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Structural Constituents of the Seagrass *Posidonia australis*

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Large amounts of seagrass, *Posidonia australis*, wash onto beaches in South Australia each year, causing substantial environmental problems. It was of interest to assess the potential for an economic use of this seagrass—such as for animal nutrition. Structural constituents of *P. australis* (green, freshly deposited, and both washed and unwashed samples from dried deposits on the beach) were examined and compared. Glucose, galactose, and mannose were the dominant sugars (>10 g kg^{-1} of dry matter) in the soluble fraction of nonstarch polysaccharides in all seagrass forms. The content of the insoluble constituents of the nonstarch polysaccharides was significantly higher than soluble nonstarch polysaccharide constituents ($P < 0.01$). Data showed that the major constituents of the *Posidonia* cell wall are cellulose and lignin (190−209 and 145−154 g kg^{-1}, respectively). The crude protein content of *Posidonia* ranged from 54 to 61 g kg^{-1}. Results showed no biologically significant compositional differences between the four different forms of seagrass tested. Dry, unwashed seagrass, which is readily available in large quantities and easily harvested, may have potential as a foodstuff for ruminant animals.

KEYWORDS: Seagrass; animal nutrition; cell wall composition; nonstarch polysaccharides

INTRODUCTION

The ocean, which covers about 71% of our planet's surface, supports many different kinds of plant life, including various types of large algae, known as seaweeds, which grow freely in shallow waters around the world. Also conspicuous on many coasts are the marine angiosperms, comprising seagrasses and saltmarsh plants, and to a lesser extent the marine lichens (*1*−*3*). Throughout the world, including Australia, marine flora is dominated by algae and seagrasses (*4*). There are about 8000 known species of seaweed and seagrasses along the world's coastlines, and they extend out into water as deep as 270 m. The annual global seaweed harvest amounts to about 3×10^6 tons of algal fresh weight (*5*, *6*).

Australia presents a special case in terms of seagrass distribution as it has the highest number of species in the world. The Australian aquatic angiosperms are without doubt the most productive plants on the earth. In fact, the productivity of some species of seagrass may be considerably higher than the growth rate of normal pasture in the southeast of Australia (9−18 tons ha^{-1} yr^{-1}) with 430 mm of annual rainfall (*7*, *8*).

There are currently approximately 154 different species (*9*), including invertebrates and fish, which are known to consume living seagrass. The intensity of grazing varies with locality. Grazing on live seagrass appears more common in tropical waters, such as in the Caribbean (*10*). Seagrasses, therefore, could potentially offer nourishment to land-based herbivores as well, including both ruminants and nonruminants.

A number of studies have been carried out on the structure and composition of various species of seagrass. According to Brun et al. (*11*), the cell wall from rhizomes of *Halophila ovalis* and *Halodule uninervis* have noncellulosic polysaccharides containing mainly glucose and arabinose, with only a very small amount of pectin. The lignin consists mostly of nonconjugated phenols. Most seagrasses have leaf sheaths, and these are clearly differentiated from leaf blades and enclose the young, developing leaves. The fiber bundles of the sheath are lignified, and because of this they persist on the rhizomes long after the other tissues of the leaf sheath have rotted away (*12*, *13*). It has been observed that some species of seagrass have energy and nutrient levels similar to those of poor forage, and their nutritional components vary with season, species, and age (*14*−*16*). The results of several studies on the composition of different types of marine plants have determined that seagrasses have the third highest organic matter and energy content of all marine plants tested (*17*, *18*).

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Seagrasses have been regarded as an environmental problem in many parts of the world (*19*). For example, on southern Australian beaches hundreds of tons of seagrass, *Posidonia australis*, are massed each year by the action of waves, and local residents consider that this causes a variety of environmental problems, such as encouraging proliferation of hazardous insects (primarily mosquitoes) and causing navigation and fishing problems.

In spite of a significant amount of research being carried out on different species of seagrass throughout the world, there is little information comparing the nutritive value of different collected forms of seagrass, such as *P. australis*, which could possibly be used for animal nutrition (*20*).

A major characteristic of ruminant nutrition in Australia is its dependence on pasture forage (*21*, *22*). It is already well documented that annual pastures in Southern Australia have declined in productivity and quality in recent years, primarily due to the loss of leguminous plants in the sward (*23*, *24*). In order to reduce the dramatic effects of overgrazing, especially during the dry season, on both pasture quality and soil erosion, and concomitant declining animal body weight and productivity and high stock death rates, the use of supplementary protein and energy sources has become routine practice. One alternative protein and energy source that can be considered is marine plant life.

The aim of the work described in this paper was primarily to estimate the chemical composition and structural constituents of different collected forms of *Posidonia*. This information could then be used to determine the collection form most appropriate for use as a nonconventional foodstuff in animal nutrition.

MATERIALS AND METHODS

Collection of Plant Material. Four different forms of seagrass, *P. australis*, were collected from the beach at Kingston SE, South Australia, in midsummer, including (I) *P. australis* that was green in color, harvested directly from the sea at a maximum depth of 1 m; (II) fresh *P. australis*, collected from the edge of the beach, as close as possible to the water, that seemed to have been amassed by recent wave action and the color of which was mostly brown; (III) dry and washed *P. australis* collected from the beach, above the water-line, and washed three times in tap water the day after collection in order to remove surface sand, dirt, and other contaminants; (IV) dry but unwashed *P. australis*, which was used as collected, i.e., as for (III) but without washing.

Sample Preparation. After collection, each of the different forms was separately sun-dried for 24 h and then further dried in an oven at 60 °C. Approximately 500 g of each sample of plant material was ground through a 1 mm screen and further mixed, and a 200 g subsample was placed in an airtight plastic container for later chemical analysis.

Analytical Techniques. Ground samples were analyzed for nonstarch polysaccharides (NSP) and uronic acids (UA) using the modified method of Englyst et al. (*25*) and Blumenkrantz and Asboe-Hansen (*26*), respectively. Neutral detergent fiber (NDF), acid detergent lignin (ADL), and insoluble ash were analyzed according to Van Soest (*27*). Cellulose and hemicellulose were calculated using the values obtained for NDF, acid detergent fiber (ADF), and ADL (cellulose = ADF − ADL; hemicellulose = NDF − ADF). The amino acid content was determined at the South Australia Research and Development Institute (SARDI), using a Waters ion-exchange HPLC system, utilizing postcolumn *o*-phthalaldehyde derivation and fluorescence detection, following hydrolysis in 6 M glass-distilled HCl containing 0.1% phenol for 24 h at 110 °C in evacuated sealed tubes (*28*). The tannin content of the samples was determined using the vanillin/HCl method of Burns (*29*). The crude protein (CP) and total ash content of the samples were measured using proximate analysis (*30*). Soluble ash was calculated using the value obtained for the total and insoluble ash (soluble ash = total ash − insoluble ash) (*31*).

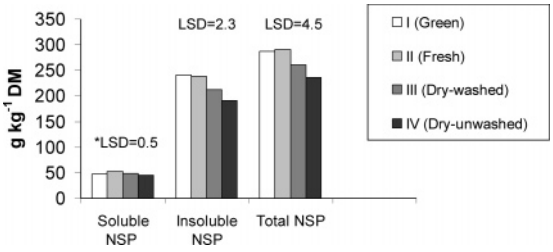

Figure 1. Comparison of soluble, insoluble, and total NSP of four collection forms of *Posidonia australis* (*LSD: least significant difference $P < 0.01$).

Table 1. Insoluble and Soluble NSP Components of Four Collection Forms of *Posidonia australis* (g kg^{-1} of DM)

	collection forms				
component	I (green)	II (fresh)	III (dry, washed)	V (dry, unwashed)	LSD ($P \leq 0.01$)[a]
			Insoluble NSP		
mannose	5.2 ± 0.3	5.5 ± 0.8	4.2 ± 0.6	7.1 ± 0.1	2.3
xylose	57.5 ± 0.5	59.4 ± 0.9	53.1 ± 0.8	43.1 ± 0.1	4.9
galactose	7.1 ± 0.4	10.9 ± 1.9	3.9 ± 0.7	6.7 ± 0.3	4.6
glucose	157 ± 15.4	150.6 ± 1.0	140.3 ± 6.6	122 ± 0.6	26.0
rhamnose	2.2 ± 0.1	2.2 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	3.0
fucose	3.6 ± 0.1	3.0 ± 0.1	3.3 ± 0.1	3.0 ± 0.1	4.0
ribose	3.3 ± 0.1	3.2 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	3.0
arabinose	4.7 ± 0.1	3.7 ± 0.1	3.0 ± 0.1	5.0 ± 0.1	4.0
			Soluble NSP		
mannose	13.9 ± 0.1	17.1 ± 0.1	13.9 ± 0.5	19.9 ± 0.1	1.0
xylose	4.9 ± 0.1	5.3 ± 0.02	8.0 ± 0.1	6.3 ± 0.1	0.5
galactose	10.1 ± 0.3	10.5 ± 0.09	1.5 ± 0.1	0.9 ± 0.2	2.0
glucose	14.2 ± 1.9	11.4 ± 0.01	17.1 ± 0.1	13.3 ± 0.1	4.0
rhamnose	0.8 ± 0.1	3.2 ± 0.2	1.5 ± 0.1	1.2 ± 0.1	5.0
fucose	1.2 ± 0.1	1.4 ± 0.1	1.8 ± 0.2	1.4 ± 0.1	0.4
ribose	0.3 ± 0.1	1.1 ± 0.7	2.4 ± 1.9	0.2 ± 0.1	4.0
arabinose	1.7 ± 0.1	1.7 ± 0.1	2.7 ± 0.2	1.9 ± 0.1	0.5

[a] Least significant difference.

Statistical Analysis. Data obtained for each collected form of plant sample were analyzed statistically using analysis of variance, and means ($n = 3$) were compared using Fisher's protected least significant difference (LSD) method at the 0.05 probability level or below.

RESULTS

The soluble, insoluble, and total NSP contents of the experimental samples are presented in **Figure 1**. The total and soluble NSP content of plant samples I, III, and IV (green; dry, washed; and dry, unwashed) were significantly less ($P < 0.01$) than that of II (fresh). Soluble NSP for all samples was less than 60 g kg^{-1} of dry matter (DM) content, whereas overall insoluble NSP in the samples was high at 200 g kg^{-1} and above. Among the different samples, dry, unwashed *Posidonia* (IV) contained significantly less insoluble NSP than the other forms ($P < 0.01$).

The soluble and insoluble NSP constituents of the experimental samples are shown in **Table 1**. For all the seagrass forms collected, glucose, galactose, and mannose were the dominant sugars in the soluble NSP fraction (more than 10 g kg^{-1} of DM), while ribose and rhamnose were present in the lowest quantities. The content of insoluble NSP constituents was significantly higher than that of the soluble NSP constituents, with glucose and rhamnose being present in the highest and lowest amounts, respectively. The insoluble NSP constituents from all four different seagrass forms collected were significantly different from each other ($P < 0.01$).

Table 2. Cell Wall Constituents of Four Collection Forms of *Posidonia australis* (g kg^{-1} of DM)

	collection forms				
constituents	I (green)	II (fresh)	III (dry, washed)	IV (dry, unwashed)	LSD ($P \leq 0.01$)[a]
uronic acid	172	177	184	186	15
tannin	17.4	17.4	18.5	18.2	ND[b]
NDF	468	465	473	452	15
ADF	351	353	359	335	4
ADL	149	154	151	145	16
cellulose	202	199	209	190	24
hemicellulose	117	112	114	117	ND
soluble ash	98	94	102	146	ND
insoluble ash	55	57	54	54	ND
total ash	153	151	156	200	ND

[a] Least significant difference. [b] Not determined.

Table 3. Amino Acid and Crude Protein Content of *Posidonia australis* (g kg^{-1} of DM Basis)[a]

	collection forms			
components	I (green)	II (fresh)	III (dry, washed)	IV (dry, unwashed)
arginine	3.13 ± 0.13	2.76 ± 0.43	2.47 ± 0.09	2.47 ± 0.09
alanine	3.06 ± 0.30	2.93 ± 0.19	2.31 ± 0.14	2.50 ± 0.01
aspartic acid	7.08 ± 2.06	5.72 ± 0.39	4.16 ± 0.20	4.49 ± 0.18
cysteine	1.62 ± 0.01	1.16 ± 0.01	0.98 ± 0.01	1.23 ± 0.01
glutamic acid	7.43 ± 1.02	7.01 ± 1.36	4.86 ± 0.27	5.08 ± 0.16
glycine	3.70 ± 0.29	3.46 ± 0.19	2.78 ± 0.16	3.03 ± 0.04
histidine	0.82 ± 0.01	0.84 ± 0.01	0.64 ± 0.01	0.65 ± 0.01
isoleucine	2.84 ± 0.33	2.65 ± 0.25	2.00 ± 0.01	1.95 ± 0.07
leucine	4.06 ± 0.33	3.65 ± 0.31	2.80 ± 0.06	2.90 ± 0.10
lysine	2.50 ± 0.49	2.38 ± 0.33	1.80 ± 0.09	1.88 ± 0.09
methionine	1.02 ± 0.01	0.78 ± 0.001	0.72 ± 0.01	0.66 ± 0.01
phenylalanine	2.38 ± 0.01	2.49 ± 0.00	2.00 ± 0.01	2.06 ± 0.01
proline	2.83 ± 0.30	2.55 ± 0.19	2.05 ± 0.12	2.08 ± 0.06
serine	3.50 ± 0.32	2.77 ± 0.16	2.10 ± 0.06	2.32 ± 0.04
threonine	2.79 ± 0.23	2.39 ± 0.17	2.05 ± 0.06	2.16 ± 0.04
tyrosine	1.03 ± 0.01	0.89 ± 0.01	0.88 ± 0.01	0.99 ± 0.01
valine	3.38 ± 0.55	4.69 ± 1.78	2.49 ± 0.06	2.49 ± 0.02
total amino acids	53.0 ± 6.3	49.0 ± 5.7	37.0 ± 1.4	39.0 ± 0.7
crude protein	61.1 ± 5.2	54.3 ± 4.9	48.1 ± 4.1	56.5 ± 5.3

[a] Data show the mean and SE ($n = 3$).

From **Table 2** it can be seen that although both dry forms contained slightly more UA than the green and fresh forms, these differences were not significant. The tannin content of the samples ranged between 17.4 and 18.5 g kg^{-1} of DM. Although the total ash content of dry, unwashed *Posidonia* (IV) was the highest (200 g kg^{-1} of DM), its insoluble ash content was not different from that of the other forms collected.

The NDF content for sample IV (dry, unwashed) was significantly less than that of the other samples ($P < 0.01$), but there were no significant differences between the other samples. The cellulose, hemicellulose, and lignin concentrations of the samples varied between 190 and 209, 112−117, and 145−154 g kg^{-1}, respectively; however, none of these differences were statistically significant. Overall the data suggests that the major constituents of the *Posidonia* cell wall are cellulose and lignin (**Table 2**).

Table 3 shows the amino acid content and CP content of the samples. The concentrations of glutamic acid and aspartic acid were the highest, while generally histidine, methionine, and tyrosine contents were the lowest. **Table 3** also shows that the mean CP content for *Posidonia* was relatively poor (48.1−61.1 g kg^{-1}).

DISCUSSION

The aim of this study was primarily to measure the chemical composition and structural constituents of different collected forms of the seagrass, *P. australis*, as indicative of its possible use as a nonconventional foodstuff in animal nutrition. Coincidentally, we could determine whether one collected form might be more useful for this purpose than others.

Various factors influence the quality of animal feed, but undoubtedly structural carbohydrate is one of the most important (*32*−*34*). Several methods are available for the determination of dietary fiber. Defining dietary fiber solely as NSP, as proposed by Englyst et al. (*25*), gives the best index of the plant cell wall polysaccharides. The method presented in this investigation, however, measured dietary fiber as the sum of monosaccharides released by hydrolysis of NSP. This not only gives separate values for total, soluble, and insoluble dietary fiber but also gives an indication of the various types of fiber by giving values for cellulose and the constituent sugars of the noncellulosic polysaccharides. The soluble NSP content of all four seagrass samples were similar, ranging between 45 and 47 g kg^{-1} of DM (**Figure 1**). This concentration of soluble NSP is similar to that reported by Pirc (*35*) for *Mediterraneas* seagrasses. The insoluble NSP content of samples I (green) and II (fresh) are slightly higher than those of samples III (dry, washed) and IV (dry, unwashed), but this is probably due to the higher content of soluble ash in (III) and (IV), which in turn, affects the proportion of total insoluble NSP (*36*, *37*).

The NDF, as representative of the fiber content of plants, was similar in all samples. The lignin content of the cell wall ranged from 145 in IV (dry, unwashed) to 154 g kg^{-1} of DM in II (fresh). This level of lignin in seagrass seems to be very high when compared with that of traditional lignocellulosic foodstuffs, which generally range from 8% to 13% of the DM (*1*). The high proportion of fiber, especially lignin, in these seagrass samples is in agreement with other studies (*38*, *39*).

All samples contained high amounts of UA. The quantitation of UA in biological materials is important in cell wall analysis, since the amount of UA relative to hexoses and pentoses is taken as an indication of the proportions of the pectic polymers, hemicelluloses, and cellulose, in the cell walls. Changes in the amount of insoluble uronides (protopectins) in forage cell walls have been widely used as an indicator of forage digestibility so that the high quantity of UA determined in the samples in this study may indicate a low digestibility of *P. australis* (*39*, *40*).

The high levels of insoluble NSP constituents and NDF indicate that the *P. australis* collected for this study was in a developed stage of maturity and aging (*41*). In general, the secretion of the plant cell wall at the surface of the plant cell depends partly on a coordinated and partly on a sequential synthesis of various materials, primarily polysaccharide and lignin. The amounts of these substances vary at different times during the growth and development of the cell (*42*). The fact that no significant differences in the cell wall content of the four collected forms of *P. australis* were detected suggests that all collection forms were derived from the same stage of plant growth.

Tannins are known to have adverse effects on animal nutrition. Thus, the high levels of tannins detected in *P. australis* may cause protein precipitation and a decrease in palatability, leading both to a voluntary decrease in food intake and to a decrease in digestibility, as compared with those of other grasses. In this study the tannin content of the different forms of *Posidonia* collected was found to be similar, ranging between 17.4 and 18.5 g kg^{-1} of DM. These values are substantially

higher than for conventional feedstuffs such as legumes and pasture grasses, which generally have tannin concentrations ranging from 1 to 4 g kg^{-1} of DM (*43*). Thus, the associated effects of tannins on protein and carbohydrate digestion would be of importance if *Posidonia* is to be considered for use as a feedstuff. The finding here that the tannin results for all four forms of seagrass studied were similar may appear to be in contrast to O'Donovan's (*44*) observation that the level of tannin is higher in plants exposed to the sun than those in the shade. This apparent disparity may be explained by the fact that the seagrass washed up on the beach had grown under water and therefore would have had the same amount of exposure to light while it was growing as that harvested directly from in the water. Alternatively, the tannins in the different *Posidonia* forms may have undergone oxidation reactions which resulted in the levels of tannins detected being similar (*24*, *43*, *45*).

The insoluble ash content of the samples ranged from 54 to 57 g kg^{-1} of DM, with soluble ash levels from 94 to 146 g kg^{-1}. The total ash content of the experimental material (151−200 g kg^{-1} of DM) is thus proportionally higher than in other plants, a result in agreement with that of Klumpp and Nichols (*46*). With such a high total ash content it would probably not be possible to feed seagrass as the sole dietary ingredient, even for ruminant animals such as cattle and sheep, but it is possible that it could be used as a part of their diet.

The mean CP content of different forms of *P. australis* found in this study (48.1−61.1 g $kg^{-1)}$ was similar to that shown by Klumpp and Van der Valk (*15*). With such a relatively low protein content, the four forms of *P. australis* examined in this study would clearly be considered to be a poor dietary protein source for animal nutrition. Feedstuffs with this amount of protein are usually classified as "poor-quality roughages" (*47*). Future investigations should therefore focus on the use of seagrass as forming just part of an animal ration rather than as the sole ingredient.

The nutritional value of *P. australis* as a possible source of specific protein constituents was investigated further by determining the amino acid composition of the four different forms. The nutritional value of a feed species is considered to be high, especially for nonherbivores, if its essential amino acid composition matches closely that of the feeding animal (*48*). The results shown here indicate that protein derived from *P. australis* is relatively deficient in certain amino acids, especially the essential amino acids cysteine, histidine, methionine, and tyrosine. There is some variation, however, between the results of this current study and those reported by other researchers such as Augier et al. (*49*), who analyzed the chemical composition of a number of species of aquatic plants, including *P. australis*. These variations could be due to many factors, including both the place and depth where the *Posidonia* was collected, the degree of development of the plants, and seasonal variation.

The data presented here indicates that aspartic acid and glutamic acid are the most abundant amino acids in all four forms of *P. australis* collected. This is in agreement with Brown et al. (*50*), who reported that the content of these two amino acids in some aquatic plants constituted 10% (or higher) of the total amino acid content. The results presented here also show, that of the essential amino acids, arginine and leucine are present in the highest amounts. Allen and Kilgore (*51*) have reported that these two amino acids plus lysine are the most common essential amino acids in aquatic plants. The relative proportions of individual essential amino acids in sample III (dry, washed) *Posidonia* were in most cases lower than in the other samples tested, probably reflecting the lower protein content of sample III.

In conclusion, the results reported here indicate that all four collected forms of *P. australis* tested are rich in NDF (452−473 g kg^{-1}) including 234−287 g kg^{-1} total NSP, 172−186 g kg^{-1} UA, and 145−154 g kg^{-1} lignin. The seagrass is also rich in ash (up to 200 g kg^{-1}) and poor in CP (as low as 48.1 g kg^{-1}). Thus, the quantity of the major nutrients in *P. australis* makes it comparable to more common lignocellulosic feed sources for ruminants. According to normal international classifications, feedstuffs with a crude fiber content of more than 180 g kg^{-1} of DM and low protein content are grouped as roughages (*52*).

While the results from this study show that there are some statistically significant differences between the four separate forms of seagrass collected in terms of their chemical constituents, from a biological point of view, however, if used as a feedstuff these measured compositional differences are unlikely to result in any significant differences in animal growth rates. Dry, unwashed seagrass (sample IV in the present study), which is readily available in large quantities and is the easiest form to harvest and process, would thus appear, from a commercial point of view, to be the form best suited for use in further studies investigating the potential of *Posidonia* as an alternative feedstuff for ruminant animals. Clearly, such studies on both the nutritive value (especially palatability and digestibility) and the possible presence of any antinutritive factors in this potential feedstuff are highly warranted. Additional studies on the possibility of improving its nutritive value, by physical or chemical means, are also indicated.

ABBREVIATIONS USED

CP, crude protein; DM, dry matter; NSP, nonstarch polysaccharides; NDF, neutral detergent fiber; ADL, acid detergent lignin; ADF, acid detergent fiber; SARDI, South Australia Research and Development Institute; UA, uronic acids.

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