POST-EXTRACTION ALGAL RESIDUE AS A PROTEIN SOURCE FOR CATTLE CONSUMING FORAGE

A Thesis

by

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ABSTRACT

Four studies were conducted to evaluate the potential for post-extraction algal residue (**PEAR**) to be incorporated as a protein source in the grazing sector of the beef cattle industry. In Experiment 1, blends of PEAR and conventional protein supplements (dried distillers' grains, **DDG**; cottonseed meal, **CSM**) were offered to steers consuming Bermudagrass to evaluate palatability of PEAR. Supplement completion, time required for consumption, and amount of supplement consumed were recorded. In Experiment 2, isonitrogenous amounts of PEAR and CSM (100 mg N/kg BW) were supplemented to steers consuming low-quality forage to compare effects on nutrient utilization. Experiment 3 evaluated the optimal inclusion rate of PEAR to steers consuming low-quality forage. Treatments included no supplemental protein, 3 levels of PEAR (50, 100, and 150 mg N/kg BW) and 1 level of CSM (100 mg N/kg BW). In Experiment 4, the effects of upstream operations on the nutritive value of PEAR were quantified.

Observations indicate PEAR may be blended with existing protein sources in the beef industry without negatively affecting palatability, but there may be palatability concerns when PEAR is offered alone. Provision of 100 mg N/kg BW of PEAR or CSM stimulated forage intake ($P \leq 0.05$) and increased N retention ($P = 0.02$) relative to unsupplemented animals. Imbalances in mineral intakes (Ca:P ratio of 8:1) were observed when PEAR was supplemented, but not CSM. Total digestible OM intake (**TDOMI**) responded quadratically ($P = 0.01$) to increasing provision of PEAR with maximization occurring when PEAR was provided at 100 mg N/kg BW. There was not a

difference in TDOMI ($P = 0.13$) at isonitrogenous levels of PEAR and CSM, indicating forage utilization was stimulated to a similar extent. Excess mineral levels and imbalances in PEAR were largely a result of cultivation, harvesting, and extraction procedures which could be controlled. Thus, there is potential to alter production streams to optimize oil yield and co-product value. Overall, our results indicate PEAR can be incorporated as a protein source in the beef cattle industry, thus increasing economic viability of biofuel production from algal biomass.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Due to a growing population and increased industrialization, global energy demands are increasing. Petroleum-based fuels are under scrutiny as the detrimental environmental consequences of fossil fuel combustion are debated. The United States currently imports at least 60% of the nation's petroleum; two-thirds of which are utilized in the transportation sector (Pienkos and Darzins, 2009). Thus, there is an immediate need to identify a biofuel feedstock capable of providing a significant amount of energy while mitigating concerns over greenhouse gas emissions. Existing biofuel crops do not have the potential to address national energy demands as they require significant

Table 1. Productivities of oil crops

	Oil yield (L/ha)	Land area needed (M ha) [a]	% of existing US cropping area [a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Coconut	2689	99	54
Oilpalm	5950	45	24
Microalgae [b]	58,700	4.5	2.5

[a] To meet 50% of US transportation fuel needs, [b]assuming oil content of 30%
Adapted from Christi (2007)

agricultural inputs in the form of high-quality land (Table 1) and water (Christi, 2007), therefore, there has been recent interest in novel biofuels. Algae is capable of converting light, water, and CO_2 into biomass on marginal, infertile land with a variety of water

sources (FAO, 2010). Microalgae has the potential to produce 40 – 80 t DM/ha/y, which exceeds the production capacities of conventional agricultural crops (Tredici, 2010; Wijffels and Barbosa, 2010). Lipid content of algae has been reported to exceed 80%, (at the expense of DM productivity), but typically averages 20 – 50% (Christi, 2007; FAO, 2010). However, the chemical composition of algae is not constant and is subject to variation between strains, batch, growth temperature, pH, mineral inputs, water, light, and degree of agitation (Chacón-Lee and Gonzáles-Mariño, 2010). For large-scale production, a lipid content of 10 – 20% is more realistic (Table 2). The protein fraction of algae is expected to be retained in the delipidated biomass, termed post-extraction algal residue (**PEAR**). Thus, there is potential to simultaneously produce bioenergy and a source of nutrients, which can help address world food demands, further alleviating pressure on natural resources (FAO, 2010). Biofuel production from algal biomass is currently limited by a lack of technology, standard industry protocols, and the resulting inability to be cost-competitive with petroleum-derived fuels (Pienkos and Darzins, 2009). Production cost estimates of algal biofuel range from approximately $4 to > $1,000 per gallon of algal oil yield (FAO, 2010). Pienkos and Darzins (2010) summarized the findings of the Aquatic Species Program, funded by the Department of Energy throughout late 1970's and early 1990's, and concluded the price of algal oil production was approximately 2 – 3 times greater than that of conventional crude oil. The algal biofuel industry is still emerging; as large-scale production becomes feasible, economic viability of biofuel production from algal biomass will likely hinge on placement and marketing of the co-product, PEAR.

Table 2. Nutritive value of algae

Species	Protein, %	Carbohydrates, %	Lipids, %
Arthospira maxima [a]	60 – 71	13 – 16	6 – 7
Anabaena cylindrical [d]	43 – 56	25 – 30	4 – 7
Chlamydomonas rheinhardii [d]	48	17	21
Chlorella vulgaris [d]	51-58	12 – 17	14 – 22
[c]	48	8	13
Chlorella pyrenoidoisa [a]	57	26	2
Dunaliela salina [d]	57	32	6
Euglena gracilis [d]	39 – 61	14 – 18	14 – 20
Nannocloropsis spp. [e]	29	36	18
Porphyridium cruentum [d]	28 – 39	40 – 57	9 – 14
[b]	34	32	7
Scenedesmus obliquus [b]	50 – 56	10 – 17	12 – 14
Spirulina platensis [a]	46 – 63	8 – 14	4 – 9
[c]	61 – 64	15 – 16	8
Spirogyra sp [d]	6 – 20	33 – 64	11 – 21
Synechococcus sp [d]	63	15	11

[a] Phang, 1992, [b] Rebolloso-Fuentes et al., 2000, [c] Tokusoglu and Unal, 2003, [d] Becker, 2007

Post-extraction algal residue in the beef industry

From a nutritive value standpoint, the protein fraction of PEAR is its most attractive attribute. Post-extraction algal residue will be utilized sparingly as human food due to sensory issues with texture, color, and smell of algae (Becker, 2007). Large volumes of PEAR could be utilized as fertilizer, but at the expense of market value (FAO, 2010). Livestock industries have gained attention as a potential market for PEAR; livestock in the United States consumed approximately 272 t protein in 2008. Non-ruminants are more sensitive to mineral imbalances which are inherent in PEAR (discussed below) than ruminants; thus, there will be less toxicity concern when PEAR

is fed to cattle versus poultry or swine. As demonstrated in Table 2, lipid content of algae species suitable for large-scale production rarely exceeds 20%, suggesting significant quantities of PEAR will be produced. The beef cattle market is relatively large; in a one-time snapshot (August 2012), there were 10.7 million cattle on feed and 30.5 million cows in the cow-calf industry in the United States (USDA-NASS, 2012), indicating the potential for large-scale nutrient utilization. The production segments of the cattle industry include the cow-calf sector, stocker operations, and feedlots.

Feedlots utilize high concentrate rations designed to minimize cost of gain and duration of fattening while maximizing animal growth. Typical feedlot rations are energy dense and may contain 70 - 85% grain (corn), 8.3 – 9.0% roughage (corn silage or alfalfa), plant-based protein (ethanol co-products), fat supplement (tallow) and minerals (Vasconcelos and Galyean, 2007). Inclusion level and market value of ingredients in the feedlot sector is driven largely by energy or protein content. From a fuel conversion standpoint, lower levels of lipid are desirable in PEAR, but would result in a lower value feedstuff when marketed to the beef feeding industry. In feedlot rations, PEAR would most likely compete with dried distillers' grains, which has a comparable protein fraction (27.4%), but greater lipid content (10.4%; Drewery et al., 2011) and OM content. Market value of dried distillers' grains in feedlot rations is $319/t (USDA-NASS, 2012), which is greater than the expected value of PEAR. There is potential for large-scale utilization of PEAR in the beef feeding industry (6.2 million t/y), but nutritive value suggests monetary contribution of PEAR to algal biofuel operations may be maximized if PEAR is marketed in an alternate segment of the beef industry.

Stocker and cow-calf operations utilize forage and commonly supplement protein as cottonseed meal or soybean meal, co-products of oil production. Cottonseed and soybean meal contain < 2% lipid, approximately 50% protein (NRC, 2000), and are priced primarily on their protein content. In a hedonic pricing model, Bryant et al. (2012) compared samples of PEAR to conventional protein supplements and corn and determined the value/t of PEAR ranged from $122 – 281, although the current value would be greater as the model was based on feed prices from 2005 - 2010. Post-extraction algal residue will likely command a higher value when marketed to cow-calf operations relative to feedlots; however, market size is smaller (3.7 million t/y). Sales of dried distillers' grains plus solubles represent 12 – 16% the revenue of a corn-based ethanol plant (Elobeid et al, 2006; Taheripour et al., 2010), which is lower than the monetary contribution from soybean meal (53% of revenue; Taheripour et al., 2010). Revenue generated from co-product sales will have a greater impact on economic viability of algal biofuel production if PEAR is priced based on protein versus energy content.

Bryant et al. (2012) reported an average ash content of 45.7% for PEAR, which is significantly greater than distillers' grains, soybean meal, or cottonseed meal (5 – 7% ash; NRC, 2000) and similar to values in Table 3. Due to lower concentrations of protein and OM, approximately double the amount of PEAR relative to cottonseed meal or soybean meal would need to be fed to achieve similar responses in animal performance. Negative implications of this scenario include 1) increased mineral excretion, resulting in an increased environmental footprint associated with beef production, 2) increased

storage and labor demands, and 3) poor nutrient stewardship due to over-supplementation of minerals. Potential for mineral toxicity and imbalance would likely have the greatest impact on acceptability and inclusion rate of PEAR in beef operations.

Table 3. Macronutrient and macromineral profile of post-extraction algal residue (PEAR) and conventional protein supplements

	PEAR, diatom		PEAR, floc.[1]		Conventional suppl[2]			Max[3]
Analysis	A	B	A	B	CSM	SBM	DDGS	
Macronutrients, % DM								
OM	57.0	58.3	59.0	56.8	93.0	93.3	94.8	----
CP	16.0	14.8	21.3	20.2	48.9	54.0	29.5	----
Macrominerals, %								
Ca	0.60	2.06	5.28	6.18	0.16	0.29	0.32	----
P	0.80	0.74	0.39	0.40	0.76	0.71	0.83	----
Na	2.63	2.89	6.45	6.61	0.03	0.01	0.24	----
S	0.85	0.83	0.93	0.87	0.26	0.48	0.40	0.40
K	1.48	1.22	0.63	0.56	1.22	2.36	1.07	3.00
Mg	0.72	0.70	0.87	0.79	0.35	0.33	0.33	0.40

[1]Floc. = flocculated
[2]CSM = cottonseed meal, SBM = soybean meal, DDGS = dried distillers grains plus solubles; values from NRC, 2000
[3]Maximum tolerable concentrations for beef cattle (NRC, 2000)

Mineral and heavy metal concentrations of PEAR samples (unpublished data) are compared to those of conventional protein supplements and the maximum tolerable concentration of minerals for beef cattle (NRC, 2000) in Tables 3 and 4. Discussion will focus on the mineral content of "PEAR, flocculated", which represent sub-samples of PEAR produced from a large-scale algal biofuel operation and utilized in feeding trials (Chapter II and III). Data of "PEAR, diatom" are presented to demonstrate the variation in the nutritive value of PEAR for different algal species, methods of processing, etc.

Although S and Al in PEAR are in excess of maximum tolerable concentrations, inclusion rate will likely not be limited if PEAR is incorporated in the grazing industry. Assuming forage S content is 0, a 590 kg cow consuming 2% of her BW daily as forage could receive 4 kg of PEAR without exceeding the maximum tolerable concentration of S intake for beef cattle. In this example, forage and PEAR were assumed to be 90% DM for simplicity, resulting in 0.25% S intake/d. Using the same parameters, daily Al intake would not exceed 300 ppm, which is clearly under the level where toxicity would become a concern (NRC, 2000).

Table 4. Micromineral and heavy metal profile of post-extraction algal residue (PEAR) and conventional protein supplements

	PEAR, diatom		PEAR, floc.[1]		Conventional suppl[2]			Max[3]
Analysis	A	B	A	B	CSM	SBM	DDGS	
Microminerals, ppm								
Cu	7	5	17	16	54	23	11	100
Fe	2330	2550	4540	5030	160	145	560	1000
Mn	124	108	72	86	12	41	28	1000
Zn	126	45	40	36	----	----	----	500
Cu	6	6	17	16	----	----	----	100
Heavy metals, ppm								
Al	288	319	4310	4130	----	----	----	1000
Ba	1	2	92	81	----	----	----	----
Cr	6	6	144	214	----	----	----	1000
Ni	13	13	41	54	----	----	----	50

[1]Floc. = flocculated
[2]CSM = cottonseed meal, SBM = soybean meal, DDGS = dried distillers grains plus solubles; values from NRC, 2000
[3]Maximum tolerable concentrations for beef cattle (NRC, 2000)

There is a marked difference in the Ca:P ratio for PEAR versus conventional supplements. A Ca:P ratio of 1 – 7:1 is considered desirable (NRC, 2000), with

reductions in nutrient conversion and animal performance associated with deviations from this ratio (Wise et al., 1963; Ricketts and Campbell, 1970). Bahiagrass hay, late vegetative Bermudagrass, and mature brome grass, all forages typical of cow-calf operations, provide a Ca:P ratio of 1.2 – 2.3:1 (NRC, 2000). As forage matures, CP and P may become insufficient to meet animal requirements; this situation is typical especially during winter months. Supplementation of cottonseed meal, soybean meal, or distillers' grains is a common management practice to address protein and P deficiencies in the basal forage. The Ca:P ratio of these conventional supplements is < 0.50:1, which complements the mineral profile of the basal forage. Provision of PEAR will over-fortify Ca relative to P; Dowe et al. (1957) demonstrated, as the Ca:P ratio increased from 1.3:1 to 13.7:1, gains decreased in growing beef calves. Thus, there is no additional benefit associated with provision of excess Ca, and supplementation of PEAR may result in a potential reduction in animal performance and increased nutrient wastage.

Protein content of PEAR (21%) is less than half that of soybean meal (54%) and cottonseed meal (49%), and slightly lower than that of distillers' grains (30%). As of July 2012, the value of soybean meal was $568/t; market value of supplements utilized in the beef grazing industry will be determined according to their protein content relative to that of soybean meal (USDA-NASS, 2012; Figure 1). In the current market, the expected value of PEAR is $218/t. As demonstrated, PEAR contains a significant ash content which may be partially attributed to algae cultivation and methods of upstream processing.

Raceway ponds, a commonly utilized "open system" for algal cultivation, allow optimal light capture and low investment costs relative to "closed systems" (photobioreactors). Open systems are influenced by environmental conditions, thus treatments are applied (high salinity, high pH, minerals) which have potential to adversely affect nutritional characteristics of PEAR. The goal of algal harvesting is to concentrate the low cell density of biomass to a point where oil extraction is possible (Pienkos and Darzins, 2009). Flocculation is a common harvesting method; however, flocculants may contain significant levels of minerals or heavy metals, which would be

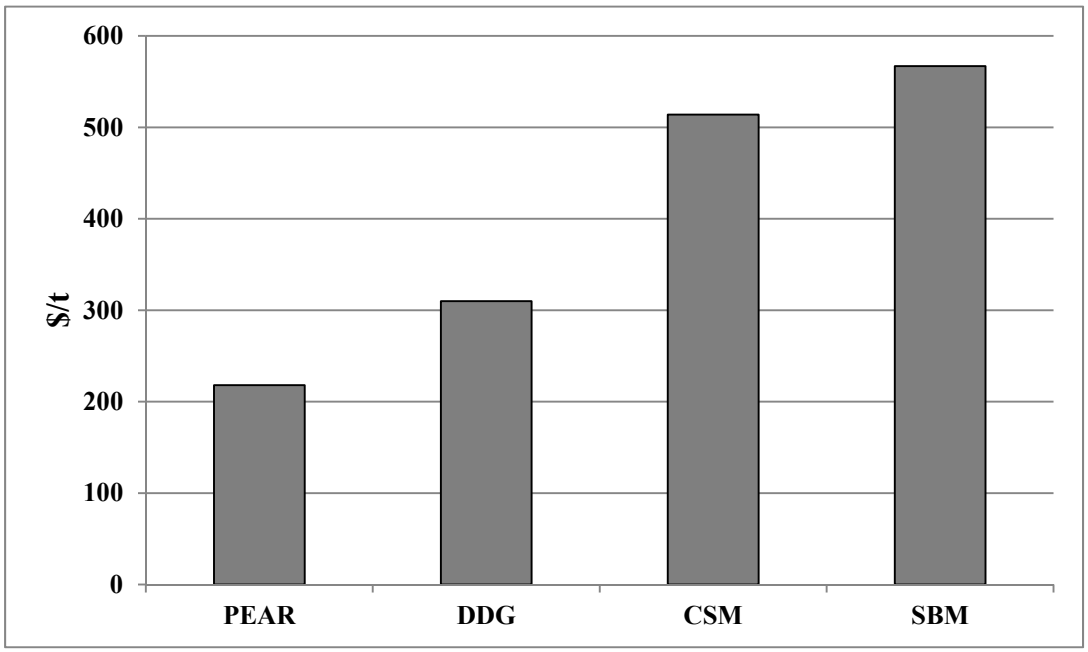

Figure 1. Market value of feedstuffs based on protein content relative to that of soybean meal. PEAR = post-extraction algal residue, DDG = dried distillers' grains, CSM = cottonseed meal, SBM = soybean meal. The NRC (2000) reported protein content was used for DDG, CSM, and SBM.

retained in PEAR. It is likely the ash content of PEAR could be controlled and minimized according to standard management practices employed in individual operations. If PEAR was priced solely on protein, decreasing ash by 10% would result in an approximate 48% increase in market value (assuming protein fully replaces the fraction of PEAR which was ash), which would increase economic viability of biofuel production from algal biomass. There are scenarios where the ash fraction of PEAR, especially the Na content, may positively affect market value. Blending cottonseed meal with salt regulates intake of supplements, resulting in decreased labor requirements (Riggs et al., 1953; Weir and Miller, Jr., 1953). In a one-time price comparison from a local agricultural supplier, cottonseed meal (36% CP) was valued at $562/t and a self-fed supplement (20% CP, 0.8 – 1.4% Ca, 0.7 – 0.9% P, 19 – 23% NaCl, 33,070 IU Vitamin A/kg) was valued at $441/t. In this scenario, the value of PEAR would increase 41% (assuming a similar value to quoted self-fed supplement) if marketed as a self-fed supplement versus priced on protein content, but would be less than if protein was increased to a similar level of cottonseed and soybean meal.

There is potential to further increase the value of PEAR in the beef industry by processing it into a form which will transition easily into existing beef feeding operations. Physical benefits of pelleting feed include less storage space, improved handling and transportation characteristics, and minimization of dust during the feeding process. Pellets have been documented in improving poultry and swine performance versus a meal (Wondra et al., 1995; Jahan et al., 2006); these observations can be attributed to decreased feed wastage, reduced selective feeding, improved palatability,

and decreased ingredient segregation (Behnke et al., 1994). Therefore, pelleted feed is expected to demand a premium in price relative to meal. The prices of cottonseed meal and a 41 range cube were compared over a 2 y period; on average, the range cube was

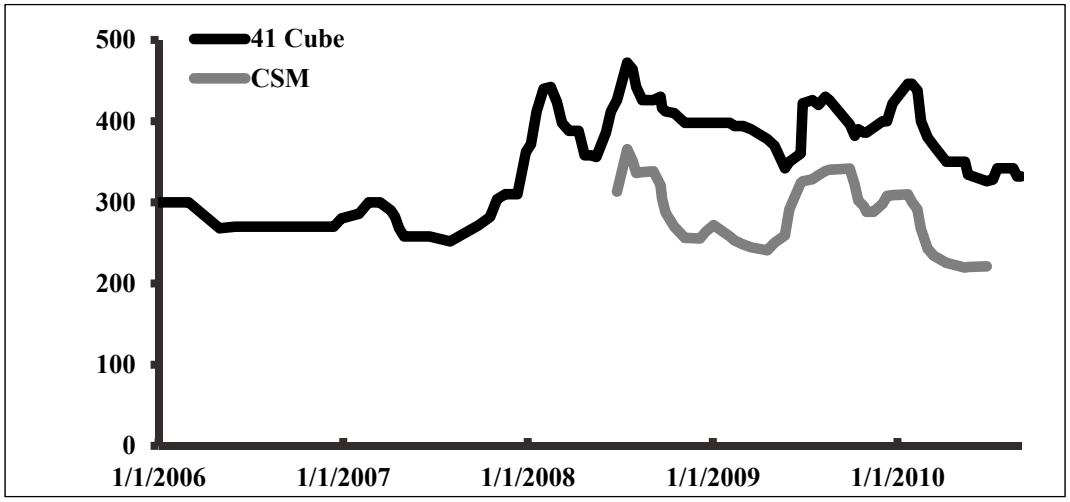

Figure 2. Prices of cottonseed meal (CSM) and a 41 range cube from a local agriculture supplier

valued an additional $123/t relative to cottonseed meal (Figure 2). As large-scale algal biofuel operations emerge, standard protocols should be developed to process PEAR into a more valuable form to further increase the contribution of PEAR to the economic longevity of algal biofuel production.

Whole algal biomass in the beef industry

The lipid profile of algae is concentrated in polyunsaturated fatty acids (**PUFA**), most notably eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**; Spolaore et

al., 2006). Fish and fish oil are well-known sources of PUFA; however, consumption of ruminant products accounts for a significant percent of fat intake in Western diets. There is interest in manipulating fatty acid composition of beef and dairy products to favor accumulation of PUFA versus saturated fat (SFA). Lipids of tissues from monogastrics fed unsaturated fatty acids closely resemble fatty acid composition of the diet, while the composition of lipids in ruminant tissues is influenced by diet, ruminal modification of fatty acids, and activity of tissue desaturases (S. B. Smith, Texas A&M University, College Station, TX, personal communication). Scollan et al. (2006) maintains the amount of omega-3s in meat can be manipulated by increasing the level of the desired fatty acid in the diet and reducing the extent of ruminal biohydrogenation. Biohydrogenation is responsible for low PUFA and high SFA in ruminant tissues and must be bypassed to significantly increase the amount of PUFA present in beef. Ruminal biohydrogenation can be bypassed if oil is provided as whole oilseed (Smith et al., 1981) or is coated in a material resistant to ruminal microbes (Ekeren et al., 1992); therefore, future investigation should focus on the bypass ability of PEAR. In-vitro studies (Ashes et al., 1992; Gulati et al., 1999) observed rumen microbes do not hydrogenate long chain omega-3 fatty acids to a great extent, concluding EPA and DHA are resistant to biohydrogenation. However, when Doreau and Chilliard (1997) supplemented fish oil to dairy cattle via rumen cannulas, they reported low concentrations of EPA and DHA in the fatty acid fraction of duodenal digesta, indicating a high degree of ruminal biohydrogenation of long-chain PUFA. Similar in-vivo studies (Lee et al., 2005; Scollan et al., 2001; Shingfield et al., 2003) confirmed these observations. These conclusions

suggest the beneficial fats present in algae would fail to enrich the fatty acid profile of beef, or the degree of enrichment would be biologically insignificant, but fail to take into consideration the potential for PEAR to bypass the rumen.

Odor and palatability issues are expected to pose challenges when whole algae is incorporated in cattle diets. High concentrations of EPA and DHA are associated with a fishy odor, which may cause reluctance in cattle to consume algae and result in residual odor and flavor in beef carcasses. Unacceptable odors have been detected in carcasses of poultry fed 2 - 4% fish oil (Dansky et al., 1962; Edwards and May, 1965), implicating a constraint on the amount of PUFA which can be added to monogastric rations. Oxidative processes associated with high concentrations of long-chain PUFA are responsible for sensory issues in the final product, and are also expected to cause off-flavors and increased rancidity in beef (Scollan et al., 2006). Supporting this observation is the work of Daley et al. (2010), where grass-fed beef retained a distinct grass flavor relative to grain-fed beef due to a higher omega-3 intake.

Therefore, feeding whole algal biomass to beef cattle will likely not result in a product which contains significant levels of omega-3 fatty acids or is readily accepted by consumers. It is expected dietary inclusion of algal biomass in cattle diets will be limited by 1) the willingness of the animal to consume algae due to organoleptic issues, and 2) the undesirable effects of algae on characteristics of the beef carcass. From an economic perspective, conversion of oil to biofuel and marketing the subsequent lipid-extracted co-product to beef cattle is likely more feasible.

Response to protein supplementation

Forage is an important source of nutrients and a valuable resource in grazing operations, but may be deficient in protein, resulting in decreased animal performance. Moore and Kunkle (1995) reviewed the relationship between forage CP content and forage OM intake; when forage contained < 7% CP (low-quality), intake was positively

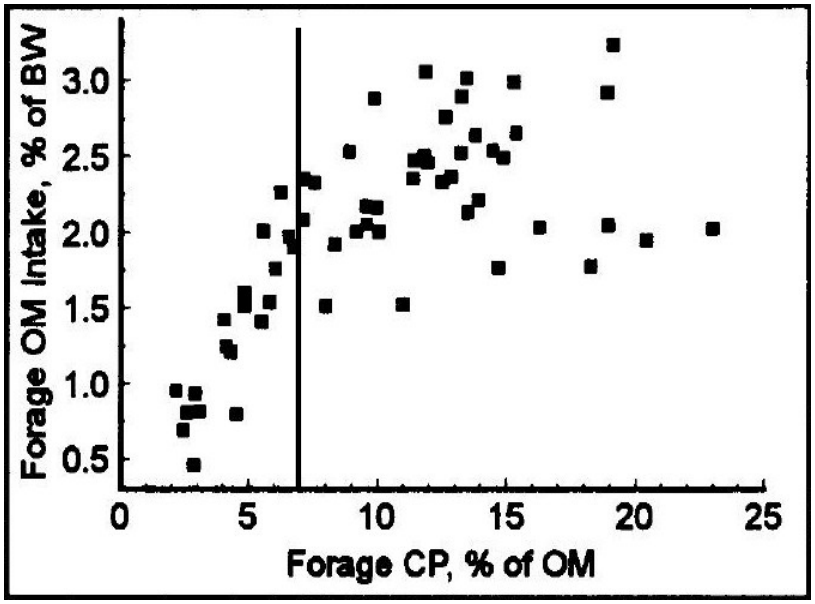

Figure 3. The relationship between forage OM intake and forage CP content. Adapted from Moore and Kunkle, 1995

related to CP content, while no relationship existed when forage CP exceeded 7% (Figure 3). Ruminally available N (**RAN**) and fermentable OM affect microbial growth (Beaty et al., 1994), with N being the first-limiting factor to microbial growth when low-

quality forage is consumed (Köster et al., 1996). As microbial N requirements are addressed, bacterial growth is promoted, resulting in increases in forage intake and microbial N flow the small intestine (Wickersham et al., 2008), improving the energy and protein status of the animal, respectively. Therefore, supplemental protein can be provided to address N deficiencies in the basal forage, increasing forage utilization and subsequent animal performance.

Degradable intake protein (**DIP**) and undegradable intake protein (**UIP)** are components of feed protein. Provision of DIP directly supplies N to rumen microbes, and is regarded as the specific component of protein limiting low-quality forage utilization (Köster et al., 1996; Mathis et al., 2000; Bandyk et al., 2001). Supplemental UIP is digested post-ruminally and contributes to the RAN supply via hepatic urea production and urea recycling. Assuming the contribution to RAN is the sum of the direct provision of N to the rumen and the amount of N recycled, it is apparent DIP is more valuable to cattle consuming low-quality forage than UIP. When UIP is provided to cattle consuming low-quality forage, urea recycled to the gut as a percent of N intake is greater than for supplemental DIP (Wickersham et al., 2008; Wickersham et al., 2009), indicating recycling mechanisms are more important when supplemental protein is provided as UIP. The NRC (2000) recommends feeding 13% fermentable OM as DIP to supply adequate RAN, but recognizes this generalization does not fit all feeding situations based on differences in digestibilities among diets and fails to take into account the amount of RAN supplied from N recycling. When cattle are consuming low-

quality forage, supplementing 9.0 – 11.6% of digestible OM as DIP adequately supplies RAN (Köster et al., 1996; Klevesahl et al., 2003; Wickersham et al., 2008).

Supplementation of DIP stimulates forage OM intake (Köster et al., 1996; Olson et al., 1999; Mathis et al., 2000), which ultimately improves animal performance. Figure 4 illustrates the effect of supplemental DIP on forage OM intake low-quality forage is consumed. In these experiments, the basal forages contained 1.9 – 5.9% CP and similar proportions of DIP. Thus, Figure 4, and the following figures throughout the text, only consider supplemental DIP. Köster et al. (1996) and Klevesahl et al. (2003) observed a plateau in forage OM intake as supplemental DIP increased. The response to supplemental protein is most dramatic at the first level of supplementation, with diminishing returns as protein provision nears the microbial N requirement. There is a threshold, the requirement, after which additional protein no longer increases forage intake. It is likely microbial N requirements were addressed in the studies of Köster et al. (1996) and Klevesahl et al. (2003). Mathis et al. (2000; Exp. 3) and Wickersham et al. (2008) observed a linear relationship for forage OM intake and level of supplemental DIP; 40 – 60% of this response was attributed to the first level of supplemental protein, with less dramatic increases at each additional increment of DIP. Supplementation of DIP resulted in an additional 34% increase in forage OM intake than UIP relative to unsupplemented animals; however, forage OM intake was stimulated when UIP was provided to cattle consuming low-quality forage (Table 5; Bandyk et al., 2001), supporting the observation that DIP is more valuable when forage N content is limiting. Mechanisms in which DIP and UIP increase nutrient supply to the host differ. Provision

of UIP indirectly supplies RAN via N recycling, increases protein flow to the duodenum, which is positively related to the ability of the animal to accommodate ruminal fill (Bandyk et al., 2001), and alleviates metabolic discomfort via removal of VFA (Wickersham et al., 2004). Degradable intake protein stimulates intake by providing a direct supply of RAN, increasing microbial CP flow to the duodenum, which accomplishes many of the same things as UIP.

Processes for increased intake when protein is supplemented to cattle consuming low-quality forage (< 7% CP) include increases in forage digestion and rate of passage

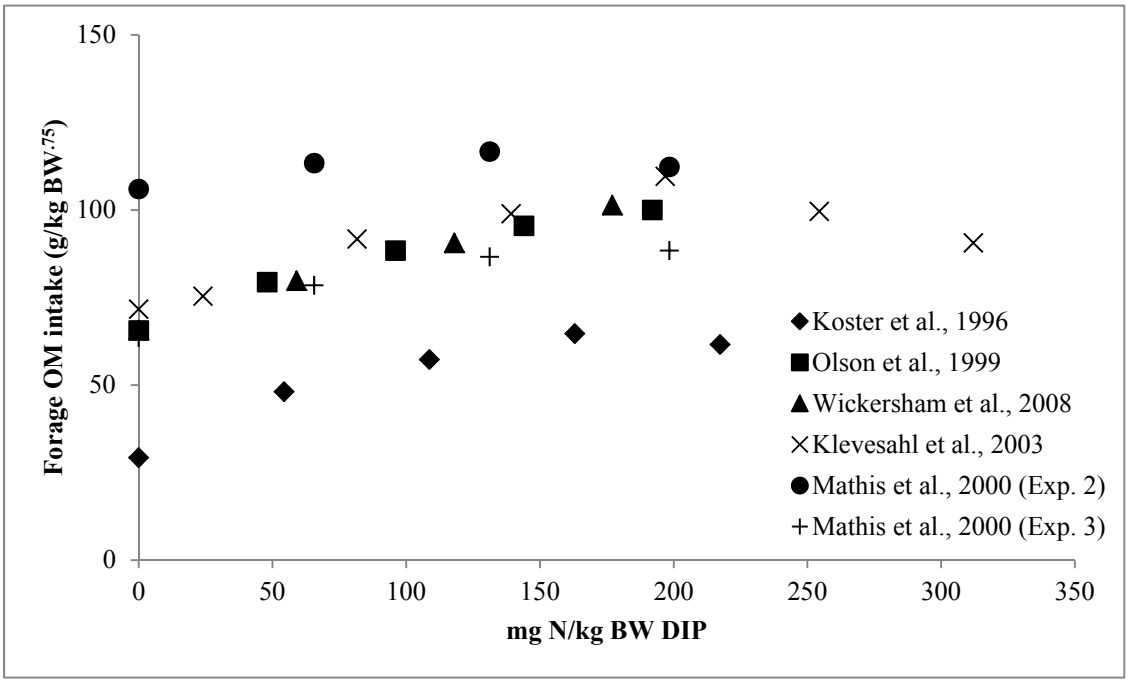

Figure 4. The response in forage OM intake to DIP supplementation in cattle consuming low-quality forage

(Ellis, 1978; McCollum and Galyean, 1985). Accordingly, total tract OM digestibility (**OMD**) often increases when supplemental N is provided to cattle consuming low-quality forage (Olson et al., 1999; Klevesahl et al., 2003; Wickersham et al., 2008). Köster et al. (1996) observed a slight depression in OMD at the level of supplementation which elicited the greatest response in forage OM intake. Rate of passage and rumen retention time within diets are negatively related; thus, there is less opportunity for celluloytic bacteria to interact with the fiber portion of digesta at greater intakes, resulting in net decreases in digestibility although forage fermentation activities are increased. Total tract digestibility is slightly greater when UIP is supplied versus DIP (Table 5; Bandyk et al., 2001), a result of increased intake associated with DIP.

Table 5. Effect of undegradable intake protein (UIP) and degradable intake protein (UIP) supplementation on steers consuming low-quality forage[1]

	CON[2]	UIP	DIP	P vs C	R vs C
Intake, g/kg $BW^{0.75}$					
Forage OM	47.8	61.0	77.4	28	62
Total digestible OM	18.9	30.1	36.0	59	91
Total tract digestibility, %					
OM	39.5	47.1	44.7	19	13
NDF	39.8	44.9	42.1	13	6
Ruminal pH	6.7	6.6	6.5	-1	-3
Fermentation parameters, m*M*					
Ammonia	0.6	1.4	4.2	133	600
Total VFA	69.9	77.6	81.5	11	17

[1]Adapted from Bandyk et al., 2001

[2]CON = no supplemental protein; UIP = 400 g/d casein infused post-ruminally; DIP = 400 g/d casein infused ruminally; P vs C = percent difference in CON and UIP, R vs C = percent difference in CON and DIP

Indicators of forage fermentation include ruminal ammonia concentrations, ruminal pH, and total VFA concentrations. Ruminal ammonia is a N source for the majority of rumen bacteria (Bryant and Robinson, 1962), especially celluloytic microbes (*Bacteroides succinogenes, Ruminococcus albus, and Ruminococcus flavefaciens*; Baldwin and Allison, 1983). Ruminal ammonia is generally regarded as indicative of RAN concentrations. Provision of DIP directly supplies RAN, thus, positive relationships between the level of supplementation and ruminal ammonia concentrations have been reported (Köster et al., 1996; Olson et al., 1999; Mathis et al., 2000; Klevesahl et al., 2003; Wickersham et al., 2008). However, if energy and N intake are synchronized, rate of ammonia uptake by microbes may be similar to rate of release and ammonia concentrations do not reflect RAN (Petit and Veira, 1994; Kolver et al., 1998). Wickersham et al. (2008) observed ruminal ammonia was 2.06 m*M* when ruminal OM and NDF digestion were greatest; at a higher level of supplementation, ruminal ammonia increased to 3.66 m*M* and was accompanied by a decrease in ruminal digestibility. Ruminal ammonia is greater when protein is infused ruminally versus post-ruminally (Table 5; Bandyk et al., 2001). Recycled urea N from absorbed metabolizable protein is responsible for increases in ruminal ammonia when UIP is supplemented, while deamination of ruminally degradable protein or provision of NPN increases ruminal ammonia for supplementary DIP.

Reductions in ruminal pH to < 6 depress growth rates of fibrolytic microbes, inhibiting fiber digestion (Stewart, 1977; Hoover, 1986). When protein (UIP or DIP) is supplemented to cattle consuming low-quality forage, ruminal pH decreases, but remains

within acceptable levels for bacteria to digest fiber (6.2 – 6.9; Köster et al., 1996; Olson et al., 1999; Mathis et al., 2000; Bandyk et al., 2001; Klevesahl et al., 2003). Decreases in ruminal pH are a consequence of increased total VFA concentrations, end-products of forage fermentation, with provisional protein (Köster et al., 1996; Olson et al., 1999; Mathis et al., 2000; Klevesahl et al., 2003; Wickersham et al., 2008). The magnitude of ruminal pH change is likely related to the extent of the effect of protein supplementation on forage utilization and, therefore, VFA concentrations (Klevesahl et al., 2003). Molar proportions of acetate decrease and proportions of propionate increase when supplemental protein is provided to cattle consuming N deficient forage (McCollum and Galyean, 1985; Chase and Hibberd, 1987, Olson et al., 1999, Wickersham et al., 2008), indicating an increase in energetic efficiency (Van Soest, 1982). Celluloytic bacteria have a requirement for branched chain VFA (Baldwin and Allison, 1983), molar proportions of which increase with DIP supplementation (Olson et al., 1999; Klevesahl et al., 2003; Wickersham et al., 2008), increasing the capacity for cellulose fermentation in the rumen. Wickersham et al. (2008) observed ruminal OM and NDF digestion was maximized at the level of supplementation where total VFA concentrations were greatest, but there was not a clear relationship between ruminal digestion and ruminal ammonia or molar proportions of VFA. It should be recognized that, at higher levels of supplementation and intake, an increased amount of forage is digested, but extent of fermentation is decreased due to increases in passage rate. Supporting this is the work of Wickersham et al. (2008) where, at the level of supplementation intake and ruminal

ammonia were the greatest, ruminal digestion was slightly depressed. The same principle can be applied to the observations of Bandyk et al. (2001) to explain decreases in total tract digestibility for DIP relative to UIP, despite an increase in ruminal ammonia and total VFA.

Protein supplementation is an efficient management practice to address N deficiencies of basal forage, increasing animal performance (Wickersham et al., 2008) and monetary returns. Supplementation of DIP stimulates forage utilization more effectively than UIP. Supplementing 9.0 – 11.6% of digestible OM as DIP maximizes total digestible organic matter intake (**TDOMI**) in cattle consuming low-quality forage (Köster et al., 1996; Klevesahl et al., 2003; Wickersham et al., 2008); however, metabolizable protein may be insufficient to maximize growth, depending on available energy intake and production stage of the animal. Thus, supplementation of UIP should be considered after RAN deficiencies are addressed, but metabolizable protein requirements have not been met by microbial protein and escape protein.

CHAPTER II

POST-EXTRACTION ALGAL RESIDUE AS A PROTEIN SUPPLEMENT FOR BEEF STEERS CONSUMING FORAGE: PALATABILITY AND NUTRIENT UTILIZATION

Overview

Market value of post-extraction algal residue (**PEAR**) will be driven by its ability to compete with commonly fed protein sources, such as cottonseed meal (**CSM**) and dried distillers' grains (**DDG**). Two experiments were conducted to evaluate potential for PEAR to be incorporated in existing beef operations. In Experiment 1, 12 steers were used in a 12 × 12 Latin square experiment consisting of 12 4-d periods to evaluate palatability of PEAR-containing supplements in steers consuming Bermudagrass. Each period included 3-d where steers were fed a test supplement and a 1-d washout where steers were fed DDG. Supplements were formulated with different carrier ingredients (DDG, CSM, or liquid supplement, **LS**) at varying levels of PEAR inclusion. Intake and time required for consumption were recorded daily. In Experiment 2, 6 steers were used in concurrent 3 × 3 Latin Square experiments to determine the effect of PEAR on nutrient utilization and mineral intake in steers consuming low-quality forage. Treatments included no supplemental protein (**CON)** and isonitrogenous levels of PEAR or CSM. Complete consumption was observed ≥ 91% of the time for DDG- and CSM-based supplements, which was markedly lower (55%) when PEAR was offered alone. However, the rate of consumption of PEAR was similar ($P \leq 0.05$) to that of CSM-based supplements. Provision of isonitrogenous levels of PEAR and CSM stimulated ($P \leq$

0.05) total digestible OM intake to a similar extent ($P = 0.98$) and OM and NDF digestion was similar for all treatments ($P \geq 0.23$). Supplemented steers retained and absorbed more N ($P \leq 0.02$) than CON. Supplementation of PEAR resulted in a Ca:P ratio of 8:1, which was the only mineral imbalance observed. Overall, our results suggest PEAR can be blended (up to 60%) with existing ingredients to create suitable protein supplements for grazing operations.

Introduction

Algal biomass is an attractive third-generation biofuel feedstock because it is more productive than land-based plants, does not require high-quality agricultural land or water, and can accumulate significant amounts of lipid (Scott et al., 2010). However, the lipid content of algae rarely exceeds 50%, indicating the co-product, post-extraction algal residue (**PEAR**), will be produced in greater quantities than oil (Becker, 2007; Christi, 2007). Therefore, economic feasibility of the emerging algal biofuel industry depends on marketing and placement of PEAR into a market of sufficient scale and value.

Significant cattle populations in the United States (one time capacity of 10.7 million feedlot cattle and 30.5 million beef cows; USDA-NASS, 2012) and the ability of ruminants to utilize co-products (distillers' grains, cottonseed meal) indicate the beef cattle industry is a potential market for PEAR. Post-extraction algal residue retains the protein fraction (25.2%) of the original biomass after oil extraction (Bryant et al., 2012) and may be a suitable protein source for beef cattle.

An initial step in determining the suitability of PEAR as a feedstuff is to quantify its acceptability and investigate the consequences of its use on nutrient utilization in beef cattle. Post-extraction algal residue contains a significant proportion of ash (24.3 – 57.6%; Bryant et al., 2012); from a feeding standpoint, this raises concerns for mineral toxicity and decreased value per ton. Accordingly, our objectives were to measure the palatability of PEAR in steers consuming a basal diet of forage (Experiment 1) and evaluate the effects of PEAR supplementation on forage utilization and mineral intake in steers consuming low-quality forage (Experiment 2).

Materials & methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University.

Experiment 1: Palatability

Twelve Angus steers (348.5 ± 8.2 kg of BW) were used in a 12 × 12 Latin Square experiment designed to evaluate the palatability of PEAR at divergent inclusion levels and with differing carriers as a supplement to cattle consuming a basal diet of Bermudagrass (Table 6). Bermudagrass was chopped through a screen (76 × 76 cm) and offered daily at 0600 h at 2% of initial BW to facilitate complete consumption. Steers were housed in individual stalls in an enclosed barn and given ad libitum access to water and commercial trace mineral blocks (composition: ≥ 96.0% NaCl, 1.00% S, 0.15% Fe, 0.25% Zn, 0.30% Mn, 0.009% I, 0.015% Cu, 0.0025% Co, and 0.001% Se; United Salt Corporation, Houston, TX).

Table 6. Composition of forage and supplements (Exp. 1)

	Composition, %		DM	OM	NDF	ADF	CP
Bermudagrass			89.9	93.3	76.1	41.9	13.3
Treatment[1]	DDG	PEAR					
100 DDG	100	0	87.6	94.4	54.5	21.0	31.5
80 DDG	80	20	88.7	86.2	--	--	29.0
60 DDG	60	40	89.8	80.6	--	--	27.9
40 DDG	40	60	90.2	73.0	--	--	22.5
	CSM	PEAR					
100 CSM	100	0	89.6	88.2	34.5	13.9	40.3
80 CSM	80	20	90.5	84.5	--	--	37.1
60 CSM	60	40	90.3	80.4	--	--	32.8
40 CSM	40	60	90.8	72.0	--	--	29.1
	LS	PEAR					
100 LS	100	0	42.7	--	--	--	--
66 LS	66	33	59.8	--	--	--	--
33 LS	33	66	72.7	--	--	--	--
100 PEAR	0	100	92.0	59.3	--	--	21.2

[1]DDG = dried distillers' grains, PEAR = post-extraction algal residue, CSM = cottonseed meal, LS = liquid supplement

Twelve consecutive 4-d periods were conducted, each period consisting of 3-d where steers were fed their assigned test supplement and 1-d where steers were fed dried distillers' grains (**DDG**) containing no PEAR. Algal biomass (genus: *Chlorella* sp.) was grown photosynthetically in an open pond, flocculated, dewatered, spray-dried, and lipid was extracted with a methyl pentane solvent. One kg of supplement was offered daily at 1130 h and rate of intake was determined by recording the time required to completely consume the supplement. After 1 h of exposure to the supplement, refusals were collected, weighed, and sampled.

Treatments were arranged as a 3 × 4 factorial with three carriers being tested: DDG, cottonseed meal (**CSM**), and a commercial liquid supplement (**LS**) and four levels of PEAR inclusion on an as-fed basis. A high production pellet mill (Pellet Pros, Dubuque, Iowa) was used to process PEAR into a manageable form, yielding 2.5 cm × 0.3 cm pellets. To facilitate pelleting, PEAR was combined with a binder (4% as-fed). Pellets were subsequently crumbled (California Pellet Mill, Crawfordsville, IN) to allow PEAR to be mixed with the other supplements and prevent sorting. In treatments where LS was the carrier, PEAR was not pelleted; however, binder was added and the PEAR/binder mixture was blended directly with LS. Samples of supplements were retained at each mixing.

Hay was dried in a forced-air oven for 96 h at 55°C and allowed to air-equilibrate for determination of partial DM. Hay and supplements were pooled across day on an equal weight basis, then ground through a 1-mm screen using a Wiley mill and dried at 105°C for determination of DM. Organic matter was determined as the loss in dry weight upon

combustion in a muffle furnace for 8 h at 450°C. Nitrogen was measured using the Elementar rapid N cube (Elementar, Hanua, Germany) and CP was calculated as N × 6.25. Analysis for NDF and ADF was performed sequentially using an Ankom Fiber Analyzer with sodium sulfite and amylase omitted and without correction for residual ash (Ankom Technology Corp., Macedon, NY). Physical structure, namely small particle size, of PEAR and LS prevented determinations of NDF and ADF.

Experiment 2: Nutrient utilization

Six steers (212.6 ± 13.4 kg of BW) fitted with ruminal and duodenal cannulas were used in concurrent 3 × 3 Latin square design experiments to determine the effect of supplemental PEAR on utilization of low-quality forage and mineral intake. Steers were housed in an enclosed barn and allowed ad libitum access to water. Chopped prairie grass (Table 7) was offered daily at 0700 h and fed at 130% of the previous 5-d average consumption. Supplements were provided just prior to feeding hay at 0700 h in feed pans within hay bunks, allowing access to the supplements throughout the day.

Treatments included a control, which received no supplemental protein (**CON**), and PEAR or CSM both provided at 100 mg N/kg BW. Level of supplementation was based on results of a related experiment (Chapter III). Species, cultivation, and harvesting method of PEAR were outlined in Experiment 1. The same process as Experiment 1 was used to pellet PEAR, but it was not crumbled as sorting was not a concern. Cottonseed meal was not pelleted.

Experimental periods were constructed as follows: 1) 8-d for adaptation to treatments, 2) 5-d for measurement of intake and digestion, and 3) 1-d for determination

Table 7. Composition of forage, supplements, and water (Exp. 2)

Item[1]	Prairie hay	PEAR	CSM	Water
	---------- DM basis ----------			
Chemical composition, %				
OM	92.3	52.8	90.9	
CP	6.7	19.1	44.9	
NDF	69.4	--	42.6	
ADF	43.2	--	23.7	
Macrominerals, %				
Ca	0.55	7.53	0.24	< 0.01
P	0.10	0.55	1.42	< 0.01
S	0.13	0.82	0.49	< 0.01
Na	0.01	5.15	0.16	0.01
Microminerals, ppm				
Fe	69	4608	272	0.02
Cu	2	31	13	< 0.01
Mn	229	131	34	0.01
Heavy metals, ppm				
Al	24	2533	199	< 0.01

[1]PEAR: post-extraction algal residue; CSM = cottonseed meal

of liquid passage flow, ruminal fermentation, and plasma urea N (**PUN**). Steers were housed in individual pens (2.1 × 1.5 m) for the first 4-d of each period, then moved to individual metabolism crates for the remainder of adaptation and throughout the collection period. Metabolism crates were designed such that urine and feces were collected into separate bins by gravity.

Calculations of intake, digestion, and N balance were made from observations on d 9 through 13. Hay, supplement, and ort samples were collected d 9 through 12 to correspond with fecal and urine samples collected d 10 through d 13. Feces and urine collected over each 24-h period were thoroughly mixed and a portion of each (3% fecal matter, 2% urine) was sub-sampled before freezing at -20°C. Urine pH was maintained below 3 by adding 400 mL of 6 *M* HCl to urine bins prior to collection.

On d 14 of each period, ruminal fermentation parameters, liquid passage flow, PUN, and plasma mineral concentrations were measured. Immediately before feeding, each steer received a 200 mL pulse dose of solution containing 17.8 g of Cr-EDTA administered ruminally (Udén et al., 1980). The solution was administered into various ruminal sites to achieve uniform dispersion. A suction strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh) was used to collect rumen fluid samples prior to feeding (0 h) and at 4, 8, 12, 16, and 20 h after feeding. A portable pH meter with a combined electrode (VWR SympHony) was used to measure the pH of each sample at the time of sampling. Subsamples of ruminal fluid were prepared and frozen at -20°C for determinations of VFA and Cr concentrations. Prior to freezing, 8 mL of rumen fluid was combined with 2 mL of 25% *m*-phosphoric acid for VFA analysis. Ten mL of

rumen fluid was frozen for Cr analysis without the addition of chemical agents. An additional sampling time occurred at 24 h for subsequent Cr analysis. Immediately after (h 0) and 4 h after treatments were offered, blood samples were collected from the jugular vein of each steer with 15 mL heparinized tubes. Blood was immediately placed on ice and centrifuged at 5000 × *g* for 15 min. Plasma was frozen at -20°C for subsequent PUN analysis.

Hay, ort, and fecal samples were dried in a forced-air oven for 96 h at 55°C, allowed to air-equilibrate, and weighed to determine partial DM. Hay and supplement samples were composited on an equal weight basis across days. Ort and fecal samples were composited by steer across days within period. Hay, ort, supplement, and fecal samples were ground with a Wiley mill to pass a 1-mm screen and analyzed for DM, OM, NDF, ADF, and N using procedures outlined in Experiment 1. Hay, ort, supplement, and water samples were digested on a graphite digestion block (SCP Science, Champlain, NY) and mineral concentrations were determined using an ICP spectrophotometer (Thermo Fisher Scientific Inc.).

Rumen fluid samples were thawed and centrifuged at 20,000 × *g* for 20 min. Volatile fatty acid concentrations were measured using a gas chromatograph with methods described by Vanzant and Cochran (1994). Plasma urea N concentrations were measured using a UV-VIS with colorimetric procedures as described by Broderick and Kang (1980). Chromium concentration was determined with an ICP spectrophotometer (Spectro Analytical Instruments Inc., Malborough, MA).

Formulas

Water consumption was not measured in this experiment; we assumed water consumption was equal to the amount of moisture excreted in the feces and urine (Table 8). Digestibilities were calculated with the following formula: [1 – (output of nutrient/intake of nutrient)] × 100. Liquid dilution rate was calculated by regressing the natural logarithm of Cr concentrations against sampling time (Warner and Stacy, 1968). Ruminal fluid volume was calculated by dividing the amount of Cr dosed by the calculated ruminal fluid Cr at h 0. Ruminal liquid turnover time was calculated as the inverse of the ruminal liquid dilution rate, and ruminal liquid flow was calculated by multiplying ruminal liquid volume by ruminal liquid dilution rate (Cochran and Galyean, 1994).

Statistical analysis

Experiment 1: Palatability

Palatability parameters were analyzed using the MIXED procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment, period, day, and treatment × day, with steer and treatment × period × steer as the random terms. The repeated term was day, with treatment × steer as the subject. The LSMEANS option was used to calculate treatment means. When complete consumption of the supplement did not occur, observations were excluded for statistical analysis of time and rate of consumption.

Experiment 2: Nutrient utilization

Intake, digestion, and N balance were analyzed using the MIXED procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment, period, and square, with steer as a random effect. Volatile fatty acid and PUN were analyzed using the MIXED procedure. Terms in the model were treatment, period, square, hour, and hour × treatment, with steer and treatment × period × steer × square included as random terms. The repeated term was hour, with treatment × steer serving as the subject. Treatment means were calculated using the LSMEANS option and contrasts included CON vs. PEAR, CON vs. CSM, and PEAR vs. CSM.

Results

Experiment 1: Palatability

Supplements containing DDG or CSM were completely consumed within the allotted amount of time (1 h) ≥ 90% of the time (Table 8). When DDG was the sole ingredient, the consumption rate was 100%, with a slight decline to 94 and 91% for 60 DDG and 40 DDG, respectively. The allotted amount of 100 CSM was consumed 97% of the time, which increased to 100% for 80 CSM and 60 CSM, and decreased to 92% for 40 CSM. There was a markedly lower rate of complete consumption for 100 LS, 59%, which increased to 88% for 66 LS, but decreased to 50% for 33 LS. Similarly, for 100 PEAR, complete consumption occurred 55% of the time within 1 h. A trend (P = 0.07) for a treatment × day interaction was apparent for the amount of supplement consumed (Table 8). This tendency is accounted for by decreasing consumption of 100 LS, 100 PEAR, and 66 LS over the duration of the 3-d period, and consistent supplement

intake across days for the other supplements. When 100 LS was initially introduced (d 1), 920 g were consumed, which decreased to 680 – 700 g on d 2 and 3. Similarly, intake of 100 PEAR decreased from 840 to 800 g between d 1 and 2, and further on d 3 (680 g). This trend was also observed for 66 LS; the supplement was completely consumed (1000 g) on d 1, with a decrease to 890 g by d 3. Averaged across days, supplement intake was similar ($P \geq 0.56$) for 66 LS and supplements containing DDG or CSM (920 – 1000g), and significantly greater ($P < 0.01$) than 100 PEAR, 100 LS, or 33 LS (770 – 780 g). The treatment × day interaction was not significant ($P = 0.11$) for the time required for complete consumption. Additionally, day was not significant ($P = 0.42$). However, supplement composition significantly affected ($P < 0.01$) time required to finish (Table 9). Instances where the supplement was not finished within 1 h were excluded from statistical analysis; therefore, results are only reflective of observations when complete consumption occurred. There was no statistical difference ($P \geq 0.23$) in time required to complete supplements containing DDG (5.55 – 5.73 min) or CSM (6.56 – 7.71 min). In contrast, supplements containing LS or 100 PEAR required significantly longer (13.00 – 15.96 min; $P < 0.01$) than DDG- or CSM-based supplements.

Rate of consumption, expressed as g of supplement consumed per min (**GPM**), was calculated solely from observations when complete consumption of the test supplement was achieved (Table 9). There was a treatment × day interaction ($P < 0.01$) and significant effect of day ($P < 0.01$) on the rate of supplement consumption. These observations were driven by 100 LS, where rate of consumption markedly declined from

Table 8. Amount consumed and percent of time test supplements were completely consumed (Exp. 1)[1]

Treatment[2]	Amount consumed, g[3]	SEM	Complete consumption, %[4]
100 DDG	1000[a]	65.2	100
80 DDG	1000[a]	65.2	100
60 DDG	990[a]	65.2	94
40 DDG	920[a]	65.2	91
100 CSM	970[a]	65.2	97
80 CSM	1000[a]	65.2	100
60 CSM	1000[a]	65.2	100
40 CSM	950[a]	65.2	92
100 LS	770[b]	65.2	59
66 LS	960[a]	65.2	88
33 LS	780[b]	65.2	50
100 PEAR	780[b]	65.2	55

[1]Within each column, means with differing subscripts differ at $P \leq 0.05$ level of significance

[2]DDG = dried distillers' grains, PEAR = post-extraction algal residue, CSM = cottonseed meal, LS = liquid supplement

[3]Amount consumed = the amount of supplement consumed on average, out of 1000 g. Trt × d: $P = 0.07$

[4]Complete consumption = % of time entire amount of offered supplement was consumed within 1 h

d 1 (118.9 GPM) to d 2 and 3 (~75 GPM). Supplement composition significantly affected ($P < 0.01$) rate of consumption. Supplements containing DDG and 40 CSM were all consumed at a rate of 174 – 193 GPM. All other CSM-based supplements and PEAR were consumed between 145 – 152 GPM. Supplements based on LS were consumed between 63 – 96 GPM.

Experiment 2: Nutrient utilization

One observation was excluded due to circumstances unrelated to the treatment. Provision of PEAR and CSM increased forage OM intake relative to CON ($P \leq 0.05$; Table 10), but did not differ ($P = 0.44$). Unsupplemented animals consumed 4.10 kg of forage OM daily, which increased to 4.75 and 4.58 kg/d when PEAR or CSM was provided, respectively. Total OM intake was similar between sources of supplemental protein ($P = 0.28$) and greater ($P \leq 0.01$) than CON. Total digestible organic matter intake (**TDOMI**), a primary measure of forage utilization, increased ($P = 0.05$) from 2.35 for CON to 2.82 kg/d for PEAR and CSM. The difference in TDOMI between PEAR and CSM was not significant ($P = 0.98$). Urinary output was greater ($P < 0.01$) for PEAR (6.90 kg/d) than CON and CSM (4.38 and 4.24 kg/d, respectively), and did not differ between CON and CSM ($P = 0.85$). There was a tendency ($P = 0.09$) for CSM to excrete more fecal water than CON, but other treatment contrasts were similar ($P \geq 0.21$).

Table 9. Time required and rate of consumption of test supplements when complete consumption occurred (Exp. 1)[1]

Treatment[2]		Time required (min)[3]	SEM	Rate of consumption (GPM)[4]	SEM
100 DDG	------when complete consumption occurred------	5.55[a]	2.18	187[a]	16.0
80 DDG		5.72[a]	2.18	180[a]	16.0
60 DDG		5.73[a]	2.18	186[a]	16.0
40 DDG		5.66[a]	2.18	193[a]	16.0
100 CSM		7.36[a]	2.18	154[b]	16.0
80 CSM		6.94[a]	2.18	152[b]	16.0
60 CSM		7.71[a]	2.18	148[b]	16.0
40 CSM		6.56[a]	2.18	174[a]	16.0
100 LS		13.50[b]	2.18	90[e]	16.0
66 LS		15.96[b]	2.18	63[d]	16.0
33 LS		15.29[b]	2.18	96[c]	16.0
100 PEAR		13.00[b]	2.18	152[b]	16.0

[1]Within each row, means with differing subscripts differ at $P \leq 0.05$ level of significance

[2]DDG = dried distillers' grains, PEAR = post-extraction algal residue, CSM = cottonseed meal, LS = liquid supplement

[3]Time required = the time, in min, required to finish entire amount of test supplement, when complete consumption occurred. Treatment × d: $P = 0.11$, effect of d: $P = 0.42$

[4]Rate of consumption = g of supplement consumed per minute, when complete consumption occurred. Treatment × d: $P < 0.01$, effect of d: $P < 0.01$.

Control steers consumed 45.3 g N/d, which was significantly less ($P < 0.01$) than those receiving either source of supplemental protein. Supplement N intake was similar ($P = 0.79$) for PEAR and CSM. Treatments were designed to be isonitrogenous; however, CSM received an additional 0.4 g supplemental N/d due to variation in the nutritive value of the supplements. Despite increased N intake with supplemental protein, fecal and urinary N excretion were not significantly affected ($P \geq 0.20$) by provision or source of protein, with the exception of a trend ($P = 0.09$) for PEAR to excrete more urinary N than CON and a trend ($P = 0.10$) for CSM to excrete more fecal N than CON. Apparently absorbed N significantly increased ($P < 0.01$) from 6.7 (CON) to 21.3 (CSM) and 26.5 g N/d (PEAR) when supplemental protein was provided. There was not a significant difference ($P = 0.20$) in the amount of N absorbed for PEAR and CSM. Negative N retentions were observed for CON (-9.3 g N/d), but not for CSM (4.5 g N/d) or PEAR (3.0 g N/d); there was not a statistical difference between sources of supplemental protein ($P \geq 0.75$). Provision of protein significantly increased ($P \leq 0.01$) urinary excretion of urea, but did not affect ($P \geq 0.28$) urinary ammonia excretion (Table 11).

Organic matter total tract digestion (**OMD**) was greatest for CSM (59.0%) and slightly decreased for CON (57.1%) and PEAR (55.9%), but there was not a statistical difference for any treatment contrasts ($P \geq 0.33$). Similarly, total tract NDF digestion (**NDFD**) was not significantly affected by provision of or source of protein ($P \geq 0.23$), ranging from 48.6 (PEAR) to 53.7% (CSM).

Table 10. Effect of post-extraction algal residue (PEAR) or cottonseed meal (CSM) on intake, digestion, and N balance in cattle consuming prairie grass (Exp. 2)

	Treatment[1]				Contrast *P*-value[2]		
	CON	PEAR	CSM	SEM	CON vs PEAR	CON vs CSM	PEAR vs CSM
No. of observations	6	6	5				
OM intake, kg/d							
Forage	4.10	4.75	4.58	0.28	0.01	0.05	0.44
Total	4.10	5.07	4.83	0.29	< 0.01	0.01	0.28
Digestible OM intake	2.35	2.82	2.82	0.21	0.05	0.05	0.98
Water excretion, kg/d							
Urine	4.38	6.90	4.24	0.81	< 0.01	0.85	< 0.01
Fecal	7.93	8.86	9.40	0.76	0.21	0.09	0.48
Total tract digestion, %							
OM	57.1	55.9	59.0	2.3	0.69	0.54	0.33
NDF	51.5	48.6	53.7	2.8	0.46	0.58	0.23
N, g/d							
Intake							
Total	45.3	71.6	70.0	4.0	< 0.01	< 0.01	0.64
Supplement	0.0	18.8	19.2	1.2	< 0.01	< 0.01	0.79
Fecal	38.6	45.1	48.6	4.4	0.22	0.10	0.53
Urinary	16.0	23.5	16.8	2.9	0.09	0.85	0.14
Apparent absorbed	6.7	26.5	21.3	2.7	< 0.01	< 0.01	0.20
Retained	-9.3	3.0	4.5	3.3	0.02	0.02	0.75

[1]CON = 0 mg N/kg of BW of supplement; PEAR = 100 mg N/kg of BW of PEAR; CSM = 100 mg N/kg BW of CSM

[2]CON vs PEAR = effect of no supplement vs PEAR, CON vs CSM = effect of no supplement vs CSM, PEAR vs CSM = effect of 100 mg N/kg BW of PEAR vs 100 mg N/kg BW of CSM

Table 11. Effect of post-extraction algal residue (PEAR) or cottonseed meal (CSM) on urinary urea and ammonia excretion in cattle consuming prairie grass (Exp. 2)

	Treatment[1]				Contrast *P*-value[2]		
	CON	PEAR	CSM	SEM	CON vs PEAR	CON vs CSM	PEAR vs CSM
No. of observations	6	6	5				
Urinary urea N excretion							
N, g/d	1.35	2.10	2.31	0.51	0.01	< 0.01	0.43
% of urinary N excretion	8.34	10.99	13.15	2.82	0.30	0.11	0.43
Urinary ammonia N							
N, g/d	0.13	0.10	0.09	0.04	0.38	0.22	0.64
% or urinary N excretion	0.81	0.45	0.54	0.19	0.01	0.06	0.44

[1]CON = 0 mg N/kg of BW of supplement; PEAR = 100 mg N/kg of BW of PEAR; CSM = 100 mg N/kg BW of CSM

[2]CON vs PEAR = effect of no supplement vs PEAR, CON vs CSM = effect of no supplement vs CSM, PEAR vs CSM = effect of 100 mg N/kg BW of PEAR vs 100 mg N/kg BW of CSM

Table 12. Effect of post-extraction algal residue (PEAR) or cottonseed meal (CSM) on ruminal fermentation parameters in cattle consuming prairie grass (Exp. 2)

	Treatment[1]				Contrast *P*-value[2]		
	CON	PEAR	CSM	SEM	CON vs PEAR	CON vs CSM	PEAR vs CSM
No. of observations	6	6	5				
PUN, m*M*[3]							
hour 0	0.87	0.84	1.32	0.22	0.10	0.04	0.54
hour 4	1.10	1.95	1.69	0.22	0.10	0.04	0.54
Total VFA, m*M*	98.5	83.0	98.7	6.30	0.11	0.98	0.10
Molar percentages							
Acetate	73.4	72.9	71.5	0.47	< 0.01	0.36	0.03
Propionate	16.5	18.1	17.3	0.42	< 0.01	0.11	0.09
Butyrate	8.72	8.77	8.39	0.11	0.77	0.06	0.04
Isobutyrate	0.60	0.71	0.61	0.03	0.01	0.89	0.02
Isovalerate	0.42	0.52	0.42	0.02	< 0.01	0.92	< 0.01
Valerate	0.38	0.45	0.44	0.03	0.04	0.07	0.68
pH	6.63	6.46	6.45	0.08	0.03	0.05	0.91

[1]CON = 0 mg N/kg of BW of supplement; PEAR = 100 mg N/kg of BW of PEAR; CSM = 100 mg N/kg BW of CSM

[2]CON vs PEAR = effect of no supplement vs PEAR, CON vs CSM = effect of no supplement vs CSM, PEAR vs CSM = effect of 100 mg N/kg BW of PEAR vs 100 mg N/kg BW of CSM

[3]Treatment × time : $P = 0.04$, effect of time: $P < 0.01$

Effects of PEAR and CSM supplementation on ruminal fermentation are summarized in Table 12. There was not a significant treatment × time ($P = 0.58$) interaction or effect of time ($P = 0.99$) on total VFA concentrations, therefore, treatment means of all sampling times are presented. Animals receiving CON or CSM had similar ($P = 0.98$) total VFA concentrations (98.5 and 98.7 m*M*, respectively) and there was a weak trend ($P = 0.10$) for total VFA concentrations to decrease for PEAR (83.0 m*M*) relative to CSM. Significant differences in molar percentages of various VFAs were present, but the biological significance is likely minor. Ruminal pH was greater ($P \leq 0.03$) for CON (6.63) than PEAR (6.46) and CSM (6.45).

Liquid passage rate (Table 13) was similar ($P \geq 0.59$) without regard to provision or source of protein. Rumen volume was significantly greater ($P = 0.03$) for PEAR (78 L) versus CON (58 L), and tended ($P = 0.06$) to be greater than CSM (63 L). Liquid flow rate was similar ($P \geq 0.16$) in all treatment contrasts, ranging from 1.34 – 1.81 L/h. Ruminal turnover time was not affected ($P \geq 0.52$) by treatment, although there were numerical differences; provision of protein decreased turnover time from 54.3 to approximately 46.3 h.

There was a treatment × hour interaction ($P = 0.04$) for PUN which was driven by a 133% increase in PUN from h 0 to 4 for PEAR and an approximate 26.5% increase for CON and CSM. At h 0, PUN concentrations tended ($P = 0.10$) to differ for CON and PEAR (0.84 – 0.87 m*M*), and were greater ($P = 0.04$) for CSM (1.27 m*M*) relative to CON. Four hours after feeding and supplementation, PEAR had approximately double the amount of PUN (1.95 m*M*) relative to h 0, while PUN increased slightly for CON

(1.10 m*M*) and less dramatically for CSM (1.69 m*M*). Overall, there was only a trend ($P = 0.10$) for PUN levels of CON and PEAR to differ, while CSM was consistently greater ($P < 0.04$) than CON and both protein sources were similar ($P = 0.54$).

Intakes of select macrominerals (Ca, P, S, and Na) are presented in Table 14; micromineral (Fe, Cu, Mn) and heavy metal (Al) intakes are presented in Table 15. Intake of individual minerals significantly increased ($P < 0.01$) for PEAR versus CON in every observation. Dramatic increases (> 1200%) were observed for total Na, Fe, and Al intake for PEAR versus CON, while relatively smaller increases (33– 300%) were observed for Ca, P, S, Cu, and Mn. The majority of the observed increase in mineral intake for Ca, Na, Fe, Cu, and Al was attributed to the supplement, while forage mineral content was responsible for increased P, S, and Mn intake. Greater mineral intakes ($P < 0.01$) were observed in all instances for PEAR versus CSM, with the exception of P and Mn ($P \geq 0.11$).

Discussion

Experiment 1 was designed to evaluate the palatability of PEAR and blends of PEAR with conventional feedstuffs in steers consuming a basal diet of forage. Our results indicate PEAR is not palatable alone or as a component of LS, but may be blended up to 60% with DDG or CSM with no adverse effect on palatability. We based our evaluation of palatability on the amount of time the supplement was completely consumed, the time required to finish the supplement, and the amount of supplement refused. In Experiment 2, we did not directly measure palatability, but did not have an issue with supplement refusals of 100% PEAR supplements. In visual observations,

Table 13. Effect of post-extraction algal residue (PEAR) or cottonseed meal (CSM) on liquid passage rate, outflow, turnover time, and ruminal fluid volume (Exp. 2)

	Treatment[1]				Contrast *P*-value[2]		
	CON	PEAR	CSM	SEM	CON vs PEAR	CON vs CSM	PEAR vs CSM
No. of observations	6	6	5				
Liquid passage rate, %/h	2.27	2.31	2.52	0.40	0.92	0.59	0.66
Liquid flow rate, L/h	1.34	1.81	1.43	0.24	0.16	0.79	0.26
Ruminal fluid volume, L	58.3	77.5	63.0	5.63	0.03	0.54	0.09
Turnover time, h	54.3	44.7	47.9	12.15	0.52	0.69	0.84

[1]CON = 0 mg N/kg of BW of supplement; PEAR = 100 mg N/kg of BW of PEAR; CSM = 100 mg N/kg BW of CSM

[2]CON vs PEAR = effect of no supplement vs PEAR, CON vs CSM = effect of no supplement vs CSM, PEAR vs CSM = effect of 100 mg N/kg BW of PEAR vs 100 mg N/kg BW of CSM

Table 14. Effect of post-extraction algal residue (PEAR) or cottonseed meal (CSM) on macromineral intake in cattle consuming prairie grass (Exp. 2)

	Treatment[1]				Contrast *P*-value[2]		
	CON	PEAR	CSM	SEM	CON vs PEAR	CON vs CSM	PEAR vs CSM
No. of observations	6	6	5				
Ca, g/d							
Intake							
Total	20.9	71.0	24.2	3.35	< 0.01	0.47	< 0.01
Supplement	0.0	46.4	0.2	2.30	< 0.01	0.94	< 0.01
P, g/d							
Intake							
Total	4.4	8.6	8.6	0.45	< 0.01	< 0.01	0.99
Supplement	0.0	3.4	3.8	0.22	< 0.01	< 0.01	0.16
S, g/d							
Intake							
Total	5.6	11.4	7.4	0.55	< 0.01	0.01	< 0.01
Supplement	0.0	5.0	1.3	0.25	< 0.01	< 0.01	< 0.01
Na, g/d							
Intake							
Total	0.2	31.6	0.7	1.26	< 0.01	0.80	< 0.01
Supplement	0.0	31.5	0.5	1.41	< 0.01	0.81	< 0.01

[1]CON = 0 mg N/kg of BW of supplement; PEAR = 100 mg N/kg of BW of PEAR; CSM = 100 mg N/kg BW of CSM

[2]CON vs PEAR = effect of no supplement vs PEAR, CON vs CSM = effect of no supplement vs CSM, PEAR vs CSM = effect of 100 mg N/kg BW of PEAR vs 100 mg N/kg BW of CSM

Table 15. Effect of post-extraction algal residue (PEAR) or cottonseed meal (CSM) on micromineral and heavy metal intake in cattle consuming prairie grass (Exp. 2)

	Treatment[1]				Contrast *P*-value[2]		
	CON	PEAR	CSM	SEM	CON vs PEAR	CON vs CSM	PEAR vs CSM
No. of observations	6	6	5				
Fe, mg/d							
Intake							
Total	227	3124	267	171.2	< 0.01	0.87	< 0.01
Supplement	0	2850	34	169.1	< 0.01	0.88	< 0.01
Cu, mg/d							
Intake							
Total	6.7	26.8	10.8	2.5	< 0.01	0.17	< 0.01
Supplement	0.0	18.7	4.2	2.1	< 0.01	0.15	< 0.01
Al, mg/d							
Intake							
Total	48.9	1621	94.3	74.1	< 0.01	0.66	< 0.01
Supplement	0.0	1557	42.1	73.1	< 0.01	0.68	< 0.01
Mn, mg/d							
Intake							
Total	842.2	1116.3	983.0	78.5	< 0.01	0.09	0.11
Supplement	0.0	78.8	10.8	5.7	< 0.01	0.17	< 0.01

[1]CON = 0 mg N/kg of BW of supplement; PEAR = 100 mg N/kg of BW of PEAR; CSM = 100 mg N/kg BW of CSM

[2]CON vs PEAR = effect of no supplement vs PEAR, CON vs CSM = effect of no supplement vs CSM, PEAR vs CSM = effect of 100 mg N/kg BW of PEAR vs 100 mg N/kg BW of CSM

steers receiving PEAR generally consumed half of the allotted amount of supplement within 10 min of feeding, and consumed the rest at a later point in time. It is important to note, however, the amount of supplement offered was less in Experiment 2 (508 – 787 g/d, depending on BW) than Experiment 1 (1000 g/d), which may have affected observations.

Decreased palatability associated with PEAR may be partially attributable to its NaCl content; PEAR in our experiment contained 5.2% Na, which was approximately 3100% greater than the Na content of CSM and DDG (Drewery et al., 2011). Reductions in feed intake have been observed when NaCl or Na-acetate were infused ruminally, a result of increased rumen osmolality (Ternouth and Beattie, 1971; Bergen, 1972; Forbes et al., 1992; Provenza, 1995). Rumen osmolality regulates feed intake if osmotic pressure exceeds 400 mOsm/kg (Bergen, 1972); however, rumen osmolality was 344 mOsm/kg when NaCl composed 5% of the diet (Garza and Owens, 1989) and exceeded 400 mOsm/kg when supplemental NaCl was provided at 9 – 11% of the diet (Bergen, 1972). In our study, Na intake was 0.55% of the total diet when PEAR was provided, which is clearly under the threshold where reductions in forage intake would be observed and in agreement with our observations of increased forage OM intake with provision of PEAR in Experiment 2. However, Na content coupled with the provision of significant quantities of other minerals may have been sufficient to cause a short-term cessation of supplement intake, followed by resumed intake at a later point. Observations of reduced consumption of PEAR on d 2 and 3 in Experiment 1 suggest an effective feedback mechanism to reduce or slow intake. Incorporation of approximately

30% NaCl in protein supplements is a viable management practice in grazing cattle operations to regulate supplement intake (Cardon et al., 1951; Riggs et al., 1953; Chicco et al., 1971). Although salt is commonly used to impede intake, the total mineral load may be the causative factor; thus, the impacts of consuming > 40% ash in supplements should be further investigated. Salt is almost completely absorbed from the rumen as it is ingested, resulting in increased blood NaCl concentrations and the need to urinate to avoid toxicity issues (Cardon et al., 1951). Therefore, water consumption is positively related to the NaCl content of the diet (Meyer et al., 1955; Rogers et al., 1979). For every 100 g of salt ingested, 4.2 L of urine are required for elimination (Cardon et al., 1951). In Experiment 2, water consumption was increased 16% for PEAR versus CSM; this increase was more significant (36%) in Chapter III at isonitrogenous levels of PEAR and CSM. We observed more dramatic increases in water consumption in Chapter III because the entire supplement was infused to the rumen at a single time point while, in Experiment 2, steers were allowed to self-regulate intake of PEAR. If we extended the time for supplement consumption from 1 h to > 2 h in Experiment 1, we likely would not have observed refusals of PEAR as there would be a longer period of time to dilute the NaCl load and rumen osmolality via water consumption.

Experiment 2 was designed to evaluate potential for PEAR to be integrated as a protein supplement in grazing cattle operations based on comparisons of forage utilization versus CSM. Additionally, there is concern the mineral composition of PEAR will limit its incorporation in beef feeding systems due to toxicity issues; thus, we investigated relationships between PEAR supplementation and mineral intake.

Protein supplementation to cattle consuming low-quality forage increases forage utilization and animal performance by providing an additional source of N to ruminal microbes (Köster et al., 1996; Mathis et al., 2000; Wickersham et al., 2008). The response to protein supplementation is dependent on the protein content of the basal forage with diminishing return expected once forage protein surpasses 7% (Moore and Kunkle, 1995). In our study, steers were allowed ad libitum access to prairie grass, which contained 6.7% CP. Supplemental protein elicited a stimulatory effect on forage OM intake, which is in agreement with previous observations (Köster et al., 1996; Olson et al., 1999; Klevesahl et al., 2003; Wickersham et al., 2008). In our study, forage OM intake increased 12 – 16% when protein (100 mg N/kg BW) was supplemented to cattle consuming low-quality forage. Wickersham et al. (2008) supplemented a similar amount of protein (118 mg N/kg BW) as casein and observed a 38% increase in forage OM intake; however, the prairie hay utilized in their study was more N deficient than ours (4.9% CP), limiting microbial growth and intake to a further extent. Our observations demonstrate provision of PEAR is effective at stimulating forage intake in steers consuming low-quality forage.

The amount of OM consumed and digested in the total tract increased with provision of protein, indicating an improvement in forage utilization. A similar TDOMI response has been observed in cattle consuming low-quality forage and receiving supplemental protein (Köster et al., 1996; Olson et al., 1999; Klevesahl et al., 2003; Wickersham et al., 2008). As expected, the degree of increase in TDOMI is dependent on the quality of the basal forage. In steers consuming 1.94% CP prairie hay,

supplementation of DIP (109 mg N/kg BW) increased TDOMI 157% (Köster et al., 1996); our observation (24% increase) was more similar to that of Mathis et al. (2000), where TDOMI increased 18% when 131 mg N/kg BW of DIP was supplemented steers consuming 5.9% CP brome grass.

Although statistically insignificant, OMD and NDFD were numerically depressed for PEAR. Rumen retention time was shortest with the largest ruminal fluid volume and liquid flow rate for PEAR. These observations were likely driven by increased water consumption and forage OM intake for PEAR relative to CON or CSM. Köster et al. (1996) and Mathis et al. (2000) observed decreased OMD and NDFD at increased levels of supplementation, which were also associated with increased forage OM intake. As digesta is retained in the rumen for a longer period of time, there is increased potential for microbes to interact with diet OM, which would be reflected in measures of total tract digestibility. Thus, the decrease in digestibility for PEAR likely resulted from competing effects of passage and digestion. Rumen fluid volume ranged 27.0 (CON) – 36.4% (PEAR) of BW, which is greater than the average observed by Olson et al. (1999) and Mathis et al. (2000), 18.7 and 18.3% of BW, respectively. Error is inherent when using a marker rather than ruminal evacuation to measure passage rate and retention time; however, there was no experimental bias between treatments, thus, we are confident the trend we observed is reflective of the differences in ruminal kinetics for CON, PEAR, and CSM.

Nitrogen excretion observations were characterized by numerical differences. There was a 22% increase in fecal N excretion when supplemental protein was provided

to steers consuming low-quality forage and a 46% increase in urinary N output when PEAR was provided. When ruminal N requirements are addressed, there is a larger increase in urinary N output than fecal N output (Bunting et al., 1989; Archibeque et al., 2001). Our observations contrast those of Wickersham et al. (2008), who observed a 57% increase in fecal N excretion and 27% increase in urinary N excretion at a similar level of supplementation (118 mg N/kg BW). In the study conducted by Wickersham et al. (2008), the increase in forage OM intake when a similar level of protein was provided was more dramatic than ours (38 versus 13%, respectively). Therefore, our observations were likely driven by: 1) the ability of our basal forage to address a majority of microbial N requirements, and 2) relatively small differences in forage intake and the amount of ingested material in the subsequent fecal output.

Supplemental mineral intakes were greater when PEAR was provided versus CSM for all minerals and heavy metals, with the exception of P. There is concern the mineral profile of PEAR will cause toxicity issues and limit inclusion rate in the beef industry; however, based on recommendations of the NRC (2000), the only intake of concern was the Ca:P ratio.

Sulfur intake as a percent of the total diet was only slightly higher for PEAR (0.19%) than CON (0.13%) or CSM (0.14%), despite a 54% increase in supplemental S intake for PEAR versus CSM. Forage S intake was greater when protein was supplemented versus CON; however, DM intake increased 18% from CON to CSM, and an additional 10% from CSM to PEAR, resulting in minimal increases in S intake as a percent of the total diet. For all treatments, total S intake was under the threshold

(0.40%) where S toxicity would become a concern (NRC, 2000). If forage intake was similar, cattle could consume double the amount of PEAR supplemented in our study without exceeding the maximum tolerable concentration. Based on observations from Experiment 1, PEAR will likely be incorporated in the grazing industry as a blended supplement. The S content of 40 CSM is 0.69%, which increases to 0.74% for 40 DDG, but is still less than 100 PEAR (0.82%). Calculations are based on S content of CSM and PEAR used in Experiment 2, and average S in DDG (Drewery et al., 2011). Sulfur content and level of PEAR inclusion are positively related; thus, S will be less restrictive if PEAR is blended with CSM or DDG, rather than fed alone.

Iron and Al intake were orders of magnitude higher for PEAR than CSM or CON; ≥ 91% of this increase was attributable to supplement consumption. Iron concentration of the diet increased from 51 ppm for CON and CSM to 545 ppm for PEAR, which was less than the maximum tolerable concentration for beef cattle (1000 ppm; NRC, 2000). There would be an approximate 39% decrease in the Fe content of a blended supplement (60% PEAR and 40% DDG or CSM) versus PEAR. Blending with other ingredients would alleviate the constraint on inclusion rate caused by Fe content of PEAR. There was a 2151% increase in Al intake for PEAR relative to CON or CSM. Beef cattle can tolerate 1000 ppm Al without toxicity issues (NRC, 2000); however, dietary Al concentration did not approach this level when PEAR was provided (283 ppm), indicating inclusion rate of PEAR in grazing cattle operations will not be limited by Al content.

The NRC (2000) recommends daily consumption of 6 g Ca and 5 g P to meet maintenance requirements of growing and finishing cattle weighing 200 kg, which is similar to the characteristics of the steers utilized in our study. Forage intake met Ca requirements, but failed to address P requirements for unsupplemented steers; supplementation with PEAR and CSM addressed these requirements. Ruminants are more tolerant of a wide Ca:P ratio than monogastrics; the feeding recommendation of Ca:P is 1:1 – 7:1 (NRC, 2000). Wise et al. (1963) observed decreases in animal performance and nutrient conversion when this ratio was imbalanced, more drastically if Ca:P was < 1:1 than > 7:1. Accounting for total intake, CON (5:1) and CSM (3:1) were within the recommended feeding ratio for Ca:P, but PEAR provided excess amounts of supplemental Ca relative to P (8:1). Call et al. (1978) did not observe a decrease in animal performance when Ca:P intake was 9:1, but there was no beneficial aspect to providing additional Ca, resulting in apparent nutrient wastage for PEAR. The Ca:P ratio of individual supplements are 13:1 (PEAR) and 0.05:1 (CSM and DDG); thus, blending PEAR with CSM or DDG would create a supplement which better compliments the Ca:P ratio of typical forages relative to 100% PEAR.

CHAPTER III

EFFECT OF INCREASING AMOUNTS OF POST-EXTRACTION ALGAL RESIDUE ON LOW-QUALITY FORAGE UTILIZATION IN STEERS

Overview

Algal biomass has been identified as a third-generation biofuel. Significant quantities of the co-product, post-extraction algal residue (**PEAR**), remain after lipid extraction. After extraction, PEAR is concentrated in protein, suggesting it may replace cottonseed meal (**CSM**) as a protein supplement. Our objective was to determine the optimal level of PEAR supplementation to steers consuming low-quality forage and compare effects of supplementation on N metabolism and forage utilization versus a CSM control. Five steers arranged in a 5 × 5 Latin square had ad libitum access to oat straw. Treatments were infused ruminally once daily and included no supplemental protein (**CON**), PEAR at 50, 100, and 150 mg N/kg BW, and CSM at 100 mg N/kg BW. Increased provision of PEAR stimulated total digestible organic matter intake (**TDOMI**) quadratically ($P = 0.01$) from 0.9 (CON) to 1.6 kg/d (100 mg N/kg BW of PEAR). Organic matter digestibility (**OMD**) increased quadratically ($P < 0.01$) with supplementation, with maximization at 50 mg N/kg BW of PEAR. At isonitrogenous levels of PEAR and CSM, TDOMI was similar ($P = 0.13$) as was OMD ($P = 0.50$). Negative N balance was observed for all treatments except PEAR provided at 100 or 150 mg of N/kg BW. Nitrogen balance was greatest (1.84 g N/d) at 100 mg N/kg BW of PEAR. There were no significant differences between PEAR and CSM supplementation in measurements of plasma urea-N, ammonia, or VFA concentrations. Our observations

suggest cattle provided PEAR utilize forage in a similar manner to those supplemented CSM, indicating PEAR has potential to replace CSM as a protein supplement in forage-based operations. Forage utilization is maximized when PEAR is provided at 100 mg N/kg BW.

Introduction

Algal biofuel production utilizes land and water unsuitable for food or feed production while maintaining a high productivity of biomass per hectare; assuming an oil content of 30%, algae can produce 58,700 L oil/ha (Christi, 2007). Most importantly, algal biomass is energy dense (20 - 75% oil; Christi, 2007), making it an attractive third-generation biofuel. Numerous species of algae can be cultivated; however, those suitable for large-scale production contain approximately 20% lipid, indicating the co-product (post-extraction algal residue; **PEAR**) will be produced in excess of the desired product, oil (Becker et al., 2007). Consequently, utilization of algae for biofuel production may not be economically or environmentally viable without the concurrent development and marketing of PEAR.

Ruminants utilize co-products of variable quality from a number of industries, indicating potential for PEAR to be successfully incorporated into the beef industry. After lipid extraction, PEAR retains its protein fraction (19 – 38% CP; Bryant et al., 2012), suggesting the potential to replace conventional sources of supplemental protein in grazing cattle operations. Benefits of protein supplementation to cattle consuming low-quality forage have been widely demonstrated in the literature (Köster et al., 1996; Mathis et al., 2000; Wickersham et al., 2004) and protein supplementation is accepted as

standard practice in the beef industry. Accordingly, the objectives of this study were to determine the effects of increasing delivery of supplemental PEAR on forage utilization in steers consuming low-quality forage and compare observations to steers supplemented with an isonitrogenous level of cottonseed meal (**CSM**).

Materials & methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Texas A&M University.

Five steers (198.2 ± 6.1 kg of BW) fitted with ruminal and duodenal cannulas were used in a 5 × 5 Latin square design to determine the effects of provision and level of PEAR on low-quality forage utilization. Treatments consisted of four levels of supplemental PEAR: 0, 50, 100, or 150 mg N/kg BW (**CON**, **50P**, **100P**, and **150P**, respectively) and one level of CSM provided at 100 mg N/kg BW (**100C**). Algal biomass (genus: *Chlorella s*p.) was grown photosynthetically in an open pond, flocculated, dewatered, spray-dried, and extracted with a methyl pentane solvent. Oat straw (Table 16) was chopped through a screen (76 cm × 76 cm) offered daily at 0700 h and fed at 130% of the previous 5-d average consumption at 0700 h daily. Prior to feeding, treatments were dosed ruminally each day by removing ruminal contents (~1 kg), placing the treatment in the rumen, and returning the ruminal contents to the rumen. Steers were housed in an enclosed barn and had ad libitum access to water and commercial trace mineral blocks (composition: ≥ 96.0% NaCl, 1.00% S, 0.15% Fe, 0.25% Zn, 0.30% Mn, 0.009% I, 0.015% Cu, 0.0025% Co, and 0.001% Se; United Salt Corporation, Houston, TX).

Table 16. Chemical composition of forage and supplements

Item	Oat straw	PEAR[1]	CSM[2]
	---------- % DM ----------		
OM	92.7	55.5	91.0
CP	4.5	17.9	42.9
NDF	80.3	--	24.2
ADF	58.3	--	16.5
Acid detergent insoluble ash	5.3	--	0.8

[1]PEAR: post-extraction algal residue (genus: *Chlorella sp.*)
[2]CSM: cottonseed meal

The five experimental periods were broken down as follows: 1) 8-d for adaptation to treatments, 2) 5-d for measurement of intake and digestion, and 3) 1-d for determination of duodenal flow, ruminal fermentation, and plasma urea N (**PUN**). Steers were housed in individual pens (2.1 × 1.5 m) for the first 4-d of each period and moved to individual metabolism crates for the remainder of adaptation and throughout the collection period. Metabolism crates were designed such that urine and feces were collected into separate bins by gravity.

Calculations of intake, digestion, and N balance were made from observations on d 9 through 13. Hay, supplement, and ort samples were collected on d 9 through 12 to correspond with fecal samples collected on d 10 through d 13. Feces and urine collected over each 24-h period were thoroughly mixed and a portion of each (3% fecal matter, 2% urine) was sub-sampled before freezing at -20°C. Urine pH was maintained below 3 by adding 400 mL of 6 *M* HCl to urine bins prior to collection.

On d 14 of each period, ruminal fermentation, duodenal flow, and liquid passage rate were characterized. Immediately before feeding, each steer received a 200 mL pulse dose of solution containing 17.8 g of Cr-EDTA solution administered ruminally (Udén et al., 1980). The solution was administered into various ruminal sites to achieve uniform dispersion. A suction strainer (Raun and Burroughs, 1962; 19 mm diameter, 1.5 mm mesh) was used to collect rumen fluid samples prior to feeding (0 h) and at 4, 8, 12, 16, 20 h after feeding. A portable pH meter with a combined electrode (VWR SympHony) was used to measure the pH of each sample at the time of sampling. Subsamples of ruminal fluid were prepared and frozen at -20°C for later determinations of VFA, NH_3 N

and Cr concentrations. Prior to freezing, 8 mL of rumen fluid was combined with 2 mL of 25% *m*-phosphoric acid for VFA and ammonia N analysis. Ten mL of rumen fluid was frozen for Cr analysis with an additional sample collected at 24 h. Whole ruminal contents (1 kg) and duodenal digesta (300 mL) were also collected at all sampling times (except 24 h after feeding) to determine duodenal flow. Duodenal contents were immediately frozen at -20°C. To isolate ruminal bacteria from whole ruminal contents, 500 mL of 0.9% saline solution was added immediately after sample collection, blended for 5 min, and strained through 2 layers of cheesecloth. The liquid fraction was frozen immediately at -20°C and the remaining material was returned to the rumen. Acid detergent insoluble ash (**ADIA**) served as an internal marker to calculate duodenal flow. Immediately after and 4 h after treatments were administered, blood samples were collected from the jugular vein of each steer with 15 mL heparinized tubes. Blood was immediately placed on ice and centrifuged at 5000 × *g* for 15 min. Plasma was frozen at -20°C for subsequent PUN analysis.

Hay, ort, and fecal samples were dried at 55°C in a forced-air oven for 96 h, allowed to air-equilibrate, and weighed to determine partial DM. Duodenal samples were lyophilized. All dried samples were then ground with a Wiley mill to pass a 1-mm screen. Hay and supplement samples were composited on an equal weight basis across days. Ort and fecal samples were composited by steer across days within period. Hay, supplement, fecal, and duodenal samples were dried at 105°C for DM determination. Organic matter was determined as the loss in dry weight upon combustion for 8 h at 450°C. Nitrogen was measured using the Elementar rapid N cube (Elementar, Hanua,

Germany) and CP was calculated as N × 6.25. Analysis for NDF was performed using an Ankom Fiber Analyzer with sodium sulfite and amylase omitted and without correction for residual ash (Ankom Technology Corp., Macedon, NY). Acid detergent fiber was also determined using an Ankom Fiber Analyzer, and ADIA was measured on hay, supplement, ort, fecal, and duodenal samples by combusting Ankom bags containing ADF residues for 8 h at 450°C in a muffle furnace.

Rumen fluid samples were thawed and centrifuged at 20,000 × *g* for 20 minutes. Volatile fatty acid concentrations were measured using a gas chromatograph with methods described by Vanzant and Cochran (1994). Ammonia N and PUN concentrations were measured using a UV-vis with colorimetric procedures as described by Broderick and Kang (1980). Chromium concentration was determined with an ICP spectrophotometer (Spectro Analytical Instruments, Inc., Malborough, MA).

To isolate ruminal bacteria, samples of ruminal contents were thawed and centrifuged at 500 × *g* for 20 min to remove feed particles from the sample. Supernatants were centrifuged at 20,000 × *g* for 20 min to pellet the bacteria. Pellets were resuspended in a 0.9% NaCl solution and centrifuged at 20,000 × *g* for 20 min. Bacterial pellets were frozen and lyophilized. Dried bacterial samples were analyzed for DM, OM, N and cytosine.

Formulas

Digestibilities were calculated by the following formula: [1 – (output of nutrient/intake of nutrient)] × 100. Duodenal flow was calculated by dividing fecal ADIA output by the concentration of ADIA in duodenal digesta. Liquid dilution rate was

calculated by regressing the natural logarithm of Cr concentrations against sampling time (Warner and Stacy, 1968). Ruminal fluid volume was calculated by dividing the amount of Cr dosed by the calculated ruminal fluid Cr concentration at 0 h. Ruminal liquid turnover time was calculated as the inverse of the ruminal liquid dilution rate, and ruminal liquid flow was calculated by multiplying ruminal liquid volume by ruminal liquid dilution rate (Cochran and Galyean, 1994).

Statistical analyses

Intake, digestion, N balance, and duodenal flows were analyzed using the MIXED procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). Terms in the model included treatment and period, with steer as a random effect. Fermentation profile variables and PUN were analyzed using the MIXED procedure. Terms in the model were treatment, period, hour, and hour × treatment, with steer and treatment × period × steer included as random terms. The repeated term was hour, with treatment × steer serving as the subject. Treatment means were calculated using the LSMEANS option and were separated using linear and quadratic contrasts for amount of PEAR provided and contrasts between 100P and 100C.

Results

Level of PEAR supplementation

Increased provision of PEAR quadratically increased forage OM intake (Table 17; $P = 0.05$), from 1.92 kg/d for CON to a peak of 2.78 kg/d for 100P. Total digestible organic matter intake (**TDOMI**) quadratically increased in response to greater levels of supplemental PEAR, from 0.91 to 1.58 kg/d for CON and 100P, respectively. By design,

total N intake increased linearly ($P < 0.01$) with increased PEAR supplementation, from 14.3 g/d for CON to 45.8 g/d when 150P was administered. Animals receiving no supplemental PEAR (CON) consumed 14.3 g forage N/day. In accordance with forage OM intake, forage N intake increased quadratically in response to provision of PEAR. As expected, fecal and urinary N excretion were closely associated with N intake, linearly increasing ($P < 0.01$) as a result of increased PEAR supplementation. Apparent N absorption increased with greater levels of supplementation in a linear fashion ($P < 0.01$), ranging from -3.33 g/d for CON to 16.6 g/d for 150P. Nitrogen retention increased quadratically ($P < 0.01$) with increased PEAR supplementation; 1.84 g N/d were retained when 100P was provided, which decreased to 0.87 g N/d at 150P. Negative N retentions were observed for steers consuming only forage (CON) and at the lowest level of PEAR supplementation (50P; -13.6 and -1.55 g N/d, respectively). Urinary output linearly increased ($P = 0.02$) from 3.47 kg/d (CON) to 6.18 kg/d (150P). Fecal moisture output also linearly increased ($P < 0.01$) by 70% from CON to 150P.

Increased provision of PEAR increased apparent OM digestibility (**OMD**) quadratically ($P < 0.01$). Unsupplemented steers (CON) had the lowest OMD (46.6%) with the greatest observation at 50P (54.6%). Steers receiving 100P and 150P had comparable OMD (50.7 and 47.3%, respectively). At 50P, apparent NDF digestibility (**NDFD**) was 55.8%, which decreased to 44.2% at 150P. Unsupplemented steers had NDFD similar to that of steers supplemented 100P (49.7 and 50.2%, respectively). Ruminal NDF digestion was not affected ($P \geq 0.43$) by level of supplemental protein.

Table 17. Effect of protein supplementation level with post-extraction algal residue (PEAR) or cottonseed meal (CSM) on forage utilization and N balance

	Treatment[1]						Contrast *P*-value[2]		
Item	CON	50P	100P	150P	100C	SEM	Linear	Quadratic	100P vs 100C
No. of observations	5	5	5	4	5				
OM intake, kg/d									
Forage	1.92	2.55	2.78	2.73	2.50	0.20	< 0.01	0.05	0.22
Total	1.92	2.72	3.11	3.22	2.74	0.21	< 0.01	0.05	0.11
Digestible OM intake	0.91	1.48	1.58	1.53	1.35	0.13	< 0.01	0.01	0.13
Moisture excretion, kg/d									
Fecal	4.65	5.49	7.43	7.93	6.72	0.76	< 0.01	0.55	0.09
Urinary	3.47	4.52	4.86	6.18	2.75	0.77	0.02	0.84	0.04
Ruminal digestion, %									
NDF	43.1	41.2	43.3	46.3	39.5	4.1	0.42	0.42	0.38
Total tract digestion, %									
OM	46.6	54.6	50.7	47.3	49.2	1.85	0.83	< 0.01	0.50
NDF	49.7	55.8	50.2	44.2	50.9	1.94	0.01	< 0.01	0.95
N, g/d									
Intake	14.3	28.0	38.3	45.8	37.9	2.09	< 0.01	0.05	0.85
Forage	14.3	19.5	21.4	20.5	19.9	1.66	< 0.01	0.04	0.41
Supplement	0.0	8.5	16.9	25.3	18.0	0.86	< 0.01	0.91	0.18
Fecal	17.7	20.3	24.7	29.3	26.4	3.67	< 0.01	0.70	0.63
Urinary	10.2	9.2	11.7	15.7	12.2	1.30	< 0.01	0.04	0.76
Absorbed	-3.3	7.7	13.6	16.6	11.6	2.17	< 0.01	0.04	0.40
Retained	-13.6	-1.55	1.84	0.9	-0.6	2.48	< 0.01	< 0.01	0.38
Duodenal flow, g N/d									
Total	27.0	37.3	48.4	39.7	46.2	3.3	< 0.01	< 0.01	0.55

[1]CON = 0 mg N/kg of BW; 50P = 50 mg N/kg of BW of PEAR; 100P = 100 mg N/kg of BW of PEAR;150P = 150 mg N/kg of BW of PEAR; 100C = 100 mg N/kg of BW of CSM

[2]Linear = linear effect of PEAR level; quadratic = quadratic effect of PEAR level; PEAR vs CSM = effect of 100P vs 100C

Table 18. Effect of protein supplementation level with post-extraction algal residue (PEAR) or cottonseed meal (CSM) on urinary urea and ammonia excretion

	Treatment[1]						Contrast *P*-value[2]		
Item	CON	50P	100P	150P	100C	SEM	Linear	Quadratic	100P vs 100C
No. of observations	5	5	5	4	5				
Urinary urea N excretion									
N, g/d	1.15	0.96	1.85	0.97	2.66	0.60	0.90	0.54	0.30
% of urinary N excretion	11.9	11.1	14.5	5.64	21.4	5.11	0.48	0.41	0.30
Urinary ammonia N									
N, g/d	0.52	0.29	0.15	0.15	0.32	0.11	0.02	0.29	0.24
% of urinary N excretion	5.33	2.92	1.16	1.04	2.92	1.37	0.03	0.37	0.32

[1]CON = 0 mg N/kg of BW; 50P = 50 mg N/kg of BW of PEAR; 100P = 100 mg N/kg of BW of PEAR;150P = 150 mg N/kg of BW of PEAR; 100C = 100 mg N/kg of BW of CSM

[2]Linear = linear effect of PEAR level; quadratic = quadratic effect of PEAR level; PEAR vs CSM = effect of 100P vs 100C

Duodenal N flow increased as level of N increased, from 27.0 g N/d for CON to 46.2 g N/d for 100C, but was not statistically significant ($P \geq 0.12$).

Urinary urea N excretion and the percentage of urinary N excretion as urea (Table 18) were not significantly impacted by level of PEAR supplementation ($P \geq 0.41$). However, urinary ammonia N excretion decreased linearly ($P = 0.02$) from 0.52 g N/d for CON to 0.15 g N/d for 100P or 150 P. Similarly, there was an inverse relationship between the percent of urinary N excreted as ammonia and level of PEAR supplementation; urinary N excretion as ammonia decreased linearly ($P = 0.03$), from 5.33 to 1.04%, as level of PEAR increased.

Plasma urea N concentrations are presented in Table 19. Plasma samples were collected 5 min after treatments were ruminally infused/just prior to feed delivery (h 0), and 4 h after feeding. There was not a significant ($P = 0.69$) treatment × time interaction, and sampling time did not significantly affect ($P = 0.13$) PUN concentrations. Supplementation did not affect PUN at h 0 or 4 ($P \geq 0.27$).

Measurements to characterize the effect of supplemental PEAR on ruminal fermentation included ruminal ammonia, VFA, and ruminal pH (Table 19). For ruminal ammonia concentrations, there was a treatment × time ($P < 0.01$) interaction (Figure 5) and a significant effect ($P < 0.01$) of time. Ruminal ammonia increased quadratically ($P = 0.05$) with increased provision of PEAR. Unsupplemented animals (CON) had the second highest average ruminal ammonia (0.99 m*M*), with the highest average concentration occurring for 150P (1.92 m*M*). For CON and 50P, ruminal ammonia spiked at h 4, then consistently decreased through h 20. Following a similar trend,

Table 19. Effect of protein supplementation level with post-extraction algal residue (PEAR) or cottonseed meal (CSM) on plasma urea N and ruminal fermentation parameters

	Treatment[1]						Contrast *P*-value[2]		
Item	CON	50 P	100 P	150 P	100 C	SEM	Linear	Quadratic	100P vs 100C
No. of observations	5	5	5	4	5				
PUN, m*M*									
hour 0	2.68	2.00	2.08	3.05	3.17	0.65	0.37	0.27	0.29
hour 4	1.90	1.59	2.25	2.82	2.81	0.65	0.37	0.27	0.29
Ammonia, m*M*[3]	0.99	0.40	0.86	1.92	1.36	0.43	0.08	0.05	0.35
Total VFA, m*M*[4]	85.8	90.0	92.6	87.7	95.3	4.19	0.61	0.21	0.56
Molar percentages									
Acetate	77.2	76.0	75.7	75.2	76.1	0.66	0.04	0.55	0.60
Propionate	16.0	16.4	16.7	17.0	16.1	0.57	0.22	0.95	0.47
Butyrate	5.26	5.84	5.89	5.78	6.01	0.21	0.08	0.08	0.61
Isobutyrate	0.64	0.72	0.72	0.80	0.69	0.02	< 0.01	0.96	0.22
Isovalerate	0.54	0.61	0.61	0.74	0.63	0.03	< 0.01	0.33	0.60
Valerate	0.32	0.39	0.42	0.47	0.40	0.02	< 0.01	0.55	0.32
pH[5]	6.41	6.58	6.66	6.69	6.41	0.07	< 0.01	0.15	< 0.01

[1]CON = 0 mg N/kg of BW; 50P = 50 mg N/kg of BW of PEAR; 100P = 100 mg N/kg of BW of PEAR;150P = 150 mg N/kg of BW of PEAR; 100C = 100 mg N/kg of BW of CSM

[2]Linear = linear effect of PEAR level; quadratic = quadratic effect of PEAR level; PEAR vs CSM = effect of 100P vs 100C

[3]Treatment × time: $P < 0.01$, effect of time: $P < 0.01$

[4]Treatment × time: $P = 0.41$, effect of time: $P = 0.48$

[5]Treatment × time: $P = 0.17$, effect of time: $P < 0.01$

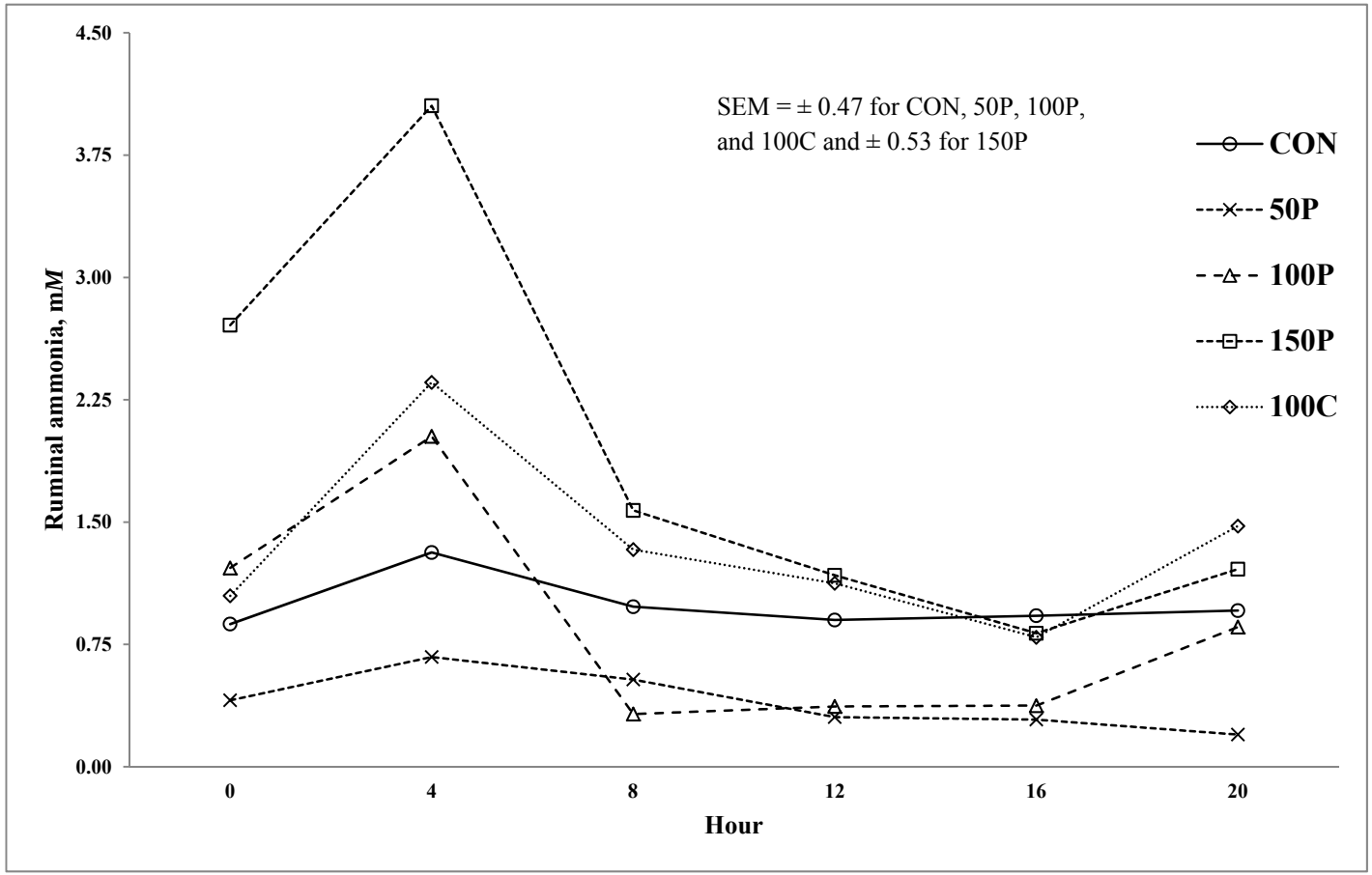

Figure 5. Effect of protein supplementation with post-extraction algal residue (PEAR) or cottonseed meal (CSM) on ruminal ammonia concentration. CON = 0 mg N/kg BW; 50P = 50 mg N/kg BW of PEAR; 100P = 100 mg N/kg BW of PEAR; 150P = 150 mg N/kg BW of PEAR; 100C = 100 mg N/kg BW of CSM. Quadratic effect ($P = 0.05$) of amount of PEAR provided daily. No significant difference ($P = 0.35$) between 100P and 100C. Effect of hour and treatment × hour ($P < 0.01$).

Table 20. Effect of protein supplementation with post-extraction algal residue (PEAR) or cottonseed meal (CSM) on liquid passage rate, outflow, turnover time, and ruminal fluid volume

	Treatment[1]						Contrast *P*-value[2]		
Item	CON	50P	100P	150P	100C	SEM	Linear	Quadratic	100P vs 100C
No. of observations	5	5	5	4	5				
Liquid passage rate, %/h	4.17	3.47	3.81	3.61	3.49	0.59	0.52	0.59	0.60
Liquid flow rate, L/h	1.70	1.59	2.78	1.97	1.60	0.54	0.36	0.46	0.08
Ruminal fluid volume, L	45.06	45.97	66.94	57.47	47.86	8.5	0.10	0.48	0.07
Turnover time, h	27.44	30.49	28.05	27.70	34.73	4.8	0.91	0.58	0.13

[1]CON = 0 mg N/kg of BW; 50P = 50 mg N/kg of BW of PEAR; 100P = 100 mg N/kg of BW of PEAR;150P = 150 mg N/kg of BW of PEAR; 100C = 100 mg N/kg of BW of CSM

[2]Linear = linear effect of PEAR level; quadratic = quadratic effect of PEAR level; PEAR vs CSM = effect of 100P vs 100C

ruminal ammonia was highest in steers receiving 100P or 150P at h 4, but the decrease in ammonia concentration at h 8 was more dramatic than observed in CON or 50P. Concentrations of ruminal ammonia decreased approximately 6-fold for 100P and 2.5-fold for 150P from h 4 to 8 and stayed low until h 20.

Total VFA concentrations were not significantly affected by treatment × time ($P = 0.41$), nor was there an effect of level of supplementation ($P = 0.21$) or sampling time ($P = 0.48$). Thus, overall treatment means are presented in the Table 19. Molar proportions of acetate decreased linearly ($P = 0.04$), but propionate proportions were unaffected ($P \geq 0.22$), and there was a quadratic tendency ($P = 0.08$) for butyrate concentrations to increase as provision of PEAR increased. Proportions of isobutyrate, isovalerate, and valerate linearly increased ($P < 0.01$) from CON to 150P. Treatment × time interaction was not significant for ruminal pH ($P = 0.17$); however, there was a treatment and time effect ($P < 0.01$). Ruminal pH linearly increased ($P < 0.01$) from 6.41 for NC to 6.69 for 150P. For all treatments, ruminal pH was slightly more acidic during the h 8 – 16 sampling times.

Liquid passage rate (Table 20) was unaffected ($P \geq 0.52$) by provision of supplemental protein. Steers consuming only forage (CON) had the fastest rate of passage (4.17%/h), which ranged from 3.47 – 3.81%/h when PEAR was supplemented. Treatment did not significantly affect ($P \geq 0.13$) turnover time; animals receiving CON, 100P and 150P had numerically similar turnover times (27 – 28 h), which slightly increased to 30.5 h for 50P. There was a trend ($P = 0.10$) for ruminal fluid volume to increase as supplementation level increased. Ruminal fluid volume was nearly identical

for CON and 50C (45 – 46 L), and substantially increased in 100P (66.9 L) and 150P (57.5 L). Although statistically insignificant ($P \geq 0.12$), steers receiving 100P had faster liquid flow rate (2.8 L/h) than CON, 50P, or 150P (1.6 – 2.0 L/h).

Comparison of PEAR and CSM

Although designed to be isonitrogenous, steers receiving 100C consumed an additional 1.1 g supplemental N/d due to variations in the chemical composition of the supplements when treatments were initially assigned.

Forage OM intake was similar ($P = 0.22$) for animals receiving 100P and 100C (2.78 and 2.50 kg per d, respectively). Similarly, TDOMI was not significantly affected ($P = 0.13$) by protein source. Urinary output was significantly greater ($P = 0.04$) for 100P than 100C while fecal moisture output tended ($P = 0.06$) to be increased for 100P. Total and forage N intake were also not impacted ($P = 0.41$) by supplement type. Accordingly, fecal and urinary N excretion were similar ($P \geq 0.63$) for 100P and 100C. Retained and absorbed N were similar ($P \geq 0.38$) between sources of protein, although a negative N retention was observed for 100C.

Organic matter digestibility was similar ($P \geq 0.50$) for 100P (50.7%) and 100C (49.2%). Observations of NDFD were similar ($P = 0.95$) at isonitrogenous levels of PEAR and CSM (50.2% for 100P and 50.9% for 100C). Although statistically insignificant ($P = 0.37$) ruminal NDF digestion was greater for 100C than 100P, 47.0 versus 43.3%, respectively. Duodenal N flows were greater for 100C (46.2 g N/d) than 100P (38.1 g N/d). However, this difference was not statistically significant ($P = 0.28$).

Urinary urea N excretion and the percentage of urinary N excretion as urea were not impacted by source of supplemental protein ($P = 0.30$); 14.5% of urinary N excretion was urea for steers receiving 100P and 21.4% for those receiving 100C. Similarly, measurements of urinary ammonia N excretion were comparable ($P \geq 0.24$) among sources of supplemental protein.

Liquid passage rate was similar ($P = 0.60$) when 100P or 100C was supplemented, 3.81 and 3.49%/h, respectively. There was not a difference ($P = 0.13$) in turnover time between isonitrogenous sources of supplemental protein, although 50P was numerically more similar to 100C than 100P.

Ruminal pH was significantly affected ($P < 0.01$) by source of supplemental protein, but the difference was biologically insignificant, 6.66 for 100P and 6.41 for 100C. Steers receiving 100P or 100C had similar ($P = 0.35$) ruminal ammonia concentrations; however, ruminal ammonia decreased more rapidly for 100P than 100C.

Discussion

This study evaluated the effects of increasing provision of PEAR on forage utilization and N balance to steers consuming low-quality forage. Additionally, one level of supplementary PEAR was compared to an isonitrogenous amount of CSM to investigate the differences in PEAR and CSM as a protein source in cattle consuming low-quality forage.

Provision of protein, as PEAR or CSM, stimulated forage intake and digestion, which is consistent with previous observations (Köster et al., 1996; Mathis et al., 2000; Wickersham et al., 2004). Our treatments were designed such that the highest level of

supplementation (150P) would exceed N requirements of the ruminal microbes; thus, we expected, and observed, a plateau response in forage OM intake, which is in agreement with similar studies (Köster et al., 1996; Klevesahl et al., 2003; Wickersham et al., 2004). Forage OM intake peaked at 100P in our study, which is slightly less than the level of supplementation required to maximize forage OM intake (128 – 192 mg N/kg BW) observed by others (Scott and Hibberd, 1990; Mathis et al., 2000; Klevesahl et al., 2003). Using quadratic regression, forage OM intake was calculated to be maximized if PEAR was provided at 110 mg N/kg BW; thus, it is likely provision of an additional level of supplementation between 100P and 150P would have further stimulated forage OM intake and our observations would fall closer to those of Scott and Hibberd (1990), Mathis et. al (2000), and Klevesahl et al. (2003). When cattle are consuming low-quality forage, 9.0 – 11.6% of TDOMI is required as DIP to address ruminal microbial N requirements (Köster et al., 1996; Klevesahl et al., 2003; Wickersham et al., 2008), which is lower than the NRC (2000) recommendation (13% TDN as DIP). At 100P, TDOMI was 0.67 kg/d greater than CON; 85% of this response (increase of 0.57 kg) was observed when the first increment of supplemental protein, 50P, was provided. This observation accentuates the stark ruminal N deficiency in cattle fed low-quality forage and the efficacy of providing small amounts of supplemental protein to stimulate forage utilization. An observed increase in TDOMI for 50P resulted from increased forage intake coupled with increased total tract OM and NDF digestion. Further provision of supplemental protein stimulated additional forage OM intake, but was accompanied by reduced digestion. Ruminal volume and liquid outflow numerically increased in 100P

and 150P relative to 50P. Accordingly, ruminal turnover time was longer for 50P, allowing increased opportunity for ruminal microbes to degrade ingested forage, thus increasing digestion. Although 50P and 100C were numerically similar in all measures of ruminal fluid passage rate, total tract digestion for 50P was greater than 100C; these observations underscore the dynamic nature of fermentation.

Ruminal NDF digestion was not statistically different for 100P and 100C, suggesting increases in total tract digestion were driven by post-ruminal changes. Based on the nature of measuring total tract and ruminal digestibility, it is likely our total tract observations are more accurate, as they are independent of markers and the inherent variability therein. At 100C, forage OM intake and TDOMI was lowest for all supplemented animals, resulting from a slower ruminal turnover time, but similar total tract digestibility to 100P. The ability of 100P to stimulate intake without negatively affecting total tract digestion is likely a direct result of its DIP content, which we assume to be a significant fraction of the protein fraction due to the physical nature of PEAR. Thus, at isonitrogenous levels of PEAR and CSM, we assume PEAR provides a greater amount of DIP to the animal relative to CSM. Bandyk et al. (2001) infused 113 mg N/kg BW ruminally and post-ruminally and observed provision of DIP increased forage OM intake 34% more than UIP, but there was not a difference in OMD or NDFD. Direct provision of N to the microbes is related to increases in protein degradability; it would be expected provision of DIP would stimulate digestion to a greater extent than UIP. However, supplementation with DIP elicits a greater response in forage OM intake than UIP. Thus, the ability of DIP to increase digestibility relative to UIP is often not

recognized at supplementation levels which address ruminal microbial N requirements due to the competing effects of intake and passage rate. Accordingly, although 100P and 150P likely provided a direct N source to the ruminal microbes (DIP), measurements of digestion were similar to that of 100C.

Nitrogen intake and excretion are positively related; however, when protein is supplemented to ruminants consuming a N deficient basal diet, the increase in fecal N excretion is more dramatic than urinary N excretion (Sarraseca et al., 1998; Wickersham et al., 2008). Increases in fecal N excretion results from increased bacterial growth and intake, both of which favor a shift away from urinary N excretion (Wickersham et al., 2008). Indigestible microbial N is excreted in the feces, largely accounting for increases in fecal N output (Wickersham et al., 2008). Unsupplemented steers consumed 14.3 g N/d and excreted approximately double this amount (27.9 g N/d), indicating body tissue protein was mobilized to address animal N requirements; Sarraseca et al. (1998) and Wickersham et al. (2008) also observed negative N retentions in ruminants consuming only low-quality forage, but the extent was less than in our study. For CON, duodenal N flow exceeded N intake, an indication of N recycling. Ammonia is detoxified to urea in the liver of the ruminant; as urea, it can be eliminated through the urine or enter the digestive tract (Huntington and Archibeque, 1999). The purpose of this mechanism is to allow the animal to retain catabolic products of protein metabolism (urea N) within the body for a longer period of time, thus allowing increased opportunity to utilize N in an anabolic manner (Huntington, 1989). Wickersham et al. (2008) demonstrated, in cattle consuming low-quality forage, ≥ 95% of urea produced in the body was recycled to the

gut. Therefore, hepatic urea production was almost equivalent to the amount of urea recycled when dietary protein is insufficient, demonstrating the efficiency of N recycling in ruminants facing a protein deficiency. At 50P, fecal N output increased minimally (2.6%) while urinary N output was depressed (- 9.8%) and there was a 38% increase in total N flow to the duodenum relative to NC. As additional protein was supplemented (100P), fecal and urinary N output increased; however, there were only minimal (2%) increases in the amount of N flowing to the duodenum, indicating the extent of N recycling was lessened when ruminal N requirements were addressed by the diet. These observations point to the remarkable ability of ruminants maintained on low-quality forage to conserve and exploit N when it is a limiting nutrient. Supplementation of 100C yielded greater duodenal N flows than any level of supplemental PEAR, likely a result of increased UIP provision.

Protein supplementation provides a source of ruminally available N to the microbes, stimulating microbial growth and fermentable OM (forage) intake. Microbial growth is dependent on a supply of ruminally available N with ammonia serving as the primary N source for celluloytic bacteria (Bryant and Robinson, 1962). In our study, level of supplemental protein and the subsequent effect on forage OM intake were poorly linked to ruminal ammonia concentration. Interestingly, average ruminal ammonia concentration was greater for CON than 50P or 100P. Köster et al. (1996) observed similar ammonia concentrations in control steers maintained on N deficient (1.94% CP) prairie hay. The relationship between protein supplementation to cattle consuming low-quality forage and ruminal ammonia concentration in our study differs

from that of Köster et al. (1996), Olson et al. (1999), Klevesahl et al. (2003), and Wickersham et al. (2008), where ruminal ammonia concentration increased as level of protein increased. We believe our results were driven by: 1) water dilution, 2) the rapidly degradable nature of PEAR, and 3) sampling time. We observed a distinct trend in ruminal ammonia for 100P and 150P, where concentrations decreased to 0.32 and 1.57 m*M*, respectively, at h 8. Assuming water consumption is equal to the amount of fecal moisture and urinary output, there was a 58 and 88% increase in water consumption from CON to 100P and 150P, respectively. From CON to 50P, there was a 23% increase in water consumption, which was greater than the increase (16%) for 100C. Thus, we believe dilution played a role in decreasing ruminal ammonia concentrations when PEAR was supplemented. These observations are supported by a faster liquid flow rate for 100P and 150P relative to 100C. Increased water consumption associated with provision of PEAR was likely driven by the Na content of PEAR (5.2%, Chapter II). Additionally, the physical characteristics of PEAR (fine, powder-like) suggest it is immediately degradable in the rumen, likely resulting in a shorter payout in ruminal versus CSM, a more slowly degraded protein source. Thus, if we sampled closer to time of supplementation (h 2), we would likely observe greater ruminal ammonia concentrations for 50P, 100P, and 150P.

Total VFA concentrations were positively related to TDOMI when PEAR was supplemented, although not statistically significant. As observed in measurements of intake, the first level of supplementation (50P) accounted for a majority (62%) of the increase in total VFA, with minor numerical increases from 50P to 100P. Provision of

100C increased concentrations of VFA to a further extent than 100P (11.1 and 7.9%, respectively) relative to CON, despite lower TDOMI and similar total tract digestibility. Ruminal fluid volume for 100C was 72% that of 100P; additionally, 100C was characterized by a longer ruminal turnover time, potentially affecting total VFA concentrations. Increased length of ruminal turnover associated with 100C was likely the primary reason ruminal NDF digestion was greater for 100C relative to 100P. Molar proportions of acetate decreased and propionate slightly increased as level of supplementary protein increased, which is in agreement with observations of McCollum and Galyean (1985), Chase and Hibberd (1987), and Olson (1999), indicating an increase in energetic efficiency (P. J. Van Soest, 1982) associated with increased provision of protein. Degradable intake protein provides a source of branched-chain AA to the rumen, which serve as precursors for branched-chain VFA (Köster et al., 1996); thus, we expected, and observed, increases in the proportions of isobutyrate, isovalerate, and valerate from CON to 150P. The amount of DIP supplemented was similar for 50P and 100C, resulting in comparable proportions of branched-chain VFA. Branched-chain VFA are required for growth of celluloytic bacteria (Baldwin and Allison, 1983), which are responsible for forage fermentation activity. The extent of ruminal NDF digestion was largely unrelated to the presence of branched-chain VFA, indicating ruminal fluid volume, passage rate, and retention time play a more significant role in regulating ruminal NDF digestion.

CHAPTER IV

EFFECT OF UPSTREAM OPERATIONS ON THE NUTRITIVE VALUE OF POST-EXTRACTION ALGAL RESIDUE

Overview

Post-extraction algal residue (**PEAR**), an algal biofuel coproduct, has potential to be marketed as livestock feed. Our objective was to quantify the nutritive value of PEAR produced from a single batch of Nannochloris oculata subjected to different methods of harvesting (centrifugation or flocculation), drying (drum-, spray-, or belt-dried), and extraction (ethanol or hexane) to determine the effect of upstream operations on downstream products. These processes resulted in 10 unique samples for determinations of nutritive value. Crude protein and lipid content (acid-hydrolysis) of samples ranged from 19 – 36% and 2 – 10%, respectively. All PEAR samples contained concentrations of S (≥0.81%) which may limit inclusion rate in cattle diets. Flocculation increased levels of Fe and Al ≥289% relative to centrifuged samples, which would further restrain the use of PEAR. Flocculated and centrifuged PEAR had Ca:P of 18:1 and 9:1, respectively, which would present formulation challenges. Excessive mineral concentrations have negative impacts on animal health, weight gain, and predisposition to disease. Additionally, excessive mineral excretion increases the environmental footprint of livestock production. Flocculation decreased S and Na concentrations by 37 and 77%, respectively, compared to centrifuged samples; however, each remained present in quantities which would impact ration formulation. Reducing mineral concentrations and increasing protein content in PEAR will increase its value and

utilization in beef cattle operations. Mineral imbalances can be partially attributed to cultivation, harvesting, and extraction protocols which can be avoided or managed, reinforcing the need for upstream operations to be aware of the downstream impacts.

Introduction

Global demands for energy are steadily increasing, emphasizing the need to identify renewable, sustainable alternatives to fossil fuels. Traditional first-generation biofuel feedstocks are limited in their production capacity, compete with food production, require significant agricultural inputs, and freshwater (Dragone et al., 2010; Sander and Murthy, 2010). Second-generation biofuel feedstocks are an attractive alternative to first-generation feedstocks because they do not strain world food markets; however, conversion of cellulosic biomass to biofuel presents expensive technological challenges which have inhibited commercial scale-up (FAO, 2008). Algal biomass, a third-generation biofuel feedstock, is not associated with the challenges first- or second-generation biofuels face (Nigam et al., 2011) and, therefore, may serve as an economically effective and environmentally sustainable feedstock for biofuel. Becker et al. (2007) suggested algae species suitable for large-scale production have a maximum lipid content of 22%, indicating the co-product (post-extraction algal residue; **PEAR**) will be produced in excess of oil yield. These observations were confirmed recently by Sander and Murthy (2010), who suggested a biodiesel to co-product ratio of 12:17; thus, economic feasibility of algal biofuel production is contingent on placement and marketing of the co-product. Potential markets for PEAR include the livestock industry, specifically beef cattle operations, as ruminants are effective utilizers of co-products.

Developing processes which optimize biofuel production and co-product value is essential for economic sustainability and effective nutrient stewardship. Hence, our objectives were to quantify the nutritive value of a single batch of algae (*Nannochloris oculata*) subjected to various methods of upstream processing (including harvesting, drying, expansion, and extraction), and determine factors which may limit inclusion of PEAR in cattle rations.

Materials & methods

Algae was harvested by centrifugation (10 -15% solids) or with the aid of a commercially available flocculent (30 - 40% solids). Three drying methods were tested; 1) steam-jacketed drum drying, 2) forced-air belt drying, and 3) spray drying. Centrifuged samples were drum- or spray-dried, while partially dried flocculated samples were belt-dried. Drum-dried samples appeared as flakes, similar to the physical composition of fish feed. An expander was also used on the spray-dried samples and a portion of the belt-dried samples to observe the influence of preparation prior to extraction. An expander cooks and agglomerates samples into a denser material, known as collets. Both harvesting methods successfully yielded collets, which appeared as smooth, long pellets. Hexane and ethanol were tested as solvents for extraction to compare extraction efficiency (data not shown). These processes yielded a total of 10 samples for nutritive analysis (Figure 6): 1) unprocessed drum-dried (**UD**), 2) unprocessed flocculated (**UF**), 3) unprocessed spray-dried (**US**), 4) centrifuged, drum-dried, ethanol extracted (**CDE**), 5) centrifuged, drum-dried, hexane extracted (**CDH**), 6) flocculated, expanded, hexane extracted (**FEH**), 7) centrifuged, spray-dried, expanded,

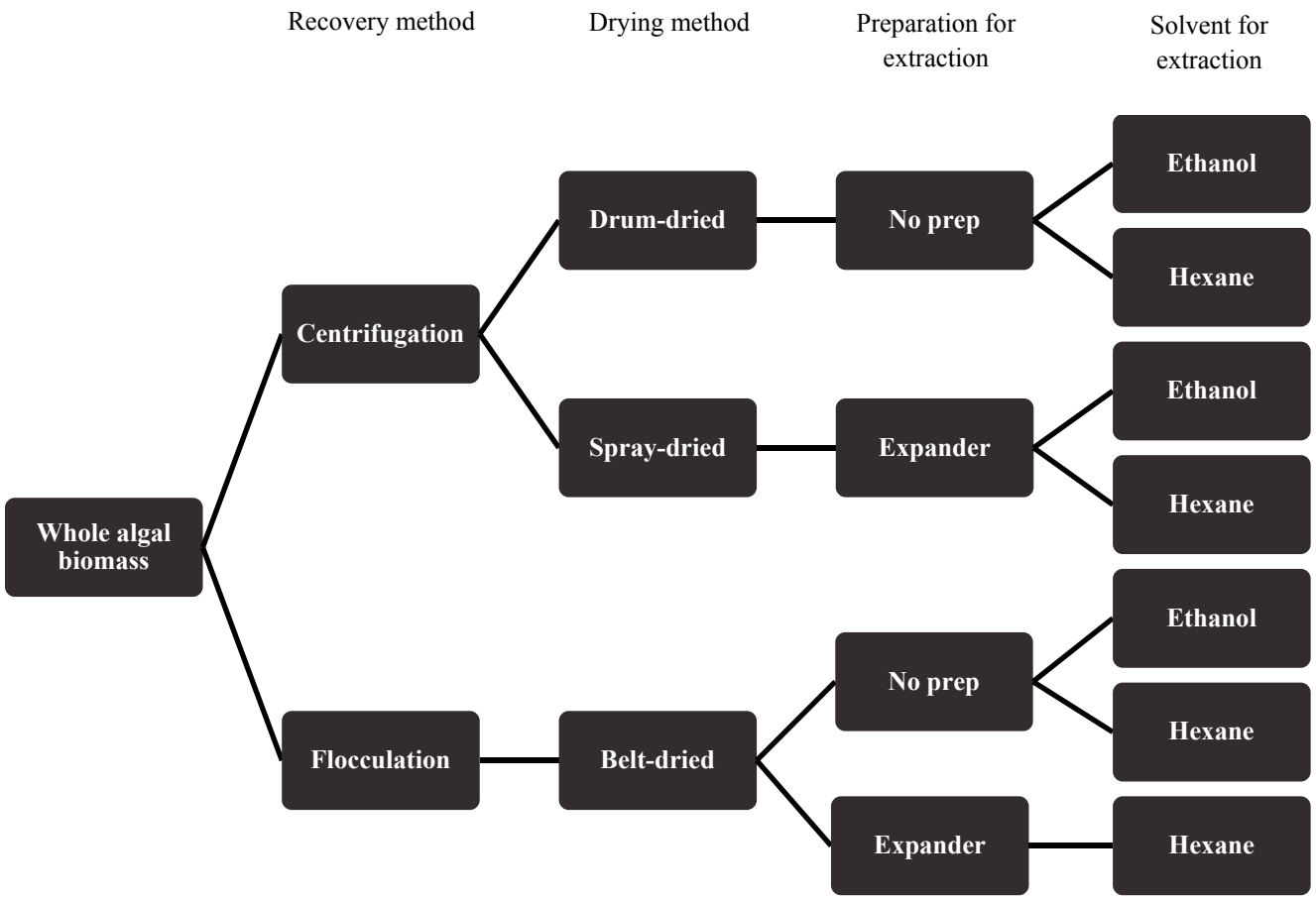

Figure 6. Methods of biomass harvesting, drying, preparation for extraction, and extraction of PEAR samples

ethanol extracted (**CSEE**), 8) centrifuged, spray-dried, expanded, hexane extracted (**CSEH**), 9) flocculated, ethanol extracted (**FE**), and 10) flocculated, hexane extracted meal (**FH**).

Samples were ground with a Wiley mill to pass a 1-mm screen and subsequently dried in a forced-air oven for 24 h at 105°C for DM determination. Organic matter was determined as the loss in dry weight upon combustion for 8 h at 450°C. Nitrogen was measured using the Elementar rapid N cube (Elementar, Hanua, Germany) and CP was calculated as N × 6.25. Lipid content was determined by ether extract (**EE**) and acid-hydrolysis fat (**AH**). Starch, macro-, micro-mineral, and heavy metal analysis was performed by a commercial laboratory (SDK Laboratories, Hutchinson, KS). Mineral analysis included Ca, P, K, Mg, Na, S, Co, Cu, Fe, Mn, Se, and Zn. Heavy metal analysis included Ag, Al, As, Ba, Be, Cd, Ch, Hg, Pb, and Ni.

Results and discussion

Complete chemical, macromineral, micromineral, and heavy metal composition are presented in Tables 21, 22, 23, and 24, respectively.

Feed DM is fractionated into ash or OM. Organic matter is further divided into protein, carbohydrate, lipids, and vitamins. Average OM content of PEAR in our study was 54.4%, with a range of 42.4 – 75.8%. Unprocessed samples averaged 54.8% OM, with a range of 46.4 – 68.2%. Soybean meal and cottonseed meal, conventional protein sources in beef cattle operations, have an ash content of 6 – 7% (NRC, 2000). These feedstuffs provide greater concentrations of usable nutrients which drive animal production (protein and energy) than PEAR. Accordingly, to achieve the same result in

Table 21. Chemical composition of algae subjected to different processing methods

Harvest	Centrifuged			Flocculated				Centrifuged		
Drying	Drum-dried			Belt-dried				Spray-dried		
Preparation							Expanded		Expanded	
Extraction		Ethanol	Hexane		Ethanol	Hexane	Hexane		Ethanol	Hexane
	UD[1]	CDE	CDH	UF	FE	FH	FEH	US	CSEE	CSEH
% DM	93.74	90.13	88.82	90.08	94.29	92.66	92.82	94.91	90.08	90.81
	----------% DM----------									
OM	46.39	75.76	50.34	49.79	50.96	42.41	52.16	68.19	56.06	53.32
CP	26.66	34.20	35.50	23.16	21.90	18.76	38.06	28.23	23.58	23.24
Lipid (EE)[2]	1.83	<0.20	0.66	1.08	0.31	0.68	1.90	0.42	2.96	2.93
Lipid (AH)[2]	10.17	2.44	5.76	2.39	2.60	3.61	3.63	10.12	9.60	8.40
Starch	4.1	5.20	4.80	2.40	2.00	2.30	2.90	2.30	4.60	4.70

[1]UD = unprocessed drum-dried; CDE = centrifuged, drum-dried, ethanol extracted; CDH = centrifuged, drum-dried, hexane extracted; UF = unprocessed flocculated; FE = flocculated, ethanol extracted; FH = flocculated, hexane extracted; FEH = flocculated, expanded, hexane extracted; US = unprocessed spray-dried; CSEE = centrifuged, spray-dried, expanded, ethanol extracted; CSEH = centrifuged, spray-dried, expanded, hexane extracted

[2]EE = ether extract, AH = acid-hydrolysis

Table 22. Macromineral composition of algae subjected to different processing methods

Harvest	Centrifuged			Flocculated				Centrifuged		
Drying	Drum-dried			Belt-dried				Spray-dried		
Preparation							Expanded		Expanded	
Extraction		Ethanol	Hexane		Ethanol	Hexane	Hexane		Ethanol	Hexane
	UD[1]	CDE	CDH	UF	FE	FH	FEH	US	CSEE	CSEH
Macromineral, %										
Ca	3.20	3.72	2.99	10.02	10.76	11.28	10.21	5.41	5.70	5.28
P	0.67	0.78	0.65	0.52	0.50	0.50	0.53	0.59	0.62	0.62
K	0.85	0.63	0.84	0.67	0.70	0.73	0.69	0.81	0.85	0.84
Mg	0.26	0.33	0.29	0.59	0.56	0.58	0.57	0.30	0.29	0.31
Na	5.06	2.55	5.25	1.12	0.95	1.05	1.17	4.72	4.81	4.95
S	1.57	1.20	1.51	0.86	0.82	0.81	0.87	1.36	1.28	1.28

[1]UD = unprocessed drum-dried; CDE = centrifuged, drum-dried, ethanol extracted; CDH = centrifuged, drum-dried, hexane extracted; UF = unprocessed flocculated; FE = flocculated, ethanol extracted; FH = flocculated, hexane extracted; FEH = flocculated, expanded, hexane extracted; US = unprocessed spray-dried; CSEE = centrifuged, spray-dried, expanded, ethanol extracted; CSEH = centrifuged, spray-dried, expanded, hexane extracted

Table 23. Micromineral composition of algae subjected to different processing methods

Harvest	Centrifuged			Flocculated				Centrifuged		
Drying	Drum-dried			Belt-dried				Spray-dried		
Preparation							Expanded		Expanded	
Extraction		Ethanol	Hexane		Ethanol	Hexane	Hexane		Ethanol	Hexane
	UD[1]	CDE	CDH	UF	FE	FH	FEH	US	CSEE	CSEH
Micromineral, ppm										
Co	0.61	0.96	0.70	1.83	1.74	2.06	1.82	0.77	0.79	0.83
Cu	2110	2920	2240	1610	1550	1560	1540	1870	2070	2010
Fe	1740	2260	1740	7400	7240	8160	6890	1780	1950	1980
Mn	43.90	60.30	46.30	126	132	142	121	51.50	57.5	55.70
Se	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
Zn	150	230	159	130	131	142	124	140	158	152

[1]UD = unprocessed drum-dried; CDE = centrifuged, drum-dried, ethanol extracted; CDH = centrifuged, drum-dried, hexane extracted; UF = unprocessed flocculated; FE = flocculated, ethanol extracted; FH = flocculated, hexane extracted; FEH = flocculated, expanded, hexane extracted; US = unprocessed spray-dried; CSEE = centrifuged, spray-dried, expanded, ethanol extracted; CSEH = centrifuged, spray-dried, expanded, hexane extracted

Table 24. Heavy metal concentrations of algae subjected to different processing methods

Harvest	Centrifuged			Flocculated				Centrifuged		
Drying	Drum-dried			Belt-dried				Spray-dried		
Preparation							Expanded		Expanded	
Extraction		Ethanol	Hexane		Ethanol	Hexane	Hexane		Ethanol	Hexane
	UD[1]	CDE	CDH	UF	FE	FH	FEH	US	CSEE	CSEH
Heavy metal, ppm										
Ag	16.90	24.30	19.20	16.30	15.90	16.10	14.60	15.80	17.30	16.80
Al	1580	2330	1820	11000	9800	11600	9500	2020	2150	2160
As	2.03	<1.5	<1.5	3.46	3.19	2.78	2.65	1.87	<1.5	<1.5
Ba	15.40	21.60	16.00	67.80	64.60	77.20	63.40	20.70	22.80	22.30
Be	0.48	0.48	0.48	0.56	0.69	0.66	0.63	0.45	0.48	0.48
Cd	0.28	0.38	0.37	0.56	0.57	0.57	0.51	0.23	0.30	0.28
Cr	5.68	9.65	6.55	17.60	17.40	23.00	26.60	8.03	12.20	12.90
Hg	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Pb	2.02	2.83	2.30	5.55	5.86	6.28	5.38	2.73	2.89	2.87
Ni	2.72	3.58	2.86	8.60	8.75	11.40	<0.5	3.76	5.76	5.90

[1]UD = unprocessed drum-dried; CDE = centrifuged, drum-dried, ethanol extracted; CDH = centrifuged, drum-dried, hexane extracted; UF = unprocessed flocculated; FE = flocculated, ethanol extracted; FH = flocculated, hexane extracted; FEH = flocculated, expanded, hexane extracted; US = unprocessed spray-dried; CSEE = centrifuged, spray-dried, expanded, ethanol extracted; CSEH = centrifuged, spray-dried, expanded, hexane extracted

animal performance as conventional supplements, PEAR will need to be fed at higher levels.

Processing method did not have a major effect on the OM content of PEAR, although ethanol-extracted samples had higher OM than hexane-extracted in all instances within processing method. The US sample had higher OM content (68.2%) than the other unprocessed samples (average of 48.2%). Accordingly, the respective processed samples (CSEE and CSHE) had higher concentrations of OM than any other sample, with the exception of CDE. Our observation of CDE is, at this point, inexplicable. Further investigation and replication is warranted to establish methods which optimize OM content, thus increasing the market value of PEAR.

Due to lipid extraction and the currently low OM fraction, the energy content of PEAR is not expected to be the primary reason for its use as a livestock feed. Crude protein in PEAR samples ranged 18.8 – 38.1%, with an average of 27.9%, which is less than the protein content of soybean meal (49.9%) and cottonseed meal (46.1%; NRC, 2000). Nutritional components of PEAR suggest its market value will be optimized within the beef cattle industry when marketed as a protein supplement to cow-calf operations, or other operations which rely heavily on forage and are deficient in protein. Protein supplementation increases forage utilization (Köster et al., 1996; Wickersham et al., 2008) and is widely accepted as a cost-effective management practice to optimize animal performance. There was little difference in the average CP for processed and unprocessed samples, 27.9 and 26.0%, respectively. The highest observation (38.1% CP) was FBEH; the other flocculated, belt-dried, or expanded samples had significantly

lower CP (average of 21.9%). The reason for greater protein in FBEH is currently unexplained. Flocculated samples not subjected to the expander had the lowest concentrations of CP (20.3%), which was 73% of the average protein in PEAR samples. Centrifuged, drum-dried PEAR contained approximately 34.9% CP, without regard to extraction solvent utilized.

Market value of PEAR in the beef cattle industry will be driven by CP concentrations; using a hedonic model, Bryant et al. (2012) demonstrated the value of PEAR will be $122 – 281/t, which was less than the value of soybean meal. Discounts in value are associated with the ash content of PEAR, resulting in lower protein content. On a nutrient basis, we expect the value of PEAR to be similar to conventional feed ingredients. Centrifuged, drum-dried ethanol extracted meal contains desirable OM and CP concentrations while maintaining the lowest amount of residual lipid (<0.20% by EE; 0.66% by AH) for all PEAR samples. These observations indicate the related processing methods (centrifuging, drum-drying, and ethanol-extracting) may optimize co-product value and oil yield. Replication is necessary to confirm these results; however, we are unaware of any ongoing efforts to describe upstream effects on the quality of PEAR. Further research to optimize the co-product and biofuel yield is clearly necessary.

Minerals are a necessary component of cattle diets, but can be provided in relatively expensive mineral supplements designed to address specific deficiencies in basal rations. Feedstuffs which lead to mineral imbalances are limited in their inclusion to avoid toxicity issues. Processed and unprocessed samples contained an imbalanced Ca:P ratio and levels of S, Cu, Fe, and Al in excess of maximum tolerable concentrations

for the total diet of an animal (NRC, 2000), suggesting inclusion level of PEAR may be constrained due to toxicity concerns.

Calcium and P are primarily used for bone growth and maintenance in the ruminant animal with a recommended feeding ratio of 1:1 – 7:1 (NRC, 2000). The P content of forage significantly decreases during the winter months, and must be provided via the supplement in grazing operations. Cottonseed meal and distillers' grains supply more P than Ca (Ca:P of 0.05 and 0.17:1, respectively; Drewery et al., 2011; Chapter II), complementing the Ca:P ratio of winter pastures while providing supplemental protein to grazing animals. Calcium content in PEAR appears to be related to flocculation, where centrifuged samples contained an average of 4.38% Ca and flocculated samples contained 10.57% Ca. Upstream processing did not appear to impact P content, which was consistent in all PEAR samples, averaging 0.61%. The Ca:P ratio of drum-dried samples was well within the feeding recommendation (5:1), but would provide an excess of Ca relative to P during winter months. This issue would be exacerbated if flocculated PEAR was supplemented (ratio of 20:1 and 9:1, respectively). Typical feedlot rations provide a Ca:P ratio of approximately 2:1 (Gaylean and Gleghorn, 2001; Vasconcelos and Galyean, 2007), suggesting the Ca:P ratio would not limit inclusion for drum-dried PEAR.

The 2000 Beef Cattle NRC lists the requirement of S for growing and finishing cattle as 0.15%, with a maximum tolerable concentration of 0.40%, and recognizes diets typical of current production systems provide sufficient S. Processed samples contained an average of 1.11% S (range of 0.81 – 1.51%), which was slightly lower than

unprocessed samples, 1.26% (range of 0.86 – 1.57). In this case, accumulation of S seems to be attributed to two sources: 1) growth of algal biomass and 2) harvesting method. Flocculated samples, including UF, contained 73% of the average S content of all algae samples, but S concentrations were still higher than that of distillers' grains (0.62%; Drewery et al., 2011) or cottonseed meal (0.49%; Chapter II). There was little interaction between other upstream processes and the resulting S content in the algae; thus, high S may be an inherent characteristic of PEAR, which is supported by the S content of PEAR utilized in our feeding trials (0.82%; Chapter II). Cases of Polioencephalomalacia (**PEM**) have been documented when cattle ingest high levels of S, via feed or water (Gould et al., 1997; Beke and Hironaka, 1991; Sager et al., 1990), and may occur with pasture or feedlot diets (Gould, 1998). Ruminal microbes convert S to hydrogen sulfide, potentially resulting in necrosis of the cerebral cortex (Gould et al., 1998; Mayland et al., 2001). Distillers' grains are notorious for containing high levels of S (0.62%; Drewery et al., 2011) from the addition of sulfuric acid to optimize the extent of fermentation during the ethanol producing process (Liu and Han, 2011). Distillers' grains have been linked to cases of PEM (Buckner et al., 2007) when fed to steers at a high inclusion rate (50%), However, distillers' grains are nearly ubiquitous in feedlot rations, but at a limited inclusion rate; the typical feedlot ration incorporates wet- or dried-distillers grains as the primary grain co-product at 16.5% of the ration (Vasconcelos and Galyean, 2007). Assuming a feedlot ration contains 0.20% S (Table 25), and distillers' grains are only utilized as the primary grain co-product, the S contribution from distillers' grains would be 51% of the ration S content. If centrifuged

Table 25. Macro- and micromineral content of diets in the feedlot and cow-calf industry

	Feedlot[1]	Feedlot[2]	Bahiagrass[3]	Bermudagrass[3]	Brome hay[3]	Orchard grass[3]	Alfalfa hay[3]
Macrominerals, %							
Ca	0.70	0.70	0.50	0.26	0.29	0.26	1.19
P	0.30	0.31	0.22	0.18	0.28	0.30	0.24
S	0.22	0.19	-	0.21	-	-	0.30
Na	0.30	0.35	-	0.08	0.01	0.01	0.07
Microminerals, ppm							
Cu	93.0	14.8	-	9.00	25.00	20.00	9.90
Fe	51.7	55.9	60.00	290.00	91.00	84.00	160.00

[1]Adapted fromVasconcelos and Gaylean, 2007
[2]Adapted from Galyean and Gleghorn, 2001
[3]Adapted from NRC, 2000

PEAR replaced distillers' grains at a 16.5% inclusion rate, the ration would provide 0.33% S (69% of S from PEAR), which would decrease to 0.24% S with incorporation of flocculated PEAR (57% of S from PEAR). The S content of forages commonly utilized in cow-calf operations can range from nearly 0 to an amount similar to that of feedlot rations (Table 5); thus, inclusion rate of PEAR could range from 10 – 36%, depending on harvesting method of PEAR and ingestion of S from the forage and drinking water.

Requirements of Cu in cattle diets vary based on the presence of S, Mo, and other minerals, but 100 ppm Cu is the recommended feeding level for beef cattle (NRC, 2000). Copper is found in higher levels in concentrates than forages; thus, feedlot diets are typically adequate in Cu availability (Table 5). Processed and unprocessed samples averaged 1924 ppm Cu, which is clearly in excess of the recommended feeding level. Ruminants store considerable amounts of Cu in the liver; upon reaching the maximum storage level, the liver undergoes hemolytic crisis and releases Cu into the circulation, potentially resulting in anorexia, jaundice, and death (Church et al., 1971; Bradley et al., 1993). As with S, Cu accumulation seemed to be linked to biomass cultivation and harvesting. Copper content was lowest in UF and the corresponding processed samples, but still averaged 1565 ppm. Unprocessed, centrifuged samples were more concentrated in Cu than UF; drying further increased Cu in PEAR, especially when spray-drying was utilized. Assuming Cu is not provided through other dietary means, which is a practical scenario in grazing operations, inclusion rate of PEAR will be limited to 4.5 – 6.5% of the diet, depending on harvesting method. Traditional feedlot rations provide 50 – 90

ppm Cu (Galyean and Gleghorn, 2001; Vasconcelos and Galyean, 2007). Distillers' grains, the component of a finishing ration PEAR may replace, contain 4.9 ppm Cu (Drewery et al., 2011). Therefore, the majority of Cu originates from other ingredients and incorporation of PEAR in an existing feedlot ration would not be an option due to extreme toxicity concerns. In Chapter II, the Cu content of PEAR we used was 31 ppm, indicating high Cu is not an intrinsic characteristic of PEAR and can be controlled. Indeed, a Cu-based product was used to control an invasive pond species in the present study, which was apparently absorbed by the biomass. This observation emphasizes the need for upstream operators to be aware of the impact their actions have on the value of downstream products and, ultimately, the ability of those products to positively contribute to the economic viability of algal biofuel.

Beef cattle ingest adequate amounts of Fe from water, soil and available feed, thus, deficiencies are not a concern (Spears, 2003). Requirement of Fe for growing and finishing cattle is 50 ppm, with a maximum tolerable concentration of 1000 ppm (NRC, 2000). Iron toxicity is characterized by a reduction in animal performance, diarrhea, effects on mineral utilization, and metabolic acidosis (Standish et al., 1971; NRC, 2000). Flocculated algae samples were high in Fe, ranging 6890 – 8160 ppm, with an average of 7423 ppm. Centrifuged samples had lower Fe concentrations (averaging 1908 ppm); there were no apparent differences between processed/unprocessed, drying method, preparation for extraction, or extraction solvent. It is apparent the flocculent used contained significant amount of Fe and, consequently, the biomass retained a significant proportion of that Fe. The Fe content of cottonseed meal (199 ppm) and distillers' grains

(52 ppm) does not pose toxicity concerns or limit inclusion rate in feedlots or grazing operations. Iron content of grazing pastures ranges from 60 – 290 ppm (Table 5); assuming the average forage contains 100 ppm Fe, inclusion rate of centrifuged PEAR will not be an issue, but flocculated PEAR will be limited to 12% of the diet. A similar inclusion rate is expected in feedlot rations, thus, PEAR could not completely replace distillers' grains. Iron content in PEAR can be controlled by either 1) utilizing a harvesting method other than flocculation, or 2) using a flocculent not highly concentrated in Fe.

Aluminum is either not required, or required in minute amounts in ruminant diets, but the maximum tolerable concentration is set at 1000 ppm (NRC, 2000). Low amounts of Al (<1,000 ppm) have little effect on mineral metabolism in ruminants, however, increasing intakes affect the utilization of certain minerals (P, F, Ca, and Mg) by forming insoluble Al complexes (Allen et al., 1984). However, when Al intake was 1200 ppm, Valdivia et al. (1978) did not observe an effect on animal performance. Aluminum concentrations were similar in centrifuged samples, ranging from 1580 – 2330 with an average of 2010 ppm, which would not limit practical inclusion rates for finishing or grazing animals. Flocculated samples contained greater levels of Al, averaging 10,475 ppm, which would limit inclusion of PEAR to 9.5%, assuming Al was not provided through other dietary means. These observations, combined with the increases observed in Fe levels associated with flocculation, suggest the utilization of flocculants containing significant amounts of Al or Fe may not be a viable protocol if PEAR is marketed as a ruminant feedstuff.

CHAPTER V

SUMMARY AND CONCLUSIONS

Conclusions

Post-extraction algal residue (**PEAR**), a co-product of algal biofuel production, has potential as a source of nutrients in the beef cattle industry. The nutritive value of PEAR suggests market value and extent of utilization will be maximized as a protein supplement in cow-calf operations. Accordingly, the broad objective of our research was to evaluate the potential for PEAR to be integrated in existing beef feeding operations. To meet our objective, we conducted a series of four projects designed to 1) evaluate the palatability of PEAR to cattle consuming forage, 2) compare the effects of PEAR on nutrient utilization versus a conventional protein supplement (cottonseed meal; **CSM**), 3) determine the optimal inclusion rate of PEAR for cattle consuming low-quality forage, 4) identify mineral toxicity or imbalances associated with PEAR supplementation, and 5) quantify the effects of upstream operations on the nutritive value of PEAR.

Forage OM intake and N balance responded quadratically in accordance with increasing PEAR provision with maximization occurring when PEAR was provided at 100 mg N/kg BW to cattle consuming oat straw (4% CP). Supplementation of isonitrogenous levels of PEAR or CSM (100 mg N/kg BW) elicited a stimulatory response on forage OM intake and N balance, but did not affect measures of digestibility when prairie grass (6.7% CP) was fed. There was not a difference in total digestible OM intake, which is a measure of forage utilization, between sources of supplemental protein, suggesting PEAR may effectively replace CSM as a protein source to grazing cattle.

Post-extraction algal residue contains a significant proportion of ash, causing concern of mineral toxicity at increased inclusion rates. Our observations suggest Cu content will be the first-limiting factor for PEAR in beef cattle diets. However, level of Cu in PEAR samples can be easily controlled during the cultivation phase. Iron and Al concentrations limit inclusion rate of flocculated PEAR, but are manageable during harvesting. High S is likely a characteristic of PEAR, and cannot be altered by upstream operations. However, of the minerals of concern, S is the least constraining to inclusion rate of PEAR. A Ca:P ratio closely aligned to nutrient requirements would likely increase the acceptability and incorporation of PEAR in grazing operations, suggesting another drawback of flocculation. When PEAR was supplemented (100 mg N/kg BW) to cattle with ad libitum access to prairie grass, the only mineral imbalance of concern was the Ca:P ratio (8:1).

Market value and extent of incorporation of PEAR in the beef industry will be heavily influenced by OM and protein content; thus, efforts should be made to decrease the ash content, minimizing toxicity concerns, while increasing the protein fraction of PEAR. Further research is necessary to fully understand the interactions and consequences of upstream processes and a method should be selected and stream-lined for optimal oil yield and nutritive value of resultant co-products. To minimize concerns of palatability and mineral toxicity, PEAR will likely be blended with an existing feedstuff to create a supplement easily incorporated in existing operations. Our observations indicate PEAR can be blended (up to 60%) with dried distillers' grains or CSM to formulate a supplement which would provide acceptable levels of protein and minerals without adversely affecting palatability.

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