consumption of the 6 birds in each pen and nutrient intakes were calculated based on feeds consumed and their nutrient concentrations.

Growth parameters

At the beginning of the experiment all 6 ducks per experiment unit were weighed individually and then weekly.

Carcass parameters

At the end of the experiment in the morning before feeding, one male and one female bird from each pen were slaughtered for evaluation of carcass traits and internal organs**.** Breast muscles were removed to measure DM, CP, EE and ash.

Economic analysis

Economic analyses were done by using current prices in Vietnamese dong (VND) to calculate the differences in total income and total expenses (including feeds, ducklings, labour, vaccines and medicines) and net profit per treatment.

Chemical analysis

Rice bran, soybean meal, the two kinds of duckweeds, feeds offered and refusals were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash by standard AOAC methods (AOAC1990). Analyses of neutral detergent fiber (NDF) followed the procedure of Van Soest et al (1991).

Statistical analysis

The data were subjected to analyze of variance (ANOVA) by using the General Linear Model (GLM) of Minitab Reference Manual Release 13 (Minitab 2000).

Results and discussion

Growth performance

Chemical composition of dietary ingredients

The duckweed from the fertilized ponds (HPDW) contained about 30% more protein than the duckweed harvested from natural un-fertilized ponds (Table 3). The low calcium content of the rice bran was compensated by the high calcium content of the duckweed. Similarly the high phosphorus content of the rice bran balanced the moderately low content of phosphorus in the duckweed. These results justified the decision not to provide a mineral supplement in the diets containing duckweeds.

Item	Rice bran	Soybean meal	High protein DW	Low protein DW
DM, %	89.6	92.3	5.41	5.51
As % of DM				
OM	88.0	94.1	77.9	72.4
CP	11.0	42.2	32.4	24.9
CF	13.3	6.07	17.2	17.0
NDF	23.8	25.4	36.6	34.5
Ash	12.0	5.9	19.8	20.2
Ca	0.17	0.44	3.31	2.98
P	1.65	0.94	0.49	0.61
ME, MJ/kg	13.2	12.2	9.2	9.2

Feed intakes

Intake of rice bran, total DM and crude protein were highest for the high protein duckweed (HPDW) and lowest for the low protein duckweed (LPDW). The crude protein content of the diets was almost the same (15.1 to 15.3%).

Table 4. Daily feed intakes of local Muscovy ducks fed duckweed replacing for soybean meal

	CTL	**HPDW**	**LPDW**	SEM	P
Daily intake, g					
Rice bran	92.1^{b}	104^{a}	71.7^{c}	1.31	<0.001
Soybean meal	15.4	-	-		
HPDW	-	25.8	-		
LPDW	-	-	30.7		
Total DM	110^{b}	130^{a}	102^{c}	1.64	<0.001
OM	95.5^{b}	112^{a}	87.5^{c}	1.40	<0.001
Total CP	16.6^{b}	19.8^{a}	15.6^{c}	0.246	<0.001
Total CF	13.1^{c}	18.2^{a}	14.8^{b}	0.209	<0.001
NDF	25.8^{a}	34.1^{b}	27.7^{c}	0.409	<0.001
Ash	11.9^{c}	17.6^{a}	14.8^{b}	0.206	<0.001
Ca	0.23^{a}	1.03^{b}	1.04^{b}	0.009	<0.001
P	1.67^{b}	1.84^{a}	1.37^{c}	0.023	<0.001
ME, MJ/kg	1.41^{b}	1.61^{a}	1.23^{c}	0.021	<0.001
CP in DM, %	15.1	15.2	15.3		

abc Mean values in the same row without common letter differ at P<0.05

Daily gain and feed conversion ratio

Final live weight and daily live weight gain were highest on the HPDW diet and lowest on the LPDW diet, with the control diet being intermediate between the two duckweed diets. The differences in growth rate can be explained mainly by the differences in feed DM intake (Figure 1). As the crude protein of the diets was similar the explanation for the superiority of the high protein duckweed diet could be the result of a superior biological value of the protein in the duckweed fertilized with biodigester effluent.

Table 5. Live weights, daily gains and feed conversion of local Muscovy ducks fed duckweed as replacing for soybean meal.

	CTL	**HPDW**	**LPDW**	SEM	P
Live weight, g					
Initial	972	927	955	63.8	0.882
Final	2394^{b}	2534^{a}	2202^{c}	66.2	0.019
Daily gain	25.4^{b}	28.7^{a}	22.3^{c}	0.67	0.001
FCR	4.32	4.51	4.63	0.13	0.271

abc Mean values in the same row without common letter differ at P<0.05

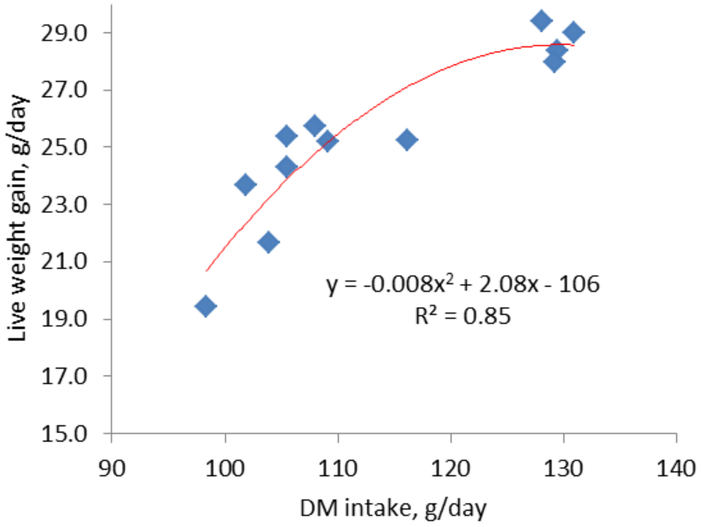

Figure 1. Relationship between DM intake and live weight gain of Muscovy ducks fed rice bran supplemented with soybean meal or high and low protein duckweed.

The better growth when rice bran was supplemented with high-protein duckweed rather than with soybean contrasts with the research reported by Bui Xuan Men et al (1996) (Table 6), where growth with 100% duckweed replacing soybean meal was 16% poorer. The differences between the two reports could be due to the quality of the duckweed. In the experiment of Bui Xuan Men et al (1996) the duckweed was produced under small farm conditions, whereas in the present experiment the high-protein duckweed was managed in an experimental farm where conditions were more controlled.

Carcass evaluation

Live weight of the slaughtered ducks differed among treatments (Table 7). When the carcass components were adjusted by covariance for differences in slaughter live weight, there were insignificant differences among the treatments other than for the weights of gizzard (which were lowest on the CTL treatment) and heart (lowest on the LPDW treatment). The lighter gizzard on the CTL diet was probably a function of the lower NDF content of this diet (23.5% compared with 26.2 and 27.2 on the HPDW and LPDW diets). This result is in agreement with a report that showed that gizzard weights for ducks increased with increasing amount of fiber in the diet (Siregar*et al* 1982; Kim Dong and Ogle 2003). There is no obvious explanation for the lower heart weight on the LPDW diet.

Table 7. Carcass parameters of Muscovy ducks fed duckweed replacing soybean meal in diets based on rice bran (Carcass dated adjusted for differences in slaughter live weight)

	CTL	**HPDW**	**LPDW**	SEM	P
Carcass, g	1600	1621	1552	28.6	0.404
Breast weight	457	483	425	10.4	0.029
Breast muscle	323[a]	344[a]	293[b]	10.0	0.047
Thigh weight	379	385	375	7.07	0.668
Thigh muscle	238	243	225	7.06	0.364
Gizzard	54.5[b]	63.4[a]	59.4[ab]	2.16	0.023
Liver	54.2	55.2	46.6	4.05	0.477
Small I, cm	186	193	186	3.71	0.281
Large I, cm	15.7	16.2	16.6	0.86	0.792
Caecum, cm	16.2	16.7	16.6	2.87	0.725
Heart, g	18.1	19.3	13.2	1.09	0.452

abc Mean values in the same row without common letter differ at P<0.05

Economic analysis and health status

Throughout the experiment, the ducks on all diets were healthy, had good appetite and low mortality (1.4%).

The feed cost and total expenses were the highest for the ducks fed rice bran and soybean meal. The highest income from selling ducks on the HPDW diet was due to the higher slaughter weight, the overall result being higher profit on the HPDW diet (Table 8). The ducks fed the HPDW diet had a natural yellow color of skin and abdominal fat that is attractive to consumers.

Table 8. Economic analysis of the effect of replacing duckweed with soybean meal (VND/bird, 21,000VND = 1USD)

Item	**CTL**	**HPDW**	**LPDW**
Total feed cost	50,600	42,232	38,205
Total expenses	154,033	144,271	139,573
Total income	179,506	190,013	165,095
Net profit	25,473	45,742	25,522

Apparent digestibility coefficients

The chemical composition of feed ingredients in the digestibility period was similar to that in the feeding period (Table 9).

Table 9. Chemical composition of feed ingredients in digestibility period (DM % is on fresh basis; other items are expressed as % of DM)

Ingredients	**DM, %**	**OM**	**CP**	**CF**	**NDF**	**Ash**
Rice bran	89.6	88.2	11.4	14.7	23.6	12.0
SB meal	92.3	94.1	42.3	6.47	25.7	5.9
HPDW	5.41	77.9	32.4	17.3	36.6	19.8
LPDW	5.51	72.4	25.1	16.3	34.5	20.2

The trends in DM intake during the measurement of digestibility were similar to those observed in the overall feeding trial (Table 10).

Table 10. Daily intakes of feeds and nutrients of local Muscovy duck fed duckweed as replacing for soybean meal (g/duck/day)

	CTL	**HPDW**	**LPDW**	SEM	P
Total DM	88.4[a]	94.5[b]	84.1[c]	2.23	0.028
OM	77.1[a]	81.4[a]	70.2[b]	1.92	0.008
CP	13.7[a]	14.7[b]	13.1[c]	0.349	0.023
CF	11.7[a]	12.3[b]	12.8[b]	0.306	0.103
NDF	20.7[a]	24.8[b]	22.6[a]	0.577	0.003
Ash	10.2[a]	12.8[b]	12.2[b]	0.341	0.001

abc Mean values in the same row without common letter differ at P<0.05

Table 11. Apparent total tract digestibility of dietary components and N-retention

	CTL	**HPDW**	**LPDW**	SEM	P
Apparent digestibility, %					
DM	74.6[a]	76.0[a]	72.5[b]	0.622	0.009
OM	77.0[a]	79.1[a]	76.0[b]	0.521	0.007
NDF	39.8[a]	48.2[b]	46.7[b]	1.264	0.002
CF	40.2	40.7	39.0	1.247	0.629
N intake, g/d	2.19[a]	2.36[b]	2.09[c]	0.055	0.020
N retention, g/d	1.75[b]	1.94[a]	1.66[b]	0.060	0.027

abc Mean values in the same row without common letter differ at P<0.05

Results for nitrogen retention supported the growth performance data with highest N retention for the HPDW diet. The higher digestibility of the NDF fraction on the duckweed diets, especially the LPDW diet, probably reflects the differences in the nature of the NDF fraction between rice bran and duckweed, as rice bran represented a lower proportion of the DM in the duckweed diets. It is probable that the NDF in rice bran, which originates mainly from the rice husk, is of lower digestibility than the NDF in duckweed.

Conclusions

- Final live weight and daily live weight gain of Muscovy ducks fed a rice bran basal diet were highest when the supplementary protein was from high protein duckweed and lowest when low protein duckweed was the supplement.
- Rice bran supplemented with soybean meal supported poorer growth than when the supplement was high protein duckweed.
- There were few differences in carcass traits when the ducks were slaughtered, except for a more attractive skin color for the ducks fed duckweed. The heavier gizzard in ducks fed duckweed probably reflected the higher fiber content of these diets.
- N retention was highest on the high protein duckweed diets and lowest for the low protein duckweed diets.
- The higher digestibility of the NDF fraction on the duckweed diets probably reflected the differences in the nature of the NDF fraction between rice bran and duckweed, as rice bran represented a lower proportion of the DM in the duckweed diets.
- The better economic results on the high duckweed diet resulted from lower feed costs and higher weight at slaughter.

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General conclusion

- It was concluded that yield and crude protein content of duckweed was optimized when the culture medium contained 12% biodigester effluent and 88% water, equivalent to 72 mg N/liter.
- The "shock" treatment of transferring the best quality duckweed to nutrient-free fresh water led to decreases in crude protein and corresponding increases in starch. However, the effects were relatively small and unlikely to have significance from the points of view of duckweed as a feed for ducks or as a substrate for ethanol production.
- Final live weight and daily live weight gain of Muscovy ducks fed a rice bran basal diet were highest when the supplementary protein was from high protein duckweed and lowest when low protein duckweed was the supplement.
- The growth rate of Muscovy ducks fed rice bran supplemented with high protein duckweed was better than those fed soybean meal
- There were few differences in carcass traits when the ducks were slaughtered, except for a more attractive skin color for the ducks fed duckweed. The heavier gizzard in ducks fed duckweed probably reflected the higher fiber content of these diets.
- N retention was highest on the high protein duckweed diets and lowest for the low protein duckweed diets. The higher digestibility of the NDF fraction on the duckweed diets probably reflected the differences in the nature of the NDF fraction between rice bran and duckweed, as rice bran represented a lower proportion of the DM in the duckweed diets.
- The better economic results on the high duckweed diet resulted from lower feed costs and higher weight at slaughter.