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Ruminal and intestinal protein degradability of various seaweed species measured *in situ* in dairy cows

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ABSTRACT

The use of seaweeds in animal diets is not new. However, little is known about the feed value of seaweed, both in terms of chemical composition and protein digestibility, and regarding variation between species and season. In this study, eight seaweed species of the genus *Acrosiphonia, Alaria, Laminaria, Mastocarpus, Palmaria, Pelvetia, Porphyra,* and *Ulva* were sampled in spring (March) and autumn (October and November) 2014 at the coast of Bodø in Northern Norway, and were analysed for chemical composition, *in situ* rumen degradability and total tract crude protein (CP) digestibility. Ash content in dry matter (DM) was generally high (overall mean 190 g/kg in DM) and varied considerably, between species ($P<0.01$) and between seasons ($P=0.02$). CP concentration in DM varied both between species ($P<0.0001$) and seasons ($P<0.01$). Highest CP in DM was found for *Porphyra* (350 g/kg DM) and lowest for *Pelvetia* (90 g/kg DM). Spring samples were higher in CP than autumn samples. The effective degradability estimated at 5% rumen passage rate (ED5) of CP varied between species ($P<0.0001$) but not between seasons ($P=0.10$). The highest ED5 of CP was found for *Alaria* (550 g/kg CP) and lowest for *Ulva* (240 g/kg CP). Digestible rumen escape protein (DEP) varied significantly between species ($P<0.0001$) but not between seasons ($P=0.06$); highest DEP was found for *Ulva* (530 g/kg CP) and *Porphyra* (500 g/kg CP). Based on our results, *Acrosiphonia, Alaria, Laminaria, Mastocarpus* and *Palmaria* can supply the rumen with high amounts of rumen degradable protein, while *Porphyra* and *Ulva* can be used as a source of digestible bypass protein. *Pelvetia* had a very low degradability and should not be used to feed dairy cows.

1. Introduction

The raising demand for both food and feed has increased the search for alternative protein sources. It is no longer environmentally sustainable to increase the land area for cultivation of feedstuffs as this will further contribute to climate

Abbreviations: DM, dry matter; OM, organic matter; N, nitrogen; CP, crude protein; aNDFom, ash free neutral detergent fiber using heat stable amylase; h, hours; min, minutes; *a*, washout fraction; *b*, insoluble but potentially degradable fraction; *c*, rate of degradation of fraction *b*; ED5, effective degradability calculated at 5% h^{-1} rumen passage rate; RDP, rumen degradable protein; DEP, digestible escape protein of the total CP intake; SID, digestibility of rumen escape protein in small intestine.

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change e.g., slash-and-burn deforestation (Steinfeld et al., 2006). The potential biomass production from the sea has renewed the interest in using seaweeds as animal feedstock. Some seaweeds are protein rich (the CP content can reach up to 470 g/kg DM e.g., *Porphyra yezoensis*; Arasaki and Arasaki, 1983; Burtin, 2003; Stadtlander et al., 2013) and could potentially be used to decrease the dependency on conventional protein feedstuffs like soybeans. The use of seaweeds in animal feeding is not new; people living on coastal areas have traditionally fed their animals with seaweeds especially during lean feed seasons (Evans and Critchley, 2014). Commercial seaweed production in 2010 reached 19 million tons mainly from seaweed farming in Asia (FAO, 2012); most of which are used for human consumption and industrial purposes, and only a small fraction is used for animal feed (Evans and Critchley, 2014). The Norwegian seaweed industry is reliant on sustainable harvesting of *Laminaria hyperborea* and *Ascophyllum nodosum* for alginate production, and for animal food and fertilizer products, respectively (Meland and Rebours, 2012).

The increasing demand for seaweed biomass for various applications generated several initiatives to cultivate seaweeds along the cold temperate to the Arctic/Boreal coastal regions of Europe (e.g., Skjermo et al., 2014). In high latitudes, the future ocean warming may be percieved to be favorable as it can increase seaweed growth and production (Krause-Jensen and Duarte, 2014). However, weather anomalies such as increasing storm surges (Roleda and Dethleff, 2011) and extreme high summer temperatures associated with global climate change can contribute to large-scale seaweed dislodgement and disappearance, reduce productivity, and increase blade erosion among different kelps (Roleda and Dethleff, 2011; Roleda, 2015 and references therein). Moreover, seaweeds have complex life cycle that are differentially susceptible to different environmental stressors (Roleda, 2015).

For the feed application of seaweed, previous studies focused on the nutritional values of single species or a mixture of different species fed to small ruminants (Ventura and Castañón, 1998). The nutritive value of seaweeds for ruminants in literature varies widely and it depends on the seaweed and animal species, and the seaweed's chemical composition. However, *in vivo* data on protein degradability of seaweeds in dairy cows are scarce (Makkar et al., 2015), to our knowledge only one study examined seaweed protein degradability in the rumen (Gojon-Báez et al., 1998). The aim of this study was to determine the *in situ* rumen and total tract digestibility of seaweed protein and to evaluate the effect of season and seaweed species on the protein value for dairy cows.

2. Material and methods

2.1. Seaweed collection and sample preparation

Eight seaweed species (the brown; *Alaria esculenta*, *Laminaria digitata*, *Pelvetia canaliculata*, the red; *Mastocarpus stellatus*, *Palmaria palmata*, *Porphyra* sp., and the green; *Acrosiphonia* sp., and *Ulva sp*.; (hereafter *Alaria*, *Laminaria*, *Pelvetia*, *Mastocarpus*, *Palmaria*, *Porphyra*, *Acrosiphonia* and *Ulva*, respectively) were sampled by hand picking in spring (March) and autumn (October and November) 2014 at the coast of Bodø in northern Norway (67°19'00"N, 14°28'60"E at low tide). *Ulva* was only sampled in autumn.

Seaweeds were thoroughly washed and cleaned of sand, epibionts, associated mesograzers and other vertebrates and invertebrates in baths of ambient seawater (salinity 34 mg/L). Clean samples were briefly washed in decreasing salinity of 30% and finally in freshwater; excess water was drained manually and samples were frozen at −20 °C until freeze drying. Dried samples were milled through a 1.5 mm screen with a cutter mill (Fritsch pulverisette 15) for *in situ* analysis, and a 1 mm screen for chemical analysis.

2.2. Animals

The experiment complied with the guidelines set out by the Danish Ministry of Justice with respect to animal experimentation and care of animals used for scientific purposes.

Three dry rumen fistulated (#1C, Bar Diamond Inc., Parma, ID, USA) Danish Holstein cows were used for rumen incubations. Cows were fed at maintenance level twice daily with ration consisting of (kg/day as fed) barley straw 2, clover–grass hay 4, concentrate mixture 2.8 and minerals 0.2. Concentrate consisted of (kg/100 kg) Soybean meal 10, barley 40, oat 40, rapeseed meal 3, sugar beet molasses 3 and minerals 4. The ration forage to concentrate ratio was 67:33, and ration concentration of CP and starch was 139 and 137 g/kg DM, respectively.

Three duodenally cannulated lactating Danish Holstein dairy cows maintained on a 60:40 forage (grass and maize silage) to concentrate ratio (DM basis) diet were used for intestinal incubations of mobile bags. All cows had free access to fresh drinking water.

2.3. Rumen in situ degradation

The seaweed samples (n = 15) were incubated in the rumen in three cows for *in situ* protein degradation at 8 time intervals (0, 2, 4, 8, 16, 24, 48 and 96 h) using Dacron bags (38 μm pore size) according to the standard NorFor procedure (Åkerlind et al., 2011). After rumen incubation, the residues were treated in a Stomacher (Stomacher® 400 Circulator, Seward UK)