Abstract

REID, JR., WAYNE STANLEY. Exploring Duckweed (*Lemna gibba*) as a Protein Supplement for Ruminants Using the Boer Goat (*Capra hircus*) as a Model. (Under the direction of Matthew Poore.)

Duckweed is the common name used to refer to members of the aquatic plant family *Lemnaceae*. Duckweed commonly grows on stagnant, nutrient enriched waters throughout tropical and temperate zones. Growth conditions include water temperatures of 6 – 33^{o}C and a wide pH range. Under ideal conditions, duckweed can double its biomass every sixteen hours to four days. Its nutrient uptake capability helps to account for a CP of 15 – 40% and high growth rate. Scientists have studied duckweed's feed attributes for fish, poultry, swine, and ruminants. A duckweed feeding trial was carried out at North Carolina State University Metabolism Educational Unit with 19 goat wethers fed four different diets. The objective of the trial was to characterize the composition of wastewater grown duckweed and evaluate its use as a protein supplement for ruminants. Our hypothesis was that duckweed is a suitable protein source for goats and will behave in a similar fashion to soybean meal. The diets included a negative control, positive control (all of the supplemental protein from soybean meal), 1/3 duckweed, and 2/3 duckweed (1/3 and 2/3 of the supplemental protein came from duckweed, respectively). The goats were fed equal amounts of hay and supplement at 4% of body weight (as fed). Duckweed exhibited a similar compositional profile to soybean meal except for being lower in CP and higher in minerals. Amino acid and protein fraction profiles were also comparable between duckweed and soybean meal.

There was no significant difference among treatments for DMI, ADF, and NDF digestibility. Nitrogen intake, N digested (g/d), and N retained (% of digested) showed no significant differences among the supplemental protein diets. Nitrogen retained as a percent of intake and N retained (g/d) tended to be slightly lower in the diets containing duckweed. Serum urea nitrogen levels also showed no significant differences for the protein diets except for a linear response ($P = 0.09$). The P balance showed no significant difference for P intake but both linear and quadratic responses for P retained (g/d), and P digested (g/d) as well as a linear response for P retained (% of digested). Similarities of the rumen pH, NH_4 and VFA data among the diets show that duckweed does not abnormally affect rumen function and is comparable to soybean meal in dietary function. Duckweed appears to be a viable source of protein and phosphorus (at lower dietary levels) supplementation for ruminants and is nearly comparable to soybean meal in its utilization.

Keywords: Duckweed, Goats, Metabolism Trial, N Balance.

EXPLORING DUCKWEED (*LEMNA GIBBA*) AS A PROTEIN SUPPLEMENT FOR RUMINANTS USING THE BOER GOAT (*CAPRA HIRCUS*) AS A MODEL

by
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Personal Biography

Wayne Stanley Reid, Jr. was born in Gastonia, North Carolina to Wayne S. Reid, Sr. and Johnnie B. Reid. Growing up, he spent much of his time outdoors where he learned to appreciate the natural world. He graduated from Belmont Abbey College in the spring of 2001 with a Bachelor of Science Degree in Biology with minors in Biological Techniques, Chemistry, and Environmental Science. He entered the Animal Science Department in the fall of 2001 in pursuit of a Master's of Science Degree. He graduated from North Carolina State University with a Master's Degree in the summer of 2004. He continued his education by pursuing a Doctor of Veterinary Medicine Degree at North Carolina State University's College of Veterinary Medicine.

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Introduction

Throughout agricultural history, people have always experimented with novel and unconventional practices to better the production and economic aspects of their operations. With current concerns of waste and by-product under-management and mismanagement, the area of alternative feeds has found itself on the frontline of improving agriculture along with the environment. Asia has long used alternative feeds in its agricultural practices due to the poor economic status of its village farmers. Village farmers use whatever is at hand to feed not only their animals, but themselves as well. In many respects, the Asian countryside is far ahead of many industrialized agricultural nations (including the United States) in its use and management of waste and waste by-products as alternative feed. Much of the research conducted on the feasibility of duckweed as a feed or feed supplement originates from Asia.

One of the most interesting alternative feeds is a small, aquatic plant known as duckweed. Duckweed is the common name used to refer to the aquatic plant family of *Lemnacea*. *Lemnacea* cosists of five genera: *Lemna, Spirodella, Landoltia, Wolffia,* and *Wolffiella* with over forty identified species. Distributed throughout the temperate and tropical zones of the world, duckweed is among the smallest of the flowering plants in the world (Skillicorn et al., 1993). Duckweed is commonly found floating on the surface of ponds, lagoons, and many other large stagnant bodies of

water. Indeed, the presence of duckweed is considered a diagnostic sign of nutrient pollution in bodies of water.

Botanical Facts

The plant itself is the smallest of the flowering plants with species measuring only 0.3 – 20 millimeters in length (Landolt, 1986). Figure 1 presents a size comparison among three separate duckweeds (*Spirdella, Lemna*, and *Wolffia*). The entire duckweed plant consists of a flat, ovoid frond. The frond has no leaf, stem, or any other specialized structures. Many species do have roots that aid in stability as well as improved nutrient uptake (Skillicorn et al., 1993). Figure 2 depicts a solitary duckweed plant. The cell walls of duckweed plants lack lignin thereby increasing their digestibility and making them an ideal feed source (Leng et al., 1995). Because duckweed is a small, floating plant, it will not survive in water moving faster than 0.3 meters per second. Duckweed spreads among bodies of water through migrating aquatic birds and floods (Skillicorn et al., 1993).

Figure 1. Size Comparison of Different Duckweeds.

This photograph depicts three distinct duckweed plants. The largest duckweed shown is *Spirodela*. The medium size duckweed is *Lemna* and the smallest is *Wolffia* (Photograph by Gerald Carr, University of Hawaii).

The ideal habitat for duckweed is the surface of brackish water which contains decaying organic matter and is sheltered from the wind (Skillicorn et al., 1993; Leng et al., 1995). Wind is considered detrimental to optimal duckweed growth since wind blows the duckweed against the shore thereby allowing some of the duckweed to dry out and die on land. Also, efficient nutrient uptake is best achieved when duckweed is spread uniformly across the surface of the water. Duckweed can grow at water temperatures between 6 – 33^{o}C (43 – 91^{o}F), however the ideal water temperature is 17^{o}C or 62^{o}F (Skillicorn et al., 1993). Duckweed is

also tolerant of a wide pH range, but does best between 4.5 – 7.5. Duckweed growth is inhibited by numerous physiological stresses including a water temperature above 35^{o}C (95^{o}F), a pH higher than 10, high concentrations of metals and ammonia, a deficiency of nitrogen, overcrowding from other duckweed plants, and competition with other plants for light and available nutrients (Skillicorn et al., 1993; Leng et al., 1995).

Figure 2. Photograph of a single duckweed plant.

This is a photograph of a single *Lemna gibba* plant. Notice the two ovoid fronds and the two roots emanating from the underside of the plant (Tsatsenko and Malyuga, 2002).

Duckweed is capable of both sexual and asexual reproduction. Sexual reproduction is rare due to the infrequent flowering of the plants. Asexual

reproduction of duckweed involves the budding of daughter fronds (leaves) from a meristematic region in a reproductive pouch located on a mother/mature frond (White and Wise 1998). A single frond is capable of reproducing 20 – 50 times during its life cycle which can span 10 days to several weeks (Skillicorn et al., 1993). Duckweed is capable of over wintering in colder climates by producing either seeds or turions. However, it should be noted that duckweed can survive several consecutive days of freezing temperatures without showing any detrimental effects. In the southern United States, duckweed is capable of growing all year long (Culley and Epps, 1973). Duckweed seeds are designed to sink to the bottom of the water source where they lay dormant until favorable growth conditions return. Seeds are resistant to both freezing and desiccation. However because sexual reproduction is rare, seeds are likewise uncommon. A more common sight is the production of turions which are dormant vegetative buds produced by the fronds of the plant. Turions sink to the bottom of the water and remain dormant like seeds, but unlike seeds, they are susceptible to freezing and desiccation (Landolt 1986).

What makes duckweed such a promising feed is its spectacular growth rate. Under ideal growth conditions of optimal nutrient availability, sunlight availability, and water temperature duckweed is capable of doubling its biomass in 16 hours to 4 days. This reproduction rate is faster than almost any other known plant, and all known forages. Anh and Preston (1997a) presented evidence that the optimum initial density for accelerated growth of duckweed is 200 – 300 g/m^2. They also

found the optimum harvesting frequency to be at 2 day intervals (Anh and Preston, 1997a). Extrapolated duckweed harvests reach amounts of 183 metric tons/ha/year of DM. However due to real world conditions (factoring in loss due to harvesting methods, run-off, and less than ideal harvesting methods), yields are closer to 10 – 40 metric tons/ha/year of DM (Skillicorn et al., 1993; Leng et al., 1995). For comparison, alfalfa can yield 11 metric tons/ha/year, hybrid bermudagrass yields 10 metric tons/ha/year, and endophyte-infected fescue yields 4.5 metric tons/ha/year (Chamblee and Green 1995).

Research

Interest concerning duckweed first surfaced in the scientific community in the late 1960's to the early 1970's. Initial tests showed that the protein content of duckweed DM commonly fell between 15 – 40%. Duckweed grown on enriched lagoons such as swine or dairy waste easily reached the 40 – 45% protein level (Landolt, 1986). Research involving duckweed originally began on two separate fronts: as a wastewater treatment and as a feed resource. Asian society has long been incorporating duckweed into their daily lives as a wet feed source for poultry and livestock, a human food source, and for wastewater treatment of both human and animal waste. Due to duckweed's prevalent use in Asian society, most of the research still originates from Asian universities and field stations.

Wastewater Nutrient Removal

Duckweed has a naturally high rate of nutrient uptake due to its accelerated growth rate. Duckweed prefers to take up nitrogen in the form of ammonium ions (Skillicorn et al,. 1993). Ammonium uptake is critically important in wastewater because ammonium increases eutrophication in open ponds and can result in the formation of nitrates if released into local groundwater (Oron et al., 1988). Due to its high nutrient uptake, duckweed is capable of tolerating the high nutrient levels commonly found in both domestic and animal wastewater. Landolt (1986) reported *Spirodela polyrrhiza* growth in the presence of 1.0 g/L nitrogen and 1.5 g/L phosphorus. Korner et al. (1998) conducted a study on the degradation of organic matter in duckweed covered versus non-duckweed covered (control) domestic wastewater system. Researchers found that after a 3 day time frame, removal efficiencies for the duckweed system were 74 – 78% while the removal efficiencies of the control system were lagging at 52 – 60%. Duckweed was found to enhance degradation of organic material in terms of chemical oxygen demand or COD (the quantity of oxygen needed for both biological and non-biological oxidation of matter in the effluent) and consequently biological oxygen demand or BOD (the quantity of oxygen that would be consumed if all the organics of the effluent were oxidized by bacteria and protozoa) over uncovered wastewater systems (Korner et al., 1998).

Alaerts et al. (1996) presented data on the performance analysis of a duckweed covered sewage lagoon. Alaerts noted that duckweed caused

concentration reductions of 90 – 97% for COD, and 95 – 99% for BOD over a 5 day time period (BOD_5), and 74 – 77% for Kjeldahl nitrogen and total phosphorus. Ninety percent of the nutrient uptake occurred in the first 7.3 days of the wastewater occupying the lagoon. Likewise, 80 – 90% of BOD load removal occurred within the first 7.3 days giving the lagoon an equivalent loading rate of 80 – 90 kg $BOD_5/m^2\ d^{-1}$ compared to a loading rate of 48 - 60 kg $BOD_5/m^2\ d^{-1}$ for the entire lagoon. This loading rate discrepancy suggests that the lagoon is capable of accommodating higher loadings and that the lagoon's design could be further optimized. Harvesting the duckweed within the first 7.3 days of retention time proved capable of removing 60 – 80% of the N and P loads or 0.26 g $N/m^2\ d^{-1}$ and 0.05 g $P/m^2\ d^{-1}$. Managing the lagoon proved to be sustainable for several years with a biomass production of 58 – 105 kg (dry weight)/ha d^{-1} or 715 – 1200 kg (wet weight)/ha d^{-1}. Table 1 provides the typical concentration reduction performance of the lagoon. Kjeldahl-N of the wastewater was reduced by 74% (10.5 mg/l inflow to 2.7 mg/l outflow). Likewise, total wastewater P was reduced by 77% (1.95 mg/l inflow to 0.4 mg/l outflow). A reduction of 99% also occurred for NH_4^+ (8 mg/l inflow to 0.03 mg/l outflow). It should be clarified however, that duckweed was not the single cause of these reductions. A mass balance showed that nutrient uptake by duckweed accounted for a 46.6% reduction in total P and a 42.5% reduction in total N (excluding NO_3^-). Duckweed harvesting accounted for only 8.1% of both N and P removal. The rest of the reduction percentages presented in Table 1 are due to percolation, sedimentation, outflow, and unaccounted losses.

Table 1. Typical inflow and outflow concentrations of a duckweed covered sewage lagoon (Alaerts et al,. 1996).

Parameter	Influent	Effluent	% Removal
BOD_5 (mg/l)	125* (80 – 160)	5* (8)	96 (90 – 95)
Kjeldahl-N (mg/l))	10.5	2.7	74
NH_4^+ (mg N/l)	8 (3 – 20)	0.03 (0.1 – 1)	99 (90 – 99)
NO_3^- (mg N/l)	0.03 (0.05 – 1)	0.05 (0.05 – 1)	NA
Total P (mg/l)	1.95	0.4	77
Ortho-PO_4^{3-} (mg P/l)	0.95 (0.5 – 2.5)	0.05 (0.06 – 0.3)	95 (90-95)

- * Calculated from COD using COD/BOD_5 ratio of 2.2 (influent) and 5 (effluent).
- Values in parentheses are based on 4 – year monitoring (1990 – 1994).
- Inffluent data for dry season has been corrected for the dilution effect due to the groundwater supply.

In 2000, a project was undertaken at North Carolina State University to select superior duckweed genotypes for the utilization of nutrients in animal wastes (Bergmann et al., 2000a). A two-step protocol was implemented to select promising duckweed geographic isolates from forty-one isolates obtained from the worldwide germplasm collection. Total protein production per culture was used for selecting the superior geographic isolates since total protein production differed 28 fold between the extremes of the forty-one isolates. These superior isolates were then reduced to three through a growth trial with full-strength swine lagoon effluent. *Lemna gibba* 8678, *Spirodela punctata* 7776, and *Lemna minor* 8627 were selected as the superior duckweed isolates (Bergman et al., 2000a).

Subsequent studies were then conducted on the superior isolates ability for nutrient removal from swine lagoon effluent (Bergmann et al., 2000b; Cheng et al., 2002). It should be noted that the duckweed used in the present feed trial is believed to originate with the *Lemna gibba* 8678 isolate that somehow escaped the swine effluent trials and found its way into a neighboring fish waste pond. A *Lemna gibba* variety of duckweed began growing on a fish waste pond adjacent to the NCSU Swine Unit while the Bergmann et al. (2000b) trial was being conducted. Table 2 shows the nutrient content of the Lemna gibba 8678 isolate along with the nutrient loss accounted for by the isolate over a 12 day period. The nutrient content of the duckweed increased with the increasing concentration levels of the swine effluent. However, nutrient uptake did not increase but decreased with the increasing concentrations of swine effluent. Only copper had a higher nutrient loss at 67% effluent than at 20% effluent. The proportion of nutrient loss began to decline at the 33% and 50% swine effluent concentration levels. Consequently, Bergmann et al. (2000b) concluded that the 8678 isolate should be grown on a swine effluent concentration of 50, 30, or 25% for the best effluent treatment and for increased duckweed biomass production.

Table 2. Nutrient content* and nutrient loss accounted for by *Lemna gibba* geographic isolate 8678 grown for twelve days** on swine lagoon effluent (Bergmann et al., 2000b).

Nutrient Content of Lemna gibba 8678							
Swine Effluent Concentration	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Cu (ppm)	Zn (ppm)
67%	5.51a	1.72a	4.00a	0.97a	0.59a	50.23a	91.25a
50%	5.73a	1.72a	3.76a	1.02a	0.59a	49.55a	94.75a
33%	5.26a	1.54a,b	4.15a	0.73b	0.53b	35.18b	69.75b
25%	5.65a	1.58a,b	4.62a	0.63b	0.47c	32.25b	64.00b,c
20%	5.07a	1.42b	4.79a	0.63b	0.49b,c	27.10b	56.00c
Nutrient Loss/Reduction Accounted for By Lemna gibba 8678							
Swine Effluent Concentration	N (%)	P (%)	K (%)			Cu (%)	Zn (%)
67%	26.1	45.5	71.5			70.9	69.7
50%	45.2	98.1	69.5			66.6	92.2
33%	56.9	67.6	77.7			36.6	98.6
25%	92.9	105.0	95.6			36.0	106.1
20%	96.3	103.7	93.3			38.8	85.3

* Values within a column with the same superscript are not significantly different according to Duncan's critical range test conducted at the 0.05 level.

** Twenty percent of the surface area was harvested every other day over the 12 day period.

Animal Feed Source

Duckweed has been fed to a variety of animals including rats (Phuc et al., 2001; Hanczakowski et al., 1995), cattle (Huque et al., 1996.; Rusoff et al., 1978), chickens (Islam et al., 1997; Samnang, 1999), ducks (Anh and Preston, 1997b; Men et al., 2001; Men et al., 2002), swine (Leng et al., 1995; Rodriquez and Preston 1996; Men et al., 1997; Lai, 1998; Gutierrez et al., 2001; Dung et al., 2002; Ly et al., 2002), sheep (Damry et al., 2001), fish (Porath and Koton, 1977; Fasakin et al.,

1999; Fasakin et al., 2001; Bairagi et al., 2002), and humans (Rusoff et al., 1980). Dry matter production of duckweed is high compared to that of other crops. Edwards et al. (1992) reported a dry matter production of 20.4 t/ha/yr for *Spirodella polyrhiza* while Oron et al. (1988) reported 54.8 t/ha/yr for *Lemna gibba*.

Duckweed is considered by many researchers to be an ideal candidate for utilization as a feedstuff for several reasons including: can be easily harvested, high protein content, low fiber and lignin content, high mineral absorption capability, extended growing and harvesting periods, nontoxic to domestic stock, and susceptible to few pests (Culley and Epps, 1973). Duckweed is considered to be easy to harvest since it floats on the surface of the water. A simple skimming device is sufficient to harvest duckweed from any body of water (Culley and Epps, 1973). Although harvesting duckweed sounds simple enough, harvesting duckweed takes either more man-power or more machinery than most articles allude to in their materials and methods section. Duckweed grown under ideal conditions on domestic or animal waste and harvested regularly will commonly have a crude fiber content of 5 – 15% and a protein content of 35 – 45% (Skillicorn et al., 1993). Anh and Preston (1996a) reported that a high level of protein (35 – 40%) in duckweed dry matter could be consistently achieved when the concentration of nitrogen in the water is kept between 10 – 30 mg/l. Duckweed contains low levels of fiber and lignin since it floats on water and does not need to stand erect like terrestrial plants. The ability of duckweed to treat wastewater through its uptake of minerals and nutrients

is well documented. Duckweed possesses an extended growing and subsequent harvesting period due to adaptation to temperate and tropical climates. The Southeast United States provides an ideal environment for duckweed. Since the winters are mild, duckweed often grows year round or only experiences short periods of dormancy (Culley and Epps, 1973). Like any other food, duckweed is only toxic/detrimental upon exceeding specific dietary levels. However, it should be noted that duckweed toxicity is still much in debate. Many researchers allude to the non-toxic effects of duckweed after only a short feed trial with little refusal or orts. Researchers are currently investigating the dietary limits at which duckweed can be added to domestic animal feed. Duckweed has an apparent immunity to most pests associated with other forages (Culley and Epps, 1973). *Spirodella, Lemna,* and *Wolffia* have only a few serious pests. Therefore unlike traditional agricultural crops, the cost for pest management of duckweed stands would be negligible (Culley and Epps, 1973).

Since duckweed grows and floats on water, water constitutes a major part of the plants composition. Culley and Epps (1973) and Skillicorn et al. (1993) considered the high water content of duckweed to be the main deterrent against its use as a mainstream feed. Duckweed stands regularly contain 92 – 95% water (Rusoff, 1980). Culley and Epps reported duckweed samples ranging from 90 – 97% water (1973). Duckweed can be fed fresh, straight off the pond or lagoon, or it can be dried and concentrated before feeding. Research has been conducted on

feeding both raw and dried duckweed, but most research in the United States deals with feeding only dried duckweed. This difference in not drying and drying duckweed is partly due to the cultural differences between agriculture in Asian countries and the United States. Much of the duckweed research in Asian countries is geared toward the small village farmer. The village farmer either has access to a private water source or in many cases a community water source. With duckweed growing on the water source, the Asian farmer has adapted his animals to eat the duckweed wet (harvested straight off the water source) and subsequently saves the farmer the time and expense of drying. The farmer also only harvests enough duckweed to feed his animals for the day. He has no need in drying and stockpiling duckweed to feed his animals later. This feeding technique is in stark contrast to farming in the United States where the majority of the feed is fed dried and harvests are as large as possible in order to accommodate stockpiling and selling of any un-needed feed.

Lawson et al. (1974) conducted research on different procedures one could use to dry duckweed (*Spirodela oligorrhiza*) including: sun drying, oven drying, pressing, parboiling, and spout bed drying (forced air drying). Lawson's findings on how drying affects the crude protein level of duckweed are presented in Table 3. The raw duckweed with no drying treatment contained 38.3% CP. Oven drying the duckweed at different depths of ½, 1, and 2 inches had no result on the crude protein of the samples (41.3 CP). Lawson does not address why the CP of his dried

duckweed sometimes had a higher CP than the raw duckweed. Although depth did not influence CP, the temperature of the oven did impact the CP of the duckweed. If duckweed is dried in an oven, it is recommended that the temperature not exceed 100^{o}C. Duckweed dried at 120^{o}C and above exhibits a definite burned appearance upon exiting the oven. However, Lawson et al. (1974) conclude that due to the high moisture content of duckweed, it would be cost prohibitive for farmers to oven dry duckweed in large quantities. Using a mechanical press under very high pressure to squeeze water out of the plant is detrimental due to the loss of nitrogen. The spouted bed drying method failed to be productive when using wet duckweed. Although slower, sun drying is the most economical method of drying duckweed, especially if time is not a major concern (Lawson et al., 1974). However, the sun dried duckweed contained a lower CP (32.4%) than oven drying at 100^{o}C (41.3%) or raw duckweed (38.3%). Upon losing its water content, duckweed presents another problem by becoming extremely lightweight thereby making stability and containment on a windy day a major concern (Culley and Epps, 1973, Lawson et al., 1974). Solar drying duckweed methods should include some form of wind barrier to help block the wind and to help catch any dried duckweed that is blown by the wind.

Table 3. Crude protein analysis of duckweed drying methods.

Treatment	Average Crude Protein*, %	Treatment	Average Crude Protein*, %
Raw Duckweed (No Treatment)	38.3	Pressed 60 psi, Oven Dried 100°C	32.1
Oven Dried 80°C	38.6	Pressed 125 psi, Oven Dried 100°C	32.7
Oven Dried 100°C, 2" depth	41.3	Pressed 250 psi, Oven Dried 100°C	29.0
Oven Dried 100°C, 1" depth	41.3	Pressed 780 psi	12.3
Oven Dried 100°C, ½" depth	41.3	Pressed 1560 psi	12.8
Oven Dried 120°C	34.1	Pressed 3125 psi	13.1
Oven Dried 140°C	39.6	Pressed 4690 psi	11.1
Sundried, 50% Relative Humidity	32.4	Pressed 6250 psi	13.1
Sundried, 54% Relative Humidity	32.4	Pressed 7810 psi	11.9
Parboiled	32.3	Liquid from Duckweed Pressed 780 psi	14.1
Pressed 250 psi, 100°C	41.1	Liquid from Duckweed Pressed 3125 psi	13.8
Spouted at 27°C	41.1		
Spouted at 50°C	39.9		

* Standard Kjeldahl. (Lawson et al., 1974)

In 1980, Rusoff et al. reported the protein and amino acid composition of four species of duckweed *(Lemna gibba, Spirodela polyrhiza, Spirodela punctata* and *Wolffia columbiana*) grown on an anaerobic dairy waste lagoon. Table 4 presents the compositional analysis of the four duckweed species. The table shows clearly that freshly harvested duckweed is commonly 94 – 96% water. The crude protein of duckweed is relatively high with a low of 25.2% for *Lemna gibba* and a high of 36.5 for *Wolffia columbiana.* Table 5 shows the essential amino acid content of the

duckweeds (mean of the 4 species) compared to the content of the FAO reference pattern, corn, and rice. The mean of the 4 duckweeds does not meet the FAO reference for Lysine, Isoleucine, or Methionine but does meet the standard for Leucine, Phenylalanine, Threonine, and Valine. The duckweed mean also shows similarity to amino acid profile of both rice and corn. The absence of the amino acid cysteine does not preclude the presence of the amino acid in the plant's concentrate, but does indicate that cysteine levels were below the detection limits (> 0.05 g/100 g of protein). Table 6 gives the amino acid profile, nucleic acid, and protein content of the different duckweed species. The data indicates that with the exception of methionine, the levels of essential amino acids present in wastewater grown duckweed meets the recommendation set forth in the FAO reference pattern. It is also evident that duckweed is a good source of the amino acid lysine, which is only present in low amounts in grains (Rusoff et al., 1980).

Table 4. Compositional analysis of duckweeds (% Dry Matter)

Species	**Dry Matter**	**Crude Protein**	**Fat**	**Crude Fiber**	**Ash**
L. gibba	4.6	25.2	4.7	9.4	14.1
S. punctata	5.2	28.7	5.5	9.2	13.7
S. polyrhiza	5.1	29.1	9.4	8.8	15.2
Wolffia columbiana	4.8	36.5	14.1	11.0	17.1

Rusoff et al., 1980

Table 5. Comparison of essential amino acids present in duckweed protein concentrate to FAO reference pattern, corn, and rice (g/100 g of Protein).

Amino Acids	Duckweed [a]	FAO	Corn	Rice
Lysine	4.0	4.2	2.3	3.2
Isoleucine	3.6	4.2	6.2	5.2
Leucine	6.7	4.8	15.0	8.2
Methionine	0.9	2.2	3.1	3.4
Phenylalanine	4.2	2.8	5.1	5.0
Threonine	3.13	2.8	3.7	3.8
Valine	4.4	4.2	5.3	6.2
Tryptophan		1.4	0.6	1.3

[a] Mean of the four species. Rusoff et al., 1980.

Table 6. Amino acid, nucleic acid, and protein composition of duckweed protein concentrate

	L. gibba	*S. polyrhiza*	*S. punctata*	*Wolffia Columbiana*	Mean
g/100 g of Protein					
Aspartic	7.12	7.55	7.38	5.63	6.92 ± 0.88 [a]
Threonine	3.20	3.45	3.31	2.55	3.12 ± 0.40
Serine	2.61	2.80	2.83	2.28	2.63 ± 0.25
Glutamic	7.60	8.00	7.69	5.76	7.26 ± 1.01
Proline	2.93	3.28	2.95	2.41	2.89 ± 0.36
Glycine	3.79	3.95	3.93	2.04	3.68 ± 0.43
Alanine	4.59	4.48	4.79	3.75	4.40 ± 0.45
Valine	4.96	4.40	4.71	3.49	4.39 ± 0.64
Methionine	0.83	0.83	1.07	0.87	0.90 ± 0.15
Isoleucine	3.87	3.75	3.76	3.06	3.61 ± 0.37
Leucine	7.15	6.85	6.88	5.83	6.68 ± 0.58
Tyrosine	2.91	3.05	3.14	2.17	2.82 ± 0.44
Phenylalanine	4.45	4.20	4.38	3.60	4.16 ± 0.39
Histadine	1.89	2.15	1.90	1.18	1.78 ± 0.42
Lysine	4.13	4.30	4.26	3.37	4.01 ± 0.43
Arginine	4.29	5.25	4.86	3.78	4.54 ± 0.64
True Protein [b]	66.32	68.29	67.80	52.77	63.80 ± 7.40
g/100 g of Dry Matter					
Nucleic Acid	6.0	6.2	6.3	6.4	6.25
Crude Protein (N x 6.25)	37.5	40.0	42.0	44.7	41.05

[a] Standard Deviation. [b] Sum of Amino Acids. Rusoff et al., 1980.

Fish

Duckweed has long been used as a feed source on Asian fish farms, but recent research is intensifying its use as a protein supplement. Porath and Koton reported that the weight of grass carp could be tripled from 100 g to 300 g in a span of only 50 days by feeding a mixture of *Lemna gibba* and *Lemna minor* (1977). Fasakin et al. used duckweed to supplement the diets of Nile tilapia fingerlings (2001). The results of the study showed that duckweed could be used to replace fish meal protein up to 10% of the diet without adversely affecting growth patterns. Due to the decreased growth rates of the fingerlings, Fasakin concluded that the complete replacement of fish meal protein by duckweed should be avoided in tilapia diets (2001). This conclusion agrees with previous work conducted by El-Sayed (1992), Almazan et al. (1986), Fasakin et al. (1999b), and Bairagi et al. (2002). In 1999, Fasakin et al. presented data to support the use of duckweed in tilapia diets, but not as the sole source of protein. They found the most cost effective (cost/unit weight gain in fish) diet to be one with 30% duckweed inclusion. Utilization of up to 30% duckweed to replace commercial fish meal was found to be cost effective for supporting both growth and profitability (Fasakin et al., 1999b).

Research conducted by Bairagi et al. (2002) compared fermented duckweed meal with raw duckweed meal as replacements for fish meal protein supplements fed to rohu fingerlings. A bacterial strain isolated from a common carp intestine was used to ferment the duckweed for a span of 15 days. Fermented duckweed diets

were found to be superior to raw duckweed diets in both growth and feed utilization efficiencies. Protein digestibility was shown to decrease with increasing levels of duckweed regardless of type (raw or fermented). Data shows that a diet including 30% fermented duckweed resulted in the best food conversion ratio and protein efficiency ratio. The 30% fermented duckweed diet yielded the rohu fingerlings with the highest carcass protein and lipid deposition. The conclusion is that fermented duckweed can be incorporated in diets up to 30% with no adverse effects while raw duckweed can only be incorporated up to 10% of the diet without showing adverse effects (Bairagi et al., 2002).

Poultry

Duckweed has been researched as a protein supplement for both chickens and ducks. Anh and Preston presented evidence in 1997 that duckweed has an equivalent biological value to that of soybean meal but duckweed protein is slightly less utilized by growing ducks than soybean protein. The lower protein utilization is probably caused by duckweed's higher fiber content, 10% for duckweed DM while only 5% for soybean DM. Anh and Preston (1997b) concluded that duckweed can totally replace soybean meal in a growing ducks diet, and can be used as the sole source of dietary protein for growing ducks. In 1999, Samnang showed that the growth rate of chickens along with the producer's profit margin can be increased by feeding duckweed.

Men et al. (2001) completely replaced commercial protein supplements used in diets for meat ducks with duckweed. No significant differences in carcass yield, chest and thigh muscle weight, and internal organ weights were found between birds on the control diet and birds on the duckweed diet. Researchers did note a poorer feed conversion rate for the duckweed diet compared with the traditionally used soybean commercial diet. Nevertheless, fresh duckweed can completely replace commercial protein supplements used for ducks with no reduction in growth performance or carcass traits. However if the duckweed is locally grown, managed, and harvested by the farm manager/owner, the savings over commercial protein supplements can reach as high as 48% (Men et al., 2001).

In 2002, Men et al. conducted a follow-up study to their 2001 research using local and exotic breeding ducks. The conclusion reached by the researchers was that duckweed could replace commercial protein supplements used in the diets of laying ducks without adversely affecting their reproductive performance. In spite of this conclusion, a reduction in the hatchability for diets where duckweed composed the major source of protein was apparent. Nevertheless when the economics of locally producing, managing, and harvesting the duckweed was factored into the equation, researchers recommended using duckweed to replace some of the commercial protein supplements but not all of the supplements. The lower hatching rate associated with the total duckweed protein diet outweighs the benefit from substituting duckweed for commercially available supplements. Economic savings

of producing, managing, and harvesting duckweed on the farm reached up to 36% over purchasing commercial supplements (Men et al., 2002).

Swine

Duckweed fed to pigs has been found to reduce back-fat deposition, improve reproductive performance, and decrease economic costs. Duckweed used to replace protein supplements for fattening pigs showed no reduction in growth rate and produced a leaner carcass with less deposition of back-fat (Van et al., 1996). Research conducted by Men et al. (1997) showed that replacing 50% of conventional protein sources such as fish meal and soybean meal with fresh/raw duckweed resulted in a larger litter size, higher litter survival rates, and heavier litter weights. Men et al. (1997) showed that by replacing half of conventional protein sources with duckweed, farmers are able to improve the reproductive performance of their sows. Gutierrez et al. presented data in 2001 that showed a 10% duckweed diet being fed without any negative effects on productive performance. It was noted by Gutierrez et al. (2001) that although pigs fed duckweed consumed more feed and grew faster, the duckweed diet was not digested as efficiently as the sorghum/soybean meal control diet. However, this discrepancy could be due to the experimental 10% duckweed diet containing 2.3% more fiber thereby altering its digestibility from the control (Gutierrez et al., 2001).

Ruminants

Little research has been conducted on the use of duckweed as a feed source for ruminants. This lack of research is due to a variety of reasons including the difficulty of harvesting sufficient amounts of duckweed for a valid feed trial. However as the interest in duckweed continues to grow, more ruminant research is starting to be conducted.

Huque et al. (1996) reported on the feed potential of *Spirodela, Lemna,* and *Wolffia* for cattle. Three rumen cannulated bulls (317.0 kg average LW) had an average duckweed consumption of 10% of their live weight (LW). Both the dry matter and crude protein of the three duckweed types were shown to be highly degradable (*Spirodela* 71% and 80%, *Lemna* 71% and 86%, and *Wolffia* 91% and 93% respectively) in the rumen over a 72 hour period. As would be expected, degradibilities decreased with reduced time in the rumen (At 48 hours: Spirodella 61% and 72%, Lemna 61% and 79%, and Wolffia 88% and 91%; at 24 hours: Spirodella 41% and 53%, Lemna 57% and 74%, and Wolffia 73% and 78% for DM and CP respectively). Huque et al. (1996) concluded that duckweed incorporated as a component of a concentrate mixture can be fed to cattle without negative results. Huque bases his conclusion on the results of a 7-day feed trial using 3 rumen cannulated bulls and the previously discussed 72-hour in situ study. Khan et al. (2002) used a similar in situ study along with an in vitro gas production study to conclude that the high protein content of duckweed along with other aquatic plants

warrants consideration of their use to supplement poor quality or deficient diets. Both studies also agree that more research on animal, specifically ruminant, response to incorporation of duckweed into the diet is needed.

O'Bryan et al. (1998) carried out a feed trial using Holstein steers in order to examine their nitrogen and phosphorus utilization when fed duckweed. The duckweed was grown in artificial wetland cells which were supplied with wastewater from an adjacent dairy farm. The duckweed diet was formulated to be both isonitrogenous and isophosphoric with the control diet. The researchers discovered that the percentage of phosphorus digested ($P<.05$), the absolute retention in g/d ($P<.01$) and the percentage of phosphorus retained ($P<.05$) were greater for the calves on the duckweed diet than for the control diet calves (O'Bryan et al., 1998). The duckweed diet yielded means of 63.89% phosphorus digested, 9.03 g/d absolute retention, and 55.41% phosphorus retained while the control diet produced means of 56.77% phosphorus digested, 7.41 g/d absolute retention, and 46.58% phosphorus retained. Nitrogen was reported to be used with equal efficiency for all variables measured in both diets. O'Bryan et al. (1998) concluded that the nitrogen and phosphorus in duckweed was able to be used with the same degree of efficiency as the nitrogen and phosphorus from other conventional feeds.

Damry et al. (2001) noticed that after a short adjustment phase, Merino sheep formed a strong preference for their duckweed diet. This preference caused the

researchers to deduce that the beneficial properties of duckweed outweighed any detrimental effects. Damry et al. (2001) reported that duckweed was more effective than urea and as effective as cottonseed meal as a protein source for wool growth. However, protein degradation data did not support the findings of Huque et al. reporting that duckweed was highly degradable. Damry's data showed rumen ammonia concentrations for duckweed similar to that of cottonseed meal which is considered a good source of 'escape' protein for ruminants. A plausible explanation is that the duckweed used by Damry had a higher resistance to ruminal microbial degradation compared to the duckweed used by Huque et al. The difference could simply be due to the varying ruminal conditions between the sheep and cattle and/or the actual residence time the duckweed spent in the rumen. It is also possible that the composition of the duckweed itself varied depending on the water source it was grown on and how the duckweed was dried before feeding (Damry et al. 2001). Huque et al. (1996) report that the duckweed used was grown on domestic wastewater and sun dried. Damry et al. (2001) report that the duckweed was sun dried but give no indication of how the duckweed was grown. Although both studies report sun drying the duckweed, variation in the temperature, weather conditions, and apparatus used could also affect the composition of the final dried duckweed. Undoubtedly more research is recommended which brings us to the present study of duckweed being fed as a protein supplement to goats.

In summary, duckweed holds great promise as an alternative feed supplement. One of the smallest plants known to man could help us produce cleaner water while at the same time providing a high quality feed for domestic stock animals (poultry, swine, and cattle). The nutrient uptake ability possessed by duckweed along with its fast reproductive rate and environmental requirements make it easy to manage. The problem with duckweed is in the harvesting of the small plants and removing the excess water. Assuming that can be done efficiently, we will be well on our way to making new strides in the supplemental feeding of duckweed.

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EXPLORING DUCKWEED AS A PROTEIN SUPPLEMENT FOR RUMINANTS USING THE MEAT GOAT AS A MODEL

Introduction

Mismanagement of industrial by-products is a concern to the world population. The agricultural industry of the United States has come under harsh criticism from many different fronts such as Robert F. Kennedy's legal attack of the conventional swine production industry (Phipps 2001). Much of the criticism centers on the use or rather the misuse of animal waste nutrients. Animal waste nutrients are an unavoidable byproduct of any animal operation, large or small. The cornerstone of the animal waste argument is how the waste is treated once it is produced. Misuse of animal waste has led scientists to investigate aquatic plants as a treatment solution. Some of these aquatic plants have presented themselves as a possible feed source for the animals producing the waste.

One of the most intriguing aquatic plants being investigated for both wastewater treatment and as an animal feed source is the plant family of Lemacea, commonly known as duckweed. The plant itself is small, measuring only 0.3 – 20 mm in size and floats on top of the water's surface (Landolt, 1986; Skillicorn et al., 1993). Duckweed can be found growing in almost any temperate or tropical climate and has the ability to double its biomass in 16 hours to 4 days under ideal weather

conditions (Skillicorn et al., 1993). The accelerated growth rate combined with the plant's nutrient uptake capability give duckweed amazing potential for wastewater treatment (Skillicorn et al., 1993). The nutrient uptake capability of this small plant also gives it the necessary composition to be used as a dietary supplemental feed source for a number of animals including poultry, swine, and cows (Rusoff et al., 1978; Skillicorn et al., 1993; Leng et al., 1995; Huque et al., 1996; Anh and Preston, 1997b). Research involving duckweed originally began on two separate fronts: as a wastewater treatment and as a feed resource. Duckweed has a high capacity for concentrating protein, phosphorus, and other minerals. According to Culley and Epps (1973), duckweed is an ideal candidate for utilization as a feedstuff for several reasons including; it can be easily harvested, it has a high protein content, it has a low fiber and lignin content, it displays high mineral absorption capability, it has extended growing and harvesting periods, it is nontoxic to domestic stock, and it is susceptible to few pests.

Duckweed grown under ideal conditions on domestic or animal waste and harvested regularly will commonly have a crude fiber content of 5 – 15% and a protein content of 35 – 45% (Skillicorn et al., 1993). Lawson et al. (1974) showed that the particular method implemented to dry the harvested duckweed could play an instrumental role in its compositional make-up. Lawson concluded that sun drying duckweed was the best economical choice since it was inexpensive to implement but yielded a quality product. Porath and Koton (1977) reported that the weight of

grass carp could be tripled from 100 g to 300 g in a span of only 50 days by feeding a mixture of *Lemna gibba* and *Lemna minor*. Anh and Preston presented evidence in 1997 that duckweed protein fed to ducks has an equivalent biological value to that of soybean meal protein but is slightly less utilized (based on live weight gain over the trial period) than soybean protein. In other words duckweed has a similar compositional profile to that of soybean meal, but the nutrients in duckweed are used at a lesser degree. Ahn and Preston surmised that the decreased utilization of duckweed protein was likely due to a lower digestibility (1997). Men et al. (2001) completely replaced commercial protein supplements used in diets for meat ducks with duckweed. No significant differences in carcass yield, chest and thigh muscle weight, and internal organ weights were found between birds on the control diet and birds on the duckweed diet. Duckweed used to replace protein supplements for fattening pigs showed no reduction in growth rate and produced a leaner carcass with less deposition of back-fat (Van et al., 1996). Men et al. (1997) showed that by replacing half of conventional protein sources with duckweed, farmers are able to improve the reproductive performance of their sows and consequently increase litter size, litter weights, and litter survival rates. Damry et al. (2001) reported that duckweed was more effective than urea and as effective as cottonseed meal as a protein source for wool growth of Merino sheep. Damry's data showed rumen ammonia concentrations for duckweed similar to that of cottonseed meal which is considered a good source of 'escape' protein for ruminants. Huque et al. (1996) reported on the feed potential of *Spirodela, Lemna,* and *Wolffia* for cattle. Both the

dry matter and crude protein of the three duckweed types were shown to be highly degradable (*Spirodela* 71% and 80%, *Lemna* 71% and 86%, and *Wolffia* 91% and 93% respectively) in the rumen of cannulated bulls over a 72 hour period (Huque et al., 1996). Based on an in situ study and a 7 day feed trial, Huque et al. (1996) conclude that duckweed incorporated as a component of a concentrate mixture can be fed to cattle without negative results. Khan et al. (2002) used a similar in situ study along with an in vitro gas production study to conclude that the high protein content of duckweed along with other aquatic plants warrants consideration of their use to supplement poor quality or deficient diets. O'Bryan et al. (1998) concluded that the nitrogen and phosphorus in duckweed fed to Holstein steers was able to be used with the same degree of efficiency as the nitrogen and phosphorus from other conventional feeds. The objective of this study was to characterize the composition of wastewater grown duckweed (*Lemna gibba*) and evaluate its use as a protein supplement for ruminants through the feeding of three duckweed diets to 20 Boer-cross goat (*Capra hircus*) wethers. Our hypothesis was that duckweed is a suitable protein source for ruminants and will behave in a similar fashion to soybean meal.

Materials and Methods

Research began with the harvesting of duckweed (*Lemna gibba*) produced at the NC A&T Swine Unit (SU). The NC A&T SU pumps their swine effluent from the hog house into an adjacent lagoon. The waste is then pumped up-hill to a holding tank where it gravity feeds into 6 wetland cells. The wetland cells dilute the waste

with fresh water and allow duckweed, cattails, and nature to purify the hog waste before returning the purified water to another lagoon. Duckweed harvested from NC A&T SU was used in chemical analysis and in a drying method comparison.

Duckweed was harvested from the wetland cells using a floating leaf skimmer (USABlueBook, Gurnee, IL). The duckweed was then placed into 5 gallon buckets that had holes drilled into the bottom for drainage. The duckweed was hand squeezed prior to being placed in the bucket and then hand pressed to eliminate as much excess water as possible. The buckets were then used to transport the duckweed to the drying site.

Figure 3. Harvesting of the duckweed*.

* The black pipe was used to draw the duckweed closer to the bank in order to increase the efficiency of the pool skimmer when there was no wind.

Due to compositional variation in duckweed caused by different drying methods,duckweed was dried using three separate methods following the initial harvest. Using the findings of Lawson et al. (1974) as a guide, we evaluated sun drying, freeze drying, and oven drying to help determine how the duckweed should be dried for use in subsequent experiments. The oven dried duckweed was spread in aluminum bake pans 1 – 1.5 inches deep. Holes were then “punched” into the duckweed to allow the hot air to get to the bottom of the pans. The pans were then placed in the oven at 55^{o}C and stirred three times daily. The duckweed dried completely within 24 hours, but remained in the oven for 48 hours. The sun dried

duckweed was dried using wooden frames with window screens stapled to the frames. The window screen holes (1mm x 1mm) allowed air underneath the wet duckweed thereby letting the duckweed dry from both the top and bottom. Twenty-four hours was sufficient to sun-dry the duckweed. Following the initial harvest, a green house was constructed at NCA&T Swine Unit which allowed subsequent duckweed samples to dry at 107 – 108^{o}F without any wind interference. Duckweed samples dried in the greenhouse were primarily from two wetland cells, and were composited over 2 week periods to include samples taken across the summer of 2002.

Due to increasing ammonia levels, the duckweed at NC A&T SU died off during harvesting. Normally, rainwater helps to dilute the nutrient concentration levels in the wetland cells of NC A&T. However, the summer of 2002 saw the climax of a 5-year drought in North Carolina thereby helping to concentrate the nutrients within the wetland cells past their normal standards. During the last days of May 2002, algae started to corrupt the pure duckweed stands within the wetland cells. The duckweed died off completely at the beginning of June. The NC A&T SU wetland cells had to be completely flushed with fresh water then plumbed in order for the swine effluent to be more effectively mixed with fresh water to achieve better control over the mixing of the cells. The duckweed began to grow back at the end of June and constituted pure stands by mid July. Nevertheless, we searched for and found an alternate source of duckweed *(Lemna gibba*) during the start of June. The

new duckweed stand was located at the waste pond of the NCSU Aquaculture Educational Unit (AEU). We used an ordinary swimming pool skimmer for the harvesting at NCSU AEU (Figure 3). Duckweed amounts for the feed trial required a larger drying operation than the green house was capable of offering. Utilizing a 24x100 foot (7.5x31 meter) roll of black plastic along with numerous 2x4's, 2x6's, and bales of hay, the fresh duckweed was dried at the NCSU Metabolism Educational Unit (Figure 4). Once the black plastic was unrolled on level ground, hay bales were strategically placed depending on the direction of the wind. The hay bales were placed inside black plastic trash bags and served to both block the wind and to catch as much dry duckweed as possible. The wooden boards were used to weight down the plastic to keep it from moving in the wind, and a border of boards was made around the entire perimeter of the plastic drying bed. Figure 4 is a photograph of the set-up used for sun drying the duckweed. Once everything was set up, the duckweed was spread as evenly as possible (<0.75 inches thick) across the surface of the black plastic (Figure). Forty-eight hours was sufficient to dry most of the duckweed, after which time it was placed in a forced air oven for 24 hours to complete the drying process. Due to the expediency and scale of drying offered by sun drying, this method was used to dry the duckweed for the trial.

Figure 4. Photograph depicting the sun drying process using black plastic, 2x4's, 2x6's, and hay bales.

The metabolism trial evaluated the duckweed (*Lemna gibba*) from the NCSU aquaculture facility as a protein supplement for goats. Experimental diets included a negative control with no supplemental protein and three diets supplemented with protein. The three protein diets contained three levels of duckweed: 0 supplemental protein from duckweed, 1/3 of supplemental protein from duckweed, and 2/3 of supplemental protein from duckweed. The Ca:P ratio of all four diets was adjusted to be constant across the diets by altering the supplemental levels of limestone or monocalcium phosphate in the diets.

The metabolism portion of the project received Institutional Animal Care and Use Committee approval and was conducted at North Carolina State University's Metabolism Educational Unit (MEU) in Raleigh, North Carolina. Nineteen of 24 goats were selected for the metabolism study. The goats were fed once daily at 8:00 AM throughout the entire trial. The goats were adapted to the duckweed diets while being housed outside. The goats were contained in a single paddock with access to fresh water and fed with 2 feed troughs. Initial feeding of loose concentrate resulted in the goats sorting through the feed and not eating the concentrate. The concentrate was subsequently pelleted, eliminating the ability to sort. The goats readily consumed the pelleted concentrate which was gradually increased with consumption. The goats were moved inside the Metabolism Unit on day 28 and assigned randomly to 1 of the 4 diets. Indoor, goats were fed individually at 4% body weight (as fed) in raised, slotted floor metabolism crates and had access to trace-mineralized salt blocks and automatic waterers. Goats were given 10 days to adapt to the metabolism crates (days 1 - 10). Concentrate in the form of pellets were fed to the goats first with a 30 to 45 minute lag before hay was fed.

Following the crate adaption phase, goats were fitted with canvas fecal-collection bags (Figure 5) and allowed 5 days (days 10 - 15) to adapt to them before initiation of a 5 day fecal collection. The fecal-collection bags consist of a collar apparatus that snaps to the actual collection bag to ensure a proper fit. The actual

collection bag contains a zipper for easier emptying. Fecal-collection bags were emptied twice daily.

Figure 5. Photograph of a goat in a wooden metabolism crate wearing a fecal-collection bag.

Collection of feed samples began on day 16. Weigh-back or ort sampling began the following day (day 17), and fecal and urine sampling was initiated on day

18. Throughout the metabolism phase, feed and orts were weighed daily along with fecal and urine output. Daily feces were weighed, mixed, and a constant percentage for each animal was taken to be dried at 55°C; this was followed by a 48-h air equilibration to determine air-dried fecal output. Daily urine output was collected in buckets containing enough 6 N HCL to keep pH < 3. Total urinary output was weighed, and a constant percentage for each animal was taken and stored frozen until analysis. Daily urine samples were pooled prior to freezing (the same percentage from each day's output) to provide a representative sample of the 5 day urine output.

One day after ending the fecal and urine collection (day 23), blood samples were obtained for serum urea nitrogen determination at 0, 4, and 8 h after feeding. Rumenocentesis was a modification of the procedure described by Nordlund and Garret (1994), which involved extracting a minimum of 5 mL of ruminal fluid from the ventral sac using a 5-cm, 14-gauge needle and a 20-mL syringe without local anesthetic. At 4 h after feeding, ruminal fluid samples were taken by rumenocentesis. Blood samples were taken from the jugular vein using 10-mL vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) with 20-gauge, 2.54-cm needles and no additives. Serum was obtained by allowing blood to clot under refrigeration for a minimum of 6 h, centrifuging tubes at 3,600 × g for 10 min, decanting into clean tubes, and then storing frozen until analyzed. Ruminal pH was determined immediately after obtaining samples using an Accumet

AP63 Handheld pH/mV/Ion Meter (Cole-Parmer Instruments, Vernon Hills, IL). Ruminal fluid samples were then placed in a crushed ice/water solution to stop fermentation and then frozen until they were thawed in preparation for analysis. On the day after ruminal fluid and blood samples were taken (day 24), goats were transported back to the NCSU Small Ruminant Educational Unit.

Preparation of Samples for Chemical Analysis

During the 5 day fecal collection period, samples of hay, supplements, and orts were taken daily, composited, and subsampled. All feed, ort, and fecal samples were ground through a 1-mm screen in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) prior to laboratory analyses.

Analysis was conducted on selected samples harvested from NC A&T SU wetland cells and two large batches of duckweed harvested at NCSU AEU. A single pooled sample of duckweed from NC A&T SU and NCSU AEU were created for amino acid analysis by compositing an equal weight from each sample obtained at each site.

Blood samples were centrifuged immediately upon returning to the lab at 3,600 x g for 10 min. The supernate was pipetted off and frozen for later analysis of serum urea nitrogen levels.

Ruminal fluid samples were thawed and centrifuged at 3,600 × g for 10 min. Supernate was mixed at a 5:1 ratio with 25% metaphosphoric acid. The mixture was covered, held at room temperature for 30 min, and centrifuged again at 3,600 × g for 10 min prior to analysis for ammonia and VFA.

Laboratory Analyses

Hay, supplement, ort and fecal samples from the fecal-collection phase were analyzed for DM, OM, Phosphorus, and Kjeldahl-N, according to AOAC (1995), and CP was calculated as percentage Kjeldahl-N × 6.25. All samples were analyzed for NDF, ADF, and acid detergent lignin as described by Van Soest et al. (1991) as modified (Komarek et al., 1994) for use in an Ankom fiber apparatus (Ankom Technology, Fairport, NY). In vitro true DM disappearance (IVTDMD) was determined using 48 hour incubation with steer ruminal inoculum and buffer (Tilley and Terry, 1963) in a batch fermenter (Ankom Technology, Fairport, NY) with NDF termination. Ruminal VFA concentrations were determined on a Varian 3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA) using a Nukol fused silica capillary column (15 m; 0.53 mm i.d.; 0.5 μm film thickness; Supelco, Bellefonte, PA). Ruminal ammonia and urinary ammonia was determined by the same colorimetric procedure used for Kjeldahl N (AOAC, 1995). Serum urea N and urine urea N were determined colorimetrically by an automated, diacetyl-monoxime method (Marsh et al., 1965). Protein fractions of the feed ingredients and drying methods were analyzed as described by Licitra et al. (1996). Duckweed harvest

samples were analyzed at Dairy One DHI Forage Testing Laboratory (Ithaca, NY) for mineral content, while the amino acid analysis was done by Woodson – Tenent Laboratories, Inc. (Memphis, TN).

Statistical Analysis

The experiment was a completely randomized design. A statistical analysis of the data was run using SAS general linear model (GLM). Three contrasts were run on intake, digestibility, nitrogen balance, urine composition, blood/serum urea nitrogen, rumen ammonia, and volatile fatty acids (VFA's) to (1) test for a difference between the negative control diet and the other three diets containing supplemental protein, (2) test for a linear trend among the three supplemental protein diets, and (3) test for a quadratic trend among the three supplemental protein diets. The model for serum urea nitrogen (SUN) included effects of diet, time, diet x time, and animal (diet) was used to test for treatment differences. Level of significance is $P < 0.10$. The experiment with drying methods was analyzed as a completely randomized design with the GLM procedure of SAS. Mean separation was done using a protected LSP test.

Results

Duckweed Harvests

Duckweed was harvested from both NCSU AEU and NC A&T SU throughout the summer of 2002. Due to increasing ammonia levels in the wetland cells (>30 ppm) at the start of June, the duckweed at NC A&T SU died. It took NC A&T several weeks to remedy the problem and for duckweed production to begin again. Table 7 presents the compositional analysis and in vitro digestibility of harvests from both NCSU and NC A&T. Duckweed appears to be an excellent source of protein with reasonable digestibility. It should be noted that the duckweed harvested on 7/16/02 and 7/19/02 at NC A&T SU was initial regrowth after the die-off thereby making any trends merely speculative. The AEU "fishbarn" duckweed was a good substitute for NC A&T SU duckweed due to their compositional similarities before the die-off that occurred at NC A&T SU. Table 8 presents the mineral analysis of duckweed harvests. Changes in nutritional composition of duckweed in the A&T harvests are probably due to the high nutrient concentrations, followed by the die-off and subsequent dilution of the wetland nutrient composition. Also, high levels of algae were found to be growing in combination with the duckweed which undoubtedly influenced the nutrient composition of the harvests. Once again, duckweed grown on fish waste had a similar profile to that of the early swine waste grown duckweed although mineral concentrations were generally lower. These two tables show that duckweed exhibited no adverse effects in the North Carolina weather and performed as well in the heat of the summer (August) as it did in the spring (April) of 2002.

Table 7. Chemical composition and in vitro true dry matter disappearance (IVTDMD) of duckweed grown on fish (NCSU AEU) and swine (NC A&T SU) waste throughout the summer of 2002.

Location	Date	% OM	% CP	% NDF	% ADF	% Cell*	% Lig*	% HemiC*	% IVTDMD
NCSU AEU	7/24/02	79.3	38.6	28.8	13.5	16.6	6.09	15.3	78.2
NCSU AEU	8/14/02	81.0	36.8	26.4	11.8	15.8	5.75	14.6	81.5
NC A&T SU									
Cell 6 Ovendried	4/20/02	76.2	38.4	31.2	16.8	15.6	6.74	14.4	76.0
Sundried Composite	4/20/02	72.7	39.3	24.4	11.8	13.6	6.40	12.6	84.2
Cell 1	5/14/02	81.9	38.4	33.8	14.9	15.4	6.86	18.9	77.2
Cell 2	5/14/02	79.9	41.0	30.1	14.5	15.8	6.34	15.6	75.7
Cell 5	Composite 6/25/02 & 7/1/02	62.5	28.8	47.4	31.4	15.3	7.99	16.0	57.9
Cell 6	Composite 6/25/02 & 7/1/02	65.5	29.7	41.8	26.1	16.3	7.17	15.7	61.9
Cell 5	Composite 7/16/02 & 7/19/02	73.6	31.0	40.1	23.1	17.3	6.91	17.1	67.6
Cell 6	Composite 7/16/02 & 7/19/02	69.8	27.9	42.2	25.0	16.8	6.43	17.2	67.2
Cell 5	Composite 8/13/02 & 8/21/02	75.8	31.9	35.5	18.7	17.9	6.48	16.8	71.3
Cell 6	Composite 8/13/02 & 8/21/02	62.1	27.6	40.9	27.3	17.0	6.68	13.6	63.0

* Cell = Cellulose, Lig = Lignin, HemiC = Hemicellulose, IVTDMD = In vitro true dry matter disappearance.

Table 8. Mineral levels of duckweed grown on fish (NCSU AEU) and swine (NC A&T SU) waste throughout the summer of 2002.

		%					ppm				
Location	**Date**	**Ca**	**P**	**Mg**	**K**	**Na**	**Fe**	**Zn**	**Cu**	**Mn**	**Mb**
NCSU AEU	7/24/02	1.45	1.52	0.46	1.78	1.59	1620	53	6	232	0.80
NCSU AEU	8/14/2002	1.47	1.89	0.51	1.96	1.81	1270	38	5	207	0.80
NC A&T SU											
Cell 6 Ovendried	4/20/2002	3.50	3.35	1.23	1.48	0.21	1620	24	6	662	0.10
Sundried Composite	4/20/2002	3.52	3.29	1.19	1.44	0.23	1420	41	6	665	0.30
Cell 1	5/14/2002	1.42	1.72	0.46	1.90	0.10	2700	19	7	540	0.40
Cell 2	5/14/2002	2.12	2.33	0.62	2.00	0.15	2370	17	6	566	0.50
Cell 5	Composite 6/25/02 & 7/1/02	1.94	1.99	0.56	1.57	0.15	5470	29	13	517	0.30
Cell 6	Composite 6/25/02 & 7/1/02	1.47	1.52	0.49	2.30	0.18	5860	21	8	460	< .1
Cell 5	Composite 7/16/02 & 7/19/02	1.18	1.49	0.41	2.72	0.18	4150	19	10	510	0.20
Cell 6	Composite 7/16/02 & 7/19/02	1.14	1.51	0.44	2.78	0.17	5380	16	8	430	< .1
Cell 5	Composite 8/13/02 & 8/21/02	2.01	2.03	0.41	3.26	0.17	3170	20	8	414	0.50
Cell 6	Composite 8/13/02 & 8/21/02	2.13	2.00	0.43	2.58	0.15	7090	23	9	304	0.10

Data from an amino acid analysis of the harvested duckweed is presented in Table 9 along with the Food and Agricultural Organization (FAO) of the United Nations reference pattern for the eight essential amino acids. The amino acid profile of the NCSU AEU fishbarn duckweed exceeds the standards set forth by the FAO except for methionine and isoleucine. However, the swine waste duckweed failed to meet the FAO pattern for methionine, isoleucine, and tryptophan.

Table 9. Amino acid levels of duckweed grown on fish and swine waste compared to the Food and Agricultural Organization (FAO) of the United Nations reference pattern for essential amino acids .

Amino Acid	Fishbarn Duckweed (g/100g Protein)	Swine Duckweed (g/100g Protein)	FAO Reference Pattern (g/100g Protein)
Tryptophan	1.45	1.38	1.4
Cystine	1.19	1.04	
Methionine	1.62	1.58	2.2
Aspartic Acid	11.69	10.68	
Threonine	3.89	3.80	2.8
Serine	3.92	3.56	
Glutamic Acid	9.66	9.07	
Proline	3.92	3.80	
Glycine	4.52	4.40	
Alanine	5.60	5.54	
Valine	4.70	4.60	4.2
Isoleucine	3.68	3.49	4.2
Leucine	6.47	6.22	4.8
Tyrosine	2.87	2.72	
Phenyalanine	4.58	4.33	2.8
Lysine	4.44	4.20	4.2
Histadine	1.54	1.34	
Arginine	5.48	4.87	

Drying Methods

Following the initial harvest, duckweed was dried through three separate procedures: sun-drying, oven-drying, and freeze-drying. The statistical analysis of the three different methods is presented in Table 10. There was no significant difference in the level of crude protein and % C protein fraction among the drying methods. The oven dried duckweed exhibited lower A and B1 levels but higher B2 and B3 levels compared with the sun-dried and freeze-dried duckweed. The % NDF and % cellulose differed between all three methods: sun-dried (24.0%, 13.8%), freeze-dried (21.4%, 13.3%), and oven-dried (26.7%, 14.1%) respectively. The % ADF of freeze-dried (11.1%) was significantly lower than the sun-dried (12.3%) and oven-dried (12.8%) duckweed. The % hemicellulose for sun-drying (11.8%) and freeze-drying (10.3%) were not significantly different from one another but both were different from the oven drying method (13.9%). The % IVTDMD for sun (81.4%) and oven (81.2%) drying were not different from each other but both methods were significantly lower from the freeze drying method (84.6%). Freeze drying appears to be the best method for drying duckweed with the least amount of deterioration to protein quality.

Table 10. Effect of Different Drying Methods (Sun Drying, Freeze Drying, and Oven Drying) on Duckweed Protein Levels and Digestibility.

	Drying Method				
Variable	**Sun**	**Freeze**	**Oven**	**SEM**	**P-value**
% CP	39.8	41.3	40.0	0.81	0.44
Protein Fractions[1]					
% A	26.8	24.8	23.0	0.91	0.13
% B1	13.0[a]	19.5[b]	11.1[a]	0.72	0.01
% B2	46.9[a]	46.8[a]	51.3[b]	0.67	0.03
% B3	6.4[a]	5.6[a]	9.8[b]	0.64	0.04
% C	6.9	3.5	4.8	1.04	0.21
% NDF	24.0[a]	21.4[b]	26.7[c]	0.56	0.02
% ADF	12.3[a]	11.1[b]	12.8[a]	0.25	0.03
% Cellulose	13.8[a]	13.3[b]	14.1[c]	0.10	0.03
% Hemicellulose	11.8[a]	10.3[a]	13.9[b]	0.49	0.03
% IVTDMD*	81.4[a]	84.6[b]	81.2[a]	0.50	0.03

[a,b,c] Means within a row that do not have a common superscript differ ($P < 0.10$).

[1] As described by Licitra et al., 1996.

* IVTDMD = In vitro true dry matter disappearance.

Experimental Duckweed Diets

The diets for this trial were formulated based on their protein levels. The nutritional analysis of diet ingredients and concentrate pellets is presented in Table 11. The wheat hay used was low in protein, but moderate in energy (8. 6 %CP, 61.7 %NDF and 32.5 %ADF) so that any protein supplement effects would be more pronounced. Soybean meal does contain more protein than duckweed, 55.4% vs. 38.2 %CP, respectively. Yet the big difference between the two is in fiber content: soybean meal 10.8 %NDF, 4.6 %ADF and duckweed 30.0 %NDF, 14.3% ADF. All dietary ingredients excluding hay were pelleted to eliminate sorting by the goats.

The pelleted concentrates are in line with one another with similar dry matters and similar protein levels with the exception of the negative control's lower protein. The negative control also shows a slight deviation in digestibility from the other diets (24 %NDF and 14 % ADF while the other diets are 19 %NDF and 10 % ADF). The protein fractions show similarities between duckweed (19.3% A, 9.4% B1, 54.9% B2, 7.4% B3, and 9.0% C) and soybean meal (15.5% A, 7.8% B1, 47.3% B2, 14.1% B3, and 8.9% C). Fractions A, B1, B2, and C are similar with B3 of soybean meal almost double that of duckweed. The IVTDMD for duckweed (76.9%) proved to be higher than the wheat hay (71.8%) but well below the soybean meal, soybean hulls, and corn (99.7%, 95.0%, and 97.1% respectively).

The ingredient and nutrient composition of the four trial diets are presented in Table 12. As planned, DM for the diets was essentially identical. The negative control diet contained no supplemental crude protein (CP) and had a lower level of CP (9.7%) than the other diets. The positive control diet contained no duckweed but had a similar level of protein (12.5 %CP) to the duckweed diets. The 1/3 duckweed diet had duckweed providing 1/3 of its supplemental CP (12.4 %CP). Likewise the 2/3 duckweed diet had duckweed providing 2/3 of its supplemental CP (12.8 %CP). The increased NDF and ADF of the negative control diet is due to the higher level of soybean hulls relative to the other diets.

Table 11. Analysis* of diet ingredients and concentrate pellets.

			Protein Fractions[1] (%)										
Ingredient	**% DM**	**% CP**	**A**	**B1**	**B2**	**B3**	**C**	**% NDF**	**% ADF**	**% Cell**	**% Lig**	**% HemiC**	**% IVTDMD**
Hay	91.7	8.6	20.2	2.0	48.0	23.4	6.4	61.7	32.5	32.0	6.20	29.2	71.8
Duckweed	92.7	38.2	19.3	9.4	54.9	7.4	9.0	30.0	14.3	16.0	6.96	15.6	76.9
Soybean hulls	90.1	14.4	24.6	5.1	47.3	14.1	8.9	61.4	43.2	44.9	4.16	18.2	95.0
Soybean meal	91.2	55.4	15.5	7.8	73.3	0.2	3.2	10.8	4.6	8.0	2.49	6.2	99.7
Corn	90.1	9.6	10.6	8.7	74.9	-0.5	6.3	9.8	2.2	5.8	2.61	7.6	97.1
Negative Control Pellet	89.2	11.0						24.1	14.2	16.9	3.04	9.9	
Positive Control Pellet	89.0	16.5						19.1	10.5	13.5	2.91	8.6	
1/3 Duckweed Pellet	88.9	16.4						19.0	10.1	12.9	2.98	8.9	
2/3 Duckweed Pellet	89.8	15.7						18.9	10.3	13.3	3.01	8.6	

* Cell = cellulose, Lig = Lignin, HemiC = hemicellulose, IVTDMD = in vitro true dry matter disappearance.

[1] As described by Licitra et al., 1996.

Table 12. Ingredient and nutrient composition of trial diets on a DM basis[1].

Ingredient, % of DM	Negative Control	Positive Control	1/3 Duckweed	2/3 Duckweed
Wheat Hay	50	50	50	50
Corn, Ground	30	30	30	30
Soybean Hulls	16.6	9.2	8.15	7.2
Soybean Meal	-	7.5	5.2	2.7
Duckweed	-	-	3.6	7.3
Molasses	1.2	1.2	1.2	1.2
Soybean Oil	0.8	0.8	0.8	0.8
Limestone	0.7	0.8	0.8	0.8
Monocalcium Phosphate	0.7	0.5	0.25	-
Calculated analyis of trial diets				
Variable	**Negative Control**	**Positive Control**	**1/3 Duckweed**	**2/3 Duckweed**
% DM[2]	90.5	90.4	90.3	90.8
% CP[2]	9.7	12.5	12.4	12.1
% NDF[2]	43.2	40.7	40.7	40.7
% ADF[2]	23.5	21.7	21.5	21.5
% Ca[3]	0.7	0.7	0.7	0.7
% P[3]	0.4	0.4	0.4	0.4

[1] All ingredients, except hay, were mixed together and pelleted.
[2] Calculated from analysis of hay and pellets sampled during digestion trial.
[3] Calculated from initial ingredient analysis.

Intake and Digestibility

Intake and digestibility data for goats are presented in Table 13. There was no significant difference among the diets for dry matter intake. There was a quadratic response in the DM digestibility of the three protein supplemented diets (P

= 0.02) with the 1/3 duckweed diet being lower, 70.0%, than the positive control, 72.2% or the 2/3 duckweed diet, 72.5%. There were also quadratic responses in the OM, NDF, and ADF digestibility of the three protein diets with the 1/3 duckweed diet being slightly lower than the positive control and 2/3 duckweed diets. The quadratic response for OM digestibility (P = 0.02) had the 1/3 duckweed diet (71.4%) lower than the positive control (73.4%) and the 2/3 duckweed diet (74.0%). The CP digestibility showed a significant difference (P < 0.001) between the negative control and the protein diets. There was also a linear decline (P = 0.07) in CP digestibility among the protein supplemented diets. NDF digestibility of 1/3 duckweed (54.6%) was lower than the positive control (59.0%) and the 2/3 duckweed (61.6%). ADF digestibility of 1/3 (53.8%) was below the positive control (59.5%) and 2/3 duckweed diet (61.5%). Cellulose digestibility showed a linear decline (P = 0.01) among the protein diets with added duckweed. Hemicellulose digestibility displayed both a linear response (P = 0.03) and a quadratic response (P = 0.005) among the three protein diets.

Table 13. Intake and digestibility in goats fed diets containing duckweed.

	Diet					Contrasts Among Diets		
Variable	**Negative Control**	**Positive Control**	**1/3 Duckweed**	**2/3 Duckweed**	**SEM**	**No Protein vs Protein[1]**	**Linear Contrast**	**Quadratic Contrast**
DMI (g/d)	593.8	600.4	584.9	590.6	31.27	0.96	0.82	0.80
DMI as % of BW	3.5	3.5	3.4	3.4	0.05	0.33	0.60	0.15
Digestibility (%)								
DM	70.6	72.2	70.0	72.5	0.69	0.26	0.77	0.02
OM	71.9	73.4	71.4	74.0	0.69	0.22	0.57	0.02
CP	53.2	64.0	61.1	61.2	1.06	< .0001	0.07	0.28
NDF	59.4	58.9	54.6	61.6	1.30	0.51	0.15	0.004
ADF	60.3	59.5	53.8	61.5	1.54	0.26	0.37	0.004
Cellulose	65.3	65.0	58.0	57.1	2.01	0.04	0.01	0.25
Hemicellulose	58.2	58.2	55.5	61.8	1.06	0.79	0.03	0.005

[1] Refers to no supplemental protein added to the negative control diet vs. the supplemental protein added to the other three diets.

Nitrogen, Urine, Phosphorus, and Serum Data

The N balance, urine composition, and blood/serum urea nitrogen (SUN) levels are presented in Table 14. As expected, the N intake ($P = 0.002$), urine N ($P < 0.001$), N retained in g/d ($P = 0.05$), N digested ($P < 0.001$) was significantly less for the goats on the negative control diet compared to the three protein supplemented diets. Nitrogen retained (g/d) also tended to exhibit a linear decrease ($P = 0.10$) among the protein supplemented diets as duckweed increased. Nitrogen retained as a % of digested ($P = 0.008$) was significantly higher for the negative control diet than for the protein supplemented diets. A significant linear decline ($P = 0.06$) is seen in the N retained as a % of intake. No significant quadratic responses occurred for the N balance. The composition of the urine from the goats also showed a significantly lower % urea of the total N ($P < 0.001$) for the negative control compared to the three protein diets. However, there was no evidence of either a linear or quadratic response among the three protein diets for urine composition.

Analysis of the phosphorus balance shows that there were no significant differences among diets for P intake, fecal P, and Urinary P. Phosphorus intake was the same for both the negative control (2.1 g/d) diet and the 2/3 duckweed diet (2.1 g/d). Urinary P exhibited a linear increase ($P = 0.05$) with increasing duckweed. Retention and digestion of P did show differences among the trial diets. The P retained (g/d) and (%) as well as the P digested (g/d) showed significant differences between the negative control diet and the protein supplemented diets as well as both

linear and quadratic responses for the protein diets. Phosphorus retained (g/d) was the same for the negative control diet (0.3 g/d) and the 2/3 duckweed diet (0.3 g/d) but the percentage of P retained was lower for the 2/3 duckweed diet (14.3%) than the negative control (16.3%). Digested P retained showed no significant difference between the negative control diet and the protein supplemented diets, but the protein diets did exhibit a linear response ($P = 0.03$).

The SUN data was taken at 0, 4, and 8 hours after feeding on the last day of sample collections. Zero hour denotes that 24 hours had elapsed since the last feeding. Each variable of the SUN data over time showed significant difference between the negative control and the protein diets: SUN at 0 hours ($P = 0.001$), SUN at 4 hours ($P < 0.001$), and SUN at 8 hours ($P = 0.005$). The only significant response among the three protein diets is a slight linear response ($P = 0.09$) for SUN at 0 hours after feeding. There was a significant time by treatment interaction in SUN levels for the diets can be seen more clearly in Figure 6.

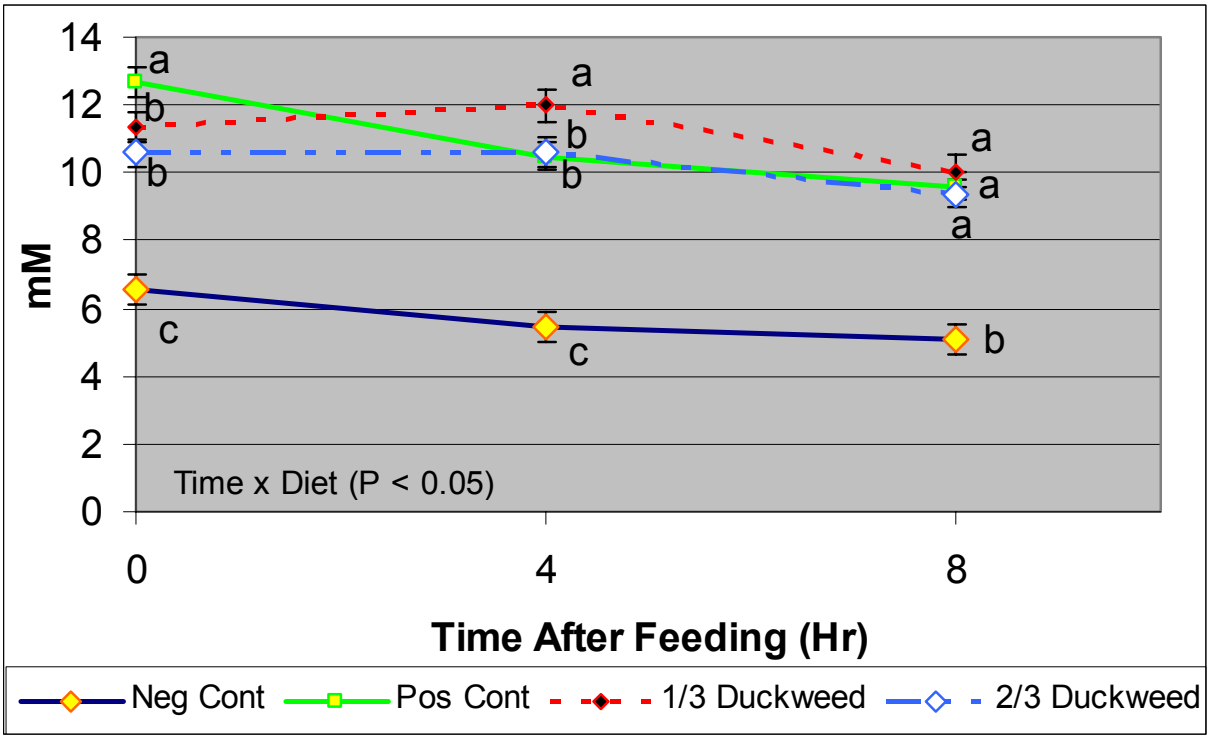
Table 14. Nitrogen balance, urine composition, phosphorus balance, and serum urea nitrogen (SUN) levels in goats fed diets containing duckweed.

	Diets					Contrasts Among Diets		
Variable	**Negative Control**	**Positive Control**	**1/3 Duckweed**	**2/3 Duckweed**	**SEM**	**No Protein vs Protein[1]**	**Linear Contrast**	**Quadratic Contrast**
Nitrogen Balance								
N Intake (g/d)	9.3	12.0	11.8	11.5	0.58	0.002	0.57	0.97
Fecal N (g/d)	4.8	4.8	5.0	4.9	0.31	0.68	0.69	0.67
Urine N (g/d)	2.0	3.7	3.7	3.7	0.18	< 0.001	1.00	0.72
% NH4 of Total N	0.9	0.9	1.0	0.7	0.27	0.96	0.60	0.52
% Urea of Total N	55.5	79.8	75.7	79.8	4.10	0.001	0.99	0.45
N Retained (g/d)	2.5	3.6	3.0	3.0	0.27	0.05	0.10	0.42
N Retained (% of Intake)	27.4	29.7	25.3	25.6	1.50	0.77	0.06	0.24
N Digested (g/d)	4.5	7.2	6.7	6.6	0.32	< 0.001	0.17	0.64
N Retained (% of Digested)	56.4	49.3	44.3	44.6	2.97	0.01	0.27	0.50
Phosphorus Balance								
P Intake (g/d)	2.1	2.3	2.3	2.1	0.11	0.31	0.22	0.43
Fecal P (g/d)	1.7	1.7	1.7	1.8	0.12	0.99	0.83	0.98
Urine P (g/d)	0.006	0.006	0.007	0.026	0.01	0.41	0.05	0.34
P Retained (g/d)	0.3	0.5	0.5	0.3	0.03	0.01	< 0.001	0.01
P Retained (% of Intake)	16.3	24.5	23.7	14.3	1.70	0.031	0.001	0.07
P Digested (g/d)	0.3	0.6	0.5	0.3	0.03	0.001	< 0.001	0.01
P Retained (% of Digested)	98.1	98.9	98.6	92.5	1.93	0.51	0.03	0.26
SUN								
SUN (mM) @ 0 hr	6.1	12.7	11.3	10.6	0.83	0.001	0.09	0.78
SUN (mM) @ 4 hr	5.5	10.5	12.0	10.6	0.92	< 0.001	0.92	0.24
SUN (mM) @ 8 hr	5.1	9.6	10.0	9.4	0.92	0.001	0.86	0.65

[1] Refers to no supplemental protein added to the negative control diet vs. the supplemental protein added to the other three diets.

[2] Refers to 0, 4, and 8 hours after feeding.

Figure 6. Serum Urea Nitrogen (mM) in Goats Fed Diets Containing Duckweed.



[a,b,c] Means within a time (0, 4, or 8 hours) that do not have a common superscript differ ($P < 0.05$).

Figure 6 clearly shows the SUN levels of the different diets over the 8 hour span that samples were taken. The negative control diet produced SUN levels that were significantly below ($P < 0.05$) the SUN values of the three protein diets at each of the three collection times. There was no significant difference between the SUN levels of the 1/3 and 2/3 duckweed diets at the 0 hour while the positive control was higher ($P < 0.05$) than the duckweed diets. However, at 4 hours after feeding, the all soybean meal (positive control) diet sharply declines so that it and the 2/3 duckweed diet are not significantly different from each other thereby leaving the 1/3

duckweed diet significantly higher than the other diets. At the 8 hour sampling, the 1/3 duckweed diet also declined rather sharply resulting in the positive control, 1/3, and 2/3 duckweed diets not being significantly different from one another.

Ruminal Ammonia and Volatile Fatty Acid Data

Analysis of the ruminal ammonia and volatile fatty acid (VFA) data is presented in Table 15. Ruminal pH showed no significant responses among the trial diets. There was a significant difference in the level of ruminal ammonia ($P = 0.02$) between the negative control diet and the three protein diets, but no linear or quadratic response among the protein diets. The only significant difference for the VFA data is a slight difference ($P = 0.08$) in the level of isobutyrate between the negative control diet and the protein diets. There is no evidence of any significant response for ruminal ammonia or VFA's as influenced by protein source.

Table 15. Ruminal ammonia and volatile fatty acids (VFA) in goats fed duckweed as a protein source.

Variable	Negative Control	Positive Control	1/3 Duckweed	2/3 Duckweed	SEM	No Protein vs Protein[1]	Linear Contrast	Quadratic Contrast
Rumen pH	6.0	5.8	5.9	6.1	0.11	0.71	0.14	0.56
Rumen NH4 (mM)	8.3	12.6	12.1	14.9	1.66	0.02	0.32	0.43
VFA's								
Total (mM)	94.8	99.3	101.7	94.3	6.44	0.62	0.58	0.56
Acetate:Propionate	2.3	2.3	2.5	2.3	0.18	0.90	0.88	0.45
Moles/100 Moles								
Acetate	57.6	59.1	59.4	59.3	1.30	0.27	0.91	0.90
Propionate	25.8	26.4	24.6	25.9	1.72	0.94	0.86	0.49
IsoButyrate	0.3	0.4	0.4	0.4	0.04	0.08	0.92	0.71
Butyrate	14.9	12.5	14.2	12.9	1.65	0.36	0.88	0.50
IsoValerate	0.5	0.6	0.6	0.6	0.09	0.22	0.85	0.62
Valerate	0.9	1.0	0.8	0.8	0.08	0.89	0.20	0.67

[1] Refers to no supplemental protein added to the negative control diet vs. the supplemental protein added to the other three diets.

Discussion

Duckweed Harvests

The actual harvesting of the duckweed went well during the trial, but is not as easily accomplished as some researchers suggest. Part of the problem arose from the sheer amount of dried duckweed needed for the feeding trial. We experimented with different harvesting methods with the ordinary pool skimmer being the most consistent and practical tool available for the job (Figure 3). Using 5 gallon buckets to help press the water out of the fresh duckweed was extremely helpful in reducing excess, free water.

The 38.2% crude protein, 30.0% NDF and 14.3% ADF of the harvested duckweed is consistent with previous research (Culley and Epps 1973; Men et al., 2001; Ly et al., 2002). The mineral analysis of the harvested duckweed is also in line with previous reports (Culley and Epps 1973; Men et al., 2001). Men et al. (2001) reported on duckweed grown on wastewater from an experimental pig farm an had lower values for Ca (0.71% of DM), P (0.62%), and Na (0.14%) but had a higher value for K (4.92%). Culley and Epps (1973) reported on duckweed harvested from an anaerobic swine waste lagoon during the winter of 1970 – 1971, in Louisiana. Their duckweed was not higher in any minerals than our duckweed, but was lower in Ca and Mg. The levels of P and K in their duckweed coincided with our values. This shows that the composition of duckweed will vary depending on the

nutrient content of the water it is grown on. Table 13 shows this fact clearly using the initial harvests from NC A&T SU and NCSU AEU. These harvests show marked differences between their compositions due to the different nutrient levels in the water. While the duckweed at NC A&T SU was dying off and then re-growing over the summer, its composition shows dramatic differences in digestibility as well as nutrient composition. Since duckweed has such amazing nutrient uptake capabilities, it is possible that duckweed could possibly serve some role as a supplement capable of being grown to meet specific dietary guidelines.

Rusoff et al. (1980) reported that duckweed grown on dairy waste lagoons had the potential to be an effective protein supplement to grains for both animal and human consumption. Our data supports the conclusion that duckweed can effectively supplement protein deficient diets. The amino acid profile for the fish and swine waste duckweed (Table 14) was similar to the dairy waste grown Lemna gibba (37.5% CP) profile presented by Rusoff et al. in 1980 (Table 6). Glutamic and Aspartic acid values for duckweed grown on fish waste was more than 2 and 3 grams/100 grams of protein higher than values reported by Rusoff et al. (1980). This variation could be due to the nutrient make-up of the water source or the protein make-up of the specific strains of *Lemna gibba*. The duckweed for this trial failed to meet the FAO ideal essential amino acid pattern for methionine and isoleucine (fish waste duckweed), and methionine, isoleucien, and tryptophan (swine waste duckweed), which is also in agreement with Rusoff et al. (1980). Our amino acid

profile was also similar to the *Lemna minor* profile reported by Phuc et al. (2001). Although no details are given concerning the water used to grow the duckweed, the majority of the amino acids in Phuc's profile were higher than our reported values. Only three (aspartic acid, glutamic acid, and glycine) are lower than our profile values. Our amino acid profile also agrees with Phuc et al. by being similar to the amino acid profile of alfalfa and soybean meal. The only major difference is that soybean meal possesses roughly twice as much glutamic acid as duckweed or alfalfa.

Drying Methods

Before harvesting for the trial began in earnest, different methods of drying the duckweed were tested for efficiency and compositional effects. Duckweed was dried using sun, freeze, and oven drying (Table 10). Our findings concerning drying duckweed agreed with those reported by Lawson et al. (1974). Lawson et al. presented data on sun and oven drying but not freeze-drying. We found freeze drying to be the best method for preserving the compositional make-up of the duckweed. Significant differences among drying methods were evident in both the protein composition and the digestibilities. The 81.4% and 81.2% IVTDMD for sun and oven-drying, respectively, agrees with the 79% IVTDMD reported by Dung et al. (2002) for *Lemna minor*. Sun-drying and oven-drying produce similar profiles although oven drying degradability of the protein present in the duckweed more than sun-drying. Oven-drying produced the lowest B1 fraction with the highest B2 and B3

fractions suggesting that the heat of the oven drying procedure altered the protein make-up of the duckweed. Neither sun-drying nor oven-drying increased the C fraction above freeze-drying thereby indicating that changes in the protein would not be detrimental to protein digestibility. Sun-drying and oven-drying are both capable of drying the duckweed within a 24 hour time frame, but the amount of duckweed capable of being sun-dried was much greater than the amount allowed by the oven space. Lawson et al. (1974) found the cost of oven-drying prohibitive while we found the amount of sample capable of being oven dried as prohibitive. Agreeing with Lawson et al. (1974) and Culley and Epps (1973), we conclude that sun drying is the most economical method of drying duckweed especially when time is not a major concern. Sun drying did present a unique problem – protecting the dried duckweed from the wind. Yet the sheer amount of duckweed needed for the trial demanded that we use the sun drying method. The amount of dried duckweed blown by the wind was reduced through the use of a wind break constructed of bales of hay and wooden boards (Figure 4).

Experimental Duckweed Diets

During an adaptation period, duckweed was fed to the goats as part of a loose concentrate (all diet ingredients except hay were mixed together as the concentrate). At first there was a slight aversion to the duckweed by the goats, as evidenced by them sorting through the concentrate and leaving the duckweed in the bottom of the feeders. After 3 to 4 days, most goats adapted to the

taste/smell/texture of the duckweed and readily accepted it as part of their diet. However, some goats continued to sort the concentrate. Pelleting the concentrate prevented the goats from sorting out the duckweed. The goats consumed the pellets with no sorting, and no noticeable orts. During the metabolism phase, the goats were offered feed at 4% body weight (as fed) with the diet being divided into half hay and half pelleted concentrate. While in the metabolism crates, all of the goats ate their allotted concentrate pellets within 30 minutes. The majority (roughly 75%) of the hay was ingested by noon. These findings agree with those of Damry et al. (2001) and Huque et al. (1996) in that duckweed is palatable and readily eaten by ruminants when pelleted.

The diets were primarily formulated for protein level. The DM, NDF, ADF, Ca, and P values were similar across diets (Table 16). The Ca:P ratio was kept at or as close to a 2:1 ratio as possible by adjusting the amount of limestone and monocalcium phosphate present in the diets. The main difference among the diets is the lower CP value (9.74%) for the negative control compared to the positive control, 1/3 duckweed, and 2/3 diets (12.45%, 12.39%, and 12.10% respectively). The negative diet represented a maintenance diet while the other diets accounted for maintenance plus activity and growth. The slightly higher NDF (43.16%) and ADF (23.48%) of the negative control are due to the higher level of soybean hulls compared to the other diets.

Intake and Digestibility

The goat NRC (1981) reports the nutrient requirements of a 20 kg goat at maintenance level to be 267 g TDN, 22 g CP, 1 g Ca, and 0.7 g P. The average DMI for the 5 day metabolism trial was 593 grams. There was no significant difference among DMI for the 4 diets thereby showing that duckweed, when included at 1/3 and 2/3 protein, is as palatable/acceptable as soybean meal (Table 18). The average weight of the goats was 17 kg. There was no significant difference among DMI as a % of body weight for the 4 diets. With the exception of one goat, the DMI met not only the maintenance requirement, but the requirement for 50 g gain/d (550 g DMI). The maintenance requirement of TDN for a 20 kg goat is 267 grams. With an average TDN of 434 g (assuming digestible DMI is approximately the same as TDN), the goats once again met the maintenance requirement as well as the 50 g gain/d requirement (367 g TDN) and nearly met the requirement for a 100 g gain/d (467 g). The maintenance requirement of CP for a 20 kg goat is 38 g. All of the goats met the maintenance requirement along with the 50 g gain/d requirement of 52 g with an average of 71 g CP intake. None of the goats fed the negative control diet met the 100 g gain/d requirement of 66 g. Of the 14 goats on the three protein diets, 13 met the 100 g gain/day CP requirement. The CP intake of goats on the negative control was only 58 g while the positive control, 1/3 duckweed and 2/3 duckweed diets produced intakes of 75 g, 72 g and 71 g respectively.

The NDF and ADF digestibilities show no significant differences except for a quadratic response among the protein supplemented diets. The same quadratic response is also exhibited in the DM, OM, and hemicellulose digestibility. The reason for the significant quadratic responses among the digestibility of the protein supplemented diets is unclear. Nevertheless, there is a reasonable explanation for this response. With 19 goats and 4 diets, three of the diets (negative control, positive control, and 2/3 duckweed) were fed to 5 goats while the 1/3 duckweed diet was only fed to 4 goats. Therefore, the number of goats could account for the quadratic response among the protein supplemented diets with the 1/3 duckweed diet being at a lower level than the positive control and 2/3 duckweed diet.

Comparing the apparent digestibilities of the trial diets to the digestibilities reported by Moore et al. (2002) and Luginbuhl et al. (2000) shows that our dry matter digestibilities were similar, but our fiber digestibilities were lower. Moore et al. (2002) reported apparent digestilities for both a hay with soybean meal diet (70.0% NDF, 67.0% ADF, and 71.3% cellulose) and a soybean hull diet (70.8% NDF, 69.2% ADF, and 73.4% Cellulose). Digestibilities for both diets are higher than the current trial diet digestibilities given in Table 13 which ranges from 54.6 to 61.6% NDF, 53.8 to 61.5 ADF, and 57.1 to 65.3% cellulose among the 4 diets. The apparent digestibility of the 24% whole cottonseed diet (65.0% NDF, 66.8% ADF, and 71.9% cellulose) reported by Luginbuhl et al. (2000) is also higher than the 4 trial diets. The higher digestibilities reported by Moore et al. and Luginbuhl et al. can be linked

to the higher quality hay used in their respective studies. Comparison with the findings of Moore et al. (2002) and Luginbuhl et al. (2000) show that the diets used in this trial are on a similar level of fiber digestibility with other diets fed to meat goats. Using a low quality hay to enhance any dietary effects of the 4 trial diets also lowered the digestibilities of the fiber, while the higher concentrate level used resulted in similar dry matter digestibility. It should be noted that Luginbuhl et al. (2000) saw intake and digestibility decrease with increasing cottonseeds in the diet. Although less digestible, there were no decreases in intake or NDF and ADF digestibility. Cellulose did show a linear decrease in digestibility from 65.0% in the positive control to 57.1% in the 2/3 duckweed diet but this decrease did not influence intake. It is difficult to compare the trial diets to one another due to the unexpected quadratic response of the 1/3 duckweed diet. However, the 2/3 duckweed diet exhibited a higher digestibility (61.6% NDF and 61.5% ADF) than the other three diets. Hence, duckweed fiber shows a comparable if not higher digestibility than soybean meal or soybean hulls in similarly formulated diets.

The DM digestibility of the trial diets is also similar to DM digestibility of the soybean meal/soybean hull and whole cottonseed diets (Moore et al. 2002, Luginbuhl et al. 2000). The DM digestibilities of the 4 trial diets are negative control – 70.6%, positive control – 72.2%, 1/3 duckweed – 70.0%, and 2/3 duckweed – 72.5%. Moore et al. reported the DM digestibility of the soybean meal diet at 70.8% and the soybean hull diet at 70.8%. Luginbuhl et al. (2000) reported the 24%

cottonseed diet as 67.1% DM digestible. Diets with lower levels of cottonseeds had higher digestibilities, 16% cottonseeds – 72.7% DM digestible and 8% cottonseeds – 71.9% DM digestiblity. Although the trial diets contained a lower quality hay than those used by Moore et al. (2002) and Luginbuhl et al. (2000), the diets also contained more grain which increased their DM digestibilities.

The Ca:P ratio was kept at as close to a 2:1 ratio as possible by adjusting the amount of limestone and monocalcium phosphate present in the diets. The 1 g maintenance requirement for Ca was easily met by the 4 g averaged by the goats. In fact, all of the goats met the 50, 100, and 150 g gain/d requirements (2, 2.5, 3 g respectively). The goats averaged a 2 g intake for P. The maintenance requirement of 0.7 g was met by all. Once again, the goats met the requirements of for the 50 and 100 g gain/d and nearly the 150 g gain/d requirement (2.1 g). There was no difference among diets for Ca or P intake. The Ca and P levels were high, but the diets were adjusted to keep Ca and P levels constant across diets so that any protein effect would be more apparent and not related to varying Ca or P levels.

Nitrogen, Urine, Phosphorus, and Serum Data

Nitrogen balance data (Table 14) shows many significant differences among the negative control diet and the protein diets but only one significant response among the three protein supplemented diets. The negative control diet showed a significant difference compared to the protein diets in N intake, urine N, N retained in

g/d, N digested, and N digested retained, as would be expected. The lower N intake for goats on the negative control is caused by the lower CP in the diet. There was no difference in the amount of fecal N but the urine N level was much lower in the negative control compared to the other diets. The increased N level in the urine of the negative control goats is a product of the increased SUN levels of the protein supplemented diets. There was a difference between the negative control and the other protein diets in the N retained in g/d but no difference in the N retained as a % of intake. However there was a linear response among the protein diets for the N retained as a % with the duckweed diets being lower than the soybean hull/meal diet. Nitrogen retention (g/d) also exhibited a linear trend (P = 0.01) as duckweed increased indicating that it might not be quite as efficient as soybean meal. Maye et al. (2002) reported on the N retention of soybean hull diets fed to meat goats. Varying concentrations of soybean hulls (0%, 25%, 50%, and 75%) were fed with hay with a maximum N retention of 1.96 g/d for the 75% soyhull diet. Although the 1.96 g/d is well below the protein supplemented diets (3.6, 3.0, and 3.0 g/d) and even the negative control diet (2.54 g/d) of the present study, the goats used by Maye et al. were more mature goats which were consequently heavier in size thereby accounting for part of the difference. Yet this comparison still suggests that duckweed is comparable to soybean hulls as a protein supplement for growing goats.

The N digested (4.5 g/d) of the negative control was significantly different ($P < 0.0001$) from the protein supplemented diets (7.2 g/d, 6.7 g/d, and 6.6 g/d respectively). Nutrient deficient animals will likely become more efficient at utilizing the nutrients required for survival/maintenance. Although the N digested and the N retained was significantly lower for the negative control, the N retained as a percent of digested was significantly higher than the protein diets due to the decreased urine N output. While goats on the negative control (no supplemental protein) digested and retained less N than those on the protein diets, they also excreted less N thereby increasing the N retained as a % of digested compared to that of the protein diets. Although the negative control goats were fed a deficient diet, they still met the NRC requirements for a gain of 50 g/d. This data suggest that goats, specifically growing goats, may be more capable of utilizing the protein present in low protein diets than nutritionists originally thought.

The urine composition of the trial goats (Table 14) showed a significant decrease ($P = .0002$) in urea output for goats on the negative control diet compared to goats on the protein supplemented diets. The amount of N excreted in the urine was significantly lower for the negative control diet due to its decreased level of CP. Huntington et al. (2001) reported on the effect of dietary protein on urine N in growing steers. Intakes of 100 g/d and 200 g/d of protein resulted in urine urea N levels of 25.1% and 42.7% respectively. Doubling the available dietary protein nearly doubled the amount of N found as urea in the urine. This trend is also seen in

the 4 trial diets. The negative control presented 55.5% of the N in urine as urea. As the protein increased in the protein supplemented diets, the percentage of N in urine as urea increased to 75.7% for the 1/3 duckweed diet and 79.8% for both the positive control and 2/3 duckweed diets. Increased urine urea indicates a higher level of protein or N in the diet and consequently an increased inefficiency on the animal's part to utilize the available N in the diet. There was no difference among diets for % NH_4 of the total N in the urine, which is also consistent with Huntington et al. (2001).

The linear and quadratic responses among the protein supplemented diets for P retained (g/d) and (%) as well as P digested (g/d) suggest that duckweed may be a viable source of phosphorus supplementation at lower dietary levels (1/3 duckweed diet) than presented by the 2/3 duckweed diet. O'Bryan et al. (1998) fed Holstein steers diets supplemented with duckweed or soybean meal, and showed that P digested ($P < 0.05$), absolute retention ($P < 0.01$), and percentage of P retained ($P < 0.05$) was significantly decreased for the soybean meal supplemented calves compared to the duckweed fed calves. O'Bryan reported means for the three variables of 63.89%, 9.03 g/d, and 55.41% (respectively) for the duckweed calves. When comparing the negative control to the protein supplemented diets, the findings of this trial support those reached by O'Bryan et al. (1998). Although when comparing the positive control to the duckweed diets, the current findings do not support O'Bryan's findings of there being significant difference between control and

duckweed diets. A possible explanation for this discrepancy lies in the formulation of the diets for O'Bryan's trial compared to this trial. Both trials were formulated to be isophosphoric and isonitrogenous (except for our negative control diet). However, O'bryan's trial used duckweed as only 25% of the supplemental CP. Although there is an inconsistency on the relationship between duckweed and control diets, our findings do support O'Bryan's conclusion that nitrogen present in duckweed are used with at least the same level of efficiency as nitrogen and phosphorus present in conventional feedstuffs. It appears that phosphorus is used more efficiently at lower dietary levels.

The level of serum urea nitrogen exhibited significant differences between the negative control and the protein diets during all three sampling intervals: 0 h ($P = 0.001$), 4 h ($P < 0.001$), and 8 h ($P = 0.001$) (Table 14). Increased serum urea nitrogen is a sign of inefficient nitrogen utilization, but also indicates the diets were providing adequate N for the goat's requirements. With more N in the blood, more N is passed to the liver and consequently excreted as waste. There was a linear response among the protein diets at the 0 h of sampling (Figure 6). The linear response present in the serum urea levels of goats on the three protein diets corresponds to the goat's level of N intake. Although no significant response is present in the N intake of the diets, the 0 h serum urea levels present themselves in the same decreasing order as the N intake of the protein diets. For all three sampling intervals, the negative control diet is significantly different from the three

protein diets. Although the diets start out in a descending order based on the increasing level of duckweed after 8 h neither of the protein supplemented diets produce different levels of serum urea compared to the other protein diets. Luginbuhl et al. (2000) reported the serum urea nitrogen of goats fed diets with varying amounts of whole cottonseed: 0% cottonseed (9.71 mM), 8% cottonseed (9.35 mM), 16% cottonseed (10.21 mM), and 24% cottonseed (12.14 mM). The blood samples were taken 2 hours after feeding. The serum urea nitrogens for the cottonseed diets are similar to the serum urea nitrogens of the protein supplemented diets suggesting that duckweed could be an effective replacement for whole cottonseeds without the detrimental effects addressed by Luginbuhl et al. (2000). The negative control serum urea nitrogen levels are well below those reported by Luginbuhl et al. (2000). Moore et al. (2002) reports a serum urea nitrogen level of 15.39 mM for a hay and soybean meal diet and 13.75 mM for a soyhull diet. Their serum urea nitrogen was taken at 2.4 hours after feeding which places both the soybean meal and soyhulls well above the negative control diet but similar to the positive control (12.68 mM at 0 hours and 10.48 mM at 4 hours after feeding). Once again, duckweed appears to be only slightly less utilized compared to a conventional soybean meal concentrate.

Rumen Ammonia and Volatile Fatty Acid Data

There was no significant difference for rumen pH among the diets. The rumen pH for the diets (6.0 – negative control, 5.8 – positive control, 5.9 – 1/3

duckweed, and 6.1 – 2/3 duckweed) were lower than those reported by Moore et al. (2002) for a soybean meal (6.52) and soybean hull (6.41) diet, which may be explained by the fact that our diets were 50% concentrate while they fed a lower level of concentrate. As expected, there was a significant difference in rumen ammonia levels (P = 0.02) between the negative control and the protein diets. Hence duckweed would appear to not be considerably different than soybean meal for supplying ammonia to ruminal bacteria. The ruminal ammonia levels exhibited no significant linear or quadratic trends. Except for isobutyrate being significant (P = 0.08), there were no significant differences between the negative control diet and the protein diets for the VFA's. Duckweed also produces a similar VFA profile to that of soybean meal. The VFA's also presented no significant responses among the protein supplemented diets. Moore et al. (2002) also reported VFA data for the soybean meal and soybean hull diets. The total VFA amount was higher for our diets (94.8 mM – negative control, 99.3 mM – positive control, 101.7 mM – 1/3 duckweed, and 94.3 – 2/3 duckweed) than the soybean meal (79.31 mM) or the soybean hull (89.94) diets of Moore et al. (2002). The acetate to propionate ratio was lower for our diets (2.3 mM for negative, positive, and 2/3 duckweed diets and 2.5 mM for 1/3 duckweed diet) than the soybean meal (3.06) or soybean hull (3.26) diets of Moore et al. (2002). Overall, our diets were higher in propionate and butyrate than the soybean meal or soybean hull diets of Moore et al. (2002). However, their diets were higher in acetate, isobutyrate, isovalerate, and valerate than our diets. Like the digestibilities, these concentration differences can be linked

back to the low quality hay and higher percentage of grain used in our study compared to Moore et al. (2002). Yet the similarities of the VFA data among the diets show that duckweed does not abnormally affect rumen function and is comparable to soybean meal in dietary function.

Our research agrees with Damry et al. (2001) and Huque et al. (1996) in that duckweed can be incorporated into a ruminant's diet without any detrimental effects. Damry et al. (2001) fed duckweed to sheep and reported that duckweed was a good source of undegradable protein for ruminants. This finding contradicted the earlier findings of Huque et al. (1996) which demonstrated that duckweed was highly degradable (87% for *Lemna*) when placed in the rumen of bulls for 72 hours. There are several plausible explanations for this contradiction that needs to be explored further including: duckweed drying conditions, ruminal conditions, and rumen residence time (Damry et al. 2001). Our findings support the findings of Huque et al. (1996) since duckweed closely approximates soybean meal which is generally highly degradable in the rumen.

Implications

We conclude from the above discussion that duckweed can be effectively incorporated as a component of ruminant diets assuming an efficient method of harvesting and drying can be developed. As with any new or novel feed encountered by animals, palatability may initially be an obstacle. Yet with an

adjustment period, duckweed can be successfully used as a dried feed. Dried duckweed has a compositional profile lower in CP but much higher in minerals than that of soybean meal. Wastewater grown duckweed is capable of serving as both a source of N and P. Duckweed is nearly as effective as a protein source for ruminants as soybean meal. Further studies are required to better detail N utilization by growing goats because of the high level of retention on the negative control diet. Further investigations into appropriate levels of dietary duckweed supplementation for different ruminant species, and the possible exploitation of duckweed's nutrient uptake are also needed.

Conclusion

Although the plant family of *Lemnaceae* contains the smallest flowering plants in the world, they possess huge potential as a source of feed. Since duckweed floats on the surface of slow moving bodies of water, it is easily skimmed and harvested. Duckweed can be dried by a variety of methods but sun drying is the most economic and efficient method. However during sun drying special care must be taken to account for any wind. Once dried, duckweed has a compositional profile similar to that of soybean meal. The DMI, N balance, urine composition, serum urea nitrogen, ruminal ammonia, and VFA data shows that duckweed behaves in much the same way as soybean meal. Mineral analysis shows that wastewater grown duckweed can serve as both a source of nitrogen and phosphorus (at lower dietary levels). Exploitation of duckweed's natural nutrient uptake ability holds promise of

the manufacturing of a biological agent that could be grown on specific media to in order to produce an organic feed that meets the strict dietary requirements of certain animals. The N balance for this trial shows that goats may be capable of better utilizing the N in protein deficient diets than originally thought and that certainly warrants a more detailed investigation. The overall conclusion of this work is that duckweed, although small in stature, possesses a high potential as a feed source.

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Appendix A:

Description of Setbacks Faced During the Summer of 2001

This is a small summary paper written at the end of the summer (2002) to describe the problems that occurred during the trial so that minor details would not be lost over time.

We started with 24 weanling goats (including 4 spares) which were moved into pens at the Metabolism Unit on May 22nd. The 24 goats were to be split up into two 12 member groups with a two week delay between the two groups. Each group was to have a week and a half adjustment period before being assigned to a trial diet. The four original diets included a positive and negative control and two duckweed diets which differed in the quality of protein contained in the duckweed (1 high protein duckweed [40%] and 1 low protein duckweed [38.37%]). The first problem we ran into dealt with the diets. The duckweed with the higher protein level (duckweed provided by Louis Landesman) was deemed unusable due to its swine feces odor and its limited availability. We therefore decided to use the duckweed we were harvesting from NC A&T SU (38.37%) at two different levels in the diets. We did not make an immediate decision concerning the levels, but instead chose to wait and see how the goats reacted to the duckweed in their diets.

The second setback we faced occurred about one week after the goats entered the pens. We had 4 goats with diarrhea on May 30th. When the goats first entered the pens, we fed them a diet consisting of pressed hay and pellets fed ad libitum (10% refusal) once a day. We then moved the goats to a diet of pressed hay and corn concentrate after the first few days. After we realized we would not be able to use the high quality duckweed, we fed the goats a one day diet consisting of hay with duckweed mixed into the concentrate. The duckweed was not well accepted by the goats. Most either picked all the concentrate out that they could, leaving the duckweed in the bottom of the feeder or ate no concentrate at all. Unfortunately, the diarrhea occurred around the same time that we first fed the duckweed. After a few days, the majority of the goats had some degree of diarrhea from loose stool to watery feces. Upon noticing the diarrhea, we immediately started giving the goats varying doses of Corrid, a commercial coccidiostat. The Corrid treatment continued for roughly one week. We did send one goat back to the Small Ruminant Unit due to its low of intake and slow diarrhea recovery. On June 5th, we believed the worst of the diarrhea to be behind us so we started to feed four new diets to which the goats had been previously randomly assigned. These new diets consisted of a corn totally mixed ration (TMR) - the wheat hay was ground up and mixed in with the corn concentrate, duckweed TMR - the wheat hay was ground up and mixed in with a mixture of corn and duckweed concentrate, duckweed grain - the hay was pressed but not ground keeping it separated from the corn/duckweed concentrate, and

pressed wheat - the hay was pressed and fed with a straight corn concentrate. After a couple of days of feeding these diets, we began to experiment with varying levels of the TMR's mixed together, mostly feeding ¼ the amount of corn or duckweed grain as duckweed TMR. This was done in order to give us a better idea of how the level of duckweed and particle size would affect the goats. Yet we faced another problem on a different front.

We originally started harvesting our duckweed at NC A&T SU in Greensboro, NC. We harvested twice a week (every Monday and Thursday) off of 5 wetland cells. Our harvest was two five-gallon buckets per cell resulting in 10 buckets, 50 gallons, roughly 180 pounds of wet duckweed. From each harvest we received roughly 10 to 12 pounds of duckweed. Still, we hit yet another setback. The ammonia levels in the wetland cells grew too high for the duckweed to tolerate resulting in a massive die off of duckweed and a consequent algae bloom. In less than a week all of the duckweed was dead. Tony Grubbs, Research Technician at NC A&T, was helping us to harvest the duckweed and dry it. We reworked a dilapidated greenhouse at NC A&T SU in order to sun dry the duckweed, but when the duckweed died, priorities changed. Tony ended up replumbing the wetlands so that either waste water or fresh water could be pumped into the cells thereby ensuring nutrient/mineral level control. Nonetheless, we were not out of the woods yet.

After assigning the goats to the new diets, the goat's intake continued to climb (their intake had been on an upward trend since recovering from their bout with diarrhea). Quite a few goats were increasing their intake at 120% daily instead of 110%. On June 15th, we switched the goats to their randomly assigned trial diets. We also moved another goat back to the Small Ruminant Unit due to its poor performance. We had met earlier that week and decided that given their negative reaction to 100% duckweed, it would be better to feed a positive and negative control along with 1/3 and 2/3 levels of duckweed. A 1/3 or 2/3 duckweed diet refers to a diet in which 1/3 or 2/3 of the supplemental protein in the diet comes from duckweed. We also decided to feed the four diets as TMR's. However, upon feeding the new diets to the goats we hit another setback. Some goat's intake dropped off immediately upon receiving the new diets, but a few goats had a delayed reduction in intake. By June 21st, all but three or four of the goats had reduced intakes. Roughly half of the goats fell below what we had deemed as the minimal level of intake and several goats had intakes less than 100 grams per day. On June 24th, all the goats were switched back to a diet of pressed wheat hay. Nevertheless, their intakes did not increase but instead held steady at their various levels. Some goat's intake level even continued to drop. We then decided to move the herd outside where we hoped social interaction would bring intake back up. On July 2nd, we moved 20 goats to a paddock outside and our two worse cases back to the Small Ruminant Unit. We built a small corral to aid in feeding and confining the animals when the need arose. We also brought in two trough feeders to put our

concentrate in. We began by feeding 5000 grams (11 pounds) of pressed wheat hay and 3000 grams of concentrate. We started with a simple corn concentrate, but started reintroducing duckweed back into the concentrate on July 7th. The level of duckweed was slowly increased at two day intervals until reaching the 2/3 duckweed level on July 17th. Goat #2096 showed very little interest in the corn concentrate upon being brought outside, and no interest in the duckweed concentrate. Due to its persistent refusal of the duckweed concentrate, Goat #2096 was moved back to the Small Ruminant Unit.

The purpose of this study was to evaluate duckweed (*Lemna gibba*) as a protein supplement for ruminants through the feeding of three duckweed diets to 20 Boer goat (*Capra hircus*) wethers. However, the feed trial experienced setbacks that consequently altered the final study. The original plan called for feeding one duckweed species (*Lemna gibba*) at differing protein levels (a low protein [38%] duckweed harvested in conjunction with NC A&T Swine Unit and a high protein [42%] duckweed obtained from continued management of the *Lemna gibba* 8678 isolate. During the summer of 2002, the NC A&T SU duckweed died off through increasing ammonia levels concentrated in part by the climax of a 5 year drought in North Carolina. Since production of the 8678 isolate was insufficient to provide enough duckweed to supplement 20 goats, we searched for and found an alternate source of duckweed at the waste pond of the NCSU Aquaculture Educational Unit (AEU). The duckweed growing on the fish waste pond at the NCSU AEU appeared

spontaneously during the time Bergmann et al. was conducting research at the adjacent NCSU Swine Unit. It is therefore our belief that the *Lemna gibba* harvested from the fish waste pond at the NCSU AEU is in fact some of the 8678 isolate or a close variety thereof. The study started with 24 goats, 20 trial goats and 4 extras. However one week prior to the moving the goats into the metabolism crates to begin the feed trial, the goats exhibited signs of an intestinal parasite infestation thereby resulting in feed refusal. The goats were treated for parasites and showed marked improvement over the following weeks but experienced feed refusal a month later. The goats were then moved outside for one month and the entire concentrate was pelleted (previously the duckweed was manually mixed with corn pellets to form a loose concentrate). In total, 5 goats were returned to the NCSU Small Ruminant Educational Unit (SREU) due to severe parasite infection, feed refusal, and reduced growth compared to other goats. Ultimately, the study evaluated duckweed (*Lemna gibba*) grown on a pond receiving waste nutrients from an agricultural facility as a protein supplement for goats. Experimental diets included a negative control with no supplemental protein and three diets supplemented with protein. The three protein diets contained three levels of duckweed: 0 supplemental protein from duckweed, 1/3 of supplemental protein from duckweed, and 2/3 of supplemental protein from duckweed.