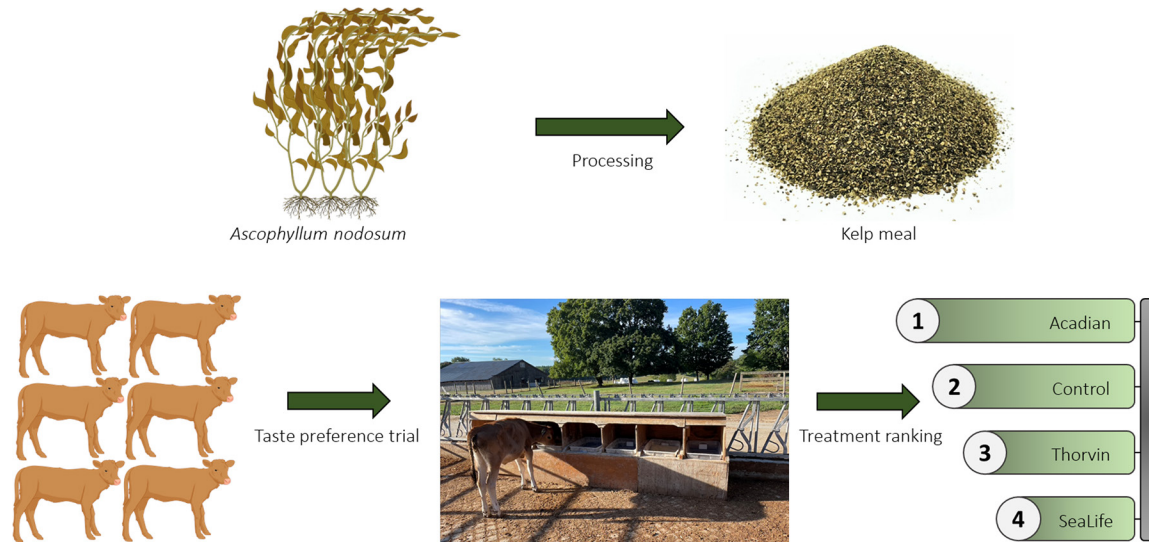


Evaluating taste preference of different sources of *Ascophyllum nodosum* meal in dairy heifers

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Graphical Abstract

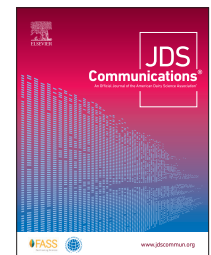


Summary

The brown seaweed *Ascophyllum nodosum*, popularly known as kelp meal, is extensively used in organic dairy farms in the United States, and several brands are on the market. However, differences in harvesting and processing methods adopted in the industry can affect the nutrient profile and concentration of bioactive compounds present in *A. nodosum* and ultimately affect taste preference in ruminants. We compared the taste preference of a diet containing a ground corn-based concentrate mash and this same concentrate supplemented with 3 sources of *A. nodosum* meal (Acadian, SeaLife, or Thorvin) using 6 Jersey heifers. The diets containing Acadian and SeaLife were the most and least preferred, respectively, indicating that heifers were able to distinguish between different sources of the same seaweed species.

Highlights

- *Ascophyllum nodosum* meal is widely used in organic dairies.
- Harvesting and processing methods may alter the nutrient profile of *A. nodosum*.
- Heifers differentiated *A. nodosum* meal sources based on taste preference.
- Acadian *A. nodosum* meal was the most preferred source by Jersey dairy heifers.



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Evaluating taste preference of different sources of *Ascophyllum nodosum* meal in dairy heifers

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Abstract: We evaluated the supplementation of different sources of the brown seaweed *Ascophyllum nodosum* (ASCO) meal on taste preference in dairy heifers during a sequential elimination experiment. Six organic certified Jersey heifers averaging (mean ± standard deviation) 16 ± 1.15 wk of age and 92 ± 9.88 kg of body weight at the beginning of the study were used. Treatments consisted of a ground corn-based concentrate mash without seaweed supplementation (control), or this same concentrate mash supplemented with 57 g/d of ASCO meal obtained from Acadian Seaplants (Acadian Kelp), North American Kelp (SeaLife Kelp), or Thorvin Inc. (Thorvin for Animals). The experiment was conducted with 1 heifer enrolled at a time for 11 d each (n = 66 d total) with the feeding regimens distributed as follows: d 0–2 (adaptation phase), d 3–6 (feeding segment 1), d 7–9 (feeding segment 2), and d 10–11 (feeding segment 3). During feeding segment 1 (d 3–6), the control diet was the most consumed treatment resulting in a total dry matter intake of 22.6 kg followed by Acadian, Thorvin, and SeaLife with 17.7, 13.2, and 11.0 kg, respectively. However, Acadian was selected as the most preferred treatment during feeding segment 1 for a total of 11 d, with control, Thorvin, and SeaLife totaling 8, 3, and 2 d, respectively. In the final ranking of treatments, when all 3 feeding segments were included in the evaluation, Acadian was selected as the first choice by 4 heifers with a ranking of 1.67 points, on a scale ranging from 1 (most preferred) to 4 (least preferred), followed by control (2.50 points), Thorvin (2.67 points), and SeaLife (3.17 points). Overall, the treatments containing Acadian and SeaLife were the most and least preferred, respectively, indicating that heifers were able to distinguish different sources of the same seaweed species based on a taste preference sequential elimination experiment.

The dried and milled product obtained from the brown seaweed *Ascophyllum nodosum* (ASCO), commonly known as kelp meal, is extensively used in organic certified dairy farms across the United States (Antaya et al., 2015; Snider et al., 2022; Reyes et al., 2023). *Ascophyllum nodosum* has a wide spectrum of bioactive compounds including antioxidants, PUFA, and phlorotannins in addition to being a rich source of iodine (Antaya et al., 2015, 2019; Makkar et al., 2016). There are several brands of ASCO meal currently available in the marketplace, with companies adopting different methods for harvesting, drying, and storing fresh ASCO removed from the sea. Therefore, it is conceivable that varying pre- and postprocessing protocols used in the seaweed industry including different harvesting techniques (e.g., suction and cutting, mechanical mowing), drying procedures (e.g., geothermal, solar, wind, mechanical air flow), and milling mesh size could change the nutrient profile and concentration of bioactive metabolites in ASCO meal, ultimately affecting taste preference in ruminants. In addition, harvesting season, geographical location of seaweed beds, algal density, herbivory, and nutrient availability can further affect the chemical profile and the concentration of phlorotannins in ASCO (Connan et al., 2004; Svensson et al., 2007), as well as the time elapsed between harvesting and processing of fresh seaweed.

Glutamic acid and the nucleotides inosinate and guanylate are known to promote the “umami” taste in human foods, which is characterized by a taste-enhancing effect modulated by interactions among these umami compounds and a prolonged aftertaste (Yamaguchi and Ninomiya, 2000). According to Yamaguchi and

Ninomiya (2000), seaweeds are one of the richest sources of Glu, suggesting that ASCO meal could establish the umami taste and increase DMI and growth of dairy heifers. However, Erickson et al. (2012) reported, during a sequential elimination experiment done with Holstein heifers, that supplementation of 30 or 60 g/d of ASCO meal to a starter grain resulted in a lower taste preference ranking than the starter grain fed alone. They concluded that the heifers may have had an aversion toward the umami taste of ASCO meal or another component originated from this seaweed. Although Erickson et al. (2012) investigated taste preference of the same source of ASCO meal fed in incremental amounts to heifers, we are not aware of any study that has compared taste preference of different sources of ASCO meal supplemented at the same dietary level.

We hypothesized that Jersey heifers would be able to distinguish, via taste preference (most to least preferred), 3 sources of ASCO meal presumably due to differences in seaweed chemical composition (e.g., AA profile) in response to varying pre- and postharvesting methods used in the seaweed industry as reported above. The objective of this study was to investigate the taste preference of 3 different sources of ASCO meal available commercially using Jersey heifers during a sequential elimination experiment.

All experimental procedures were reviewed and approved by the University of New Hampshire Institutional Animal Care and Use Committee (protocol no. 220504). This study was conducted at the University of New Hampshire Burley-Demeritt Organic Dairy Research Facility (Lee; 43°10'N, 70°99'W) from July 7 to October

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18, 2022. Environmental temperature averaged 19.5°C (minimum = 2.3°C; maximum = 36.3°C) during the length of the study. These records were obtained from the National Centers for Environmental Information (US Department of Commerce-National Oceanic and Atmospheric Administration) weather station located at the University of New Hampshire Kingman Farm (Madbury; 43°17'N, 70°93'W), approximately 12 km away from the experimental site.

Six organic certified Jersey heifers averaging (mean \pm SD) 16 \pm 1.15 wk of age and 92 \pm 9.88 kg of BW at the beginning of the study were used to investigate taste preference and DMI of different ASCO meal sources in a sequential elimination experiment (Nombekela et al., 1994; Erickson et al., 2012). Treatments were a concentrate mash without ASCO meal supplementation (control diet), or this concentrate mash supplemented with 57 g/d of Acadian Kelp (Acadian Seaplants, Dartmouth, NS, Canada; Acadian diet), 57 g/d of SeaLife Kelp Meal (North American Kelp, Waldoboro, ME; SeaLife diet), or 57 g/d of Thorvin for Animals (Thorvin Inc., New Castle, VA; Thorvin diet). The amount of ASCO meal supplemented in the present study was based on the companies' recommendation and our previous research (Antaya et al., 2015, 2019; Silva et al., 2022).

The ASCO meal sources were top-dressed to the concentrate mash and hand-mixed before each feeding. The concentrate mash contained (% as fed): 46.8% ground corn, 29.9% ground peas, 12% ground barley, 5.02% extruded soybeans, 2.88% mineral-vitamin premix, 2.05% molasses, and 1.35% NaCl. Heifers were housed in an individual pen with free-choice water and access to a calf hutch measuring 2.2 m length \times 2.7 m width \times 1.9 m height and bedded with kiln-dried sawdust. A manger (69 cm wide \times 395 cm long \times 61 cm depth) was placed on the front side of the pen. This cafeteria-style manger contains 7 slots (42 cm wide \times 47 cm long \times 27 cm depth) that were fitted with plastic containers to allow heifers to have access to all 4 treatments simultaneously. The experiment was conducted using 1 heifer per time equaling 11 d/heifer and a total of 66 nonconsecutive days including all 6 animals. As reviewed by Meier et al. (2012), evaluations lasting between 5 and 12 d resulted in the most accurate prediction of palatability in taste preference studies because at least 5 d are necessary for ruminants to balance their diet in choice situations. Four plastic containers containing the treatments and an additional empty container were positioned randomly in the middle of the manger during each feeding throughout the study. Containers were rerandomized daily to minimize potential bias. Two extra empty containers were placed at the outside slots of the manger to nullify the border effect (Erickson et al., 2012).

The amount of feed offered daily was calculated to allow approximately 10% refusals for each diet. Feed offered and refused was weighed and recorded daily at 0730 h. Feeding periods were distributed as follows: adaptation phase (d 0–2; all 4 diets offered), feeding segment 1 (d 3–6; all 4 diets offered to determine the first preference), feeding segment 2 (d 7–9; the most consumed diet in feeding segment 1 was removed and replaced by an empty container, with the 3 remaining diets offered to determine the second preference), and feeding segment 3 (d 10–11; the most consumed diet during feeding segment 2 was removed and replaced by an empty container, with the remaining 2 diets offered to determine the third and fourth preferences).

Concentrate mash and ASCO meal samples were collected once when each heifer was enrolled in the study and composited over

time for later chemical analyses. Samples were shipped to Cumberland Valley Analytical Services (Waynesboro, PA) and analyzed for DM (method 930.15; AOAC International, 2016), CP (total N \times 6.25; method 990.03; AOAC International, 2016), α -amylase, sodium sulfite-treated NDF (Van Soest et al., 1991, with modifications [Whatman 934-AH glass micro-fiber filters with 1.5 μ m particle retention]), ADF (method 973.18; AOAC International, 2016), starch (Hall, 2009), lignin (Goering and Van Soest, 1970), ether extract (method 2003.05; AOAC International, 2016), and ash (method 942.05; AOAC International, 2016). Minerals were analyzed based on AOAC method 985.01 (AOAC International, 2016), with modifications (0.35 g of sample incinerated for 1 h at 535°C followed by digestion in open crucibles for 20 min in 15% nitric acid on a hot plate, with samples diluted to 50 mL and analyzed via inductively coupled plasma mass spectrometry). The iodine concentration of mash and ASCO meal was measured by inductively coupled plasma mass spectrometry at the Michigan State University Diagnostic Center for Population and Animal Health (Lansing, MI). Feed samples were also shipped to the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO) for AA analyses done by cation exchange chromatography coupled with postcolumn ninhydrin derivatization using norleucine as the internal standard (method 982.30; AOAC International, 2016). Tryptophan was measured after alkaline hydrolysis and sulfur AA were determined following performic acid oxidation (method 988.15; AOAC International, 2016). Particle size of the ASCO meal sources was determined according to Baker and Herrman (2002) using a Ro-Tap sieve shaker equipped with 14 sieves of various sized apertures at Cumberland Valley Analytical Services. The chemical composition of the concentrate mash and ASCO meal sources is presented in Table 1.

Heifers taste preference was analyzed by ranking the consumption of the diets from the most to the least preferred. Rankings were determined by giving an arbitrary value of 1 to the most consumed diet during feeding segment 1, a value of 2 to the most consumed diet during feeding segment 2, and values of 3 and 4 during feeding segment 3 for the third and fourth preferred diets, respectively. Final rankings were summed and then divided by the number of heifers used. Data tabulation and calculations were done using Excel from the Microsoft Windows software (Microsoft Co.).

Total DMI (kg) during feeding segment 1 (i.e., d 3–6) and average DMI (kg/d) are presented in Table 2. During feeding segment 1, the control diet was the most consumed (22.6 kg), followed by Acadian (17.7 kg), Thorvin (13.2 kg), and SeaLife (11.0 kg) diets. However, Acadian was the most preferred diet as discussed in detail below. Daily DMI varied slightly across the 3 feeding segments (mean = 2.95 kg/d), indicating that the removal of the most preferred diet during feeding segment 1 had no detrimental effect on daily feed intake over time.

The total and mean number of days during which diets were selected as the first preference by individual heifers during feeding segment 1, as well as the overall ranking of treatments across all 3 feeding segments are reported in Table 3. The Acadian diet was the most preferred by heifers for a total number of 11 d, followed by the control, Thorvin, and SeaLife diets for a total number of 8, 3, and 2 d, respectively. Regarding the overall taste preference ranking when all 3 feeding segments were included in the calculation, Acadian was chosen as the first option for 4 out of 6 heifers

Table 1. Chemical composition of the concentrate mash and different sources of *Ascophyllum nodosum* meal¹ fed to dairy heifers

| Item | Concentrate mash | Acadian | SeaLife | Thorvin |
|---|------------------|----------------|----------------|----------------|
| Nutrient, % of DM (unless otherwise indicated) | | | | |
| DM, % as fed | 92.1 | 88.4 | 88.9 | 89.8 |
| CP | 16.5 | 9.40 | 11.1 | 8.60 |
| ADF | 4.80 | 20.3 | 18.5 | 19.9 |
| NDF | 10.6 | 31.3 | 23.2 | 22.6 |
| Lignin | 0.90 | 16.3 | 15.5 | 11.3 |
| Starch | 51.2 | 0.20 | 0.20 | 0.20 |
| Ether extract | 1.63 | 2.60 | 2.31 | 1.19 |
| Ash | 6.68 | 25.9 | 28.3 | 31.2 |
| Ca | 0.72 | 1.53 | 1.49 | 1.64 |
| P | 0.44 | 0.18 | 0.19 | 0.16 |
| K | 0.82 | 2.45 | 2.53 | 2.81 |
| Mg | 0.98 | 0.98 | 0.90 | 0.79 |
| I, mg/kg of DM | — | 628 | 803 | 810 |
| Mean particle size (\pm SD), μ m | — | 570 \pm 2.05 | 701 \pm 2.20 | 850 \pm 2.05 |
| EAA, % of CP | | | | |
| Arg | 6.72 | 3.13 | 2.53 | 2.72 |
| His | 2.41 | 1.32 | 1.01 | 1.04 |
| Ile | 4.03 | 3.61 | 2.84 | 2.98 |
| Leu | 8.06 | 5.78 | 4.46 | 4.66 |
| Lys | 5.66 | 4.33 | 3.34 | 3.50 |
| Met | 1.27 | 1.81 | 1.42 | 1.42 |
| Phe | 4.81 | 3.85 | 2.94 | 3.11 |
| Thr | 3.54 | 3.85 | 3.04 | 3.37 |
| Trp | 0.99 | 0.96 | 0.81 | 0.91 |
| Val | 4.53 | 4.33 | 3.34 | 3.50 |
| NEAA, % of CP | | | | |
| Ala | 4.81 | 4.93 | 4.46 | 4.27 |
| Asp | 9.55 | 8.18 | 8.11 | 7.51 |
| Cys | 1.56 | 2.05 | 1.62 | 1.81 |
| Gly | 4.10 | 4.09 | 3.14 | 3.63 |
| Glu | 17.8 | 10.4 | 19.3 | 9.71 |
| Orn | 0.07 | 0.24 | 0.20 | 0.52 |
| Pro | 5.87 | 3.13 | 2.33 | 2.59 |
| Ser | 4.17 | 3.13 | 2.43 | 2.59 |
| Tyr | 3.11 | 1.56 | 1.01 | 1.29 |
| Tau | 2.12 | 1.08 | 0.91 | 1.55 |

¹Commercial names: Acadian Kelp (Acadian Seaplants, Dartmouth, NS, Canada), SeaLife Kelp Meal (North American Kelp, Waldoboro, ME), and Thorvin for Animals (Thorvin Inc., New Castle, VA).

with a mean preference ranking of 1.67 (1 = most preferred and 4 = least preferred). The control diet was the second most preferred with 2 heifers selecting it as their first option, resulting in a mean

preference ranking of 2.50. No heifers selected Thorvin or SeaLife as their first choice, thus leading to mean preference rankings of 2.67 and 3.17, respectively. Based on these results, Acadian and

Table 2. Total DMI per treatment during feeding segment 1 (d 3–6) and mean (\pm SD) daily DMI per feeding segment in dairy heifers fed a concentrate mash without (control) or with 57 g/d of different sources of *Ascophyllum nodosum* meal (Acadian Kelp [Acadian Seaplants], SeaLife Kelp [North American Kelp], and Thorvin for Animals [Thorvin Inc.])

| Item | Days | Heifer | | | | | | Total DMI |
|------------------------------|-------|--------|------|------|------|------|------|----------------------------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| Treatment | | | | | | | | |
| Control, kg | | 1.48 | 8.80 | 0.81 | 0.66 | 3.58 | 7.23 | 22.6 |
| Acadian, kg | | 3.76 | 1.19 | 2.87 | 2.78 | 3.81 | 3.25 | 17.7 |
| SeaLife, kg | | 1.14 | 0.26 | 1.98 | 1.06 | 3.08 | 3.46 | 11.0 |
| Thorvin, kg | | 2.77 | 1.81 | 1.61 | 1.45 | 3.02 | 2.55 | 13.2 |
| Feeding segment ¹ | | | | | | | | Mean ² \pm SD |
| 1, kg/d | 3–6 | 2.29 | 3.02 | 1.82 | 1.49 | 3.37 | 4.12 | 2.69 \pm 0.91 |
| 2, kg/d | 7–9 | 2.67 | 3.55 | 4.25 | 1.67 | 2.74 | 3.55 | 3.07 \pm 0.82 |
| 3, kg/d | 10–11 | 2.60 | 3.67 | 3.75 | 1.77 | 2.98 | 3.81 | 3.10 \pm 0.74 |

¹The first 2 d of the study were used as an adaptation phase.

²Calculated as the sum of daily DMI per feeding segment divided by the number of heifers.

Table 3. Total number of days in which treatments were selected as the first preference during feeding segment 1 (d 3–6) and ranking of treatments over all 3 feeding segments (d 3–6, d 7–9, and d 10–11) in dairy heifers fed a concentrate mash without (control) or with 57 g/d of different sources of *Ascophyllum nodosum* meal (Acadian Kelp [Acadian Seaplants], SeaLife Kelp [North American Kelp], and Thorvin for Animals [Thorvin Inc.]

| Item | Treatment | | | |
|-------------------|-----------|------------------------------------|---------|---------|
| | Control | Acadian | SeaLife | Thorvin |
| Heifer | | | | |
| 1 | 0 | 3 | 0 | 1 |
| 2 | 4 | 0 | 0 | 0 |
| 3 | 0 | 3 | 1 | 0 |
| 4 | 0 | 3 | 0 | 1 |
| 5 | 1 | 2 | 0 | 1 |
| 6 | 3 | 0 | 1 | 0 |
| Total days | 8 | 11 | 2 | 3 |
| Mean ¹ | 1.33 | 1.83 | 0.33 | 0.50 |
| Heifer | | Ranking of treatments ² | | |
| 1 | 3 | 1 | 4 | 2 |
| 2 | 1 | 2 | 4 | 3 |
| 3 | 4 | 1 | 3 | 2 |
| 4 | 4 | 1 | 2 | 3 |
| 5 | 2 | 1 | 4 | 3 |
| 6 | 1 | 4 | 2 | 3 |
| Sum | 15 | 10 | 19 | 16 |
| Mean ³ | 2.50 | 1.67 | 3.17 | 2.67 |

¹Calculated as the sum of days in which treatments were selected as the first preference during feeding segment 1 (d 3–6) divided by the number of heifers.

²Ranking of treatments: 1 = most preferred; 2 = second preferred; 3 = third preferred; and 4 = least preferred.

³Calculated as the sum of rankings divided by the number of heifers.

SeaLife were the most and least preferred diets, respectively, with control and Thorvin placing between both.

According to Yamaguchi and Ninomiya (2000), umami is a characteristic taste imparted by Glu and the 5'-ribonucleotides inosinate and guanylate, which together play key roles in the taste, palatability, and acceptability of foods by humans. Some seaweed species are known to contain high concentration of Glu (Yamaguchi and Ninomiya, 2000), suggesting that ASCO meal could promote the umami taste when fed to dairy cattle. Erickson et al. (2012) observed that the control diet (starter grain) was the first option for all 6 Holstein heifers (6 wk old) enrolled in their study (mean preference ranking = 1), with the ASCO meal source SeaLife supplemented at 30 or 60 g/d averaging 2.83 and 2.16, respectively. Results from Erickson et al. (2012) suggest that the potential umami trait attributed to ASCO meal promoted aversion instead of a taste-enhancing effect in Holstein calves even though these authors did not measure the concentration of Glu in the seaweed fed. In the present study, Jersey heifers chose Acadian as their most preferred diet and SeaLife as the least preferred, indicating that these 2 sources of ASCO meal used appear to have unique characteristics that modulated (positively or negatively) taste preference in heifers. As shown in Table 1, the concentration (% of CP) of Glu was numerically greatest in SeaLife, thus coinciding with the poorest taste preference ranking associated with this ASCO meal source. However, Glu concentration was similar between Acadian and Thorvin despite Acadian being more preferred by heifers than Thorvin, implying that a minimum amount of Glu is needed to establish taste aversion. Nevertheless, we cannot conclusively as-

sociate Glu concentration with either taste enhancement or avoidance based on the available data and results.

The seaweed industry adopts various pre- and postharvesting protocols to produce ASCO meal such as different harvesting methods (e.g., suction and cutting, mechanical mowing), drying procedures (e.g., geothermal, solar, wind, mechanical air flow), and milling mesh size that can change the nutrient profile and chemical characteristics of the final product including the concentration of bioactive compounds such as phlorotannins. *Ascophyllum nodosum* is one of the richest sources of phlorotannins, an oligomer of phloroglucinol with antimicrobial and antiherbivory properties (Svensson et al., 2007; Wang et al., 2009). It has been also reported that the concentration of phlorotannins present in ASCO varies in response to season, herbivory, tide depth, and nutrient availability (Connan et al., 2004; Svensson et al., 2007). Therefore, phlorotannin levels in ASCO meal are expected to differ from source to source. Although phlorotannins were not measured in the ASCO meal supplements used herein, the possibility of their involvement on modulation of taste preference in ruminants as reported for terrestrial tannins cannot be disregarded. Specifically, terrestrial tannins are known to form complexes with glycoproteins present in the saliva of ruminants, ultimately reducing the flavor and consumption of feed as reviewed by Besharati et al. (2022). As phlorotannins resemble terrestrial tannins in their ability to bind to proteins and carbohydrates (Ragan and Glombitza, 1986), it is conceivable that phlorotannins could also bind to salivary glycoproteins and promote taste aversion.

We hypothesized that heifers would be able to separate via taste preference the sources of supplemental ASCO meal possibly in response to differences in seaweed chemical composition due to varying pre- and postharvesting methods used in the seaweed industry. However, the differences in chemical composition among the 3 sources of ASCO meal used in the current experiment were relatively small and likely had minor influence on taste preference outcomes apart from Glu concentration as discussed above. Mean particle size averaged 570, 701, and 850 μm for ASCO meal sourced from Acadian Seaplants (Acadian Kelp), North American Kelp (SeaLife Kelp Meal), and Thorvin Inc. (Thorvin for Animals), respectively (Table 1). Despite a noticeable difference in granulometry when comparing these 3 sources of ASCO meal, it is unlikely that particle size played a role in sorting behavior that could have favored or disfavored a given diet.

In summary, the Acadian diet was the most preferred by Jersey heifers based on the mean ranking of treatments across all 3 feeding segments (d 3–6, d 7–9, and d 10–11). Our results further showed that heifers were able to discriminate, via taste preference, 3 different sources of ASCO meal produced from the same algal species. Therefore, taste preference and, potentially DMI, can be affected positively or negatively depending on the source of ASCO meal purchased by dairy producers.

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Nonstandard abbreviations used: ASCO = *Ascophyllum nodosum*.