Duckweed protein supports the growth and organ development of mice: A feeding study comparison to conventional casein protein

B. Roman, R.A. Brennan, and J.D. Lambert

Abstract: As global population growth and meat consumption increases, sustainable alternatives to conventional protein-rich fodder crops for livestock are needed to reduce negative environmental impacts. Duckweed, a small floating aquatic plant, can generate 5 to 10 times higher protein yields than conventional land-grown crops. Although some *in vivo* feeding trials with duckweed have been conducted, those measuring animal weight are limited, and those examining organ development are nonexistent. To secure broad acceptance of new protein sources, such controlled studies are critical. This study measured the food intake, growth, and final organ and adipose tissue mass of male CF-1 mice fed a semi-purified diet containing casein or diets in which 10% or 25% of the casein was replaced with duckweed protein (DWP). Proximate analysis showed that the DWP preparation used contained 39.9% protein (w/w), and contained all of the essential amino acids with Met as the limiting amino acid. The average growth rates were not significantly different among the treatment groups: 0.21 g/day; 0.24 g/day; and 0.25 g/day for the control, 10%, and 25% DWP protein diets, respectively. The daily food intake of both DWP diets was 6.5% to 8.0% higher than the control diet, but feeding efficiency did not differ among diets. The relative weight of the liver, spleen, kidneys, heart, and epidydimal fat, and colon length were not significantly different between treatment groups. The results from this study show that replacement of up to 25% dietary casein with DWP has no adverse effects on the growth rate and final organ and adipose tissue weights of laboratory mice.

Keywords: Duckweed, in vivo animal trial, plant protein, sustainable protein

Practical Application: Duckweed can produce 5 to 10 times more protein per area than land-grown crops such as soybean. In this study, up to a 25% replacement of casein with duckweed protein had no observable effect on the growth or organ development of laboratory mice. Thus, duckweed has the potential to be used as a protein supplement for livestock, poultry, and fish, thereby decreasing environmental impacts from land-grown crops used for animal feed.

1. INTRODUCTION

Due to global population growth and increasing meat consumption in developing regions, the demand for animal-derived proteins is expected to double by 2050 (FAO, 2011). This increase in animal production will have compounding effects that are expected to negatively impact the environment: increased livestock production will require the conversion of forests, wetlands, and grasslands into grazing lands, essentially converting CO_2 sinks into greenhouse gas producing areas. Furthermore, feeding livestock takes significant quantities of plant protein, with 85 million tons of plant protein required to produce 64 million tons of livestock protein annually (Steinfeld et al., 2006), concomitantly increasing land use, nutrient loads in water bodies, and reducing water availability, soil fertility, and biodiversity (Roser & Ritchie, 2017). Potential solutions to alleviate the environmental impacts of animal agriculture include decreasing animal protein (i.e., meat) consumption, and switching to more sustainable sources of plant protein to meet the demands for food and fodder. It is estimated that alternatives to animal protein could claim up to one-third of the market by 2054 (Lux Research, 2014). Indeed, young consumers are increasingly looking to replace animal-derived proteins with plant-based proteins in their diets, with 46% agreeing that meat alternatives are healthier than meat (McCarthy & Dekoster, 2020; Scott-Thomas, 2015). Moreover, a study conducted on Dutch consumers found that when the nutritional and sustainability benefits of a nontraditional meal ingredient were presented, consumers had a more positive evaluation of the meal, given that sensory properties like taste are satisfactory (de Beukelaar et al., 2019).

Many attempts have been made to find abundant and inexpensive protein-rich substitutes for conventionally grown feedstocks. For example, insect meal has been used in poultry, rabbit, pig, and fish diets (Adeniji, 2007; Adeniji, 2008; Duwa et al., 2014; Adewolu et al., 2010), but the protein content is low (<20% dry matter [DM]) (Lundy & Parrella, 2015). Soy, wheat, corn, sorghum, peas, and lupin have been used as supplements in fish meal (Allan et al., 2000; Burel et al., 2000; Kaushik et al., 1995), but due to their lower nutritional value, only a small portion of these plant-based proteins are typically blended with conventional meat-based feed. Another emerging protein source is algae: 24 million tons are currently farmed globally, with the majority being used in health foods, cosmetics, and animal feed. Although some microalgae contain nearly 50% protein DM, algae protein development has been hampered by high production costs and technical challenges (Henchion et al., 2017). Clearly, additional research and development of sustainable protein sources is required to support growing demand.

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Duckweed is a small floating aquatic plant, that when grown under ideal conditions, can double its mass every 16 to 48 hr and accumulate a crude protein content of up to 45% of the DM, making it an ideal candidate for a sustainable protein (Leng, 1999). In fact, it has been determined that duckweed grown on partially treated municipal sewage can generate protein yields that are 5 to 10 times higher than conventional land grown crops, such as soybeans, maize, and oats (Roman & Brennan, 2019). In addition to its prolific growth rates, another benefit of duckweed is the ease with which it can be skimmed from water and used for beneficial purposes. Although algae and seaweed have long been used as components of plant-based nutrition (De Pinto & Verhoff, 1977), harvesting algae from water can be difficult and energy-intensive, and seaweed is only sustainably available in coastal regions. Duckweed, however, can grow anywhere there is water: various genera of duckweed (most common: *Lemna*, *Spirodela*, and *Wolffia*) are found worldwide, and the dominant genera in the region depends on the water quality, topography, and climate (Leng, 1999).

Although the *quantity* of protein producible by duckweed is well documented, less work has been done on the *quality* of duckweed protein (DWP). Several studies have examined the amino acid composition of various duckweeds and concluded that based on its amino acid profile, the quality of DWP is suitable for consumption by fish, poultry, cattle, and humans (Appenroth et al., 2018; Hanczakowski et al., 1995; Sharma et al., 2019). Duckweed can also accumulate trace minerals (K, Ca, Mg, Na, Fe) which are often deficient in the feed available to small livestock farmers (Leng, 1999), as well as micronutrients (iodine and vitamin A) which are commonly deficient in the diets of malnourished people (Valdimirova & Georgiyants, 2014). Although duckweed can provide a source of minerals and vitamins, it is generally only regarded as a protein supplement due to the high protein yields that can be achieved. Duckweed feeding trials have been conducted with pigs, ruminants, poultry, and fish, with varying results. In some studies, duckweed has been shown to be able to supply all protein requirements for certain animals with no adverse health effects (Cheng & Stomp, 2009), while in other studies, small additions of duckweed into animal diets has been shown to decrease their weight gain and food intake (Sonata et al., 2019). The confounding results in prior duckweed feeding studies are likely due to several factors, with arguably the most important being the broad range of nutritional quality of the duckweed used, which is highly dependent on its growth medium. In addition, no studies to date have examined organ development in animals fed a duckweed supplemented diet, which is critical for securing broad acceptance of protein supplements.

In this study, DWP was extracted, analyzed, and formulated into two diets in which 10% and 25% of the protein content was derived from DWP, and the balance was casein. These duckweed-supplemented diets were compared to a conventional casein diet and fed to 30 CF-1 male mice for 4 weeks. Body weight gain, food intake rate, and final organ weight were analyzed.

2. MATERIALS AND METHODS

2.1 Preparation of duckweed and diet formulation

The duckweed used in the study was purchased from a commercial DWP distributor in the United States that promotes the plant as a protein supplement that can be added to human food products, and is a polyculture of 14 different species from three genera, including one *Spirodela*, eight *Lemna*, and five *Wolffia* species. The conditions for its cultivation and drying are proprietary information and were not reported by the manufacturer; however the biomass characteristics of the raw duckweed powder are shown in Table S1. To enable a more accurate comparison of DWP to casein, the duckweed was defatted and polyphenols removed by extracting sequentially with hexane and ethyl acetate, respectively. In brief, 50 g of dried duckweed was combined with 200 mL solvent for 10 min, draining the solvent through a Buchner funnel, and repeating. Extraction with each solvent was performed a total of three times. Proximate analysis and total amino acid analysis were performed on the defatted/polyphenol-depleted duckweed using Association of Analytical Chemists, International (AOAC)-methods (The University of Missouri Agriculture Experiment Station Chemical Laboratories, Columbia, MO, USA). A study of human food sources of nutrients in the United States showed that no source of protein accounts for more than 20% of the total protein intake (dairy, poultry, and beef each account for less than 20%; O'Neil et al., 2012). Based on this, we selected replacement levels of 10% and 25% as they represent amounts that are potentially relevant to human consumption. The diets: AIN93G (casein); AIN93G + 10% DWP replacement (4.3% duckweed mass addition); and AIN93G + 25% DWP replacement (10.7% duckweed mass addition) were prepared by Research Diets Inc (New Brunswick, NJ, USA). Diets were matched for energy, macronutrient, and micronutrient composition (Table 1).

2.2 Mouse feeding experiment

The feeding study was approved by the Pennsylvania State University Institutional Animal Care and Use Committee (Protocol No. 45380). Male CF-1 mice (5 weeks old, 33.3 ± 2.4 g at Day 1) were purchased from Charles River Laboratory (Wilmington, MA, USA). Mice were housed with 12 hr light/dark cycles held at 18 °C to 23 °C and 50% to 60% relative humidity, and allowed to acclimate to the room for 1 week prior to starting the study. Mice were randomized by body weight ($n = 10$ per diet) to each diet. The body mass of each mouse was recorded twice a week for 4 weeks. Food consumption was measured twice weekly. Fresh water was provided *ad libitum*. At the end of the feeding study, the mice were anesthetized and euthanized by exsanguination. The blood samples were centrifuged at 3,200 *g* for 15 min to separate the plasma which was then stored at −80 °C for future analysis. The liver, spleen, lungs, kidneys, heart, and epididymal (epi) fat were removed, rinsed with ice-cold 0.9% NaCl, trimmed of connective tissue and fat, blotted dry, and weighed. Organ and adipose tissue masses were normalized to final body weight. The length of the colon was also recorded. Feeding efficiency was calculated as the ratio of rate of body weight gain (g/d) to food intake (g/d), representing the change in body weight as a function of the mass of food consumed.

2.3 Statistical analysis

The growth rate, food intake rate, and organ masses were compared separately using a one-way ANOVA and Tukey post-test in Minitab to determine differences for each variable depending on the diet ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 DWP quality

Proximate analysis showed that the defatted/polyphenol-depleted duckweed preparation used in our experiments contained 39.9% protein, 14.2% fiber, and 1.1% fat (Table 2). Amino acid analysis showed that the material contained all the essential amino

Table 1–Composition of diets used in this study.

	AIN93G (control)		AIN93G + 10% DWP (4.3% mass)		AING93G + 25% DWP (10.7% mass)	
Ingredient	**mass (g)**	**Energy (kcal)**	**mass (g)**	**Energy (kcal)**	**mass (g)**	**Energy (kcal)**
Casein	200	800	180	720	150	600
L-Cystine	3.0	12	3.0	12	3.0	12
Corn starch	397	1,590	397	1,590	397	1,590
Maltodextrin 10	132	528	132	528	132	528
Sucrose	107	428	107	428	107	428
Cellulose	50	0	32	0	5.0	0
Soybean oil	70	630	70	630	70	630
T-Butylhydroquinone	0.01	0	0.01	0	0.01	0
Mineral mix	3.5	0	3.5	0	3.5	0
Calcium carbonate	12.5	0	10.9	0	8.4	0
Potassium phosphate	6.9	0	6.4	0	5.8	0
Potassium citrate	2.5	0	2.3	0	2.1	0
Sodium chloride	2.6	0	2.6	0	2.6	0
Vitamin mix	10	40	10	40	10	40
Choline bitartrate	2.5	0	2.5	0	2.5	0
Duckweed	0	0	43	79	108	199
Total	1,000	4,028	1,003	4,027	1,007	4,027

Table 2–Proximate analysis and amino acid composition of the duckweed protein isolate used in this study.

Proximate analysis		**Amino acid composition (mg/g)**					
(%)		**Essential**		**Nonessential**		**Conditionally essential**	
Crude protein	39.9	His	8.9	Ala	24.1	Arg	24.9
Crude fat	1.1	Ile	20.3	Asp	35.3	Cys	4.7
Crude fiber	14.2	Leu	36.1	Glu	43.1	Pro	17.9
Ash	5.4	Lys	26.4	Gly	21.1		
Moisture	14.4	Met	7.9	Ser	15.3		
		Phe	23.0	Tyr	14.0		
		Thr	17.4				
		Trp	6.8				
		Val	24.9				

Table 3–Essential amino acid (EAA) composition of protein isolate from duckweed used in this study and other common plant-protein sources. Values are represented in grams per 100 g raw material.

	EAA content of protein isolate (g/100 g)								
EAA	**Duckweed**	**Oat**	**Lupin**	**Wheat**	**Soy**	**Brown rice**	**Pea**	**Corn**	**Potato**
His	0.9	0.9	1.2	1.4	1.5	1.6	1.6	1.1	1.4
Ile	2.0	1.3	1.5	2	1.9	2.3	2.3	1.7	3.1
Leu	3.6	3.8	3.2	5	5	5.7	5.7	8.8	6.7
Lys	2.6	1.3	2.1	1.1	3.4	4.7	4.7	1	4.8
Met	0.8	0.1	0.2	0.7	0.3	2	0.3	1.1	1.3
Phe	2.3	2.7	1.8	3.7	3.2	3.7	3.7	3.4	4.2
Thr	1.7	1.5	1.6	1.8	2.3	2.3	2.5	1.8	4.1
Trp	0.7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Val	2.5	2	1.4	2.3	2.2	2.7	2.7	2.1	3.7

EAA values for other common plant-protein sources from Gorissen et al., 2018; N/A, not analyzed.

acids (EAA) in appreciable amounts, and all but two nonessential (Asp and Sec) and one conditionally essential (Gln) amino acids (Table 2). The EAA composition of DWP was compared to other common plant-protein sources (Table 3), and the mass required to meet the WHO recommended daily EAA intake for a 70 kg adult human was calculated for each protein source (Table 4). The limiting EAA for duckweed is Met, and assuming that duckweed is 100% digestible, it would require 92.2 g of defatted/polyphenol-depleted duckweed powder/day to meet adult human requirements (Table 4). Compared to the mass required of the limiting EAA for the other plant-protein sources, defatted/polyphenol-depleted duckweed out-competes all but brown rice and potato protein isolates (Table 4), suggesting that duckweed is an ideal candidate as a protein supplement for a human diet.

3.2 Body weight gain and relative organ weights

There was no significant difference in body weight or rate of body weight gain for the mice fed different diets (Figures 1A and 1B, $P = 0.559$). The relative masses of the liver, spleen, kidneys, heart, and epididymal fat were not different among the mice treated with the three different diets (Figure 1C). The lungs of the mice fed the 25% DWP diet were significantly larger (about 20%) than those of the mice on the control diet ($P = 0.028$). This difference could be due to chance, since it has been reported that

Table 4–Mass of protein isolate required to meet the daily recommended essential amino acid (EAA) intake for an adult human by the World Health Organization. Values are represented in grams per 70 kg body weight per day. Bolded and underlined values indicate the limiting essential amino acid.

	Mass to meet the daily human EAA requirement (g/70 kg bw/d)								
EAA	Duckweed	Oat	Lupin	Wheat	Soy	Brown rice	Pea	Corn	Potato
His	78.7	77.8	58.3	50.0	46.7	43.8	43.8	63.6	50.0
Ile	69.0	107.7	93.3	70.0	73.7	60.9	60.9	82.4	45.2
Leu	75.6	71.8	85.3	54.6	54.6	47.9	47.9	31.0	40.7
Lys	79.5	161.5	100.0	**<u>190.9</u>**	61.8	44.7	44.7	**<u>210.0</u>**	43.8
Met	**<u>92.2</u>**	**<u>728.0</u>**	**<u>364.0</u>**	104.0	**<u>242.7</u>**	36.4	**<u>242.7</u>**	66.2	**<u>56.0</u>**
Phe	76.1	64.8	97.2	47.3	54.7	47.3	47.3	51.5	41.7
Thr	60.3	70.0	65.6	58.3	45.7	45.7	42.0	58.3	25.6
Trp	41.2	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Val	73.1	91.0	130.0	79.1	82.7	**<u>67.4</u>**	67.4	86.7	49.2

Daily recommended EAA intake for humans from WHO, 2007; N/A, not analyzed.

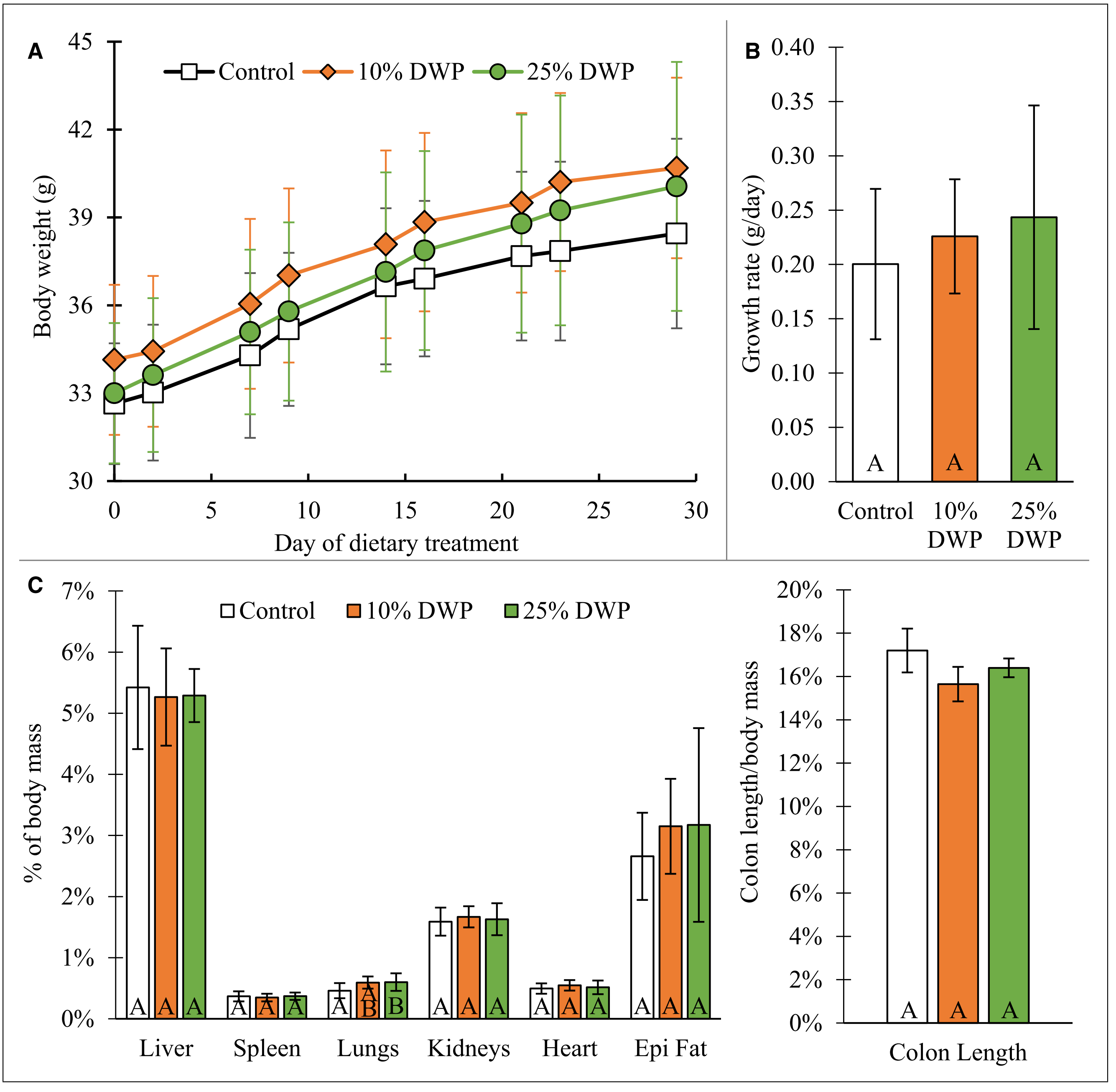

Figure 1–(A) Body mass of mice fed three diets over four weeks: control (casein protein); 10% DWP (casein + 10% duckweed protein); and 25% DWP (casein + 25% duckweed protein). (B) Average growth rate of mice fed three different diets. (C) Organ percentage of body mass from the mice fed three diets after 4 weeks ($n = 10$ per diet; 95% CI).

Table 5–Review of duckweed feeding studies published since 1990 (adapted from Sonta et al., 2019). Bolded Positive/Negative/Neutral in the result column indicates the effect on including duckweed in the diet compared to the conventional diet.

Animal	Diet	Result	Citation
Broiler chickens	Titan broilers fed commercial diet supplemented with 0, 100, 150, 200, 250, or 300 g/kg DW	**Negative**: Chickens fed diets over 150 g/kg DW showed decreased body weight gain and feed intake. Chickens fed the no DW diet reached much higher body weight gains and feed intake.	Haustein et al. (1992)
	Titan and Arbor Acres broilers fed maize diets supplements with 0%, 10%, 15%, or 25% dried DW	**Positive (≤15% DW supplement)**: Body weight gains and feed intake were comparable up to 15% DW, but deteriorated at 25% DW.	Haustein et al. (1994)
	Vencobb broilers sesame oil cake diet supplemented with 0%, 3%, 6%, or 9% dried DW	**Positive (≤6% DW supplement)**: Final body weight and feed conversion were higher in the 3% and 6% DW diets. Poorest results were observed by the 9% DW supplement diet.	Ahammad et al. (2003)
	Gallus domesticus fed commercial diets supplemented with 0%, 5%, 10%, 15%, or 20% dried *Lemna*	**Neutral**: *Lemna* addition into the diets had no effect on the average daily gain, feed consumption, or feed conversion.	Putra and Ritonga (2018)
	Vencobb broilers fed commercial diets supplemented with 0%, 4%, 8%, or 12% dried DW	**Negative**: Increasing % of DW in diet decreased body growth, feed conversion ratio, and protein and energy intake	Kabir et al. (2005)
Laying hens	Star Cross Brown laying hens fed rice polish and fish meal diet replaced with 0, 50, 70, 110, 130, and 150 g/kg dried DW.	**Negative**: No differences were observed between all groups for body weight, egg weight, and livability. Feed intake, egg production, and feed conversion ratio all decreased with increasing DW in diet.	Akter et al. (2011)
	Hy-Line laying hens fed soybean meal diet replaced with 0% or 12.5% DW.	**Neutral**: Egg production and nutrient composition of eggs were similar between the two diets, except omega-3 acids were higher in the DW diet than in the soybean diet.	Anderson et al. (2011)
	Lohmann Brown layers fed a commercial diet supplemented with inorganic salt (C) or DW (E)	**Positive**: Egg quality parameters were improved in the DW diet and birds had lower Cd in yolk and Pb in blood when fed the DW diet.	Witkowska et al. (2012)
Ducks	Muscovy ducks fed rice diet supplemented with: E1) 80% water spinach; E2) 80% DW; and E3) 35% water spinach and 45% DW	**Positive**: Daily weight gain was lowest in E1 and highest in E2. Feed conversion ratio was higher in E2 compared to E1 and E3. The use of DW in duck diets was advised.	Ngamsaeng et al. (2004)
	Xingding ducks fed commercial diet supplemented with fresh (wet) DW: (C) commercial compound feed; (E1) 50% C + fresh collected DW *ad libitum* (E1); 50% C + DW forage (E2); and (E3) DW lagoon forage.	**Negative**: All E3 ducks died within the first 3 weeks, and was concluded that DW cannot be used as the sole feed for ducks. Final body weight and daily weight gain were significantly lower in the E groups compared to C. DW inclusion in the diet caused a decrease on the growth of the ducks.	Khanum et al. (2005)
	Jinding ducks fed mustard oil cake diet replaced with 0%, 5%, 10%, or 15% dried DW.	**Negative**: Body weight gain, egg weight, and feed conversion ratio were similar between groups, but egg production decreased with increasing % DW in diet.	Khandaker et al. (2007)
Pigs	Piglets fed soybean diets supplemented with 0%, 20%, 40%, 60% dried DW	**Positive**: Daily body weight gains were significantly higher in pigs fed 40% and 60% DW diets compared to the 0% (control soybean diet) and 20% DW diets.	Moss (1999)
	Pigs fed sorghum and soybean meal (C) replaced with 10% dried DW (E).	**Neutral**: Weight gain, feed conversion ratio, and final body weight were similar between groups.	Gutierrez et al. (2001).
	Pigs fed sweet potato vines (C) or fresh DW (E)	**Positive**: Body weight, live weight gain, and feed conversion ratio were higher for the DW diet.	Du (1998)
	Pigs fed control diet compared to DW diet: C) 60% broken rice; 33% rice bran; % fish meal; and 2% soybean meal and E) 69% cassava root; 8.6% DW; and 22.4% supplements	**Neutral**: Pigs fed the C diet had higher final body weight, but the feed conversion ratio was similar. The carcasses of E had thinner backfat than C.	Van et al. (1997)
Ruminants	Merino ewes fed oaten chaff diets supplemented with DW: C) 700 g/day oaten chaff E1) 630 g/day oaten chaff + 50 g/day dried DW E2) 540 g/day oaten chaff + 100 g/day dried DW E3) 630 g/day oaten chaff + 1 kg/day fresh DW	**Neutral**: Hair coat parameters (wool yield, rate of wool elongation, fiber diameter) did not differ among the groups. It was concluded that DW is a valuable source of protein for the ruminants.	Damry et al. (2001)
	Boer goats fed soybean meal supplemented with 0%, 33%, and 66% dried DW	**Neutral**: Nitrogen intake, excretion, and serum urea level, phosphorous intake and excretion, and rumen pH, ammonium, and volatile fatty acids were similar for all diets. It was concluded that DW is nutritionally comparable to soybean meal.	Reid (2004)

(*Continued*)

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Table 5–(Continued).

Animal	Diet	Result	Citation
Fish	Nile tilapia fed fish meal (C) supplemented with 20% (E1) or 40% (E2) dried DW and 20% (E3) or 40% (E4) fresh DW	**Neutral**: Weight gains were significantly higher in the C and E3 diets. The remaining diets showed similar weight gains. Feed conversion ratio and protein efficiency were similar for all groups. Fish fed E diets had greater phosphorous and protein content and lower lipid content than fish fed the C diet. Fish fed the C diet had higher dry matter content and lower ash content than fish fed the E diets.	El-Shafai, et al. (2004)
	Tilapia fed commercial feed supplemented with 0% (C), 50% (E1), and 100% (E2) dried DW	**Neutral (≥50% DW supplement)**: Final body weight of fish fed the C and E1 diets were similar, but fish fed the E2 diet had lower final body weight.	Tavares et al. (2008)
	Carp (*Cyprinus carpio* L.) fed a commercial diet supplemented with 0%, 10%, and 30% dried *Lemna*	**Positive**: Carp showed increasing survival rate with increasing *Lemna* % in diet. Final weight and growth rate were similar for all diets.	Sirakov and Velichkova (2018)
	Vundu fed fish meal supplemented with 0%, 10%, 20%, and 30% dried DW	**Negative**: Growth rate, body weight, and feed conversion ratio were similar for 0 and 10% DW fed fish, but were lower for 20 and 30 % DW fed fish.	Effiong et al. (2009)
Shrimp	Pacific white shrimp fed fish meal replaced with 0%, 5%, 15%, 25%, or 35% fermented DW.	**Positive**: Survival rates were similar in all groups. The best growth efficiency was observed in shrimp fed the 35% DW diet.	Flores-Miranda et al. (2015)
This study	CF-1 male mice fed casein diet supplement with 0%, 10%, and 25% DW protein (DWP)	**Neutral**: Mice body mass growth was similar for each diet and food intake rate was higher for both DWP diets.	This study

there is no difference in the size of lungs from obese mice (>50 g) and lean mice (<30 g) (Guivarch et al., 2018), which is a much larger gap in body mass than that observed between the control- and DWP-fed mice in this study. The average colon lengths of the mice fed the three diets were not significantly different (Figure 1C). There were no gross differences in organ morphology between treatment groups. These results indicate that DWP can support normal growth and development of major organs. To our knowledge, this is the first study to examine the effect of DWP on organ weights.

A number of duckweed feeding studies were conducted in the 1970s and 1980s (reviewed in Culley et al., 1981). Table 5 summarizes the results and limitations of studies conducted since 1990. Studies in nonruminant mammals have focused on pigs. Consistent with our present results, one study with piglets found that replacing up to 60% of soybean meal mass in their diets with duckweed mass resulted in similar or greater growth (Moss, 1999). While the studies in piglets are generally positive, with duckweed substitution yielding equivalent or superior increases in body weight, most are confounded by the use of different base diets between treatment groups, poorly characterized nutrient content for the overall diet, and/or incomplete characterization of the duckweed used in the studies.

Similar confounding issues exist in studies with other agricultural species. For example, two studies examined the impact of dried duckweed on body weight gain in broiler chickens, but found different results. One study found that the chickens fed corn-based diets supplemented with 10% and 15% duckweed showed comparable bodyweight gain to the chickens fed a solely corn-based diet (Haustein et al., 1994), while another study found that chickens fed an undefined commercial diet supplemented with 4%, 8%, and 12% duckweed had decreasing body weight as the percentage of duckweed increased (Kabir et al., 2005). Without clearly defined basal diets, it is impossible to determine the reasons for the differences observed between these studies.

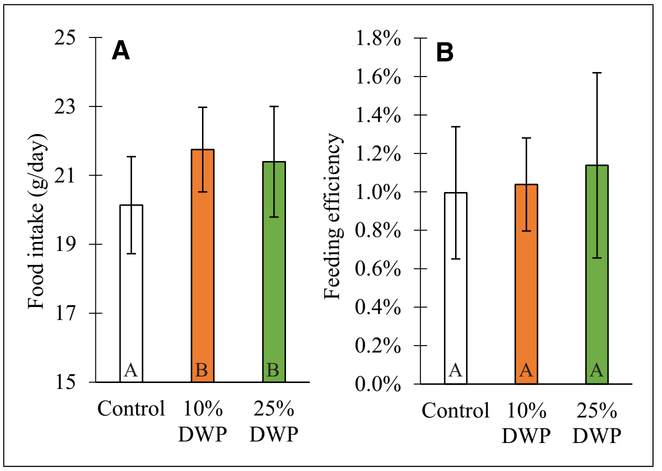

Figure 2–(A) Food intake rates and (B) feeding efficiency of the three diets ($n = 16$ per diet for food intake; $n = 10$ per diet for feeding efficiency; 95% CI).

It is well-established that duckweeds can exhibit a large range of nutritional characteristics depending on the nutrient content of their growth medium, with protein content ranging from 15% to 45% and fiber content ranging from 5% to 30% (Leng et al., 1995). A strength of the current study is the use of a well-defined duckweed supplement and a consistent basal diet, allowing a more robust comparison between duckweed-derived protein and other protein sources. Thus, future duckweed feeding studies in agricultural species should formulate duckweed into diets based on a percentage of *protein* substitution, rather than a percentage of *mass* substitution to accurately represent the effect of DWP diets on animal growth.

3.3 Food intake and feeding efficiency

Food intake of the DWP diets were significantly greater than the control casein diet ($P = 0.006$, Figure 2A). The feeding efficiency of the diets were not significantly different ($P = 0.678$, Figure 2B), however, indicating that there was no difference in utilization of protein and nutrients between the diets. Furthermore, there was no trend present ($R^2 = 0.43$) for the rate of body weight gain against the mean daily food intake rate for each group, establishing that the DWP diets provided no additional nutritional benefit.

4. CONCLUSION

Compared to conventional land grown crops, duckweed can produce larger quantities of protein for animal feed, which makes it a prime candidate to meet growing global demands for animal-derived proteins. This study showed that replacement of up to 25% of casein in the basal diet with DWP had no adverse effects on the growth or organ development in mice. Future studies investigating the effect of duckweed diets on the development of agricultural animals should use a consistent basal diet and substitute duckweed for other protein-rich feed based on protein content rather than total dry mass. Finally, given the growing demand for plant-based proteins for human consumption, studies that examine the palatability and ingredient functionality of DWP are needed.

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AUTHOR CONTRIBUTIONS

B. Roman–Collected and analyzed data, interpreted results, and drafted the manuscript.

R.A. Brennan–Responsible for article review and revision.

J.D. Lambert–Responsible for experimental supervision and article review.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1–Biomass characteristics of the duckweed powder that was purchased and used for this study (data from FDA GRAS Notice No. 742).

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