

Aquatic Plants from Minnesota

Part 4 - Nutrient Composition

by

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FOREWORD

This bulletin is published in furtherance of the purposes of the Water Resources Research Act of 1964. The purpose of the Act is to stimulate, sponsor, provide for, and supplement present programs for the conduct of research, investigations, experiments, and the training of scientists in the field of water and resources which affect water. The Act is promoting a more adequate national program of water resources research by furnishing financial assistance to non-Federal research.

The Act provides for establishment of Water Resources Research Centers at Universities throughout the Nation. On September 1, 1964, a Water Resources Research Center was established in the Graduate School as an interdisciplinary component of the University of Minnesota. The Center has the responsibility for unifying and stimulating University water resources research through the administration of funds covered in the Act and made available by other sources; coordinating University research with water resources programs of local, State and Federal agencies and private organizations throughout the State; and assisting in training additional scientists for work in the field of water resources through research.

This Bulletin is number 56 in a series of publications designed to present information bearing on water resources research in Minnesota and the results of some of the research sponsored by the Center. The Bulletin is concerned with the results of a survey of selected aquatic plants in Minnesota conducted in anticipation of finding compounds which might be useful in medicine. The survey is part of a research project aimed at finding a nutritional, medicinal or industrial use for the unwanted aquatic plants in lake shoreline areas. It is possible that some aquatic plants may contain industrially useful gums-mucilages, or new useful antimicrobial, anticoagulant, or antineoplastic therapeutic principles. If a good industrial, medicinal, or nutritional use for aquatic plants can be discovered, the results of the research could provide an economic incentive for aquatic plant collection and control. The successful completion of the project may significantly assist the State and Nation in partially solving their lake pollution problems.

This Bulletin is related to the following research project:

OWRR Project No.: A-025-Minn.

Project Title: Alleviation of Lake Pollution by Utilization of Aquatic Plants for Nutritional, Medicinal or Industrial Purposes

Principal Investigator: E. John Staba, Dept. of Pharmacognosy, College of Pharmacy, University of Minnesota

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Publication Abstract:

Samples of 22 freshwater aquatic plants were analyzed to determine their potential feeding value for ruminants. Proximate analyses (mean-SD), on a dry matter basis were: crude protein, 12.7[±]4.4%; ether extract, 1.46[±].98%; crude fiber, 19.2[±]6.4%; ash, 2.05[±]1.24%; and NFE 64.6[±]6.5%. NDF, ADF and ADL contents averaged 41.6[±]13.4%; 32.0[±]9.6% and 6.35[±]2.76%, respectively. Mineral contents (mean-SD) of the 22 aquatic plants were: P, 0.25[±]0.19%; Ca, 1.83[±]1.68%; K, 1.54[±]0.92%; Na, 0.30[±]0.25%; Mg, 0.31[±]0.16%; Fe 924-730 ppm; Zn, 80.6-96.6 ppm; Cu, 13.8-34.0 ppm; Mo, 19.7-9.7 ppm and Mn, 269-152 ppm. Van Soest's estimated apparent digestibility averaged 63.0[±]8.3%.

Publication Descriptors: *Aquatic Plants/ *Protein/ *Fiber/ Nutrients/
Forage/ Carbohydrates/ Minerals

Publication Identifiers: *Ruminants/ *Proximate Analyses/ *Digestibility/
Palatability/ Lipids/ Van Soest Analysis

I. INTRODUCTION

A. Objective of the study.

The nutritive value of aquatic plants is of interest from several standpoints. They serve as the main source of food for a variety of aquatic fauna, including the insects among the invertebrates. Diets of vertebrates such as moose, muskrat and beaver, as well as many water-fowl, are chiefly composed of aquatic vegetation. Therefore, it would appear that aquatic plants may have a valuable potential as a livestock forage.

Before practical use can be made of this fresh-water aquaculture, detailed knowledge must be known about their nutritional composition. Three previous bulletins have been concerned with their: (1) chemical composition, (2) pharmacological properties, and (3) antimicrobial effects. The purpose of this study was to determine the nutritive value of aquatic plants by determining their chemical and biochemical composition.

B. Review of Nutrient Analyses

The nutritive value of aquatic plants was determined by proximate, Van Soest and mineral analyses. A brief explanation of terms and which nutrients are determined by each analyses will be presented here. Laboratory procedures will be discussed under Materials and Methods.

1. Carbohydrates

The analysis of feeds for individual carbohydrates is a long and tedious task. Therefore, in a routine feed analysis they are determined as two groups: crude fiber (CF) and nitrogen free extract (NFE). The separation is obtained by a chemical method devised over 100 years ago by Henneburg in Germany and known as the Weende method. The Weende series of analyses is often referred to as the proximate analysis.

The proximate analysis determines moisture, crude protein (CP), crude fiber (CF), ash and ether extract (EE). Nitrogen free extract (NFE) is calculated as 100 minus the percentages of ash, crude fiber, crude protein, moisture, and ether extract.

Crude fiber is primarily defined as the structural and protective parts of the plant. Chemically, crude fiber consists of cellulose and lignin, although a considerable part of the lignin passes into the NFE. Crude fiber was originally defined as the indigestible portion of the plant. This premise has long been known to be false as herbivores partially digest cellulose.

Nitrogen free extract was originally defined as the available carbohydrates. Sugars, starches and the material classified as hemicellulose chemically defined the NFE. Since NFE is determined by difference, instead of directly, it includes all the cumulative errors of the other determinations.

Although the Weende method is an empirical one and doesn't provide for a clear separation into chemical groups, it is useful because of its quick and simple distinction between more and less digestible carbohydrates. This distinction is not absolute in that crude fiber is partially digested by the microorganisms in the digestive tract of herbivores and NFE contains complex polysaccharides (lignin) which are completely indigestible.

Because of the limitations of the Weende method, analyses have been developed which partition plant dry matter into two fractions based on nutritional availability: (1) cell wall constituents and (2) cell wall contents. The cell wall constituents or the fibrous fraction is limited in digestibility by the concentration of lignin and cellulose. The cell contents composed of lipids, soluble carbohydrates, protein and other water soluble material are highly digestible. This classification of feed constituents is known as the Van Soest method. Table 1 shows the relationship between the Weende and Van Soest analyses.

Table 1. Relationship Between Weende and Van Soest Analyses in Classifying Forage Organic Matter.^{a/}

Components	Van Soest fraction	Weende fraction	Nutritional availability	
			Ruminant	Nonruminant
Lipids	Cell contents (soluble in neutral detergent)	Ether extract	Virtually complete	Highly available
Sugars, organic acids and water solubles		Nitrogen free extract		
Starch				
Nonprotein nitrogen				
Soluble protein				
Pectin				
Hemicellulose	Cell-wall constituents		Partial	Low
Cellulose	(fiber insoluble in neutral detergent)			
Lignin				
Keratin				
Heat-damaged protein				
Protein attached to the cell wall				
Hemicellulose	Soluble in acid detergent		Partial	Very low
Heat-damaged protein	Insoluble in acid detergent (acid detergent fiber)		Indigestible	Indigestible
Lignified nitrogen compounds			Indigestible	Indigestible
Keratin			Indigestible	Indigestible
Cellulose		Crude fiber	Partial	Very Low
Lignin			Indigestible	Indigestible

^{a/} This table is taken in part from Maynard and Loosli (14) and Van Soest (24).

2. Crude Protein

Crude protein is defined as nitrogen times 6.25. The term crude protein therefore includes amino acids, amides, ammonium salts and many other non-protein nitrogen compounds in addition to true protein.

Since the microbes in the rumens of ruminant animals are able to utilize non-protein nitrogen as well as true protein for essential and nonessential amino acid synthesis, no categorizing of the nitrogen fraction was conducted.

3. Lipids

In the proximate analysis, lipids are determined by ether extraction of a moisture-free sample. Ether, in addition to lipids, extracts pigments such as chlorophyll, xanthophylls and carotene along with aromatic esters, aldehydes and traces of other substances. Thus, ether extract is not an accurate measure of the fat content of a feed.

4. Minerals

All animals must have an adequate and well balanced supply of minerals to maintain a healthy conditions. Most livestock forages contain adequate amounts of calcium and trace minerals, but lack phosphorus. The cereal grains, which are usually fed along with forages, are good sources of phosphorus and tend to compliment the forages. Ash or total mineral content, along with individual mineral analyses, were conducted so as to compare the aquatic plants with commonly fed forages.

C. Literature Review

Considerable interest in the nutritional properties of freshwater aquaculture occurred in the 1930's. This interest was prompted by drought conditions which occurred in the midwest at that time. Gortner (9), in an attempt to find a different source of forage for livestock during the drought, characterized lake vegetation as having high ash and protein contents, while being very low in crude fiber. Gortner summarized the high ash content as due to incrustations, while the high protein reflected the washing of fertile top soil into the lake. The low crude fiber content was assumed to be the result of water buoyancy replacing the need for cellulose and lignin supporting materials. Gortner (9) projected that this high protein, high lime, low fiber aquatic vegetation may make a very good "concentrate" for animal feeding.

Nelson and Palmer (17) conducted complete chemical analyses of *Elodea canadensis*, *Myriophyllum spicatum* and *Vallisneria spiralis*. Vitamin, protein, ash and proximate analyses as reported by Nelson and Palmer are shown in table 2. Nelson concluded that these 3 plants should be highly nutritive due to their high protein, high carbohydrate and low crude fiber content. The greatest difference among these 3 plants occurred in their protein quality. The protein in *Myriophyllum* and *Vallisneria* had a low digestibility and biological value, while *Elodea* has a biological value of 42 and apparent digestion coefficient of 70 percent.

Table 2. Chemical Composition of *Elodea canadensis*, *Myriophyllum spicatum* and *Vallisneria spiralis*.^{a/}

Item	<i>Elodea canadensis</i>	<i>Myriophyllum spicatum</i>	<i>Vallisneria spiralis</i>
Dry matter, %	7.52	13.32	5.15
		Dry basis	
Ash, %	21.87	13.83	15.64
Crude protein, %	26.81	25.83	15.15
Ether extract, %	3.53	2.47	4.28
Crude fiber, %	15.39	14.13	15.82
N-free extract, %	32.40	43.74	49.11
P, %	0.57	0.42	0.21
Na, %	0.50	0.75	2.39
Mg, %	0.65	0.74	0.76
K, %	3.65	1.87	6.77
Ca, %	2.80	2.77	1.55
Mn, %	0.331	0.513	0.039
Fe, %	0.408	0.066	0.045
B-carotene, mg%	22.2	21.4	17.5
Vitamin A, IU/gm	370.1	356.6	291.7
Vitamin B, Chase Sherman units/gm	3.76	2.61	1.83
Ascorbic acid, mg%	10.5	24.8	75.7
Vitamin C, IU/gm	2.1	5.0	15.1
Vitamin D, IU/gm	0.0	0.0	trace
Vitamin E, %	0.0	0.0	present
Vitamin B ₁₂ , Borguin-Sherman units/gm	8.68	7.60	5.73
Biological value of protein, %	42.71	0.0	0.0
Coefficient of apparent protein digestibility, %	70.28	27.44	28.60

^{a/}Data from Nelson and Palmer (17)

According to Nelsons data, it appears that aquatic plants are good sources of vitamins. Bailey (1) has also reported that aquatic plants are a good source of xanthophyll. He reported an average value of 380 mg xanthophyll per pound of dehydrated product. These plants also averaged 19% crude protein and 12% crude fiber. Magnesium, calcium, and phosphorus contents were also high.

Harper and Daniel (10) have shown a wide range in individual minerals among different plant species. Nitrogen and phosphorus values varied considerably as soil conditions were changed, while calcium values varied due to incrustations attached to the plant. Other workers (8, 11, 18, 19) have also reported that the composition of aquatic plants changes with: stage of maturity, depth and bottom conditions and the nutrient status of the water.

Boyd (3) reported that aquatic plants were found to be richer in crude protein than the average high quality roughage. Crude fat appeared

to vary with species, although submersed plants usually contain less fat than emergent plants. Boyd also stated that tannin percentages could be used as a digestibility as to be of little food value. Most high quality roughages contain less than 2 to 3% tannin, while most aquatic plants above 15% crude protein have tannin levels less than 5 percent.

A series of articles by Boyd (4, 6) suggested that dehydrated aquatic plants could be used for fodder, but that the time of harvesting and moisture, and crude protein contents should be given careful attention. Boyd (5) has also studied the interspecies variation in chemical composition.

Schuette, et al, (20, 21, 22, 23) determined the chemical composition of 6 aquatic plants from Lake Mendota and 1 plant from Green Lake, Wisconsin. Results of proximate and mineral analysis are shown in table 3.

Table 3. Chemical Composition of Aquatic Plants From Lake Mendota and Green Lake, Wisconsin.^{a/}

Analyses ^d	Clado- phora ^b	Myrio- phyllum ^b	Vallis- neria ^b	Potamo- geton ^c	Castalia ^b	Najas ^b	Chara ^c
Ash, %	26.53	20.72	25.18	11.42	11.21	19.16	41.22
Crude protein, %	18.19	18.75	11.80	8.02	17.38	11.62	4.50
Ether extract, %	2.00	2.44	0.73	0.91	2.54	1.63	0.76
Crude fiber, %	17.33	15.01	14.00	18.85	19.70	18.41	9.32
Pentosans, %	9.10	7.70	6.88	10.50	11.95	8.45	4.70
N-free extract, %	26.85	35.38	41.41	50.30	37.22	40.23	39.50
SiO ₂ , %	7.08	1.96	5.45	0.78	0.32	1.89	0.83
Fe ₂ O ₃ , %	0.49	0.08	0.81	0.11	0.09	0.40	0.06
Al ₂ O ₃ , %	1.30	4.25	0.57	0.23	0.08	0.25	0.81
Mn ₂ O ₄ , %	0.75	trace	0.52	0.08	0.09	0.05	0.08
CaO, %	3.25	4.28	8.16	3.38	1.89	8.56	3.78
MgO, %	1.62	1.34	1.87	1.38	0.75	1.61	1.19
Na ₂ O, %	--	--	0.81	0.26	1.20	1.05	0.35
K ₂ O, %	--	--	5.48	2.08	2.72	2.19	0.58
Cl, %	0.14	1.62	1.32	0.56	0.40	0.51	0.29
S, %	0.64 ^e	1.32 ^e	0.85	0.82	0.37	0.48	0.27
P, %	0.32 ^f	1.17 ^f	0.23	0.13	0.27	0.30	0.06

^a Results tabulated from data presented by Schuette, et al (20, 21, 22, 23)

^b Plants from Lake Mendota

^c Plants from Green Lake

^d All analyses presented on an air dry basis

^e Values reported as percent SO₄

^f Values reported as percent P₂O₅

II. MATERIALS AND METHODS

A. Plant Collection and Identification

Plant materials used in this study were collected from various lakes in Minnesota during August and September 1968. Specimens representing the collections were pressed between blotters and carefully dried at 50°C. Taxonomic identification was made by Dr. Robert C. Bright, Assistant Professor in Limnology, University of Minnesota, in the field and later confirmed by Dr. Gerald B. Ownbey, Professor and Curator of Herbarium, University of Minnesota. One voucher herbarium from each plant has been deposited at the Botanical Museum, Harvard University, Cambridge, Massachusetts. Representative plants were also preserved in jars containing water, 95% ethanol, formaldehyde (6:3:1) and 5% glycerine. Copper ion at 0.02 ppm was added to help retain the original color of plants.

A list of those plants collected, representing one algae, seventeen monocots and four dicots, is shown in table 4. The gross appearance and general ecology of the plants are discussed alphabetically. Numbers preceding the genus species names will be utilized in tables throughout this dissertation instead of the full names of plants.

Table 4. List of Plants Collected.^a

Class	Family	Genus	Species	Authority	Lake ^b
Algae	Characeae	Chara	vulgaris		Melissa*
Monocoty- ledons	Alismataceae	Sagittaria	cuneata	Sheldon	Itasca
	Alismataceae	Sagittaria	rigida	Pursh	Minnetonka
	Araceae	Calla	palustris	L.	Itasca
	Cyperaceae	Carex	stricta	Willd	Minnetonka
	Cyperaceae	Eleocharis	smallii	L.	Pine*
	Gramineae	Zizania	aquatica	L.	Itasca
	Hydrocharitaceae	Anacharis	canadensis	(Michx.) Rich.	Minnetonka
	Hydrocharitaceae	Vallisneria	americana	Michx.	Pine*
	Lemnaceae	Lemna	minor	L.	Itasca
	Najadaceae	Potamogeton	amplifolius	Tuckerm.	Pine*
	Najadaceae	Potamogeton	natans	L.	Itasca
	Najadaceae	Potamogeton	pectinatus	L.	Minnetonka
	Najadaceae	Potamogeton	richardsonii	(Benn.) Rydb.	Melissa*
	Najadaceae	Potamogeton	zosteriformis	Fern.	Minnetonka
	Sparganiaceae	Sparganium	eurycarpum	Engelm.	Pine*
Sparganiaceae	Sparganium	fluctuans	Robins	Pine*	
Typhaceae	Typha	augustifolia	L.	Pine*	
Dicotyle- dons	Ceratophyllaceae	Ceratophyllum	demersum	L.	Minnetonka
	Haloragaceae	Myriophyllum	exalbescens	(Fern.) Jeps.	Minnetonka
	Nymphaeaceae	Nuphar	variegatum	Engelm.	Minnetonka
	Nymphaeaceae	Nymphaea	tuberosa	Paine	Minnetonka

a Total plants were collected and studied except in case of *Nymphaea tuberosa* and *Nuphar variegatum* where only stems and leaves were collected.

b Asterisk - Collection made in September 1968; Non-asterisk - Collection made in August, 1968.

1. *Anacharis canadensis*: Submerged. Stems are so branched that they often form large masses. Leaves (2 mm) are whorled, usually three in each whorl.
2. *Calla palustris*: Emergent. Perennial herbs with petioled basal leaves and solitary spathes, leaves are either ovate or subrotund (5-10 cm).
3. *Carex lacustris*: Emergent plants grown in swamps and marshes, leaves are stout usually with conspicuous cross-serrate and with numerous elevated nerves.
4. *Carex stricta* (Niggerhead): Emergent plants grown in marshes, and swamps, stems are in a dense tussock and sheaths smooth on the side opposite the leaf.
5. *Ceratophyllum demersum* (Coontail): Submerged. Stems with whorls of stiff, forked leaves and leaflets with toothed or serrated margins on one side only. They are freely branched, forming large masses and are found in quite water.
6. *Chara vulgaris* (Muskgrass): Submerged green algae. The plant possesses a musky odor and is made up of stems bearing whorled, smooth brittle branches, easily snapped with a slight pressure.
7. *Eleocharis smallii* (Spike rush): Erect and emergent. Rhizomes are often conspicuous and the leaf sheaths are obliquely truncate and firm.
8. *Lemna minor* (Duckweed): They represent the smallest of the aquatic plants (2-4 x 1.5-3 mm). They have no true leaves nor stems, but the floating green plant body usually possessing a tiny root that penetrates the water. They may grow sufficiently dense to prohibit sunlight from penetrating the water- thus killing algae and other aquatic plants.
9. *Myriophyllum exalbescens* (Fern) (Water-milfoil): Submerged herbs in quiet water. Leaves are feather-like with one central axis and branches in whorls around the stem.
10. *Nuphar variegatum* (Yellow water lily): Floating leaves are heart-shaped with veins radiating from the mid-rib nearly to the margin without forking. The floating flowers are attractive and yellow.
11. *Nymphaea tuberosa* (Water lily): Floating circular leaves possess much-forked veins radiating to the margin. Flowers with green sepals and white petals and are long-petioled (usually striped).
12. *Potamogeton amplifolius*: Those belonging to *Potamogeton* sp. (Pondweed) are plants with usually both floating and submerged leaves scattered along the stem, and with midribs evident when held against bright light. For *Potamogeton amplifolius*, the submerged leaves (8-20 cm) are falcately folded, and floating leaves (5-10 cm) are elliptic.

13. Potamogeton pectinatus: Leaves are all submerged, narrowly linear (3-10 cm x 0.5-1.5 mm). Lower part of stem is simple or sparingly branched with elongated internodes, while the upper part is freely dichotamously branched. Therefore, the appearance of a bunch of rounded threadlike leaves as they float in the water is very characteristic.
14. Potamogeton richardsonii: Stems are freely branched and densely leafy. Leaves are lanceolate to nearly linear (3-12 cm x 5-20 mm).
15. Sagittaria cuneata (Water plantain): Emergent plants rooted to the substratum and extending upward out of the water. Leaves are long-petioled and sagittate with variable sizes (6-18 x 1-10 cm).
16. Sagittaria rigida (Stiff wapato) Emergent plant with leaf blades scarcely wider than the petiole, erect and stiff. Usually found in somewhat limey soils.
17. Sparganium eurycarpum: Stout and emergent (5-12 dm). Leaves are shallowly and broadly triangular in cross section (8 dm x 6-12 mm). They grow in mud or shallow water.
18. Sparganium fluctuans: Floating plants with slender, elongate stems (15 dm), leaves are flat, thin, alternate, translucent with cross reticulate and with sheathing bases.
19. Typha augustifolia (Cat-tail): Erect, colonial herbs with long, linear leaves sheathing at the base. Flowers are densely crowded in long, cylindric, terminal spikes. They grow in marshes.
20. Vallisneria americana: Perennial herbs with very thin, long, ribbon-like, basal submersed leaves (2 m x 3-10 mm). They are found in quiet water area.
21. Zizania aquatica (Wild rice): Robust annual grasses usually 2-3 m high. They are found in marshes and shallow water with tall culms and wide flat blades.

B. Methods of Analyses

Plants listed in Table 4 were harvested and allowed to air dry (app. 90% DM). Samples were ground through a 20 mesh screen and stored in sealed bottles. Analyses were conducted using A.O.A.C. methods with permissible variations.

1. Proximate Analysis. Procedures and Reagents. Dry matter and Ash

Procedure:

1. Weigh approximately 2 gm. of sample (exact wt. must be recorded) into oven dry silica glass ashing crucibles.

2. Dry to a constant weight (12-24 hr.) at 95-100 degrees C. in still heat oven or if forced air or vacuum oven is available drying temperature can be decreased to 70-75 degrees C.
3. Transfer crucibles directly from oven to dessicator, cool and weigh as rapidly as possible to prevent moisture uptake. Do not leave dessicator open while weighing.
4. Place crucibles in muffle furnace and set at 600 degrees C. Ash at this temperature for 2-4 hours.
5. Remove crucibles and weigh as described in 3.

Calculations:

$$\% \text{ D.M.} = \frac{\text{oven dry sample weight}}{\text{sample weight}} \times 100$$

$$\% \text{ Ash (as received basis)} = \frac{\text{ash weight}}{\text{as received sample wt.}} \times 100$$

$$\% \text{ Ash (D.M. basis)} = \frac{\text{ash weight}}{\text{oven drv sample wt.}} \times 100 \text{ or } \frac{\% \text{ ash as received basis}}{\% \text{ drv matter}} \times 100$$

Ether Extract

Reagents.

1. Diethyl ether (C.P. grade, anhydrous)

Procedure.

1. Weigh 2 grams air dry sample, into tared 15 cm filter paper (number 1) and paper clip.
2. Fold filter paper to seal sample into center of paper and clip shut. (Be sure sample will not fall out).
3. Dry at 100°C for 12 hr., cool in dessicator and weigh.
4. Arrange approximately 40 sample packets into a 3" x 12" cylinder made of cheese cloth. Seal both ends and side of cheese cloth cylinder with string.
5. Place cheese cloth bag in soxhlet extractor and extract for 48 hours. Invert after 24.
6. Remove samples, allow ether to evaporate under a hooded area, place in drying oven and dry as in step 3.

$$\% \text{ EE (air dry)} = \frac{\text{weight of oven dry sample + paper + clip} - \text{clip after extraction}}{\text{weight of sample + paper + paper clip} - \text{paper \& clip}} \times 100$$

$$\% \text{ EE (D.M. basis)} = \frac{\% \text{ EE Air dry}}{\% \text{ D.M.}} \times 100$$

Crude Fiber:

Reagents:

1. 1.25% (0.255 N) H_2SO_4 .
2. 1.25% (0.313 N) NaOH .

3. Gooch crucibles, High form, 30 ml capacity with coarse porosity.
4. Filtering cloth - dress linen with 45 threads/inch.

Procedure:

1. Place 200 ml. of 1.25 % H₂SO₄ into digestion beakers and bring to boil. Put 200 ml. of 1.25% NaOH into another set of beakers and place these on the crude fiber apparatus under medium heat.
2. Place ether extracted samples in empty digestion beaker (3rd set) and slowly pour hot acid over sample. Put beaker back on digestion heater and bring to low boil. Record time and boil for 30 minutes; periodically washing sample from sides of beaker, if necessary. Subsequent samples should be started at certain time intervals (i.e. 5 minutes), to allow time for changes required in step 4.
3. After boiling for 30 minutes, remove beaker and filter contents through a linen filtering cloth placed over a suction funnel. Suction can be supplied by water faucet suction or vacuum line. Rinse beaker with warm water, pouring all rinse over the filtering cloth. Rinse sample with water until free from acid. There is no set volume of rinse to be used. Three or four rinsings should be adequate.
4. Pour 200 ml. of the preheated 1.25% NaOH into a wash bottle; set the base beaker aside or refill with base. Rinse sample off the filtering cloth into the same beaker with the hot base. Be sure that all of the sample is rinsed off the cloth. The last part of the base can be used to rinse any residual sample from the side of the beaker.
5. Place beaker containing base and sample back on digestion rack, bring to a slow boil and boil for 30 minutes. Remove subsequent samples and handle in a similar manner.
6. After samples have boiled in base for 30 minutes, filter through Gooch crucibles under suction. Carefully rinsing all fiber from beaker. Rinse fiber thoroughly with hot water.
7. Leave sample on suction until water is removed.
8. Dry sample in drying oven until oven dry. This usually can be accomplished by drying overnight.
9. Remove samples, place in dessicator, cool and weigh.
10. Ash samples in muffle furnace, cool, and weigh.
11. Record loss in weight as crude fiber weight.

Calculations:

$$\% \text{ Crude fiber (air dry basis)} = \frac{\text{crude fiber weight}}{\text{air dry sample wt.}} \times 100$$

(sample weight ether extraction)

$$\% \text{ Crude fiber (D.M. basis)} = \frac{\% \text{ crude fiber (air dry basis)}}{\% \text{ dry matter}} \times 100$$

or $\frac{\text{crude fiber weight}}{\text{D.M. weight (sample from ether extract)}} \times 100$

Crude Protein:

Reagents:

1. Concentrated H₂SO₄
2. Catalyst - 9 parts by weight Na₂SO₄ and 1 part by weight CuSO₄.
3. Indicator - 0.5 g. methylene blue and 0.75 g. methyl red in 600 ml. ethanol.
4. Saturated NaOH solution. Pelleted or flaked NaOH and distilled H₂O should be used. Sp. gr. should be 1.36 or higher.
5. 2% boric acid solution. 2 g. boric acid crystals dissolved in distilled water and diluted to 100 ml.
6. Dilute HCL - 0.2 - 0.1 N HCL, but N must be known exactly.
7. Glass beads.
8. Granulated zinc - 20 mesh

Procedure:

1. Weigh approximately 2 g. sample into Kjeldahl flask. Add about 10 g. catalyst, 2-3 glass beads and 40 ml. conc. H₂SO₄.
2. Digest to clear blue-green color, then boil for an additional 30 minutes. Digestion requires 2-4 hours, depending on type of sample.
3. Cool to room temperature; dilute with 250 ml. distilled water.
4. Cool and cautiously layer sufficient concentrated NaOH on the bottom of the flask to neutralize acid and make contents highly basic. 70-100 ml. is usually sufficient depending on amount of acid used in step 1.
5. Add small amount of granulated zinc, connect to distillation apparatus, gently mix flask contents and distill into 50 ml. 2% boric acid solution containing 2-3 drops of indicator. The containers to collect the distillate should be in place before the flasks are placed on the distillation apparatus.
6. Collect approximately 200 ml. of distillate.
7. Titrate the distillate to neutrality with the dilute HCL.
8. Accurately record amount of HCL required for neutralization.
9. Run a blank - include all reagents used for sample. Note - approx. 20 ml. more saturated NaOH is required to neutralize the blank before distillation.

Calculations:

$$\% \text{ Protein (air dry basis)} = \frac{(\text{ml HCL} - \text{ml blank}) (\text{N HCL}) (.014) (6.25) \times 100}{\text{sample weight}}$$

$$\% \text{ Protein (D.M. basis)} = \frac{\% \text{ protein (air dry basis)}}{\% \text{ dry matter}} \times 100$$

% N is calculated by above manner, except the factor 6.25 is omitted from the equation.

Nitrogen-free Extract:

$$\% \text{ N.F.E. (air dry basis)} = \% \text{ DM} - [\% \text{ E.E.*} + \% \text{ C.F.} + \% \text{ ash} + \% \text{ C.P.}]$$

* all percentages are air dry basis

$$\% \text{ N.F.E. (dry matter basis)} = \frac{\% \text{ N.F.E. air dry basis}}{\% \text{ D.M.}} \times 100$$

2. Van Soest Analysis. Procedures for Lignin, Cellulose, Hemicellulose

Use only samples that have been dried at less than 65° C. and ground through 20-30 mesh (1 mm).

Reagents:

1. Acetone - color free
2. Acid detergent solution. Add 20 gm. cetyl trimethylammonium bromide (tech. grade) to 1 l. of 1 N. H₂SO₄.
3. Reagent grade decahydronaphthalene.
4. Neutral detergent solution. To 1 l. of distilled water add 30 gm. USP sodium lauryl sulfate, 18.61 gm. Na₂H₂EDTA.2H₂O, 6.81 gm. reagent grade sodium borate decahydrate, 4.56 gm. reagent grade anhydrous Na₂HPO₄, 10 ml. 2-ethoxyethanol (purified, ethylene glycol, monoethyl ester) mix and adjust pH to 7.0.
5. Anhydrous sodium sulfite, reagent grade.
6. Sulfuric acid, 72%. Standardize to specific gravity of 1.634 at 20°C.

Procedures:

1. Neutral Detergent Fiber (cell walls or cellulose + lignin + hemicellulose)

1. Place 0.5 to 1.0 gm. sample in reflux container (sample must have been dried at less than 65°C. and ground through 1 mm. screen.)
2. Add 100 ml. neutral detergent solution, 2 ml. decahydronaphthalene and 0.5 gm. Na₂SO₃ (scoop measure).
3. Reflux for 60 min. after onset of boiling.
4. Pour into tared non-dried No. 54 filter paper and rinse reflux container with minimum amount of 80-90°C water. A filter paper blank should also be carried through steps 4-7.
5. Wash with water, then 2 X with acetone.
6. Cool, weigh (material remaining is NDF)

$$\% \text{ cell walls} = \frac{\text{wt. dried paper and HDF} - \frac{\text{final weight of paper blank}}{\text{weight of paper used with sample}} \times \text{sample weight}}{\text{sample weight}}$$

2. Acid detergent fiber (cellulose + lignin)

1. Add 1-2 gm. dry sample (dried at 65°C. or less) to reflux container.
2. Add 100 ml. acid detergent solution and 2 ml. decahydronaphthalene.
3. Reflux for 60 min. after onset of boiling.
4. Filter into tared, tall form, 30 mm., coarse porosity sintered glass crucibles.
5. Wash two times with 90-100°C. distilled water, wash sides.
6. Dry at 100°C and weigh (material remaining is ADF)

$$\% \text{ ADF} = \frac{(\text{wt. crucible and residue} - \text{wt. crucible}) 100}{\text{wt. sample dry matter}}$$

3. Acid detergent lignin:

1. Use crucible containing dried acid detergent fiber.
2. Cover contents of crucible with 10°C. 72% H₂SO₄.
3. Stir with glass rod to break up lumps.
4. Fill half-way with 72% H₂SO₄ and stir at hourly intervals for 3 hr. Temperature must be held at 20-23°C.
5. Refill with acid as it drains away. Filter and wash free of acid - rinse and remove stirring rod.
6. Dry crucible at 100°C. - weigh after cooling (material remaining is lignin).
7. Ignite at 500°C. for 2 hour.
8. Cool weigh.

$$\% \text{ acid detergent lignin} = \frac{(\text{Wt. lost upon ignition}) (100)}{\text{sample weight}}$$

$$\% \text{ cellulose} = \% \text{ acid detergent fiber} - \% \text{ acid detergent lignin}$$

$$\% \text{ hemicellulose} = \% \text{ NDF} - \text{ADF}$$

3. Mineral Analysis.

Ashed material obtained for dry matter and ash analysis in the proximate analysis was diluted with 5 ml of solution containing .5% Li - 1.5% HCl (26.5 gms Li₂CO₃, analytic grade; dissolved in 150 ml of concentrated HCl and diluted to 1 liter).

Note: Ash and digest samples in Silica glass crucibles only, as porcelain crucibles contaminate the samples. Allow the added samples to stand for 15 minutes while the ash dissolves. The dissolved ash is then decanted into polyethylene bottles, sealed and stored until ready for analysis. Approximately 2 ml of sample is required for analysis.

A Jarrell - Ash, model 66-000 direct reading emission spectrograph was used to obtain the following elements; P, K, Ca, Na, Mg Sr Fe Zn Cu, Mo Mn and B.

III. Results and Discussion

A. Proximate Analysis

Results of duplicate analysis for proximate components are presented in table 5. Alfalfa hay, average analysis, is listed as a comparison.

1. Dry Matter

Initial dry matters on individual plants were not taken. A representative aggregate sample indicated the dry matter ranged from 5 to 20 % or 10 pounds of wet plants yields 0.5 to 2 pounds of dry plants. Literature values ranged from 5 to 24 percent with the majority falling between 10 to 15 percent dry matter (1,3,9,15,17,18).

Table 5. Proximate Analyses of Aquatic Plants and Alfalfa Hay^a

Plant identification ^b	Crude protein	Ash	Ether extract	Crude fiber	Nitrogen free extract
	%				
Alfalfa hay, all analysis ^c	16.91	8.84	2.10	31.60	40.55
1	14.70	4.49	1.07	11.39	68.35
2	13.93	1.28	4.37	17.40	63.02
3	8.63	0.96	2.96	27.41	60.04
4	9.94	0.90	0.89	29.41	58.86
5	17.00	2.18	1.51	15.20	64.11
6	7.92	5.62	0.12	7.65	77.56
7	5.78	1.64	1.09	27.03	64.46
8	17.86	1.61	2.19	11.82	66.52
9	12.28	1.67	0.21	13.49	72.35
10	15.70	0.96	2.46	23.13	57.75
11	19.88	0.80	2.40	16.33	60.59
12	14.36	2.39	1.53	15.72	66.00
13	14.05	3.22	0.09	15.64	67.00
14	11.20	2.62	1.13	19.11	65.94
15	21.81	1.91	1.37	17.34	57.57
16	14.78	2.27	1.79	23.69	57.47
17	7.60	2.47	0.71	20.57	68.65
18	13.19	1.04	1.67	14.42	69.68
19	6.92	0.93	0.98	27.49	63.68
20	15.15	3.10	0.97	27.32	53.46
21	9.88	2.44	1.10	27.43	59.15

a All analyses are on a dry matter basis.

b See Section II. A.

c From Morrison (15).

2. Crude Protein

Crude protein values ranged from 5.78% for *Eleocharis smalli* to 21.81% for *Sagittaria cuneata*. These analyses indicate that many aquatic plants are lower in crude protein than alfalfa hay. Since rations for ruminant animals should contain about 12 percent protein, dried or partially dried aquatic plants would be an adequate protein source, if the protein is at least 60 percent digestible.

Crude protein values reported by other workers for some of the same aquatic plants are compared to values from the present study in table 6. Variation in crude protein values may be due to environmental variation. Harper, et al, (10) have reported that *Potamogeton folius* was low in nitrogen when grown in sandy soil and high in nitrogen when grown in dark colored, high organic soil. Washing of fertile top soil into lakes and streams apparently raises the nitrogen content of plants. Water conditions are also reported to effect nitrogen content of plants.

Other workers have reported that crude protein varies with state of maturity (1, 3, 11). First cutting plants contain higher percentages of crude protein, but yield less than second cutting plants.

Table 6. Crude Protein Contents of Aquatic Plants as Reported by various Authors.

Genus	Species	Present Study	Gortner (9)	Boyd (3)	Bailly Harper & Daniel (10)	Crouch (7)
- - - - - % crude protein - drv matter basis - - - - -						
Potamogeton	amplifolius	14.36	12.04			
	richardsonii	11.20	12.26			
	pectinatus	14.05	19.03	21.7	12.36	
Ceratophyllum	demersum	17.00		2.17		13.3
Myriophyllum	exalbescens	12.28			21.1	15.8
Sagittaria	cuneata	21.81			14.06	

3. Ash.

Considerable variations in ash contents of aquatic plants occurs, probably due to the amount of crustations, mud and soil clinging to the plants. Plants in the present study were carefully hand washed so as to remove any crustations, soil or mud. Plants directly harvested and dried to a proper moisture level will contain higher ash levels due to soil and crustations.

In the present study, all aquatic plants ash values were less than that of alfalfa. *Chara vulgaris* (a submersed plant) at 0.80 percent ash was the lowest. Submersed plants tended to contain more ash than emergent plants.

Ash values inversely reflect the caloric content of forages; the higher percent ash the lower the gross calories. Boyd (3) reported caloric values ranging from 2.58 to 4.30 Kcal/g and that there was a negative correlation between ash and caloric values. When energy values were calculated by Boyd on an ash free basis, all values were about 4.30 kcal/gram.

4. Ether Extract

Ether extract values of aquatic plants (table 5) appear to approximate those of forages, although there was considerable variation among species. Submersed plants appeared to contain less ether extractable material than emergent plants.

5. Crude Fiber

Crude fiber values ranged from 7.65% for *Chara vulgaris* to 29.41% for *Carex stricta*. Considerable variation existed among species (Table 5). Fiber values were all lower than alfalfa hay. A 28% crude fiber value is considered good for alfalfa and all except one aquatic plant had a value lower than 28 percent.

Water bouyancy probably replaces some of the need for structural material in aquatic plants, thus resulting in lower crude fiber values than that of terrestrial plants. Curde fiber contents presumably would increase with maturity, much as in the forage plants.

6. Nitrogen Free Extract

Nitrogen free extract contents of all aquatic plants were higher than that for alfalfa. These lower NFE values are probably a reflection of the lower crude fiber values.

B. VanSoest Analysis

Results of the VanSoest series of analyses on duplicate samples are shown in table 7. *Chara vulgaris*, at 16.24%, is low in cell walls, while *Zizania equatica* is high at 62.21% and closely resembles corn silage in total NDF value. Neutral detergent fiber or cell wall content is a measure of the structural portion of plants and is chemically composed of cellulose, lignin and hemicellulose. The carbohydrates of the cell wall are not completely digestible in ruminant animals because of lignification. The soluble material in the NDF determination (100 - %NDF) is almost completely digestible in both ruminants and nonruminants.

Acid detergent fiber (ADF) or lignocellulose (lignin + cellulose) analyses of the aquatic plants showed that lignin values plus cellulose values were similar to those for alfalfa. However, several plants had higher ADF values than does alfalfa.

C. Mineral Analysis

1. Macro Minerals

Macro mineral contents of the aquatic plants are shown in table 8. With an average of 1.83 percent calcium and .25 percent phosphorus, the aquatic plants are similar to terrestrial forages. Although *Anacharis canadensis*, *Potamogeton pectinatus* and *Potamogeton richardsonii* are very good sources of calcium at 5.50, 3.76 and 4.03 percent, respectively. Potassium and magnesium values are about the same in aquatic plants as in land forages, although there is probably more variability among the aquatic plant species than in the common forages. Sodium was generally higher in the aquatic plants, with an average value of .3 percent compared to .16 percent for alfalfa. The values reported here approximate those reported by other workers for the same plant species. (7, 10, 17, 20-23).

2. Trace Minerals.

Aquatic plants appear to be excellent sources of the trace minerals (table 9). Iron values were very high, with *Sagittana rigida*, *Sparganium eurycarpum* and *Zizania equatica* being over 2,000 ppm of iron. Zinc and manganese contents of the aquatic plants were also higher than that for alfalfa. Molybdenum values tend to be high and could partially cause some interference with copper metabolism, as copper values were lower than that of alfalfa.

Table 7. Results of VanSoest Analyses of Aquatic Plants and Alfalfa Hay^{a,b}

Plant identification ^c	NDF	ADF	ADL	Hemicellulose	Cellulose
				(NDF-ADF)	(ADF-ADL)
	%				
Alfalfa	40.2	25.1	5.3	15.1	19.8
1	26.85	26.04	3.79	0.81	22.25
2	26.88	26.47	8.13	0.41	18.34
3	57.24	39.41	5.23	17.83	34.18
4	59.68	42.87	8.14	16.81	34.73
5	42.26	35.53	8.20	6.73	27.33
6	16.24	14.85	2.26	1.39	12.59
7	59.10	42.24	4.84	16.86	37.40
8	35.84	18.83	4.63	17.01	14.20
9	42.24	30.83	4.31	11.41	26.52
10	33.58	29.69	7.36	3.89	22.33
11	23.72	20.65	4.95	3.07	15.70
12	37.58	36.99	9.89	0.59	27.10
13	48.50	32.67	7.29	15.83	25.38
14	40.78	34.14	8.60	6.64	25.54
15	33.35	29.49	3.09	3.86	26.40
16	39.14	39.07	11.58	0.07	27.49
17	60.22	42.16	4.73	18.06	37.43
18	44.18	20.11	3.39	24.07	16.72
19	58.16	38.97	8.06	19.19	30.91
20	41.04	28.80	3.30	12.24	25.50
21	62.21	53.87	12.13	8.34	41.74

a Values presented by VanSoest (24). Crude fiber value of this alfalfa was 23.5% .

b All analyses are on a dry matter basis.

c See Section II A.

Table 8. Macro Mineral Contents of Aquatic Plants and Alfalfa Hay^a

Plant identification ^b	Ca	P	K	Na	Mg
	%				
Alfalfa hay ^c	1.64	0.26	1.77	0.16	0.32
1	5.50	0.02	1.09	0.517	0.560
2	1.02	0.60	3.19	0.128	0.298
3	0.32	0.17	0.72	0.023	0.138
4	0.28	0.17	0.52	0.020	0.140
5	2.46	0.22	1.29	0.336	0.686
6	0.42	0.19	0.84	0.176	0.085
7	1.95	0.17	1.00	0.759	0.399
8	2.58	0.17	1.20	0.755	0.457
9	0.92	0.26	1.35	0.766	0.205
10	0.52	0.23	1.62	0.508	0.151
11	2.76	0.13	1.56	0.165	0.440
12	1.01	0.83	2.91	0.172	0.232
13	3.76	0.34	1.99	0.107	0.200
14	4.03	0.17	1.33	0.139	0.500
15	0.78	0.55	2.82	0.391	0.354
16	0.99	0.31	1.82	0.243	0.432
17	0.59	0.29	1.12	0.097	0.166
18	0.77	0.27	2.11	0.397	0.264
19	0.69	0.17	0.88	0.117	0.177
20	1.82	0.16	3.75	0.571	0.298
21	0.93	0.17	0.62	0.250	0.295

a All analyses are on a dry matter basis.

b See Section II A

c Values reported by NAS-NRC (16).

Table 9. Trace Mineral Contents of Aquatic Plants and Alfalfa Hay^a

Plant identification ^b	Fe	Zn	Cu	Mn	Mo
	ppm				
Alfalfa hay ^c	200	17.0	13.7	52	--
1	10	0.0	0.0	128	9.4
2	1231	465.5	5.7	498	23.8
3	522	19.7	5.8	203	10.1
4	861	22.6	5.0	185	15.2
5	546	48.1	6.1	281	18.9
6	1586	37.8	8.8	329	32.6
7	534	42.5	5.6	175	18.9
8	248	48.6	6.6	103	13.2
9	243	87.6	4.6	190	9.7
10	583	18.7	7.2	115	10.2
11	391	97.8	7.7	112	14.4
12	1651	135.1	7.9	595	41.0
13	1171	84.3	8.2	535	21.0
14	218	16.5	8.3	109	11.6
15	1904	81.5	11.8	161	28.2
16	2083	145.0	165.6	236	30.2
17	2056	37.6	8.9	435	29.6
18	342	166.8	3.6	484	13.6
19	1647	48.4	5.8	502	23.6
20	323	53.0	6.4	176	12.8
21	2112	83.6	12.7	189	36.5

a All analyses on a dry matter basis.

b See Section II A.

c Values reported by NAS-NRC (16).

The mineral composition of aquatic plants is influenced by soil and water conditions (4, 10). Stage of maturity may also alter mineral composition. It thus appears, that for a given aquatic plant mineral analyses will be quite variable. However, it appears that aquatic plants are equal to or greater than conventional forages in mineral composition. Nelson (17) reported that sulfur may be limiting in aquatic plants as sulfur containing amino acids were almost nonexistent.

IV. CONCLUSION

The results of these analyses suggest that aquatic plants may be a useful forage for ruminants. Although considerable variation existed among the various species studied, many of the plants were high in crude protein and low in crude fiber indicating a high nutritive value. Also, estimates of hemicellulose, cellulose and lignin content suggested that many of the plants should be highly digestible. Van Soests estimated apparent digestibilities, calculated from NDF, ADF and ADL contents, averaged 63 percent, a respectable forage digestion coefficient.

Although the aquatic plants appear to be highly digestible, palatability must also be considered. Crouch (7) has fed dehydrated aquatic plants to finishing steers and found satisfactory performance when they were fed at low levels. When fed at high levels palatability problems developed. Studies are now underway to investigate this problem of palatability more closely.

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