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Nutritive comparison of ruminant feed, integrating crab and lobster meal

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Abstract

In attempts to increase livestock production and decrease waste products from the seafood industry, crab meal and lobster meal were tested as a potential cattle feed ingredient. Jonah crab waste, Atlantic Red crab waste, soybean meal, and blood meal were collected from various vendors and utilized in an in vitro study, an in situ study, and a nitrogen analyzer to determine crude protein in each feed. After allowing the samples to sit in a DAISY incubator for 48 h, mimicking a rumen environment, degradability for the soybean meal, blood meal, lobster meal, and crab meal were 100.13%, 76.36%, 43.77%, and 45.80% respectively. After separate samples sat in a live rumen for 48 h, the degradability results were 95.7%, 76.36%, 38.7%, and 44% respectively. Although the degradability in the blood meal varied highly between the two study methods, the crab, lobster, and soybean meal degradability were similar in both in vitro and in situ. It was determined that the crab and lobster meal have a lower degradability percentage than soybean and blood meal, as well as a lower crude protein component. However, these feeds were an excellent source of calcium, which is an important nutrient for milk production, and could be a great way to reduce waste from the seafood industry.

Introduction

As the human population increases each second, there is an increase in demand for agricultural products such as meat, milk, and cheese. In order to increase the amount of products from the farm animals, there must be enough high-quality feed for the animals to eat to produce those products. Looking specifically at dairy cattle, there are a wide variety of feed options to incorporate into their diets. Protein is often the most limiting and expensive feed ingredient, with most diets often including soybean meal, canola meal, and possibly blood meal. No matter the combination of feed matter used to make the diet, it must be balanced in order to meet all of the cow's nutritive requirements. In general, the diet of a lactating cow should contain approximately 3.5-4% of total dry matter (DMI), at least 19% acid detergent fiber (ADF), 25-28% neutral detergent fiber (NDF), 16-18% crude protein (CP), 4-6% lipid, and varying amounts of minerals and vitamins (Ontario Veterinary College 2015).

Crab and Lobster Industry Waste

It is estimated that every year, 6-8 million tons of crab, lobster, and shrimp are wasted globally (Yan and Chen 2015). By-products from these have the potential to provide feed for livestock. When considering global fish production as a whole, production reached 167 million tons in 2014. Although crustacean animals (shrimp, crabs, lobsters, etc) only account for 9% of this total production weight, the crustaceans make up the dominant portion of the waste due to their high shell yield and low meat content. (Yang et al. 2019). According to the NOAA Fisheries commercial fishing landings database, 134.6 million pounds of American lobster were caught, and 44 million pounds of Alaska snow crab were caught, both in 2021 (NOAA Fisheries). Compared to other fisheries, the crab and lobster industries produce more waste, as only 15% of

the weight is used for human consumption (Patton et al. 1974). The rest, 85% is inedible to humans and is wasted.

Decaying crab waste is harmful for the environment, as it releases ammonia and nitrates in the air, evaporates, and seeps into the soils. Large concentrations of these chemicals in the soil can pollute the water, including freshwater wells used for drinking water, causing a human health issue (Leffler 1997). In terms of carbon dioxide (CO2) emissions, shrimp and lobster production and waste accounts for 22% of CO2 emissions in the world, even though the production only accounts for 6% of seafood (Davis 2018). Due to the rapid protein degradation and ammonium production in these waste products, there needs to be a focus in preservation to maintain the level of protein in the fresh waste (Evers 1995). One step to control fishery pollution is the shell biorefinery initiative. Shell biorefinery, although not a new concept, is gaining attention in the media and research. Shell biorefinery is the concept of converting chitin, a major component of crustacean shells, into nitrogen-containing chemicals that can be repurposed (Hulsey 2018).

Chitin: What is it? Is it digestible?

The second most vital natural polymer in the world is chitin. Chitin has a derivative called chitosan that is a useful biopolymer used in the food industry, medicine, and agriculture (Shah et al. 2022). Similar to cellulose, vertebrates are unable to digest chitin on their own, but some have symbiotic bacteria or protozoa that can break down chitin into glucose for the animals to use (Biology Dictionary 2017). Ruminants, such as dairy cattle have a symbiotic relationship with their gut bacteria, which allows them to break down and use protein and energy from the chitinous materials to create high quality animal products for human digestion (Patton et al. 1974). The enzyme specifically used to digest this chitin is known as chitinase (Hamid et al

2013). Considering crab meal is the most readily available chitinous source (Patton et al. 1974), this material is one of the potential meals used for the feed study.

A commonly used substance derived from chitin is chitosan, which is the second most common polysaccharide in nature. In previous studies, chitosan has been added to beef and dairy cattle diets to improve both rumen fermentation and digestibility (Shah et al. 2022). Various studies have also supported that supplementing chitin and chitosan into the ruminant diet increased production performance of dairy cattle, improved their feed intake, digestibility, rumen fermentation, and bacterial community (Shah et al. 2022). Specifically, a 2021 study utilized 40 Holstein dairy cows randomly assigned to five treatments: control (no chitosan), supplementation with 500mg/kg dietary DMI of chitosan, supplementation with 1000mg/kg dietary DMI of chitosan, supplementation with 1500mg/kg dietary DMI of chitosan, and supplementation with 2000mg/kg dietary DMI of chitosan. After 10 weeks total, results indicated that the addition of chitosan to the diet linearly increased the milk yield and lactose yield of the dairy cattle (Zheng et al. 2021).

Previous studies of crab and lobster meal in livestock

There have been limited studies evaluating the incorporation of crab and lobster meal into livestock diets. However, the studies that have been done create a solid base line for our experiment. Researchers have previously determined the components of crab meal in terms of crude protein, fat, crude fiber, ash, and dry matter. In a previous study, Velez et al (1991) determined the rumen degradation of the crab meal was 35.9% dry matter and 50.2% crude protein (Velez et al. 1991). Previous researchers Patton et al (1974) determined that crab meal consisted of 32.7% crude protein, 8% fat, 12.9% crude fiber, and 35% ash (Patton et al. 1974). Researchers Patton and Chandler (1974) also compared solubility and crude protein

degradaibility in water and in a cow's rumen. Their main goal was to determine the feasibility of feeding the chitinous compound to ruminants by testing if the material was successfully digested. The experiment resulted in a solubility of 27% in water and 42% in the rumen, and 37.5% crude protein in water and 50.2% in the rumen (Patton and Chandler 1974). There have been minimal research studies on lobster meal.

Although the solubility and crude protein digestibility of the crab and lobster meal increased when present in the rumen during the study by Brundage et al (1984), the main concern with feeding crab and lobster meal to livestock is getting the animals to consume it. There is a concern that the feed has limited palatability due to its large amount of chitinous material (Brundage et al. 1984). If the livestock will not ingest the crab and lobster meal, they will not get the benefits of the feed in the first place. A 1995 study offered multiple diets to a group of beef steers and measured their intake and rate of gain. Results showed that as crab meal replaced 33%, 66%, and 100% of soybean meal, the beef steers rate of gain progressively decreased. In other words, as more crab meal was added to the diet, the beef steers consumed less and gained less weight. However, they determined if the crab meal was pelleted with either alfalfa or barely meal, the intake increased significantly. When pelleted with alfalfa, intake increased from 1.22 kg per day to 1.64 kg per day. When pelleted with barley, intake increased from 1.55 kg a day to 1.80 kg a day. Overall, Nicholson et al (1995) determined that crab meal is a useful supplement for beef steers, but only if they are convinced to consume it (Nicholson et al. 1995).

A few more studies were conducted to test the results of cattle consuming crab meal. A 1980 study provided a supplemental crab meal diet, and a non-supplemented diet of soybean meal. It was determined that the cows who consumed the supplemented diet had a larger intake

of crude protein (Brundage et al. 1984). Another study from 1974 used ten 24-week old calves fed two different diets: a basal diet and a 20% crab meal diet. At the end of the study, urine samples were collected and tested. The results show urine output was higher in the calves that consumed the 20% crab meal ration, and their nitrogen retention was less in comparison to the calves that consumed the basal diet (Patton et al. 1974). Once again, there is a lack of research to test the palatability of lobster meal, as well as the results of ruminants consuming lobster meal in their diet.

This specific study

The purpose of this study is to determine the digestibility rate of lobster and crab meal in an artificial rumen, and comparing it to the digestibility rate of blood meal and soybean meal in the artificial rumen. Based on these results, we will be able to determine how well the crab and lobster feed will digest in an actual rumen of a dairy cow, and compare the digestibility with their commonly-fed feeds. To support or dispute the results of the artificial rumen, samples are also suspended in a rumen via an in situ study for up to 48 h.

Methodology:

This study involved four different potential cattle feeds, with two control feeds and two experimental feeds. The two control feeds were soybean meal and blood meal, where the two experimental feeds were crab meal and lobster meal. Soybean meal was purchased from Blue Seal Feeds, blood meal was purhcased from Poulin Grain, and the lobster and crab meal were purchased from Ocean Extracts.

In Vitro True Digestibility - DAISY Incubator

Considering four diets were being tested, we prepared 11 separate samples of each, 44 total, into F57 filter bags. 48 bags were used total, as there were four blank bags to use as a

control in the incubator, and each was numbered from 1 to 48. All 48 blank bags were soaked in acetone for five min, removing any microbial digestion inhibitors, and completely air dried. The empty bags were weighed, tared, and then weighed again containing approximately 0.25 g of the specified sample (except the four blank bags). The bags were heat sealed to prevent any loss of sample.

A buffer solution was prepared for each digestion jar in the incubator. Buffer solution B and buffer solution A were separately made, and combined into a 1:5 ratio of 266 mL B and 1330 mL A in each jar. While planning to use three digestion jars in the incubator, a total of 800 mL of solution B and 4000 mL of solution A were made. Buffer A required a combination of 40 g of KH_2PO_4, 2 g of $MgSO_4$, 2 g of NaCl, 2 g of Urea, and 0.4 g of $CaCl_2$. Distilled water was added until buffer A had a volume of 4 L total. Buffer B required a combination of 12g of Na_2CO_3, and 0.8g of Na_2S. Distilled water was added until solution B had a volume of 0.8 L. Using a stirring plate and magnet, the solutions were mixed until completely dissolved, and separated into their 1:5 ratio in each of the three digestion jars.

While the buffer solution was contained in the three digestion jars, they were placed in the heated Daisy Incubator (Ankon Inc, Macedon, NY), allowing the temperature to equilibriate for 30 min. An industrial blender was filled with 39°C water, and immediately closed to retain heat. At least 2000 mL of rumen inoculum was removed from an approved cannulated-cow of choice, placed in the blender and the lid was closed between adding rumen samples. Two fistfuls of fibrous material from the rumen were added into the blender. Back in the lab, CO2 gas was added to the blender full of rumen inoculum, and blended on high for 30 sec. The blended digesta was filtered through four layers of cheeseclothe into a flask. 400 mL of blended rumen inoculum was added to each of the three digestion jars. Each digestion jar was purged with CO2

for 30 sec. The feed sample bags were added into each jar and covered. Samples were inoculated with rotation in the Diasy at 39°C for 48 h.

At completion of the incubation, the jars were removed, the fluid was drained and bags were rinsed with cold tap water until the water ran clear.

For our specific experiment:

- Bags 1-11 were the samples with crab meal
- Bags 13-23 were the samples with lobster meal
- Bags 25-35 were the samples with blood meal
- Bags 37-47 were the samples with soybean meal
- Bags 12, 24, 36 and 48 were the blank bags
- Digestion Jar 1 contained samples 1, 2, 3, 4, 13, 14, 15, 16, 25, 26, 27, 28, 37, 38, 39, 48
- Digestion Jar 2 contained samples 40, 41, 42, 43, 36, 35, 34, 33, 23, 22, 21, 20, 5, 6, 7, 8
- Digestion Jar 3 contained samples 9, 10, 11, 12, 17, 18, 19, 24, 30, 29, 31, 32, 44, 45, 46, 47

Fiber Analysis

After rinsing the 48 bags from the incubation, 24 bags were placed into the ANKOM fiber analyzer, as well as 2L of Na2SO3 solution. After the process, the samples were removed and gently pressed to remove any excess water. The samples were placed in a beaker of acetone for 3 min, then allowed to air dry. Each of the bags sat in the oven for 24 h (55℃), cooled to ambient temperature, and weighed each bag for their final weights.

Calculations

$$C_1 = \frac{blank\ bag\ final\ oven{-}dried\ weight}{blank\ bag\ original\ weight}$$

$$\%NDF = \frac{100\ x\ (W_3 - (W_1 x C_1))}{W_2}$$

$$\%IVTD = \frac{100-(W_3-(W_1 x C_1))}{W_2} x100$$

$$\%IVTD_{DM} = \frac{100-(W_3-(W_1 x C_1))}{(W_2 x\, DM)} x100$$

Where W_1 = the weight of the empty bag

W_2 = the weight of the sample

W_3 = the weight of the bag after In Vitro and ND treatment

C_1 = the blank bag correction

DM = dry matter

In Situ True Digestibility

In situ dry matter digestibility was used to compare lobster and crab meal to soybean and blood meal using a 4-by-4 Latin square design. All samples were dried for 48 hous and ground. 112 dacron bags were washed in acetone for 5 min to remove any surfactant that inhibits microbial digestion. Each bag was weighed, 5 grams of sample were added, and the bags were heat sealed. A total of 28 dacron bags were prepared for each feed type: lobster, crab, soybean, and blood. All bag samples were soaked in warm water (39℃) for 20 min. 9 bags were randomly taken from each type of feed (36 bags total) to not be placed in the rumen, representing the 0 h samples. All other samples were placed in a nylon mesh bag and submerged in the rumen of four different multiparous lactating holstein cows. At 2, 4, 8, 24, and 48 h , 2 bags were removed from the rumen of each cow (8 bags per removal time), removing 2 samples of each feed type. Each was submerged in a 10 liter bucket of water and rinsed in cold tap water until the water ran clear. Each bag was placed into a forced-air oven (55°C) to completely dry. At 12 h, 9 bags were pulled out of each rumen (36 bags total), submerged, rinsed, and dried. Dry matter degradability was calculated by determining loss from the bags suspended in the rumen.

LECO - Nitrogen Analyzer

The LECO nitrogen analyzer used infrared absorption and thermal conductivity in order to measure combustion gases in a sample (LECO Analysis). For this study, it was used to determine the presence and concentration of nitrogen (University of Nebraska-Lincoln 2013). A sample size of 0.25 grams was loaded, heat sealed, and purged of any atmospheric gases. The samples were placed in a 950°C furnace and flushed with pure oxygen to cause rapid combustion. The samples were moved through a furnace filter and a thermoelectric cooler to remove any moisture and carbon dioxide. After homogenizing the combustion product, the final product is measured and presented as precent nitrogen (% N) and percent crude protein (% CP).

Results and Disucssion

In Vitro

The results of the In Vitro study not only explain the degradability of each meal sample in the artificial rumen, but also compares degradability across samples. As seen in **Table 2** and **Figure 1**, out of the four feed samples, soybean meal degraded the most in the artificial rumen (100.13%), followed by blood meal (76.36%), crab meal (45.80%), and lobster meal (43.77%).

The final three columns in **Table 2** are comparative p-values between diets. There were differences ($p < 0.05$)between the degradability of soybean meal and rumen undegradable protein, and between blood meal and crustacean meals. However, there was not a difference between the degradability of lobster meal and crab meal. Rumen undegradable protein is the portion of dietary protein that is not degraded by microorganisms in the rumen, and is instead passed into the small intestine for digestion and absorption (Hersom and Carter 2020). In this study, soybean meal is rumen degradable protein, which is protein that is easily degraded into available nitrogen in the rumen (Jenkins 2015). The other three feeds (blood meal, crab meal,

and lobster meal) are rumen undegradable protein sources. The significant difference discovered between soybean meal and rumen undegradable protein coorelated well with the data seen in **Table 2**, considering the soybean meal degraded more than twice as much as the other feed.

In Situ

The in situ results were normally distributed, and displayed in **Table 2**. When analyzing the degradation process of each feed from 0 h to 48 h in the rumen, each feed degraded more as it sat in the rumen. The standard error throughout the in situ experiment remained small, supporting that these results were accurate representations of the average rumen digestibility. When comparing the feed samples to each other, soybean meal and rumen undegradable protein were different ($p < 0.05$), whereas blood meal compared to crustacean, and lobster meal compared to crab meal were not significantly different.

LECO

The crude protein levels of the feed products were determined to be 26.8% for crab meal, 19.7% for lobster meal, 83% for blood meal, and 46.3% for soybean meal. Determining the crude protein level in each diet was important since it is an important factor in a cow's diet. High dietary crude protein is seen to promote growth and high profitability while still protecting the physiology of the animal (Xia et al. 2018). In simplest terms, crude protein is the measurement of all protein in a specific feed based on the amount of nitrogen in the feed multiplied by 6.25. The multiplier 6.25 is used because 6.25 is the average grams of protein that contain 1 gram of nitrogen. This is referenced as "crude" protein because although it measures all nitrogen in the feed as protein, the truth is not all nitrogen is exclusively protein. This measurement tends to overestimate the actual protein content of a feed (Wattiaux et al. 2015).

Crude protein is essential for the diet, as it promotes ruminal fermentation and greater nutrient digestibility (Xia et al. 2018). Specifically, protein supplies the rumen microorganisms with ammonia and nitrogen in order to ferment feeds to make volatile fatty acids and microbial cell protein that are required for digestion (Hayes). Ruminal fermentation is essential for the cow's health, but also plays a large role in this degradation study. When cattle feed does not provide enough protein, the rumen microorganisms are not being provided enough ammonia and nitrogen to function, the microorganisms activity decreases, and the digestion rate of the cow decreases (Hayes). This protein deficiency would be observed in the cow as loss of body condition, decreased reproduction, decreased profitability, and even as extreme as death.

Compared to previous studies determining nutrative value in cattle feeds, there was variation in crude protein composition in lobster, crab, blood, and soybean meal. In this study, the crude protein of crab meal was determined to be 26.8%, whereas previous studies resulted in 50.2% (Velez et al, 1991), and 32.7% crude protein (Patton et al, 1974). One discrepency in the data that may have caused the variation of crude protein in crab meal, was the type of crab that was used in each experiment. In Patton's experiment from 1974, they used the *Callinectes sapidus sp*, and Velez's 1991 experiment does not specify the type of crab used. Two examples of the protein content from different crab meals were from the blue crab (*Callinectes sapidus*) and the mud crab (*Scylla serrata*). The average protein content in blue crabs found in a 2023 study was about 19% (Tufan 2023), whereas the mud crab in a 2022 study had an average protein content of 15.08% (Islam et al. 2022). In this experiment testing degradability between crab, lobster, soybean, and blood meal, the crab samples were from both the Jonah crab (*Cancer borealis*) and Atlantic red crab (*Chaceon quinquedens*).

Another component to consider is how mature the samples were prior to the experiment. This is more essential for the soybean meal as it is a forage. As forage matures, there is a decrease in nutritive value and digestibility. Therefore, as the forage matures, components such as water, protein, minerals, vitamins, and nonstructural carbohydrates decrease (Oregon State). Depending on the maturity of the soybean used in the experiment, the degradability in both the in vitro study and in situ study may change, as well as the crude protein percentage in the sample. The high crude protein percentage in blood meal is as expected, due to its resistance to rumen degradability. Blood meal has shown to escape ruminal degradation by rumen microorganisms, and has a high percentage of dietary protein that reaches the small intestine (Waltz et al. 1989). One portion of dietary crude protein is undegraded protein, which resists microbial attack in the rumen. Blood meal has 82% undegraded protein (Schloesser 1991).

In Vitro vs In Situ

In all four feed samples, degradability was higher in the artificial rumen than in the actual rumen. The difference is not significant in the soybean, lobster, or crab meals, but it is significant in the blood meal. As seen in **Table 3**, more than double the amount of blood meal was degraded in the artificial rumen compared to the actual rumen of the cow. This difference also affected the significance between blood meal and crustacean, making it a significant difference in vitro, but not significant in situ. The results differences between methods can be explained by the accuracy of the method based on previous studies, the slight differences between each study, as well as potential sources of error in each study.

When it comes to the in vitro method, using the Daisy incubator, there are some aspects that can lead to inaccurate results. Although the Ankom Daisy is efficient due to its ability to test multiple samples at once, it is easy to use, and inexpensive, it has been demonstrated to give

higher degradation values than what realistically occurs in a rumen (Tassone et al. 2020). For example, adding rumen inoculum is a very important step for in vitro fermentation studies. Inoculum is a population of microorganisms or cells that is being introduced to the fermentation medium (Soof et al. 2011). The rumen microorganisms are responsible for providing enzymes necessary to digest and ferment the feed consumed by ruminants, as well as providing the animal with energy (Cammack et al 2018). Specifically, rumen microorganisms are known to digest up to 80% of the digestible dry matter in the rumen (Moran 2005). Although adding rumen inoculum to the artificial rumen is necessary for fermentation to occur, inoculum is also the greatest source of uncontrolled variation in fermentation systems (Tassone et al. 2020).

Inoculum quality and composition is not guaranteed to be equal across species, breeds, or even individual animals (Tassone et al. 2020). One reason for the degradability differences between the in vitro and in situ method could be due to the different cows inoculum used. It is also important to consider that the inoculum used for the in vitro study was a random collection of that cow's inoculum. This means the sample of inoculum taken could have contained a high or low abundance of rumen microbes, as compared to the abundance throughout the entire cow's rumen inoculum during the in situ experiment. It is also important to consider that the bovine's diet highly influences their rumen microbiota, further influencing their ability to degrade food during fermentation (Hasan and Yang 2019). Although the two studies did not utilize the same cow, all of the cows' diets were essentially the same, but cannot be guaranteed to be 100% identical. Furthermore, the rumen microbes may not be identical between cows due to previous diets and lifestyles.

Another important concept was that in the Daisy incubator, all four feed sample bags were placed in all three digestion jars together, instead of having one feed type in each digestion

jar. There have been studies that support samples can affect one another when they are incubated in the same digestion jar in the Daisy incubator. During the incubation, soluble matter can be released from one feed sample into the rumen fluid, and affect the microbial activity and degradation of all of the other samples around (Fure 2019).

There were also different sample bags used in the in vitro method than in situ method. In vitro utilized Ankom F57 filter bags, which have 25 micrometer pores (ANKOM Technology). In situ utilized dacron bags, which have an average of 52 micrometer pores (Stern et al. 1983). Considering the in situ filter bags have larger pores, it would be expected that material would filter out of the sample bags throughout the incubation, leading to a smaller sample size at the end of the experiment and a resulting high degradability. However, when analyzing the results as seen in **Table 3**, the in situ method resulted in less degradability of the blood meal, or a larger sample size at the end of the experiment, but relatively similar degradability of the other three meals compared to the in vitro.

It has been discovered that when testing dry matter degradability in vitro and in situ, the degradability is higher in vitro. In a 2019 study, barley was tested using both methods, and compared degradability based on particle size, determined by the grain being rolled, hulless, or unprocessed. At 48 h, in vitro degradability results were 73% rolled, 81% hulless, and 73% unprocessed. At 48 h, in situ degradability results were 50% rolled, 71% hulless, and 38% unprocessed. The in vitro results all had similar degradability, all within 15% of each other. However, in situ results were much more diverse, as much as 40% degradability difference between particle size (DeFeo et al. 2019). The 2019 study is a great example of how results differ between in vitro and in situ studies. The higher degradability in the in vitro studies are compatible with the current experimental results.

A potential explanation for the large degradability difference of blood meal in vitro and in situ could be due to clotting or sticking of the feed sample. In a 1981 study, corn gluten meal was tested for its degradability in a dacron bag. The degradability was underestimated due to the viscous nature of the corn gluten meal in the bag. The tendency for the corn gluten meal to stick together decreased the surface area exposure of the sample, preventing particle movement out of the bag (Stern et al. 1983). It is possible a situation like this could have occurred in the blood meal of the in situ method, preventing particle movement out of the bag.

Potential Limitations of Crab and Lobster Meal

When considering the general requirements for a lactating cow's diet, the diet should contain 3.5-4% total dry matter, at least 19% acid detergent fiber, 25-28% neutral detergent fiber, 16-18% crude protein, 4-6% lipid, and a variety of minerals and vitamins (Ontario Veterinary College 2015). When analyzing the results of this study, there will have to be further experiments to find the proper balance of adding lobster and crab meal while still maintaining a healthy diet for the animal. Previous studies have discovered high concentrations of calcium and low concentrations of phosphorus in crab meal. The high concentration of calcium is a limiting factor to the inclusion of crab meal, as not more than 10% should be added to the animal diet (Vijayalingam and Rajesh 2020). Calcium requirements differ based on the stage of life the cow is in. For example, a cow 2-3 weeks before calving should be receiving less than 100 grams of calcium a day, whereas a non-lactating cow only needs 21 grams of calcium a day (Dupchak). Calcium requirements are much higher in lactating cows, as the concentration of calcium in their milk is high. The fast demand of calcium postpartum is the main reason cows develop hypocalcemia in their lifetime (McArt 2019). Due to the increased calcium demand during lactation, it would be safer to feed crab or lobster meal to lactating cows than to dry cows.

Although the high concentration of calcium in crab meal is potentially problematic if not balanced correctly, the low concentration of phosphorus is beneficial. When excess phosphorus is added to a cow's diet, such as over 0.5% for dry cows, they are at risk for hypocalcemia. Otherwise, a low phosphorus diet, such as 0.2% for dry cows, is protective and safe (Van Suan 2022). Hypercalcemia (too much calcium) shows signs of laying their head on the ground, depressed, slightly comatosed, and even a heart attack (St Boniface Farm Vets, Singh 2021). Hypocalciumia, also known as parturient paresis, can show signs such as tremors, head bobbing, restlessness, inability to stand, loss of consciousness, and even death (Oetzel 2022).

Potential Sources of Error

As with any experiment, there are various potential sources of error that could have occurred and skewed the results. It was possible that in both the in vitro and in situ studies, the bags were not completely dried prior to weighing to test dry matter degradability. If the bags were not completely dry, their final weight would have been larger than it should have been if the bag was dry, and made the degradability results lower than reality. It is also difficult to properly and confidently compare the four types of cattle feed (lobster meal, crab meal, soybean meal, and blood meal) as they all came from different sources. Soybean meal was collected from Blue Seal Feeds, blood meal was collected from Poulin Grain, and the lobster and crab meal were from Ocean Extracts. It is very possible that each source's harvesting and packaging process is different, affecting the quality of the product and the degradability in the rumen or artificial rumen.

Finally, the buffers made in the in vitro study may not have been as precise as desired. The instructions provided chemical amounts for four digestion jars, when the experiment was planning on utilizing four digestion jars. After calculating the final amounts of each chemical for

four digestion jars, the buffers were made. However, there were only enough buffer solutions to use three digestion jars, meaning there was a miscalculation.

Next steps - Palatable feed study

Review literature

Crab and lobster processing plants produce a large amount of waste. In fact, ¾ of the initial king crab that is caught is wasted, with only ¼ being used for their intended production of human food. Thirty percent of this waste could be used to process crab meal and feed cattle (Brundage et al. 1981). Although this seems like an easy way to recycle by-products from these industries, as well as provide more food to cattle, there are some concerns with adding them into feed. For example, it has been determined that there is limited palatability in crab and lobster meal, making the cattle hesitant to eat it. Additionally, there are extremely large amounts of chitinous materials in the feed, where some of it might be degraded by rumen microorganisms (Brundage et al. 1981). Chitinous material is composed of both chitin and chitosan (Fernandez and Ingber, 2013). Both of these components have commercial importance as their nitrogen content is high, and they have great abilities in biodegradability and absorption, as well as contain antimicrobial properties (Shah et al, 2022). Studies have concluded that supplementation of chitin and chitosan enhanced the production performance of dairy cattle, improved the feed intake and digestibility, and improved the rumen fermentation and bacterial community. These components can be used to improve dry matter intake, feed digestibility, rumen fermentation, bacterial community, production performance, body weight gain, and more (Shah et al, 2022).

The main purpose for this experiment would be to incorporate crab and lobster meal into dairy cattle diets. This is due to the materials' high nutritive value in terms of crude protein, crude fiber, and more. It has been determined that crab consists of 36% crude protein, with

35.9% dry matter (Velez et al, 1991). 12.9% of the crab meal consists of chitin, which is one of the most abundant polysaccharides in the world (Patton and Chandler, 1974) and is a component of the chitinous material that leads to increased production, dry matter intake, and more.

An experiment from 1974 conducted by researchers at Virginia Tech consisted of three trials using 26 male Holstein calves in total. The goal was to evaluate and determine the nutritive value of dried crab meal when incorporated in ruminant feed, as well as determine how well the cattle digest and utilize it. The nutrient analysis of crab meal is in **Table 1**, and compared to standard feed components blood meal and soybean meal. Crab meal was determined as 32.7% crude protein, 0.8% fat, 12.9% crude fiber, and 35% ash. As compared to soybean meal and blood meal, crab meal has less crude protein and fat, but more crude fiber and ash. (Stein et. al)(feedinamics).

The experiment design was a 3-by-3 replicated Latin square design. Six, 12-week old calves (74 kg BW) were evaluated for one week. At week three, each calf was housed in a metabolism stall, where their feces and urine was collected for five days. After testing the fecal and urine samples, it was discovered that there were no differences between the diets in terms of digestibility, except that the nitrogen digestibility was higher in those that were fed the crab meal rations (Patton et al, 1974).

Past Experiment #1

Researchers in Louisiana in 1990 conducted an experiment with 20 4-12 month old Holstein heifers, studying a 10-week growth trial. There were three different diets offered, with the positive control being soybean meal, and the experimental feeds of crab waste meal and crawfish waste meal. They initially weighed the amount of total feed offered to each calf, provided the feed for five h of feeding, and removed the feed to weigh the amount of orts after

five h. The results displayed the intake for dry matter and acid detergent fiber for the diet with crab waste meal was not significantly different from the soybean meal diet. However, those that consumed the crab waste meal diet consumed significantly less crude protein than the soybean meal diet, considering the soybean meal has a higher percent of crude protein as seen in **Table 1**. Overall, this experiment determined that adding about 20% of crab meal to a normal cattle diet did not cause a significant difference in feed intake, daily gain, feed conversion, nitrogen retention, or digestibility of dry matter, crude fiber, or nitrogen (Velez et al, 1991).

Past Experiment #2

A study by researchers at the University of Alaska conducted an experiment in 1980 with 30 Holsteins assigned to five diet concentrates in six blocks of five animals at five weeks of lactation. The five diets were constructed as followed:

Negative control: 51% corn, 0% soybean meal, 0% crab meal

High soybean meal: 33% corn, 18% soybean meal, 0% crab meal

Low soybean meal: 52% corn, 9% soybean meal, 0% crab meal

High crab meal: 30% corn, 0% soybean, 23% crab meal

Low crab meal: 41% corn, 0% soybean, 11% crab

The negative control was unsupplemented to access the response to protein irrespective of source or percent. The feed was weighed before being provided to the animals, and fed twice a day, rotating the diets between each individual animal in the groups. After the feeding times, the orts were weighed to determine how much feed was remaining, as well as what components of the feed was remaining. Overall, it was determined that the rejection of concentrates was affected when king crab meal was included in the diet. The cows rejected .5 and .2 kg a day when consuming the high and low crab meal diets, but rejected none of the negative control, or high

and low soybean meal diets. However, while analyzing the nutrient uptake from each diet based on the orts remaining, the protein intake was lower in the negative control ration, calcium and phosphorus intake was higher with king crab meal diets, and resulting milk production was lowest for those animals consuming the negative control diet (Brundage et al. 1981).

Past Experiment #3

A 2011 study focused on measuring the various intake rates when adding kelp meal to the cattle diet. This experiment used the sequential elimination procedure, which tested the palatability of increasing amounts of kelp meal in the diet. The overall goal was to determine if calves prefer the taste of kelp added to starter grain. The test subjects were six 6-week old Holstein calves, and 3 diets: the control starter grain (containing liquid molasses, pellets, and steam-flaked corn), 30 g/d of kelp meal added to starter grain, and 60 g/d of kelp meal added to starter grain. In this particular study, one calf was fed at a time using a feeding manger in their pen that contained seven total containers, three of which contained the starter grain and treatments. The feed was offered and the orts were weighed at 7:30 am for half the calves and 9:30 am for the second half of the calves. All three of the diets were offered for seven days, using the first two days to adapt to the various diets, and the final five days for data collection. After day three, the diet with the greatest consumption was removed to determine the second most palatable feed (Erickson et al 2012).

At the end of the study, it was seen that the control starter grain was the most palatable feed for all six calves, leading to the highest consumption rate. The 60g/d kelp meal added to the starter grain was the second-most palatable feed to five of the cows, but the 30 g/d kelp meal added to the starter grain was the second-most palatable feed to one of the cows. When analyzing the number of days that each treatment was preferred by each heifer, there was not a significant

preference between the 30 g/d kelp meal addition or the 60 g/d kelp meal addition. According to the researchers final data, there was only one more day the heifers preferred 60 g/d of kelp over 30 g/d of kelp, so the mean difference is not significant (Erickson et al. 2012).

Proposed Experiment

After researching and analyzing past experiments with similar intake and nutrition measurements, an ideal experiment would use the sequential elimination procedure. We would use six Holstein heifers at the Fairchild Dairy Farm at the University of New Hampshire. There would be a total of 5 diets involved: a control, 30 g/d of crab meal, 60 g/d of crab meal, 30 g/d of lobster meal, and 60 g/d of lobster meal. The main data collection of this experiment would be to measure how quickly the calves ate the feed. In this case, the feed would be weighed before they are provided the feed, the cavles would be given a strict time limit to consume, and then the feed would be weighed after the time is up. Our control substance would be a mixed ration containing canola meal.

The experiment would occur in four full weeks, with four treatment rotations, and data collection starting on day three to allow for adjustment. Week one, all of the heifers would have their own access to the control treatment and both variations of crab meal additive (30 g/d and 60 g/d). Week two, they would have access to the control treatment and both variations of lobster meal additive. Week three, they would have access to the control treatment, 30 g/d of lobster meal and 30 g/d of crab meal. Week four, they would have access to the control treatment, 60 g/d of lobster meal and 60 g/d of crab meal. This way, each treatment would be compared to each other at one point in the experiment. The treatments would then be ranked among each other, from most palatable to least palatable.

Some considerations that still have to be made are the following:

1. How long should the experimental diets be available to the calves? What is a reasonable amount of time for the animals to potentially finish all their feed if palatable, but also give them enough time to test out the new feed and open up to eating it? 5 min? 10 min? All day?
2. If leaving the feed out only for 5-10 min, do we need to make the calves hungry prior? Do we take away any other source of feed for a period of time? For how long do we take away their feed if at all? If left out all day, no access to any other feed besides the experimental diet provided.
3. The experiment would provide more information about the potential use of crab and lobster meal in cattle feed if we were also measuring the ability of the feed to be digested in the heifers rumen. Can we find a way to test the orts to determine what material and components were left behind by the cattle? Test their urine and/or feces to determine what nutritive components were not digested and instead were excreted as waste? Can we test a blood sample to determine what nutrients the body was able to use?

Significance/Meaning/Implications

This experiment would be a significant study because although some other scientists have conducted studies on cattle intake of crab meal, there are limited studies, potentially even none, about cattle intake of lobster meal. Being able to successfully incorporate these feed types into the cattle's diet would be very beneficial not only for their health and development, but also for the environment by limiting waste from the lobster and crab industries. I think this study could be very influential, and lead to more future experiments in terms of feeding dairy cattle various forms of animal-by products that are legal and beneficial for their health and growth.

Conclusion

The purpose of this experiment was to determine the practicality of integrating crab meal and lobster meal into the diet of a dairy cow. Not only would this diet integration provide a new resource of energy for the animal, as well as help keep up with the growing human population, but as well as decrease the wate from the crustacean industry. Based on the results, crab and lobster meal is not nearly as degradable as soybean or blood meal, and does not provide as much crude protein. However, utilizing these crustacean feeds in cattle diets not only helps convert chitin into consumable products for humans, but also decreases the waste produced by the seafood industry.

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Tables

Table 1. The average crude protein, fat, crude fiber, and ash content in three main protein sources for cattle diets: Crab meal, dehulled soybean meal, and blood meal (Stein et al.) (Feedinamics).

	Crab Meal	Dehulled Soybean Meal	Blood Meal
Crude Protein	32.7%	47.5%	87.7%
Fat	0.8%	Less than 2%	1.9%
Crude Fiber	12.9%	4%	0.8%
Ash	35%	6.27%	3.1%

Table 2. In Vitro and In Situ Dry Matter Degradation Results

	SBM	BM	LM	CM	SE	SBM vs RUP	BM vs Crust	LM vs CM
In Vitro Dry Matter Degradation	100.13	76.36	43.77	45.80	2.8448	<0.001	<0.001	0.6155
In Situ Dry Matter Degradation:								
0 Hours	39.9	14.5	13.4	14.4	0.78	<0.01	0.52	0.43
12 Hours	61.2	23.4	23.7	26.4	1.15	<0.01	0.28	0.13
48 Hours	95.7	31.6	38.7	44.0	0.01	<0.01	0.62	0.31

* SBM = soybean meal. BM = blood meal. LM = lobster meal. CM = crab meal. SE = standard error. RUP = rumen undegradable protein. Crust = crustacean.
* SE In Situ was not transformed from 1/square root

Table 3. Comparing In Vitro to In Situ Results at 48 hours of degradability.

	SBM	BM	LM	CM	SE	SBM vs RUP	BM vs Crust	LM vs CM
In Vitro	100.13	76.36	43.77	45.80	2.8448	<0.001	<0.001	0.6155
In Situ	95.7	31.6	38.7	44.0	0.01	<0.01	0.62	0.31
% Difference	105%	242%	113%	104%				

*% Difference = (Degradability of In Vitro)/(Degradability of In Situ) x100

Figures

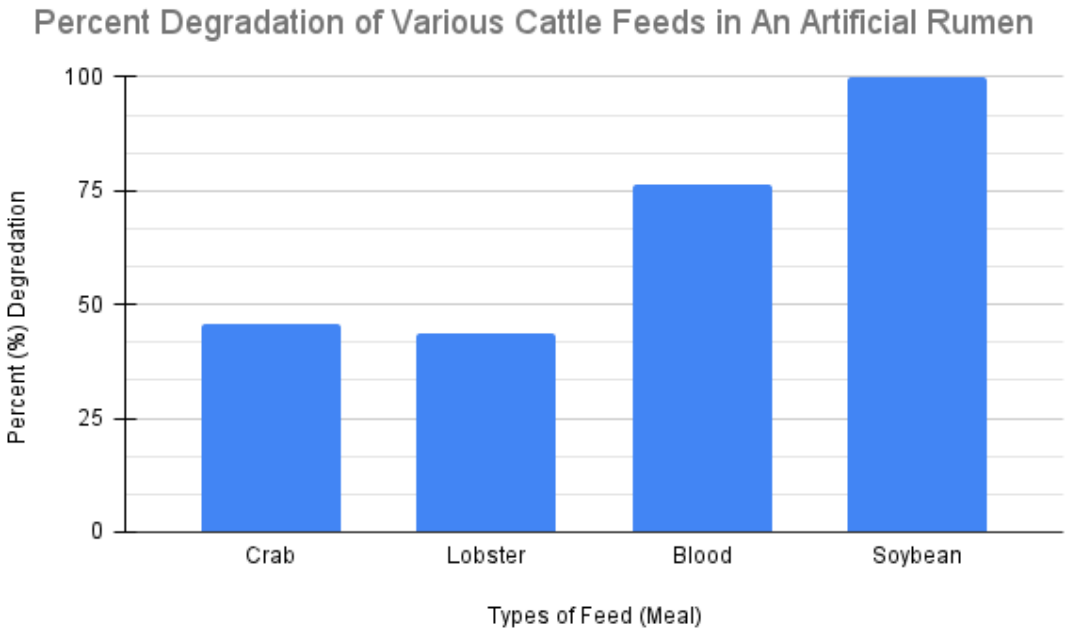

Figure 1. Comparing the percent degradation of crab, lobster, blood, and soybean meal in an artifical rumen. The artificial rumen used was the DAISY Incubator.