



November 2024

Sprouted Grain Feed Supplements Quantifying and Verifying Results

Livestock producers face daunting problems in maintaining their herds and businesses. The ever-increasing capital required to buy needed equipment, increasing restrictions on water use, changing weather patterns along with the ups and downs of the economy demand new ways of doing business to succeed, if not just to survive.

If that wasn't enough, consumers are more vocal about where their food comes from and how animals were raised, cared for and what they were fed. Along with consumer demands, large companies like McDonalds are demanding their suppliers move to sustainable practices.

Indoor Farms Corporation plans to play a meaningful role in leading the way in addressing many of the challenges noted above. Our process of growing a highly nutritious, all natural, food supplement to feed both beef and dairy cattle is very successful in meeting these demands.

Many of our customers and investors have asked for details regarding how our NovaGreens™ and NovaJuice™ sprouted grain feed supplements provide such significant benefits for livestock. With this in mind, we have brought together a collection of published and unpublished studies, articles and reports from universities and industry experts that quantify and verify the solutions we offer to the cattle industry.

John T Golle
Chairman and CEO
Indoor Farms Corporation
www.indoorfarms.us

Conversation with Utah State regarding cattle health improvements when Novagreen is part of mix.

Brady Blackett, National Sales Manager for Indoor Farms Corp, spoke with Zach Crump, PhD Animal Science (Utah State), on November 20, 2024 about his thoughts regarding cattle health improvements when NovaGreen™ is part of the TMR (total mixed ration).

In the feed trial that we conducted on the campus of Utah State in Logan, Utah, no health benefits were measured as part of the feed trial. However, one thing that was observed was decreased VFA (volatile fatty acid) production in the rumen. This can drive the health benefits of finishing cattle. Lowering the VFA's is generally good because too much can cause the rumen PH to lower, which leads to poor digestion in cattle and decreased feed intake. Furthermore, too much VFA leads to rumen acidosis. Therefore, the NovaGreen promotes a healthy rumen microbiome which is why we see improved feed to gain ratio and decreased DMI (dry matter intake) while still seeing cattle performance be unchanged.

Here is a deeper look into that study. Note, that the economics of the study were not completed by Utah State. These numbers were completed by Brady Blackett, Dr. Sanjeeva Kandola, Cumberland Valley Analytical, and Dr. Robert Harding, DVM, using the ration components and feed prices at the time the trial was completed in May 2023.

Utah State University Feed Trial - Logan, Utah

Two phases to the trial. A backgrounding phase (56 days) & finishing phase (130 days). The trial began in November 2022, ended in May 2023, and consisted of 60 SimAngus crossbred steers of similar genetics grouped by age and size. All intake and feed data were collected using Vytelle feed bunks to measure and monitor feeding behavior and intake. The purpose of the trial was to understand the effects of feeding sprouted barley on growing and finishing beef cattle.

Backgrounding Trial (56 days):

- Control group starting weight: 724 lbs. - Day 56 weight 844 lbs. - ADG 2.13
- Test group starting weight: 725 lbs. - Day 56 weight 852 lbs. - ADG 2.26
- The test group consisted of a group that received a 60% inclusion rate, a 40%inclusion rate, and a 20% inclusion rate. The 60% inclusion rate group had an ADG of 2.36 and responded best during this feeding period.
- Overall, the test group had a 5.7% ADG advantage.

Finishing Trial (130 days):

- Ration Component Prices per ton in UTAH at the time of the trial on a Dry Matter basis:
 - Mineral Package: \$1400
 - Sprouted barley fodder: \$500
 - Rolled Barley: \$350
 - Alfalfa Hay: \$311
 - Corn Silage: \$187

- Ration Profile:
 - Control Group:
 - Ration components as a % of Dry Matter & Pounds:
 - 80.5% rolled barley, 8.75% corn silage, 8.46% alfalfa hay, and 2.31% mineral package.
 - 22.9 total pounds of dry matter per head. Rolled Barley 18.4 lbs., corn silage 2 lbs., alfalfa hay 1.94 lbs., 0.53 lbs. mineral package.
 - Test Group:
 - Ration components as a % of Dry Matter & Pounds:
 - 67.9% rolled barley, 21.3% barley sprout, 8.48% alfalfa hay, and 2.31% mineral package.
 - 18.1 total pounds of dry matter per head. Rolled barley 12.3 lbs., barley sprout 3.9 lbs., alfalfa hay 1.5 lbs., 0.42 lbs. mineral package.

- Cattle Performance:
 - Control Group:
 - Beginning live weight - 844
 - Ending live weight - 1322
 - Total pounds gain: 478
 - ADG: 3.68
 - Feed to Gain ratio: 6.22:1

- Test Group:
 - Beginning live weight - 852
 - Ending live weight - 1307
 - Total pounds gain: 455
 - ADG: 3.50
 - Feed to Gain ratio: 5.17:1

- Feedlot Economics:
 - Control Group:
 - Daily ration cost: \$4.08
 - Cost head/130 days: \$530.40
 - Cost per pound of gain: \$1.10
 - Test Group:
 - Daily ration cost: \$3.64
 - Cost head/130 days: \$473.20
 - Cost per pound of gain: \$1.04

- Carcass Results:
 - Control Group:
 - HCW - 791
 - Dressing % - 59.8%
 - USDA YG - 2.30
 - Marble score - 427
 - 50% graded USDA Choice or better
 - 13.3% qualified for a Quality Program
 - Test Group:
 - HCW - 793
 - Dressing % - 60.6%
 - USDA YG - 2.79
 - Marble score - 450
 - 65% graded USDA Choice or better
 - 25% qualified for a Quality Program

Summary: Both groups could have used another 45 days on feed, but we were limited in when we could process them at the JBS Hyrum Plant and have access to the production floor to take samples. The test group of steers on a 20% inclusion rate of sprouted barley gained 5% LESS per day which was not statistically relevant while eating 21% LESS Dry Matter - this is a big deal. The overall cost of the ration per head/day was 10.7% CHEAPER for the test group while the test group had a Feed to Gain Ratio that was 17% BETTER, with the cost per pound of gain being 5.5% LESS in favor of the test group. The test group produced Carcass grades of USDA Choice or better that were 23% HIGHER than the control group while also qualifying 47% MORE carcasses into a JBS Quality Program. In this study, we can conclude that the control group that was fed sprouted barely is more sustainable. They ate less and performed the same while lowering the cost per pound of gain. Meanwhile, the feed was grown using a technology that will produce the same amount of dry matter while using 95% less water.

Hydrogreen Feed Stats:

- Hydrogreen completed their study finishing Angus cross steers for market. Here are some stats from their study.
 - 9% increase in average daily gain - could translate into getting cattle to market faster and on feed in less time.
 - 4% improvement to feed conversion ratio in finishing calves on sprouted barley - this means that they ate 4% less feed per pound of gain.
 - 6% improvement to carcass yield grade - depending on your market, this could improve the number of cattle qualified for premium markets.
 - 4% reduction to live abscess. Healthier Cattle are more profitable cattle. Fewer cattle are being treated and less chance of disease spread.
 - 47% increase to Papillae area. The papillae which is essentially the lining of the cow's rumen. It was found to be healthier and better developed. This acts as a sponge and allows cattle to absorb nutrients that are converted to energy, energy makes pounds, and pounds are what pays you. This function of the cow's digestive system also guards against toxins and thus aids in the overall health of the cattle.
- 40% reduction in enteric methane emissions.

As a summary of Hydrogreen research, below is an attached copy. Also attached is the published report that is found in the Journal of Dairy Science by Cornell University on high-producing dairy cattle with both barley and wheat sprouts. The summary at the end tells what the main finding was with that feed trial. We also really like the attached abstract that Hydrogreen has shared with us.

No gain in pounds of milk produced. The cows that were used in the study are some of the highest producing Holstein cows in the world. However, the win in this study is that when sprouted barley or wheat was part of the diet the cattle produced the same amount of milk while consuming LESS total dry matter. Depending on the cost of the ration, this could be an economic win which ultimately is what it all boils down to for any agricultural sector because margins are generally tight. Dairy folks look at what's called Income Over Feed Cost (IOFC). When Dr. Harding (Renaissance Ag) looked at this in house, it was an economic advantage for sprouted barley by 20%. Cornell did not publish an economic report. It's worth noting that comparing sprouted barley vs wheat showed that wheat contributed more to body weight gain over barley in this particular study. One other small win, at least in my mind, the cattle on sprouts consumed 4 gallons less water per day.

Look at that alone big picture. The average lactating dairy cow consumes 30-50 gallons per day. If we split the difference and call it 40 and reduce it down to 36 a herd of 1000 dairy cattle go from consuming 40,000 gallons to 36,000 gallons of water per day - a 10% reduction in water used. Depending on the cost of their water, this may or may not be an economic win. However, in states like Utah, Idaho, Arizona, and New Mexico where many dairy cattle reside in very large herds - this looks good in their efforts to become more "sustainable".

What is DCAD? What and Why DCAD is important

In a conversation with Sanjeewa Ranathunga, PhD in Animal Science. Incorporating NovaGreen into a dairy cow's diet will lower the DCAD levels of the ration. A ration with lower DCAD is of particular interest to dairymen to feed during transition periods as well as dry cows. These diets for dry cows help prevent milk fever and subclinical hypocalcemia during transition periods.

What is DCAD? What and Why DCAD is important



 **hydrogreen**
NUTRITION TECHNOLOGY

A DIVISION OF CUBICFARM SYSTEMS CORP.

Animal Science Feed Trial Summary



HYDROGREEN BEEF GROWING EXPERIMENT



BACKGROUNDING EXPERIMENT OVERVIEW



- **Design:** Replicated pen study investigating influence of HG inclusion on the background feedlot performance. Four pens (n=40) balanced for age, size, sex, and genetic background (angus composite).
 - **Variables of Interest:** Feedlot performance (weight gain, feed efficiency, cost of gain), blood serum parameters, and nutrient digestibility
- Comparison Periods:**
- 05/05/21 – 08/31/21



BACKGROUNDING EXPERIMENT OVERVIEW

Table 1. Nutrient composition of total mixed treatment diet (HG included) and control diet along with hydroponically sprouted cereal grain product (HG)

	HydroGreen	Control (% of DM)	Sprouted Grain
Dry Matter, %	54.4	61.6	22.4
Crude Protein	14.0	14.0	15.7
Neutral Detergent Fiber	38.3	38.3	24.3
Acid Detergent Fiber	25.6	30.3	13.2
WSC*	13.2	7.3	49.2
Net Energy of Gain**	4.2	4.2	5.9

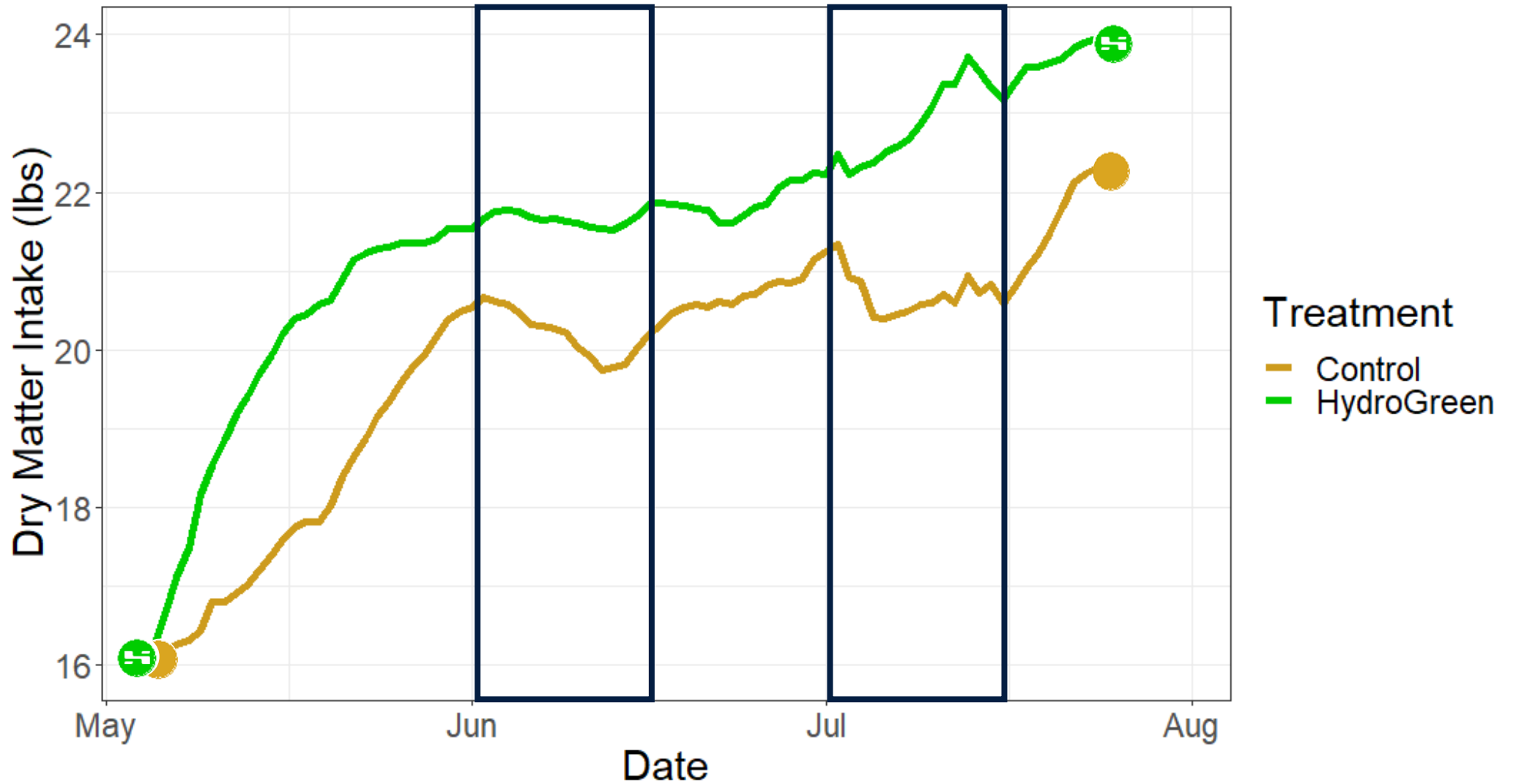
*Water Soluble Carbohydrates, **MJ/kg





BACKGROUNDING EXPERIMENT

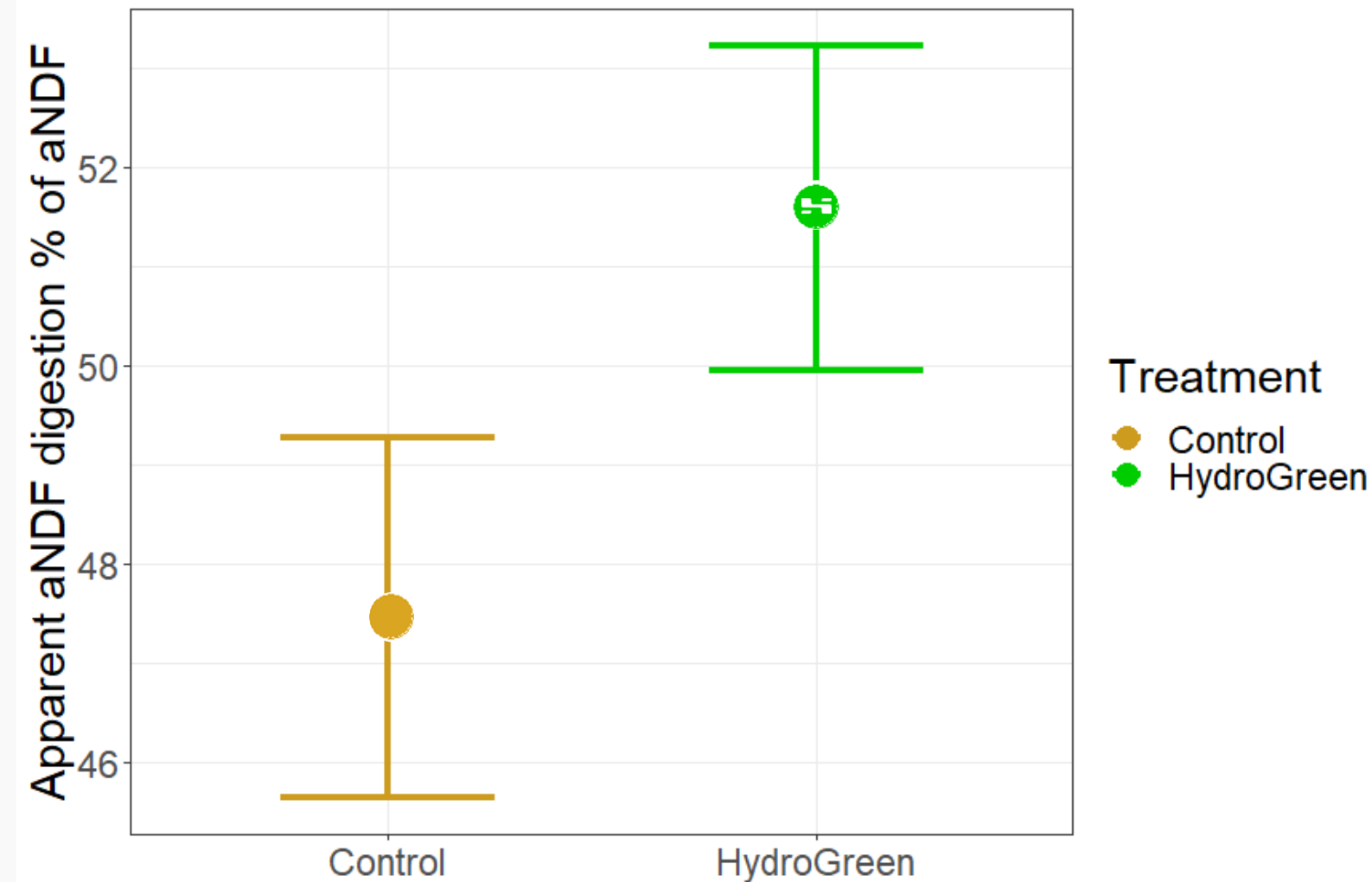
DAILY DRY MATTER INTAKE





BACKGROUNDING EXPERIMENT

APPARENT NDF DIGESTION



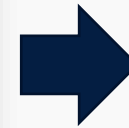
- Apparent NDF digestion is estimated to significantly increase 4% (+9%) with the addition of HG to the diet.
- NDF digestion is positively correlated to milk production, DMI, and daily rate of gain metrics
- A 1% increase in NDF digestion has been reported to increase milk production by 0.5 lbs and DMI by 0.4 lbs (Oba et al., 1997).



BACKGROUNDING EXPERIMENT

BLOOD CHEMISTRY STATISTICAL ANALYSIS

Variable	HydroGreen	Control	SE	Contrast	pval	Unit
Urea Nitrogen	22.2	17.2	0.8	5.0	0.00	mg/dL
Creatinine	1.10	1.04	0.04	0.06	0.20	mg/dL
Glucose	69.6	65.8	1.6	3.8	0.13	mg/dL
Anion Gap	22.3	23.3	0.7	-1.0	0.30	mmol/L
ALP	94.3	102.5	8.4	-8.2	0.55	U/L
Beta Carotene	1.51	1.42	0.9	0.09	0.54	ug/mL





BACKGROUNDING EXPERIMENT

STATISTICAL ANALYSIS RESULTS

Week 0 - 12

Variable	HydroGreen	Control	SE	df	Contrast	pval	unit
Daily Rate of Gain	2.54	2.28	0.07	11	0.25	0.03	lb/day
Dry Matter Intake	21.8	20.0	0.12	237	1.8	0.00	lb/day
Feed Conversion Ratio	7.61	8.70	0.54	2	-1.10	0.29	DMI/DRG
Cost of Gain	0.73	0.81	0.05	2	-0.08	0.40	\$/lb
NDF Digestion	52.1	47.5	2.12	10	4.6	0.15	%

Week 0 - 8

Variable	HydroGreen	Control	SE	df	Contrast	pval	unit
Daily Rate of Gain	2.79	2.37	0.06	11	0.44	0.00	lb/day
Dry Matter Intake	21.0	19.5	0.11	237	1.6	0.00	lb/day
Feed Conversion Ratio	7.46	8.98	0.65	2	-1.52	0.24	DMI/DRG
Cost of Gain	0.72	0.84	0.06	2	-0.12	0.31	\$/lb
NDF Digestion	49.7	46.1	2.29	10	3.6	0.26	%



TRANSITION COW OBSERVATION





TRANSITION COW OBSERVATION OVERVIEW

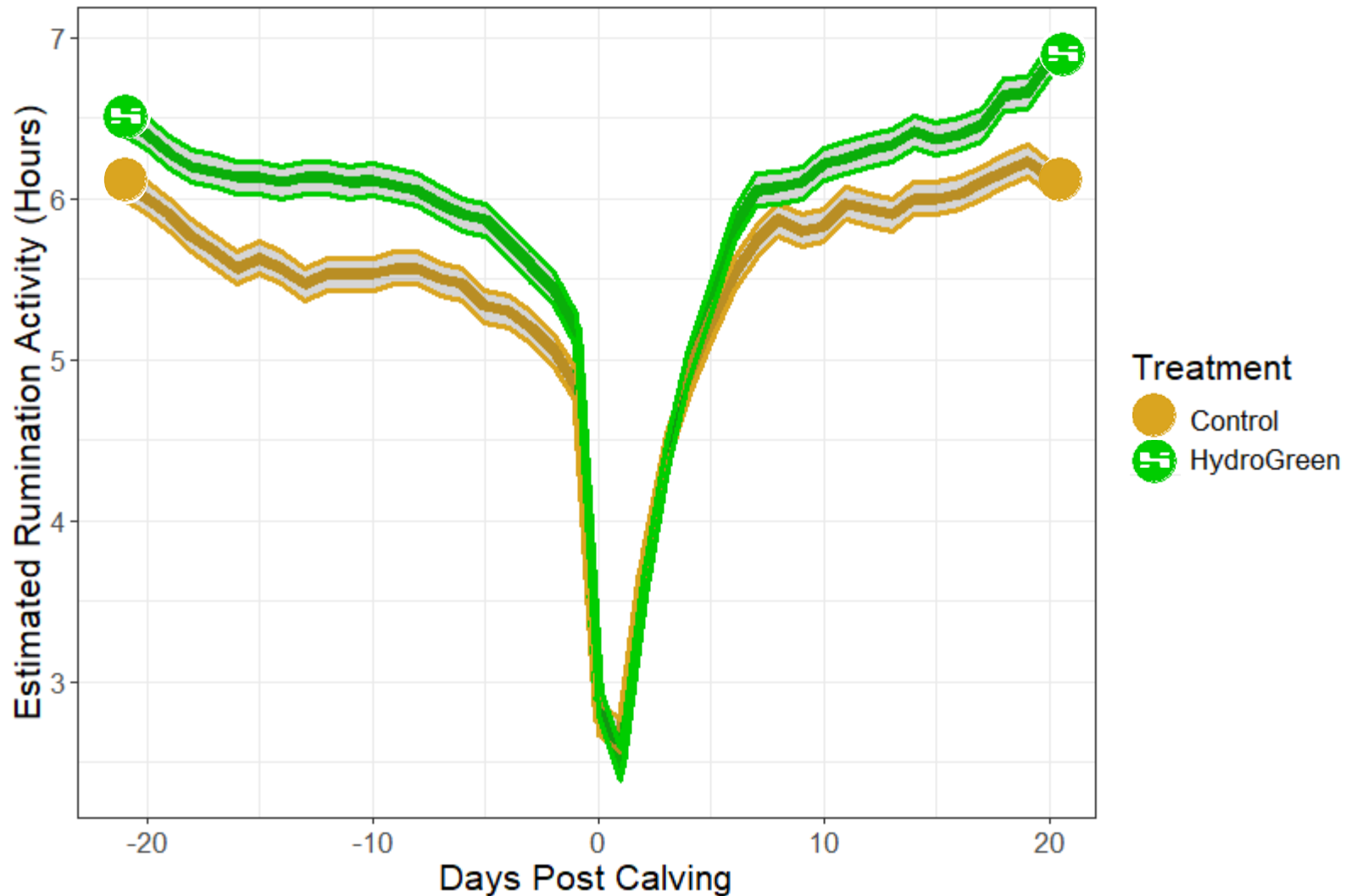


- **Design:** Crossover observation without replication investigating influence of HG inclusion on the dynamic close-up pen (n=160). HydroGreen and control diets held consistent for energy, fiber, and crude protein.
- **Variables of Interest:** Feeding behavior, along with health, and fertility and milk production post calving
- **HydroGreen Periods:**
 - 04/07/21 – 06/15/21: 570
 - 09/13/21 – 10/04/21: 172
 - Total: 742
- **Control Periods:**
 - 03/15/21 – 04/06/21: 181
 - 07/01/21 – 09/12/21: 602
 - Total: 783



TRANSITION COW OBSERVATION

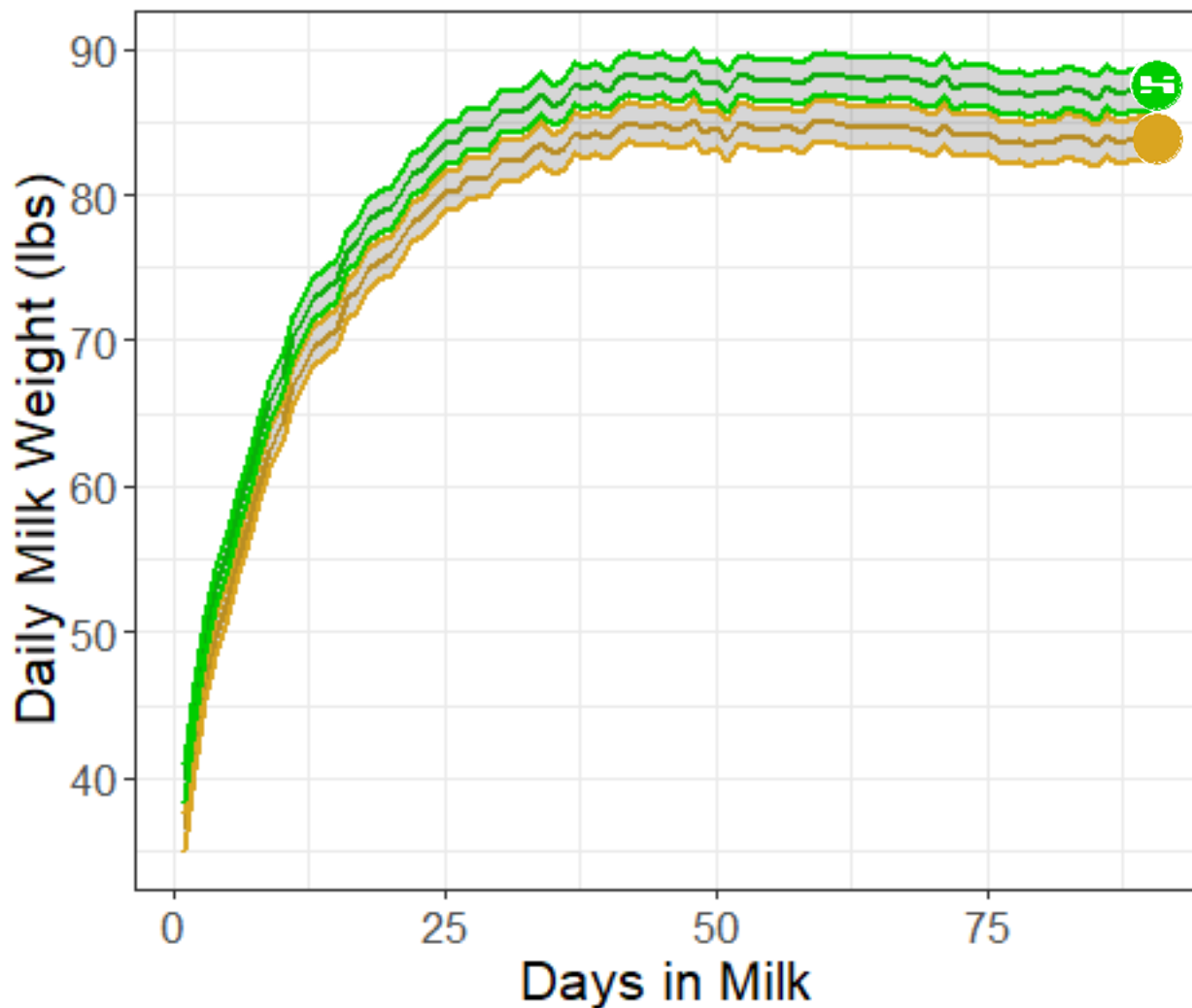
DRY MATTER INTAKE / RUMINATION ACTIVITY



- Dry matter intake and rumination activity **increased 12%** immediately
- Increases support improvements in **milk production**, cow **health** and cow **fertility** after calving.



TRANSITION COW OBSERVATION PEAK MILK PRODUCTION



Treatment

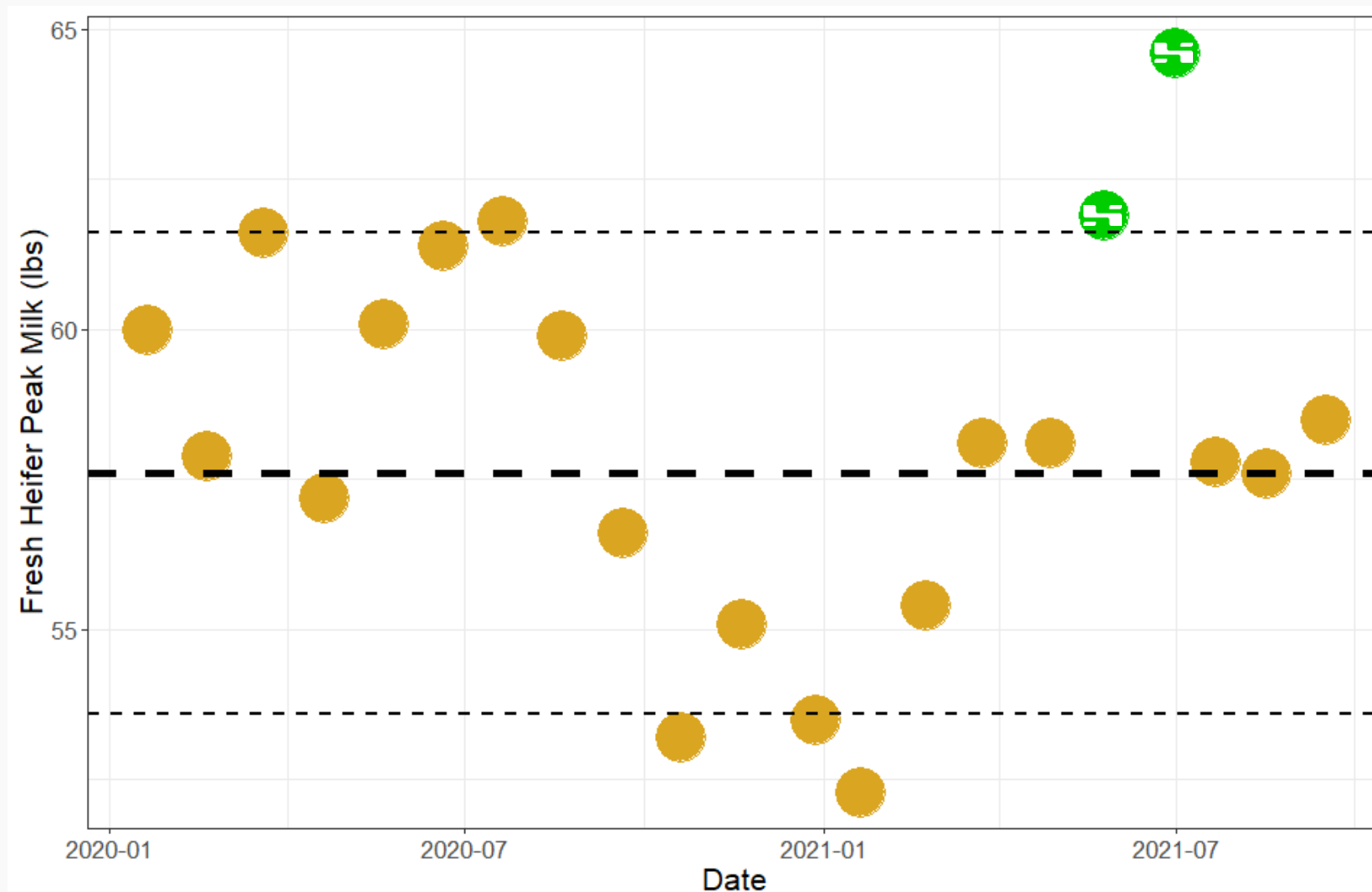
- Control
- HydroGreen

- Across all lactation groups, daily milk weights **increased an estimated 5%** adjusted for seasonality when cows transitioned with HydroGreen.
- Every pound of increased peak milk translates to roughly **200 pounds** additional milk per lactation **+\$126 USD**
- Analysis performed on **89,028** daily total cow combinations from 03/01/21 to 08/01/21



TRANSITION COW OBSERVATION PEAK MILK PRODUCTION

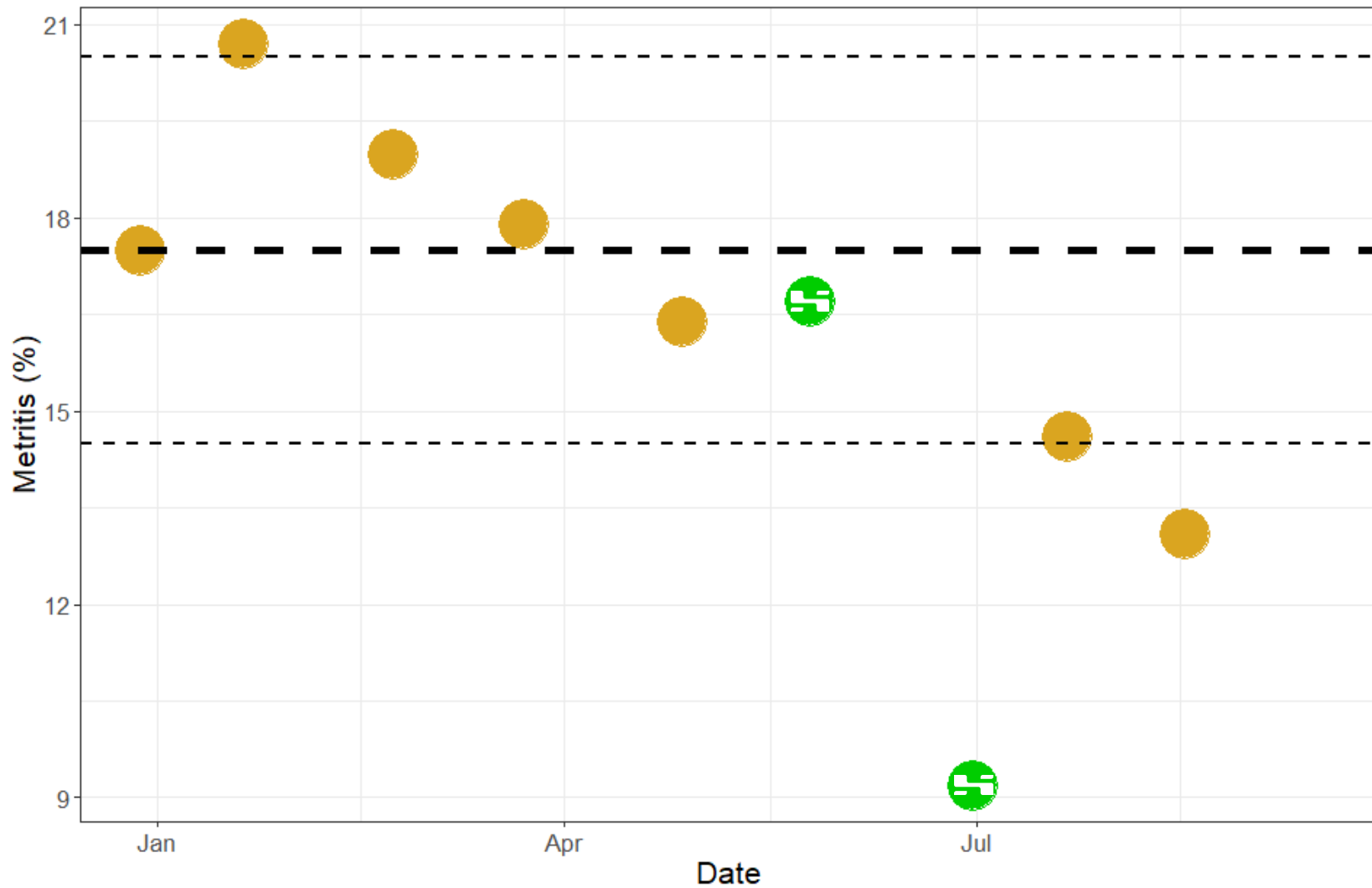
- **+16% improvement** from the 18-month average was observed in June within the fresh heifer group.
- Every pound of increased peak milk translates to roughly 200 pounds additional milk per lactation (**+\$128 USD**)





TRANSITION COW OBSERVATION

HEALTH TRENDS / METRITIS INCIDENCE



- Metritis is the most common ailment to dairy cattle post calving.
- The incidence of metritis dropped significantly in June.
- The cost per case of metritis is estimated to be **\$358 USD**

<https://www2.zoetisus.com/conditions/dairy/metritis>



BREEDING HEIFER OBSERVATION





BREEDING HEIFER OBSERVATION

OVERVIEW



- **Design:** Crossover observation without replication investigating influence of HG inclusion on the dynamic breeding pens (n=280). HydroGreen and control diets held consistent for energy, fiber, and crude protein.
- **Variables of Interest:** Feeding behavior, blood serum chemistry, and fertility rates
- **HydroGreen Periods:**
 - 06/15/21 – 07/28/21
 - Total: 280
- **Control Periods:**
 - 08/01/21 – 09/15/21: 284
 - Total: 284



BREEDING HEIFER OBSERVATION

BLOOD CHEMISTRY & PERFORMANCE

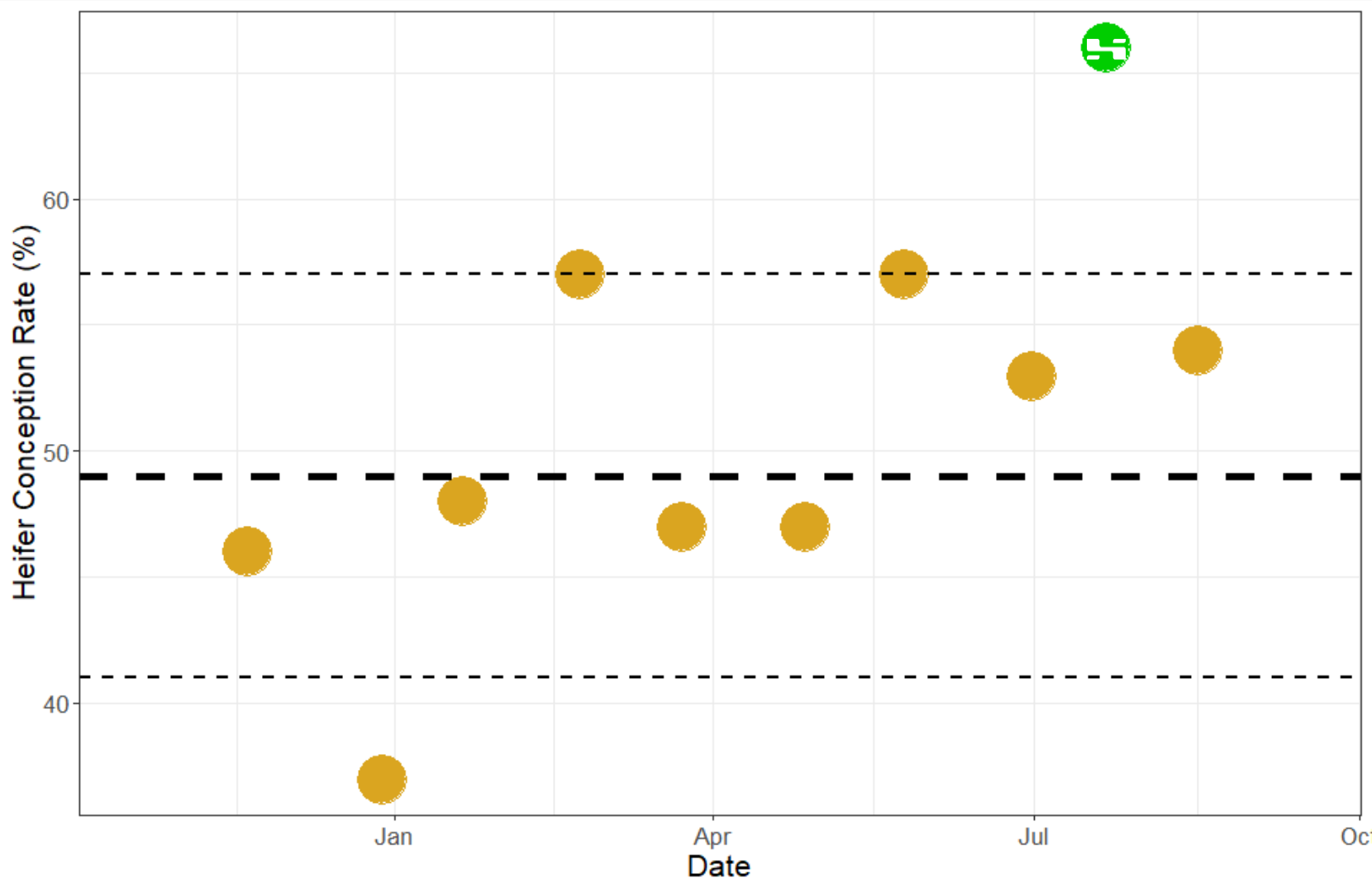


Variable	HydroGreen	Control	SE	df	Contrast	pval	Unit
Eating	496	467	4.2	451	29	0.000	minutes
Heat Index	7.5	6.8	0.2	451	0.6	0.013	N/A
Times Bred	1.8	2.2	1.0	560	0.4	0.017	count
Activity	313	305	1.7	451	8	0.000	minutes
Anion Gap	14.7	19.3	0.45	28	-4.60	0.000	mmol/L
TCO ₂	29.4	25.6	0.55	28	3.89	0.000	mmol/L
TMR uNDF/ fecal uNDF	56.7	48.0	1.2	19	8.6	0.000	NA
pdNDF digestion	85.4	78.4	2.55	19	7.03	0.081	%



BREEDING HEIFER OBSERVATION REPRODUCTION RATES

- **Conception rates** calculated on per cycle basis have increased **17%** from the six-month average.
- The annual estimated value of each percentage point increase is **\$66 per cow**



<https://hoards.com/article-4704-boosting-preg-rates-pays-multiple-dividends.html>



HIGH LACTATION GROUP EXPERIMENT





HIGH LACTATION GROUP EXPERIMENT OVERVIEW



- **Design:** Randomized block design without replication investigating influence of HG inclusion on the high group pens (n=344) balanced for age, production and components.
- **Variables of Interest:** Feeding behavior, milk production, milk components, feed efficiency, nutrient digestibility, and methane emission
- **Comparison Period:**
 - 10/14/21 – 12/14/21
- **Carryover Period:**
 - 12/15/21 – 01/02/22



HIGH LACTATION GROUP EXPERIMENT TMR ANALYSIS: WEEK 41-50

Variable	HydroGreen	Control	SE	df	Contrast	pval	Unit
DM	46.5	47.8	0.2	74	-1.3	0.000	%
CP	17.5	17.4	0.1	74	0.2	0.091	% DM
ADF	14.6	14.5	0.2	47	0.1	0.609	% DM
aNDF	27.6	27.6	0.4	74	0.0	0.985	% DM
Lignin	2.5	1.9	0.0	47	0.6	0.000	% DM
Starch	25.6	26.3	0.2	47	-0.7	0.030	% DM
Sugar (WSC)	9.0	7.9	0.1	47	1.1	0.000	% DM
NEL	0.74	0.74	0.0	47	0.0	0.378	Mcal/lb
Fumonisin	0.289	0.300	0.004	13	-0.012	0.109	ppm
DON	0.180	0.201	0.014	13	-0.021	0.300	ppm
Aflatoxin	0.000	9.33	5.10	13	-9.33	0.233	ppb

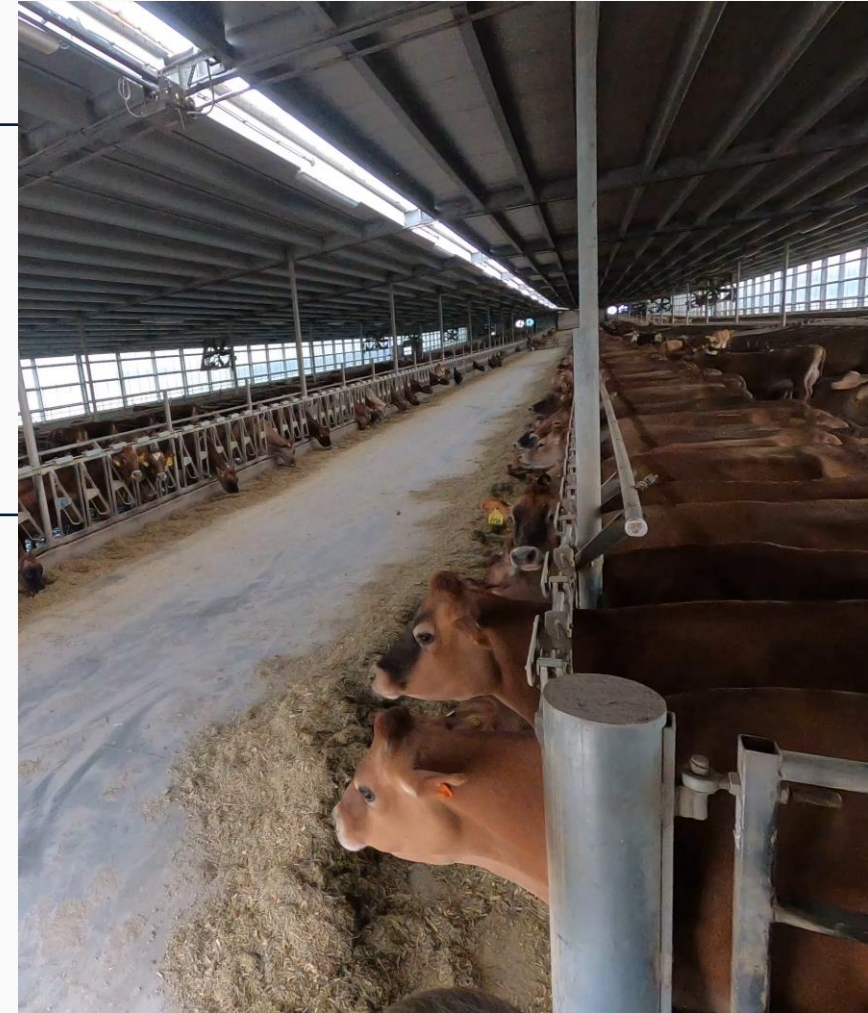




HIGH LACTATION GROUP EXPERIMENT

FEEDING BEHAVIOR & DIGESTIBILITY: WEEK 41-50

Variable	HydroGreen	Control	SE	df	Contrast	pval	Unit
Eating	5.4	4.9	0.02	65	0.5	0.000	Hours
Rumination	6.6	6.4	0.02	65	0.2	0.000	Hours
Chewing	12.1	11.3	0.05	65	0.8	0.000	Hours
Active	1.6	1.8	0.01	65	-0.2	0.000	Hours
Highly Active	3.7	3.9	0.02	65	-0.2	0.000	Hours
OMD	66.2	66.9	1.2	24	-0.8	0.678	%
NDFD	45.8	41.7	1.4	24	4.1	0.044	%
pdNDFD	71.7	67.2	1.9	24	4.4	0.177	%
NDFD_IV_240	76.0	73.9	0.3	47	2.1	0.000	%





HIGH LACTATION GROUP EXPERIMENT

PRODUCTION ANALYSIS: WEEK 41-50

Variable	HydroGreen	Control	SE	df	Contrast	pval	Unit
Milk	80.7	78.7	0.14	20470	2.0	0.000	lbs
Fat	5.14	5.04	0.004	1248	0.10	0.000	%
Protein	3.71	3.71	0.003	1248	0.00	0.649	%
ECM	103.0	99.5	0.18	1248	3.5	0.000	lbs
MUN	12.83	12.29	0.043	1248	0.54	0.000	%
Intake	53.1	53.7	0.25	62	-0.6	0.065	lbs
Feed Efficiency	1.94	1.85	0.01	62	0.09	0.032	lbs/intake
ECM/\$	13.8	13.7			0.12		lbs/\$
Methane ¹	357	454	3.0	20182	-97	0.000	g/day
MECM*	3.47	4.56			-1.09		CH ₄ ³ /ECM



¹Estimation adopted from Song et al., 2018 and Wu et al., 2014

*CH₄³/ECM

Social benefit of the methane flux reduction valued at **\$293 year⁻¹ cow⁻¹****

**The social cost per metric ton of methane has been estimated at \$8,290 tonne⁻¹ (Erickson et al., 2021).



HIGH LACTATION GROUP EXPERIMENT

PRODUCTION ANALYSIS: HG WITHHELD WEEK 51-52

Variable	Control (HG)	Control	SE	df	Contrast	pval	Unit
Milk	78.0	77.6	0.4	1680	0.4	0.404	lbs
ECM	98.6	98.1	0.5		0.5		
Intake	54.4	53.9	0.6	14	-0.5	0.603	lbs
Feed Efficiency	1.81	1.82	0.8	14	0.0	0.543	lbs
Methane ¹	442	444	7.3	1315	-2	0.877	g/day
Methane ²	463	464	3.6	6632	-1	0.478	g/day
Methane ³	453	454			-1		g/day
MECM*	4.59	4.62			-0.02		CH ₄ ³ /ECM



¹Estimation adopted from Song et al., 2018 from nostril measurement mean

²Estimation adopted from Wu et al., 2014 from parlor measurement

³ Mean of both methods

*CH₄³/ECM



TRANSITION CALF EXPERIMENT





TRANSITION CALF FEEDING EXPERIMENT OVERVIEW



- **Design:** Randomized block design investigating influence of HG inclusion on eight calf pens (n=80) balanced for age.
- **Variables of Interest:** Feeding behavior, weight gain, skeletal development, feed efficiency, blood serum parameters, nutrient digestibility, and cost of gain
- **Feeding Period:**
 - 11/09/21 – 12/14/21
- **Observation Period:**
 - 12/15/21 – 12/31/25



TRANSITION CALF FEEDING EXPERIMENT TMR ANALYSIS: WEEK 42-50

Variable	HydroGreen	Control	SE	df	Contrast	pval	Unit
DM	62.6	79.3	3.0	26	-16.7	0.000	%
CP	18.7	21.5	0.5	26	-2.8	0.000	% DM
ADF	17.8	11.0	1.2	10	6.8	0.007	% DM
aNDF	32.0	23.4	1.3	26	8.5	0.000	% DM
Lignin	4.3	4.0	0.1	10	0.3	0.107	% DM
Starch	20.8	25.1	1.1	26	-4.4	0.011	% DM
Sugar (WSC)	10.9	10.7	0.1	10	0.2	0.331	% DM
NEG	0.47	0.54	0.01	10	-0.06	0.009	Mcal/lb
NEM	0.75	0.82	0.01	10	-0.07	0.009	Mcal/lb





TRANSITION CALF FEEDING EXPERIMENT

BLOOD SERUM & DIGESTIBILITY ANALYSIS: WEEK 44-50

Variable	HydroGreen	Control	SE	df	Contrast	pval	Unit
Blood Urea N	15.3	12.2	0.92	23	3.0	0.005	mg/dL
TCO ₂	32.7	30.9	0.93	23	1.8	0.094	mmol/L
Anion Gap	17.9	19.6	0.54	23	-1.6	0.015	mmol/L
Glucose	93.1	79.1	2.92	23	14.0	0.000	mg/dL
Total Bilirubin	0.13	0.17	0.02	23	-0.04	0.052	mg/dL
pdNDFD	73.6	69.3	3.5	14	3.9	0.571	%
NDFD_IV_240	74.1	70.2	0.6	14	3.9	0.004	%

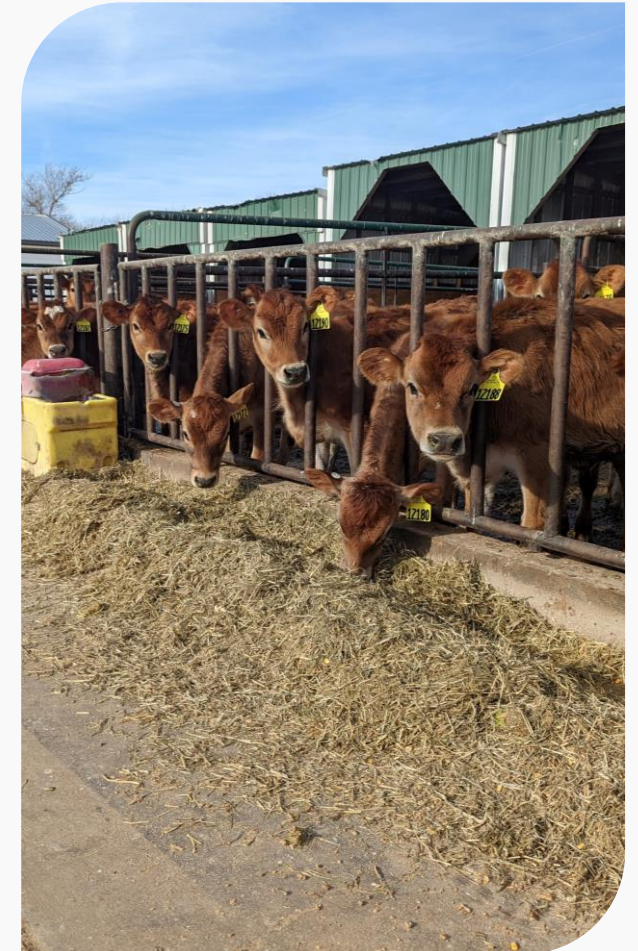




TRANSITION CALF FEEDING EXPERIMENT

PERFORMANCE ANALYSIS: WEEK 44-50

Variable	HydroGreen	Control	SE	df	Contrast	pval	Unit
Weight	276	270	2.6	75	6.2	0.102	lbs
Height	39.6	37.8	0.2	75	1.8	0.000	inches
Length	34.1	33.5	0.3	75	0.6	0.189	inches
ADG	2.4	2.2	0.1	75	0.2	0.102	lbs/day
Intake	6.9	6.5	0.2	16	0.9	0.115	lbs/day
Feed Efficiency	0.35	0.34		16	0.01		ADG/DMI
Mcal Efficiency	0.73	0.61		16	0.12		ADG/Mcal
Cost of Gain	\$0.47	\$0.63			\$0.16		\$/ADG



Increased wither height estimated increase in first lactation milk production by 5% (**\$194 cow⁻¹ year⁻¹**) (Bar-Peled, et al., 1997)



BEEF FINISHING EXPERIMENT



BEEF FINISHING EXPERIMENT OVERVIEW



- **Design:** Replicated pen study (n=4) investigating influence of HG inclusion on Angus x Holstein beef cattle (n=244) balanced for sex, incoming weight ($\mu=388$ kg), genetic background, and age.
- **Variables of Interest:** Feedlot performance, feeding behavior, rumination activity, rumen pH, activity, drinking patterns, meat carcass quality, blood serum parameters, nutrient digestibility, and cost of gain
- **Feeding Period:**
 - 02/02/22 – 08/19/22



BEEF FINISHING EXPERIMENT

ANIMAL BEHAVIOR METRICS

Statistical analysis of daily animal behavior metrics: including estimates and standard deviation analysis of rumination activity, general activity, drinking events, reticulum temperature and heat index.

	HydroGreen	Control	SEM	DF	Contrast	p-value	Unit
Estimate							
Rumination	317	311	2	179	6	<0.00	minutes
pH	5.94	6.14	0.01	179	-0.20	<0.00	
Activity	279	272	2	179	7	<0.00	minutes
Drinking Events	10.4	10.7	0.1	179	-0.3	0.002	count
Temperature	39.3	39.3	0.1	179	0.0	0.989	°C
Heat Index	2.02	1.80	0.05	179	0.22	<0.00	
Standard Deviation							
Rumination	54	61	2	179	7	<0.00	minutes
pH	0.27	0.42	0.02	179	-0.39	<0.00	
Activity	213	205	5	179	8	0.127	hours
Drinking Events	4.00	3.79	0.08	179	0.21	0.007	count
Temperature	0.92	0.93	0.02	179	-0.01	0.771	°C
Heat Index	10.8	10.1	0.5	179	-0.7	0.194	



BEEF FINISHING EXPERIMENT

FEEDLOT PERFORMANCE METRICS

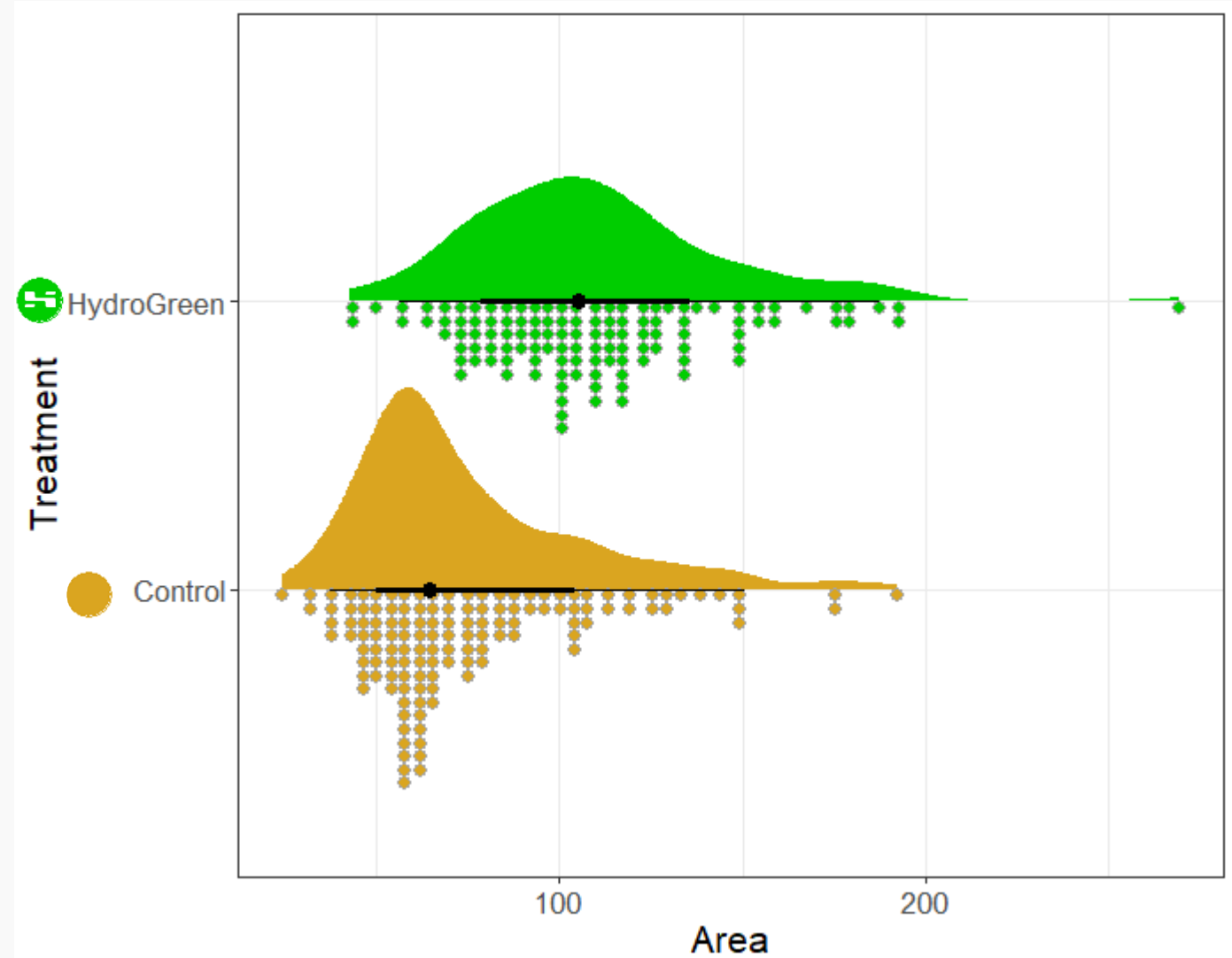
Statistical analysis of feedlot performance: daily rate of gain (DRG), feed conversion ratio (FCR), dry matter intake (DMI) along with apparent organic matter (OMD), crude protein (CPD), starch (StarchD), Fat (FatD), and neutral detergent fiber (NDFD) digestibility. Reticulum pH, methane flux, methane flux per unit intake, and methane flux per kilogram gained reported.

Variable	HydroGreen	Control	SEM	DF	Contrast	p-value	Δ	Unit
DRG	3.3	3.0	0.04	3	0.13	0.070	+9%	lbs day ⁻¹
FCR	6.85	7.10	0.20	3	-0.25	0.417		DMI DRG ⁻¹
DMI	22.7	21.5	0.11	3	0.55	0.006	+6%	lbs day ⁻¹
OMD	72.6	64.6	2.9	9	8.0	0.023		%
CPD	63.0	57.0	4.5	9	6.0	0.219		%
StarchD	90.5	80.5	2.6	9	10.0	0.005		%
FatD	75.9	60.4	6.8	9	15.4	0.052		%
NDFD	51.7	44.3	6.0	9	7.5	0.250		%
Reticulum pH	5.84	6.22	0.06	21	-0.38	<0.00		
Methane flux	175	282	17	3	-107	0.024	-38%	g day ⁻¹
Methane / DMI	17.0	29.0	2.1	3	-12.0	0.038	-41%	g DMI ⁻¹
Methane / ADG	117	206	2	3	89	<0.00	-43%	g DRG ⁻¹



BEEF FINISHING EXPERIMENT

RUMEN WALL EVALUATION





BEEF FINISHING EXPERIMENT

MEAT QUALITY METRICS

Statistical analysis of meat quality: finished weight, carcass weight, dressing percentage, ribeye area, yield grade, marbling score, fat thickness, liver abscess incidence, papillae area, methane flux, and methane emissions per finish weight (MFW) and carcass weight (MCW) reported.

Variable	HydroGreen	Control	SEM	DF	Contrast	p-value	Δ	Unit
Finished Weight	1451	1413	7.2	75	17	0.025		lbs
Carcass Weight	855	851	5.5	75	1.6	0.783		lbs
Dressing	59.0	60.4	0.5	75	-1.4	0.006		%
Ribeye Area	14.0	13.9	1.8	75	0.6	0.756		in ²
Yield Grade	3.04	3.22	0.11	75	-0.18	0.103		
Quality Grade	1.90	1.87	0.08	75	-0.03	0.760		
Marbling	558	565	42	75	-7	0.872		
Fat Thickness	0.5	1.39	0.12	75	0.20	0.101		in
Liver Abscess	13.5	17.9	8.5	75	-4.4	0.602		%
Papillae Length	15.0	12.3	0.8	23	2.7	0.002	+22%	mm
Papillae Width	7.3	6.0	0.4	23	1.3	0.002	+22%	mm
Papillae Area	110	75	8.0	23	35	<0.00	+47%	mm ²
Methane flux	175	282	17	3	-107	0.024	-38%	g day ⁻¹
MFW	106	174			-31		-39%	g lbs ⁻¹
MCW	179	291			-51		-39%	g lbs ⁻¹



BEEF GROWING EXPERIMENT





BEEF GROWING EXPERIMENT OVERVIEW



- **Design:** Replicated pen study (n=4) investigating influence of HG inclusion on Angus x Holstein beef cattle (n=462) balanced for sex, incoming weight ($\mu=410$ lbs), genetic background, and age.
- **Variables of Interest:** Feedlot performance, feeding behavior, rumination activity, activity, drinking patterns, blood serum parameters, skeletal development, nutrient digestibility, and cost of gain
- **Feeding Period:**
 - 09/23/22 – 12/16/22



BEEF GROWING EXPERIMENT

FEEDLOT PERFORMANCE METRICS

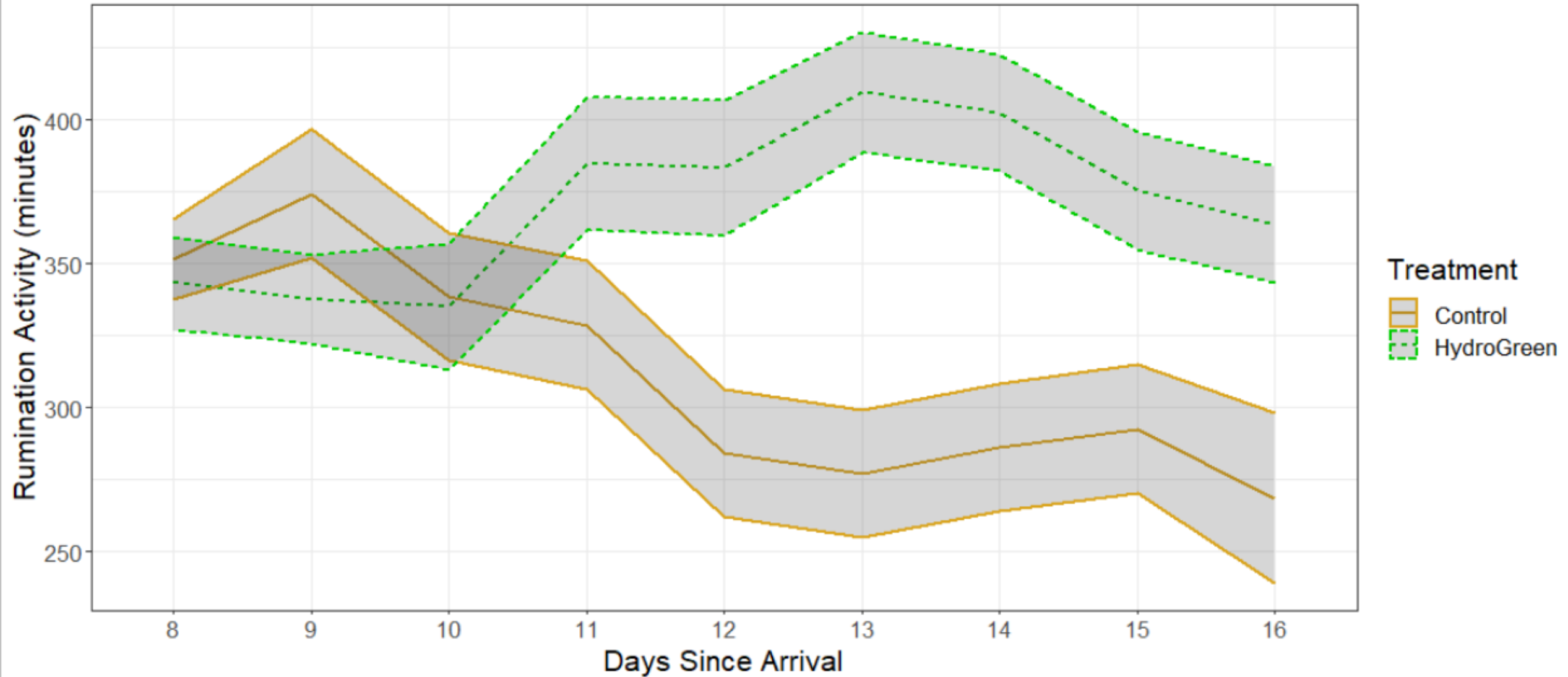
Statistical analysis of feedlot performance: daily rate of gain (DRG), feed conversion ratio (FCR), dry matter intake (DMI) along with bolus metrics, wither height and growth in terms of height. Methane flux, methane flux per unit intake, and methane flux per kilogram gained reported.

Variable	HydroGreen	Control	SEM	DF	Contrast	p-value	Δ	Unit
DRG	2.25	1.85	0.10	207	0.40	<0.001	+22%	lb day ⁻¹
FCR	5.2	6.6	0.5	207	-1.4	0.005	-21%	
DMI	11.6	12.2	0.3	78	-0.5	0.438		lb day ⁻¹
Rumination	355	303	11	39	52	<0.001		minutes
Activity	9.1	8.0	1.8	39	1.1	0.372		hours
Temperature	39.4	39.0	0.1	39	0.4	<0.001		°C
Drinking	8.5	7.6	0.3	10	0.9	0.016		count
Events								
Start Wither	43.3	44.3	0.2	224	-1.0	<0.001		inches
End Wither	45.1	45.5	0.3	164	-0.6	0.041		inches
Growth	0.04	0.03	0.0	164	0.01	0.228		in day ⁻¹
Methane flux	107	160	17	17	-53	0.010	-33%	g day ⁻¹
Methane / DMI	9.2	13.1			3.9		-30%	g lb ⁻¹
Methane / DRG	47.6	86.5			38.9		-45%	g lb ⁻¹



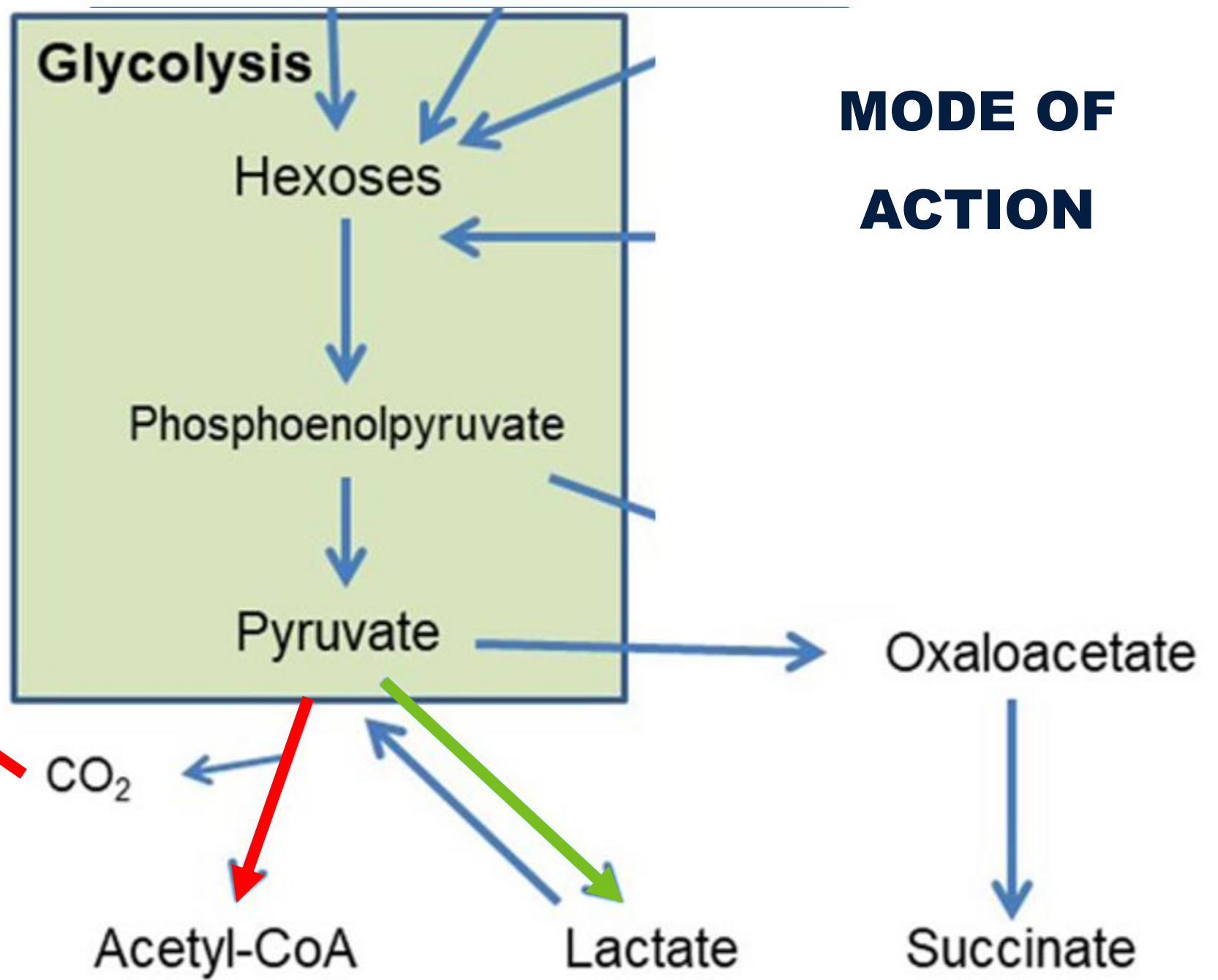
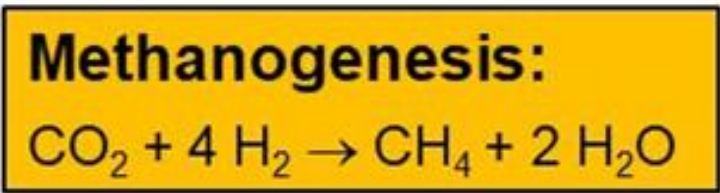
BEEF GROWING EXPERIMENT

FEEDLOT PERFORMANCE METRICS





MODE OF ACTION





FERMENTRICS





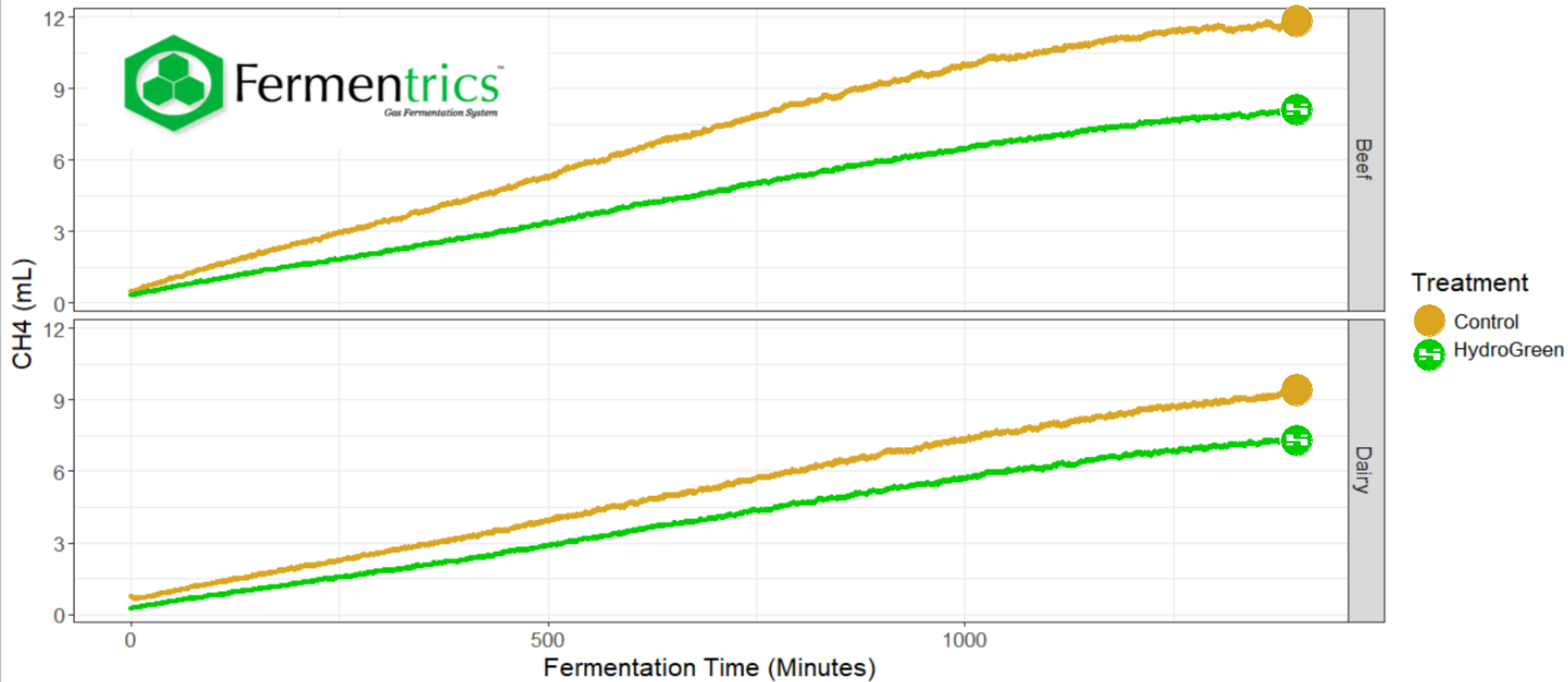
FERMENTRICS EXPERIMENT OVERVIEW



- **Design:** Randomized complete block (n=2) investigating influence of HG inclusion on invitro fermentation characteristics. Four replications, two applications (Dairy + Beef), diets balanced for metabolizable energy, crude protein and aNDF content.
- **Variables of Interest:** The FG-7 measures pH, CH₄, H₂, and NH₃ at 30 second time intervals during a 24hr fermentation. VFAs composition at 3hr and 9hr replicated timepoints.
- **Sampling Period:**
 - 04/15/22 – 04/30/22



FERMENTRICS EXPERIMENT FERMENTATION CURVE





FERMENTRICS EXPERIMENT

INVITRO FERMENTRATION METRICS

Variable	HydroGreen	Control	SEM	DF	Contrast	p-value	Δ	Unit
pH	6.21	6.29	0.03	9989	-0.08	<0.00		
Gas Flux	53.2	68.3	0.3	9989	-14.9	<0.00	-22%	mL
H ₂	0.74	0.71	0.01	9989	0.03	<0.00		ppm
CH ₄	4.63	7.09	0.02	9989	-2.46	<0.00	-35%	mL
NH ₃	251	246	0.33	9989	-5	<0.00		ppm
Acetic Acid	2420	1904	132	10	516	0.003	+27%	ppm
Propionic Acid	1566	1184	71	10	383	0.001	+32%	ppm
Butyric Acid	729	568	31	10	161	<0.00	+28%	ppm
Lactic Acid	50	13	16	10	37	0.042	+285%	ppm
Valeric Acid	155	123	7	10	32	0.002	+26%	ppm

Statistical analysis of invitro fermentation investigation: pH, gas flux, dihydrogen flux (H₂), methane flux (CH₄), ammonia (NH₃), acetic acid, propionic acid, butyric acid, lactic acid and valeric acid reported.

Effects of hydroponically sprouted cereal grains on beef performance, apparent nutrient digestibility, and enteric methane emission

S.Jenkins¹, E. Slack¹

¹HydroGreen Global, CubicFarm Systems Corporation, Sioux Falls, SD.

The increasingly negative influence of abiotic factors on crop production, coupled with the fragility of the global animal feed supply chain, accentuates the importance of investigating hydroponically sprouted cereal grains (HG) as beef cattle feedstuff. Angus x Holstein cattle (n = 244) averaging 388 kilograms in live weight were randomly assigned to pens in a commercial beef feedlot setting. Treatment pens (n = 4), blocked by sex, were randomly assigned a dietary treatment consisting of a control and a treatment group (15 % HG in a DM basis) for a 20-week comparison period. Diets were balanced for metabolizable energy, neutral detergent fiber, fat, and crude protein content. Nutrient digestibility was assessed through biweekly manure sampling. Individual body weight was assessed every six weeks with a livestock weight system (EziWeight7I, Tru-Test, College Station, Texas). Enteric methane emissions were assessed twice weekly with a laser methane sensor (LMmBE, Tokyo Gas Engineering Solutions, Tokyo, Japan). Reticulum pH was recorded every 30 seconds on a subset of animals (n = 8) with a smart bolus (pH Bolus, smaXtec, Ainring, Germany). Hydroponically sprouted grain inclusion resulted in statistically significant (p < 0.10) changes to daily rate of gain, dry matter intake and enteric methane production (Table 1). Significant reductions in methane flux coupled with the improved feedlot performance highlight the potential feeding value of HG as a novel agricultural technology solution.

Table 1. Daily rate of gain (DRG), feed conversion ratio (FCR), dry matter intake (DMI) along with apparent organic matter (OM), crude protein (CP) and neutral detergent fiber (NDF) digestion. Reticulum pH, methane flux, and methane flux per kilogram weight gained reported.

	Treatment	Control	SEM	DF	Contrast	p-value	Unit
DRG	1.68	1.52	0.05	3	0.17	0.068	kg day ⁻¹
FCR	5.34	5.61	0.17	3	-0.27	0.241	DMI DRG ⁻¹
DMI	8.97	8.53	0.11	3	0.44	0.043	kg day ⁻¹
OM digestion	73.0	67.2	4.9	3	5.8	0.385	%
CP digestion	66.3	55.0	6.0	3	11.3	0.234	%
NDF digestion	57.0	50.3	8.7	3	6.7	0.537	%
Reticulum pH	5.70	6.15	0.10	3	-0.45	<0.00	
Methane flux	174	288	2	3	-114	<0.00	g day ⁻¹
Methane / DMI	19.4	33.8	0.1	3	-14.4	<0.00	g DMI ⁻¹
Methane / ADG	104	190	2	3	86	<0.00	g DRG ⁻¹

SEM = Standard error of the mean (SE), DF = degrees of freedom.





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Replacing conventional concentrates with sprouted barley or wheat: Effects on lactational performance, nutrient digestibility, and milk fatty acid profile in dairy cows.

Yu Zang,^{1,2} Andrew T. Richards,¹ Nirosh Seneviratne,¹ Fabian Andres Gutierrez Oviedo,¹ Rob Harding,³
Sanjeeva Ranathunga,³ and Joseph W. McFadden^{1*}

¹Department of Animal Science, Cornell University, Ithaca 14853

²Laboratory of Metabolic Manipulation of Herbivorous Animal Nutrition, Yangzhou University, Yangzhou 225009

³Renaissance Ag, Vineyard, 84058

ABSTRACT

Finite natural resources, rising human population, and climate change pose challenges to traditional crop production. Hydroponically grown fodder (i.e., sprouted grains) can be an alternative feed source for dairy cows; however, only sprouted barley has been investigated in low-producing cows. We aimed to evaluate the impact of replacing conventional concentrates with sprouted barley or wheat, grown using hydroponics, on milk production, nutrient digestibility, and milk fatty acid profile in high-producing cows. Twenty-four multiparous Holstein cows [3.25 ± 1.33 lactations; 102 ± 23 d in milk (DIM); 49 ± 4 kg/d of milk] were used in a replicated 3×3 Latin square design with 21-d experimental periods. Following a 2-wk covariate period, cows were fed 1 of 3 experimental diets: a total mixed ration (1) without sprouted grains (Control), or with (2) 10% sprouted barley (Barley) or (3) 10% sprouted wheat (Wheat) on a dry matter (DM) basis. Experimental diets were formulated to be isoenergetic and isonitrogenous with sprouted grains that replaced ground corn, soybean meal, canola meal, and dextrose. Sprouted grains were grown using a semi-automatic hydroponic system and harvested after 6 d of growth. Data and sample collection occurred during the last 3 d of the covariate and experimental periods. Wide ranges were observed for the DM percent of sprouted grains (12.1 to 22.9% and 13.3 to 25.7% for barley and wheat, respectively) and the ratio of sprouted fodder to seed (0.67 to 1.07 for both barley and wheat). Feeding sprouted grains did not modify yields of milk or energy-corrected milk (ECM); however, dry matter intakes (DMI) were lower for Barley, relative to Control. Feed efficiencies

were greater for Barley than for Control (1.49 ± 0.03 vs. 1.43 ± 0.03 for milk yield/DMI; 1.85 ± 0.03 vs. 1.73 ± 0.04 for ECM/DMI). Yields and concentrations of milk components (i.e., fat, true protein, and lactose) were not impacted by treatment. Milk urea-N concentrations were greater for Wheat, relative to Control or Barley. Body weight (BW; 752 ± 3 vs. 742 ± 3 kg) and BW gain (6.53 ± 2.99 vs. -9.33 ± 2.91 kg/21 d) were higher for Wheat than for Control. Apparent total-tract digestibility of organic matter was greater for Wheat, relative to Barley. Digestibilities of neutral detergent fiber and starch were higher for Wheat and Control, relative to Barley, and crude protein digestibility was greater for Wheat, relative to Barley and Control. Rumination and physical activity were not impacted by treatment. In summary, replacing traditional concentrates with sprouted grains grown using hydroponics improved milk production efficiency (barley sprouts) or enhanced body weight gain (wheat sprouts). A life cycle assessment needs to be conducted to determine the net impact of this feeding strategy for the dairy industry.

Key words: conventional concentrate, dairy cow, hydroponic, sprouted grain

INTRODUCTION

The global demand for dairy products is expected to increase by ~35% between 2017 and 2030 (IFCN, 2018), due to an increasing human population, urbanization, and income growth (OECD/FAO, 2019). However, livestock production systems, including dairy, represent the largest user of land resources, with pasture and arable land for animal feeds together accounting for 77% of total agricultural land (Ritchie and Roser, 2013). The livestock sector is also responsible for ~29% of global water consumption (Hoekstra and Mekonnen, 2012). It is foreseeable that the use of finite natural resources will increase to meet the projected demand for milk.

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*Corresponding author: Joseph W. McFadden, 507 Tower Road, Ithaca, NY 14853, USA. Phone: (607) 255-9941, Email: McFadden@cornell.edu

Moreover, global livestock production contributes about 14.5% of anthropogenic greenhouse gas emissions, with dairy representing 30% of these emissions (Gerber et al., 2013; Opio et al., 2013). Scientific consensus states that greenhouse gas emissions will contribute to the warming of our planet by 1.1 to 5.4°C over the next century (Dijkstra et al., 2012). Therefore, innovative feeding strategies are required to reduce the utilization of natural resources and environmental impacts and improve the resilience to climate change and milk production efficiency.

In recent years, hydroponic fodder production systems have received increasing attention (Ahamed et al., 2022). Sprouted grains grown using hydroponics involve germinating seeds in a water growth medium for 5 to 10 d, and the mat composed of germinated seeds, interwoven white roots, and green shoots can then be fed to dairy cattle. When compared with soil-grown crop production, a hydroponic growing system is a year-round and weather-independent operation. Hydroponic applications produce a greater amount of fodder over a shorter duration using less land and water, no pesticides, and no herbicides (Sneath and McIntosh, 2003). Compared with intact grains, sprouted grains have elevated concentrations of CP, NDF, fat, and sugars on a DM basis (Hafla et al., 2014; Soder et al., 2018). Although sprouting improves the nutritive profile of grains, there were not net increases in nutrients with the exception of sugars derived from starch degradation. Around 15% of DM mass (mainly starch) was lost during the sprouting process (Chavan et al., 1989). During sprouting, complex nutrients (e.g., starch, protein, and lipids) are converted to more simple and digestible fractions such as sugars, peptides, AA, and fatty acids (**FA**) under elevated enzyme (e.g., amylase, protease, lipase) activity (Sneath and McIntosh, 2003; Nemzer and Al-TaHER, 2023). Higher concentrations of vitamins, minerals, and omega-3 FA are observed in sprouted grains versus grain seeds; however, these nutritional improvements of sprouts are subordinate when using as animal feeds (Sneath and McIntosh, 2003).

Only a few studies have been conducted to explore the effects of feeding hydroponically-grown sprouted grains to lactating dairy cows (Soder et al., 2018; Lawrence, 2019). Yields of milk and milk fat did not change when sprouted barley replaced cracked corn in diets fed to cows; however, DMI and milk protein yield decreased (Soder et al., 2018). In another study, DMI and yields of milk and milk components were maintained in cows fed barley sprouts at 8% of ration DM in partial replacement of ground corn and soybean meal (Lawrence, 2019). Apparent total-tract digestibility of DM and OM tended to be greater in cows fed barley sprouts (Lawrence, 2019), which may be attributed to

the release of soluble carbohydrates and N that enhance ruminal fermentation (Pond et al., 1984).

Although there are some merits to the studies of Soder et al. (2018) and Lawrence (2019), we must be cautious about interpreting such findings because (1) cows used produced 12 to 30 kg/d of milk and were not representative of modern high-producing Holstein cows; and (2) only sprouted barley was studied, and there were no comparisons among various plant species. Early focus was on barley sprout probably due to high fresh weigh, low feed cost and mold score, and its availability (Soder et al., 2018). Sprouted wheat being higher in CP and sugars is a strong justification for study, although early attention has centered on barley sprouts. Taken together, our objective was to evaluate the effects of sprouted grains (i.e., barley and wheat) grown using a semi-automatic hydroponic system on milk production efficiency and nutrient utilization in modern Holstein cows fed a conventional TMR.

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee (protocol no. 2021–0081) at Cornell University (Ithaca, NY). The study was conducted in a tie-stall barn equipped with individual feed tubs and water bowls at the Cornell University Dairy Research Center (Harford, NY) from August 26 to November 11, 2022.

Cows and Experimental Design

Twenty-four multiparous Holstein cows at (mean \pm SD) 102 \pm 23 DIM, 49 \pm 4 kg/d of milk, and 742 \pm 68 kg of BW at the beginning of the study were enrolled. Cows were fed a common diet with 62.5% forage (i.e., corn silage and grass haylage) and 37.5% concentrate (mainly ground corn and soybean meal) during a 2-wk covariate period. The ingredient and nutrient composition of the common diet are presented in Supplemental Table 1 (<https://doi.org/10.6084/m9.figshare.24921795.v1>). Following the covariate period, cows were blocked by DIM and milk yield, and within each square, assigned randomly to treatment sequences in a replicated 3 \times 3 Latin square design. Squares were balanced for potential first-order carryover effects in subsequent periods as each treatment immediately preceded and followed each other once in individual squares (Williams, 1949). Each experimental period lasted 21 d, with the first 18 d used for diet adaptation and the last 3 d for data and sample collection. Dietary treatments were (1) a TMR without sprouted grains (Control), or a TMR composed of (2) 10% barley sprouts (Barley; DM basis) or (3) 10% wheat sprouts (Wheat). Experimental

diets were formulated using AMTS.Cattle.Professional (Agricultural Modeling and Training System, LLC) to be isocaloric and isonitrogenous for meeting the nutrient requirements of a lactating dairy cow averaging 120 DIM, weighing 730 kg of BW, consuming 28 kg/d of DM, and producing 50 kg/d of milk with 3.99% fat, 2.91% true protein, and 4.80% lactose. Experimental diets contained (DM basis) 36.4% brown midrib (BMR) corn silage, 12.1% grass haylage, and 48.5% concentrate or concentrate plus sprouted grain. The Barley and Wheat diets were achieved by partially replacing conventional concentrates (i.e., soybean meal, soybean hulls, canola meal, and dextrose) in the control diet with sprouted barley and wheat at 10% of ration DM, respectively. The inclusion rate herein was determined with the considerations of optimal diet DM concentration (no less than 45%) and comparisons with previous findings (e.g., the inclusion rate = 8% ration DM). The concentrate of each diet was mixed and delivered by Purina Animal Nutrition (Trumansburg, NY). Dietary ingredients were mixed and offered as TMR once daily at 0600 h using a Super Data Ranger mixer (American Calan Inc.). Refusals were collected and weighed daily before the morning feeding. Feed offered was adjusted daily to allow for 5 to 10% refusals, with individual feed intakes recorded over the duration of the study.

Sprouted barley and wheat were grown using a semi-automatic hydroponic fodder system (FodderBox T-126; Figure 1) provided by Renaissance Ag (Vineyard, Utah). The sprouting or germination process lasted 6 d. On d 1 (0800 h), approximately 50 kg of barley and wheat seeds [about 4.5 and 4.9 kg/tray for barley (11 trays/d) and wheat (10 trays/d), respectively] were weighed out and spread evenly on trays, and trays were then placed inside the hydroponic system. On d 7 (0630 h), hydroponically grown fodder (i.e., sprouted barley or wheat) was harvested and mixed with other ingredients. The daily routine included pulling sprouted grains from the trays into a cart, weighing out the required amounts, shredding sprouted grains using a Chipper-Shredder SC262 (MacKissic Inc.), cleaning trays using soap and bleach solution, weighing out new seeds on cleaned trays, and putting trays inside the hydroponic system after pushing other trays forward. For the hydroponic system, the temperature was maintained at ~21°C, sprinklers spread water for 16 s every 1 h, and LED lights at the harvesting end were on at all times. A chlorine tablet was added to the water tank on a weekly basis to prevent mold, and the water filter was replaced every month.

Cows were milked 3 times per day at 0600, 1400, and 2200 h, with milk yields recorded at every milking throughout the study. Body weights were recorded at the end of the covariate period and each experimental

period. Daily feed offered and refusals for each cow were noted to calculate feed intake. Rumination and physical activity were recorded using the Allflex collar (Allflex Livestock Intelligence) over the duration of the study (Schirmann et al., 2009). Water meters (AS200U-75; DAE Controls LLC) were installed for 18 out of 24 cows (i.e., 9 squares) to measure water intake during the last 3 d of the covariate period and each experimental period.

Samplings and Analyses

Corn silage, grass haylage, and the concentrate mix of the common diet were collected once per week during the covariate period. During the experimental periods, forages (i.e., BMR corn silage and grass haylage) and 3 concentrate mixes were collected once per week, and sprouted grains were sampled every day. These samples were dried for 72 h at 55°C in a forced-air oven (VWR Scientific) for determination of DM to adjust the TMR, on an as-fed basis. Moreover, samples of the common diet and refusals were taken daily for the last 3 d of the covariate period. During the experimental periods, samples of BMR corn silage, grass haylage, and 3 concentrate mixes were collected on d 20, and experimental diets, refusals, and sprouted grains were taken daily on d 19 to 21. These samples were dried at 55°C for 72 h, composited by period, ground to pass through a 1-mm screen using a Wiley mill (A. H. Thomas Co.), and then stored in zip-lock bags until shipped for chemical analyses at Cumberland Valley Analytical Services (Waynesboro, PA). Another set of experimental diets and refusals from d 19 to 21 were composited by period and analyzed for particle size distribution using a Penn State Particle Separator (Heinrichs and Jones, 2022).

Individual ingredients in experimental diets (i.e., BMR corn silage, grass haylage, 3 concentrate mixes, and sprouted grains) were analyzed for DM (method 930.15; AOAC 2000), CP (method 990.03; AOAC 2000), soluble protein (Krishnamoorthy et al., 1982), NDICP (total residue from the NDF procedure analyzed for N using a Leco FP-528 Nitrogen Combustion Analyzer), ADICP (total residue from the ADF procedure analyzed for N using a Leco FP-528 Nitrogen Combustion Analyzer), aNDFom [method of Van Soest et al. (1991) with the addition of α -amylase and sodium sulfite], ADF (method 973.18; AOAC 2000), lignin (Goering and Van Soest, 1970), ethanol soluble carbohydrates (Hall et al., 1999), starch (Hall, 2009), ether extract (method 2003.05; AOAC 2000), ash (method 942.05; AOAC 2000), and minerals including Ca, P, Mg, K, S, Na, Cl, Fe, Mn, Zn, and Cu (method 985.01; AOAC 2000). Moreover, TMR (i.e., the common diet and 3 experimental diets) and refusals were measured for DM,

A. Hydroponic fodder system



B: Inside seeding end



C. Inside harvesting end

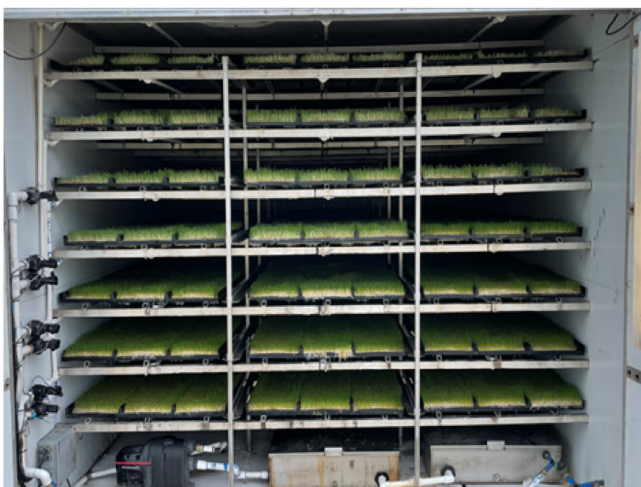


Figure 1. The semi-automatic hydroponic fodder system provided by Renaissance Ag (Vineyard, Utah).

CP, aNDFom, ADF, starch, ash, and undigestible NDF at 240 h of in vitro incubation (uNDF_{240}), following the procedures mentioned above.

Milk samples were collected using automatic samplers over the last 3 d of the covariate period and each experimental period and transferred into 2 50-mL tubes: one was preserved with 2-bromo-2-nitropropane-1,3 diol, and the other one was blank centrifuge tube. Preserved milk samples were stored at 4°C until shipped overnight in cold ice packs to Dairy One DHIA (Ithaca, NY) for the analyses of fat, true protein, lactose, TS, and MUN by Fourier transform infrared spectroscopy and that of SCC by a Fossomatic instrument based on a flow cytometry method. Milk samples without preservatives were stored at -20°C until being used for lipid extraction according to the method developed by Feng et al. (2004). Briefly, milk samples were thawed in a water bath at 37°C and composited by cow within period in proportion to milk fat yield at each milking. Compositated milk samples were then centrifuged at $17,800 \times g$ for 30 min at 4°C to obtain the fat-cake layer, which was transferred to 1.5-mL microcentrifuge tubes and stored at -80°C for the analysis of the FA profile. Total lipid of fat-cake (320 ± 10 mg) was extracted using n-hexane/isopropanol solution (3:2, vol/vol) as described by Lock et al. (2013). Samples were analyzed in an Agilent 8890 GC system equipped with a flame-ionization detector (FID), autosampler, a split/spitless injector, and a CP-Sil 88 column (100 m \times 0.25mm internal diameter and 0.20- μm film thickness). Hydrogen was used as the carrier gas at a flow rate of 1 mL/min and for the FID at 40 mL/min and N makeup gas at 30 mL/min, and the injector and detector temperature were kept at 250°C. The oven temperature program was set up as follows: initial temperature of 80°C for 1 min, raising to 215°C at a rate of 2°C/min, and held for 21.5 min (Duplessis et al., 2022). For each GC analysis, 1 μL of sample was injected and a 1:100 split ratio was used. Individual peaks were identified using reference standards (GLC reference standard 463, GLC reference standard 481-B, and octadecadienoic mixture #UC-59M), and short-chain fatty acid methyl ester was corrected for mass discrepancy using response factors reported by Ulberth and Schrammel (1995).

Blood samples were taken from the coccygeal blood vessels using 15% EDTA vacutainer tubes approximately 3 h after morning feedings on the last 2 d of the covariate period and each experimental period. Tubes were immediately placed in a bucket with ice, and blood samples were then transported to the laboratory and centrifuged at $2,171 \times g$ for 20 min at 4°C. Plasma glucose was measured using an enzymatic kit (#997-03001 Autokit Glucose; Wako Chemicals USA Inc., Richmond, VA). Rumen fluid was sampled about

3 h after morning feedings via an esophageal tubing on the last 2 d of the covariate period and experimental periods. We discarded the first 200 mL of rumen fluid to avoid saliva contamination and then collected approximately 100 mL of rumen fluid. Following collection, the pH was immediately measured using a pH meter, and 10 mL of sample was added into a 15-mL centrifuge tube with 200 μ L of 50% sulfuric acid for the analysis of ammonia N. Rumen ammonia N was determined using a colorimetric assay performed on a microplate spectrophotometer (Spectra max 190, USA) as described by Chaney and Marbach (1962).

Spot samples of feces were collected directly from the rectum or during voluntary defecation every 9 h over the final 3 d of the covariate period and experimental periods. The 8 time points were as follows: 0330, 1230, and 2330 h (d 19), 0730 and 1530 h (d 20), and 0030, 0930, and 1830 h (d 21). Approximately 200 g of fecal samples were obtained during each sampling point, transferred into 4-L storage bags to obtain a composite sample (wet weight) by cow within period, and stored at -20°C until further processing. Samples were thawed at room temperature, placed in aluminum trays, dried at 55°C for 72 h, and ground to pass through a 1-mm screen. Dried fecal samples were shipped to Cumberland Valley Analytical Services for the analyses of DM, CP, aNDFom, ADF, starch, ash, and uNDF₂₄₀ as done for feed samples. Moreover, uNDF₂₄₀ was used as the intrinsic marker to estimate fecal output of DM [i.e., (uNDF₂₄₀% in the diet \times DMI) \div uNDF₂₄₀% in feces] and apparent total-tract digestibility of nutrients.

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc.) according to the following model:

$$Y_{ijkl} = \mu + S_i + C_{j(i)} + P_k + T_l + S_i \times T_l + \text{Cov}_{ijkl} + e_{ijkl}$$

where Y_{ijkl} = dependent variable, μ = overall mean, S_i = fixed effect of square ($i = 1$ to 8), $C_{j(i)}$ = random effect of cow nested within square ($j = 1$ to 24), P_k = fixed effect of period ($k = 1$ to 3), T_l = fixed effect of treatment ($l = \text{Control, Barley, and Wheat}$), $S_i \times T_l$ = interaction between square and treatment, Cov_{ijkl} = pre-trial value for each response variable used as a covariate, and e_{ijklm} = residual error. Normality of residuals and homogeneity of variances were checked with normal probability and box plots and plots of residual versus predicted values, respectively. Outliers were removed from statistical analyses when studentized

residuals were >3.0 or < -3.0 . All results are reported as LSM and SEM, with the greatest SEM values shown in Tables 4 to 8. The treatment effect was tested using ANOVA. Least squares means were separated using Tukey's procedure when a significant F-test ($P \leq 0.05$) was detected. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Hydroponically Grown Fodder or Sprouted Grains

Daily DM concentration of sprouted grains over the duration of the study is presented in Figure 2A. The DM levels of sprouted barley and wheat ranged from 12.1 to 22.9% (mean = 16.0%) and from 13.3 to 25.7% (mean = 18.6%), respectively. The wide range of DM concentration of sprouted grains may result from sampling errors (i.e., samples were not representative), different intervals between the sampling time and the watering time (samples were always collected at 0630 h, however, watering time was not tightly controlled), and differing amounts of water used during each watering event. Standard deviation and CV for DM of sprouted barley were 2.4 percent units and 15%, respectively, and those of sprouted wheat were 3.0 percent units and 16%, respectively. In comparison, the CV for DM concentration of corn silage and grass haylage used in experiment diets was 9 and 5%, respectively. Thus, sprouted grains exhibited greater variability in DM% than conventional forages fed in the present study. Furthermore, a much larger DM% variability was observed for sprouted grains than for some common concentrates. For instance, soybean meal contained 91.3% DM with 0.8 percent units of SD and 0.9% of CV, and ground corn had 89.2% DM with 1.2 percent units of SD and 1% CV (Zang et al., 2023). Diets with more consistent ingredient composition and nutrient profile can optimize production performance in dairy cows (Sova et al., 2014). It is important to note that substituting sprouted grains for conventional concentrates may result in larger day-to-day variation in TMR composition due to the greater variability in DM%. Moreover, grain quality and variety, seed density, temperature, sampling locations within the hydroponic system, and watering systems all can impact DM% of sprouted fodder. Standard sampling procedures for sprouted fodder need to be developed, and the watering system of hydroponic systems should be closely monitored for accurate DM% measurements of sprouted grains.

Daily ratio of sprouted grains to seeds is presented in Figure 2B. In the present study, the mean ratio of sprout to seed was 0.84 for both sprouted barley and wheat, indicating that around 16% DM of seeds (mainly

starch) was lost to support the growth during the 6-d germination process. Similarly, DM loss of barley grain was 18% during a 7-d sprouting with only water at 21°C and 9.4% for a 5-d germination without nutrients at 22°C (Chung et al., 1989; Sova et al., 2014). As for wheat, the germination of 5 to 7 d resulted in a 17% DM loss (Chavan et al., 1989). Obviously, DM loss resulting from germination is against the concept of developing a sustainable dairy industry. Further research is required to explore ways to reduce DM loss, possibly through advanced nutrient recycling and light management (Ahamed et al., 2022). However, a net loss of nutrients during sprouting is unavoidable, which is the major drawback of this technological approach.

Ingredients and Experimental Diets

The ingredient and nutrient composition of experimental diets are shown in Table 1. It appears that experimental diets had numerically less starch but more sugars, relative to typical dairy rations in North America (NASEM, 2021). The nutrient composition of ingredients used in experimental diets is presented in Table 2. Sprouted wheat contained numerically higher concentrations of CP (14.9 vs. 12.0%) and starch (33.4 vs. 26.0%) and numerically lower level of aNDFom (19.9 vs. 29.7%) than sprouted barley. The particle size distribution of experimental diets and refusals are shown in Table 3. Compared with Control, Barley and Wheat diets had numerically higher proportions of particles >3.18 mm due to the inclusion of sprouted grains. No sorting was observed among cows fed different diets, indicating that shredded sprouted grains can be mixed well with other ingredients. Before the study, we compared the diets including shredded versus intact sprouted grains and noticed that small chunks of hydroponically-grown fodder appeared in both diets and refusals when non-shredded sprouted grains were used. Thus, we decided to use a chipper-shredder to avoid sorting and ensure TMR consistency.

Lactation Performance and Nutrient Digestibility

Effects of replacing conventional concentrates with sprouted barley or wheat on water intake, DMI, milk yield and composition, and milk N efficiency are shown in Table 4. Water intake was not affected by treatment and averaged 139 L/d. Dry matter intake decreased by 1.6 kg/d (i.e., -5%; $P < 0.001$) with feeding Barley versus Control, and tended ($P = 0.07$) to be 0.9 kg/d lower for Wheat than for Control. This may be related to physically effective NDF (peNDF) in the diet. A review by Zebeli et al. (2012) illustrated that DMI may be limited when peNDF inclusive particles >8 mm

(i.e., peNDF_{>8}) beyond 14.9% of ration DM. Dietary peNDF_{>8} concentrations in the current study were 12.7, 16.3, and 15.8% for Control, Barley, and Wheat, respectively. Fish and DeVries (2012) compared 2 diets with the same ingredient composition and different DM concentration (61.7 vs. 51.9%) and reported that water addition did not affect DMI in lactating Holstein cows. This finding suggests that changes in dietary DM concentration resulting from the inclusion of sprouted grains may not play a role in DMI responses.

Although DMI was lower for Barley versus Control, Barley cows produced as much milk as Control cows (45.8 vs. 46.1 kg/d; $P = 0.69$) and thus had higher feed efficiency expressed as milk yield/DMI (1.49 vs. 1.43 kg/kg; $P < 0.01$; Table 4). We speculated that this positive response may have resulted from higher nutrient digestibility in cows fed the Barley diet. However, the apparent total-tract digestibility of aNDFom and starch was lower for Barley versus Control while that of CP and ADF was similar (discussed below). This outcome may be related to differences in ruminal fermentation, intestinal digestion and absorption, and even hind-gut fermentation; however, the exact mechanisms are uncertain. Concentrations and yields of milk fat, true protein, and lactose did not ($P \geq 0.16$) differ among treatments, which may be attributed to similar nutrient composition of experimental diets. Compared with Control and Barley, Wheat increased ($P \leq 0.05$) MUN from 10.2 to 10.8 mg/dL. The Wheat diet contained the highest proportion of soluble protein, on a DM basis (7.9% vs. 7.3% for Control and 7.6% for Barley). There may not be adequate ruminal fermentable energy available to capture ruminal ammonia N derived from RDP including soluble protein, and ammonia N are converted to urea N in the liver (Arunvipas et al., 2008). Milk N efficiency was higher for Barley than for Control (30.5 vs. 28.8%; $P < 0.01$), demonstrating the potential of feeding sprouted barley as an alternative feed to conventional concentrates for improving N utilization in dairy cows.

Effects of replacing conventional concentrates with sprouted barley or wheat on BW, ruminal pH, rumination, physical activity, plasma glucose concentration, ruminal ammonia N concentration, and apparent total-tract digestibility of nutrients are presented in Table 5. Cows receiving sprouted barley and wheat, on average, gained BW by +3.8 kg/21 d whereas Control cows lost about 9 kg of BW during each experimental period ($P < 0.05$ for Barley and Wheat vs. Control). This difference can be partly explained by higher feed intake on an as-fed basis (63.6 vs. 77.2 and 76.3 kg/d for Control vs. Barley and Wheat, respectively; $P < 0.001$). There were no differences in BW (747 vs. 752 kg; $P = 0.21$)

and BW gain (+1.1 vs. +6.5 kg/21d; $P = 0.39$) between Barley and Wheat.

Ruminal pH, rumination, and physical activity averaged 6.49, 554 min/d, and 414, respectively and were not ($P \geq 0.34$) affected by treatment (Table 5). Rumination time is strongly correlated with NDF intake ($r = 0.35$ to 0.54) (Yang et al., 2001; Yang and Beauchemin, 2007; 2009). The differences in aNDFom intake among treatments were relatively small (<0.6 kg/d), which may explain similar ruminating time across diets. Digestibility of DM had ($P < 0.001$) the highest value for Wheat (77.7%), the lowest value for Barley (74.2%), and the middle value for Control (76.2%). Wheat had higher CP digestibility compared with Control and Barley (74.6 vs. 71.7 and 71.1%, respectively; $P \leq 0.001$). Digestibilities of OM and ADF were higher with feeding Wheat versus Barley ($P \leq 0.01$), and those of aNDFom, and starch increased for Wheat and Control than for Barley ($P \leq 0.03$). It appears that Wheat and Barley cows had overall the highest and lowest nutrient digestibility, respectively, indicating that sprouted wheat may be more digestible than sprouted barley,

and the digestibility of conventional concentrates is in the middle. However, the digestibility of individual ingredients including sprouted grains was not measured using in vitro or in situ technique in the current study.

Milk FA Profile

The concentrations and yields of milk SFA in cows fed experimental diets are presented in Supplemental Tables 2 and 4, respectively (<https://doi.org/10.6084/m9.figshare.24921795.v1>). The proportions of C10:0, C12:0, *iso* C13:0, *iso* C14:0, C14:0, *iso* C15:0, *iso* C16:0, C17:0, C19:0, C21:0, C22:0, C24:0, total branched-chain FA, and de novo synthesis of FA were not significantly impacted by treatment. Compared with Barley and Control, Wheat had decreased proportions of 4:0, 6:0, 8:0, and 18:0 in milk ($P \leq 0.02$) but increased proportions of 5:0, 7:0, 9:0, 11:0, 13:0, and 15:0 in milk ($P \leq 0.02$). Barley and Wheat had higher milk proportions of *anteiso* 15:0, C16:0, and *anteiso* 17:0 than Control ($P \leq 0.05$). Barley had a lower proportion of *anteiso* C13:0 ($P \leq 0.04$) but a higher proportion of C20:0 ($P < 0.001$) than Wheat and Control. The milk proportion of total odd-chain FA was higher for Wheat than for Barley and Control (2.79 vs. 2.48 and 2.48%; $P < 0.001$), whereas that of preformed FA was lower with feeding sprouted grains versus conventional concentrates ($P \leq 0.01$).

Elevated milk proportions of *anteiso* 15:0 and *anteiso* 17:0 with feeding the Barley and Wheat diets may be related to increased supplies of Ile from sprouted grains versus conventional concentrates. Isoleucine can be first converted to keto- β -methylvalerate via the catalysis of BCAA aminotransferase, then 2-methylbutyryl-CoA by branched-chain α -keto acid decarboxylase, and ultimately to either *anteiso* C15:0 or *anteiso* C17:0 via BCFA synthetase (Vlaeminck et al., 2006). Moreover, cellulolytic *Prevotella* strains are enriched in *anteiso* C15:0, and the amylolytic bacteria including *Succinivibrio dextrinosolvens*, *Succinimonas amylolytica*, *Ruminobacter amylophilus*, *Selenomonas ruminantium* and *Streptococcus bovis* are relatively abundant in linear odd-chain FA (Vlaeminck et al., 2006). Taken together, differences in milk proportions of odd-chain and branched-chain FA suggest that the microbial population in the rumen may have been modified by feeding sprouted grains versus conventional concentrates. The lower proportion of preformed FA in milk fat in cows fed sprouted grains may have resulted from decreased dietary concentration of crude fat for Wheat and Barley versus Control.

The concentrations and yields of milk UFA in cows fed experimental diets are presented in Supplemental Tables 3 and 5, respectively (<https://doi.org/10.6084/>

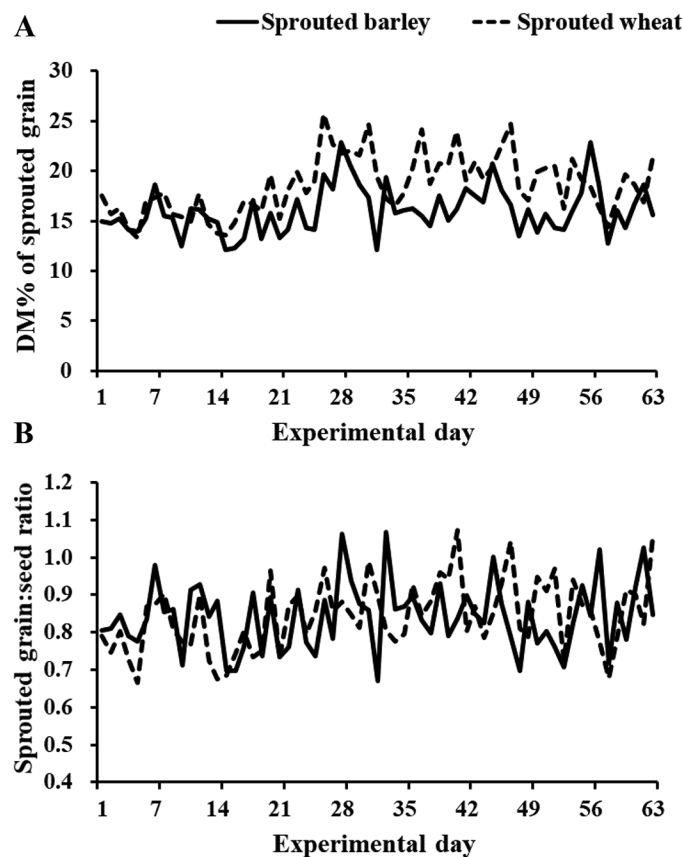


Figure 2. Daily DM% of sprouted grains (A) and the ratio of sprouted grains to seeds on a DM basis (B) over the duration of the study.

Table 1. Ingredient and nutrient composition of the experimental diets (% of DM, unless otherwise noted)

Item	Diet ¹		
	Control	Barley	Wheat
Ingredient, % of DM			
BMR Corn silage	36.4	36.4	36.4
Grass haylage	12.1	12.1	12.1
Sprouted grains	—	10.0	10.0
Ground corn	12.1	9.25	9.14
Soybean meal, 47.5% CP	11.9	11.0	10.6
Soybean hulls	6.58	2.83	4.00
Wheat middlings	5.86	5.86	5.86
Citrus pulp	4.34	4.34	4.34
Canola meal	2.68	2.31	1.89
Dextrose	2.35	0.28	0.00
Palmitic acid-enriched supplement ²	1.50	1.50	1.50
Calcium carbonate	1.43	1.43	1.43
Sodium bicarbonate	0.78	0.78	0.78
Blood meal	0.71	0.71	0.71
Salt white	0.40	0.40	0.40
Rumen-protected Met and Lys supplements ³	0.21	0.21	0.21
PAN Dairy VTM	0.20	0.20	0.20
Magnesium oxide	0.14	0.14	0.14
Magnesium sulfate	0.13	0.13	0.13
Rumen-protected Met supplement ⁴	0.11	0.11	0.11
Selenium 06	0.04	0.04	0.04
Live yeast ²	0.03	0.03	0.03
Nutrient composition, % DM			
DM, % of fresh matter	62.6	55.0	55.2
CP	16.3	16.0	16.2
Soluble protein, % CP	44.5	47.8	48.6
aNDFom	30.5	30.7	30.3
ADF	18.6	18.7	18.4
Lignin	2.68	2.53	2.33
Starch	20.6	21.0	21.7
ESC	7.59	7.41	7.27
Ether extract	4.28	3.98	4.07
Ash	7.79	10.2	8.64
Ca	1.14	1.19	1.28
P	0.40	0.42	0.41
ME, Mcal/kg of DM	2.75	2.66	2.72

¹Control = diet without sprouted grains; Barley = diet with 10% sprouted barley substituted for conventional concentrates; Wheat = diet with 10% sprouted wheat substituted for conventional concentrates.

²Live yeast was provided by Levucell SC2 (Lallemand Animal Nutrition).

m9.figshare.24921795.v1). Treatment had no effects on milk proportions of *cis*-13 C16:1, *trans*-4 C18:1, *trans*-5 C18:1, *trans* 6–8 C18:1, *trans*-11 C18:1, *trans*-15 C18:1, *trans*-11, *trans*-13 C18:2, *cis*-8, *cis*-11, *cis*-14 C20:3, *cis*-5, *cis*-8, *cis*-11, *cis*-14, *cis*-17 C20:5, and *cis*-7, *cis*-10, *cis*-13, *cis*-16, *cis*-19 C22:5. However, feeding sprouted grains increased milk proportions of *cis*-12 C18:1, *cis*-14 C18:1, and *trans*-12 C18:1 ($P \leq 0.02$) and decreased those of *cis*-9 C18:1, *trans*-9 C18:1, *cis*-6, *cis*-9, *cis*-12 C18:3, and *cis*-13 C22:1 ($P \leq 0.03$), relative to conventional concentrates. Compared with Barley and Control cows, Wheat cows had elevated milk proportions of *cis*-9 C14:1, *cis*-9 C16:1, *cis*-9 C17:1, *cis*-11 C18:1, and *trans*-10 C18:1 ($P \leq 0.02$). Relative to Control cows, cows fed the Wheat diet produced increased milk proportions of *trans*-9 C16:1 and *cis*-13 C18:1 ($P = 0.04$) and lower proportion of milk *cis*-11 C16:1 ($P = 0.05$).

Barley versus Control cows had lower milk proportion of C10:1 ($P < 0.01$) but elevated milk proportion of *cis*-11 C20:1 ($P < 0.01$). Barley cows also had lower milk proportion of *cis*-9, *trans*-11 C18:2 than Wheat and Control cows ($P \leq 0.05$). Milk proportions of *cis*-9, *cis*-12 C18:2 and *cis*-9, *cis*-12, *cis*-15 C18:3 were highest for Control, lowest for Wheat, and intermediate for Barley ($P \leq 0.02$).

Milk proportions of total n-3 FA decreased for Wheat and Barley, relative to Control (0.374 and 0.395 vs. 0.415 g/100 g of FA, respectively; $P < 0.01$). The ratio of n-6/n-3 FA decreased with feeding sprouted Wheat and Barley though, relative to Control (4.35 and 4.31 vs. 4.68, respectively; $P < 0.001$). Some cellular and animal models have shown the detrimental effects of high n-6/n-3 FA ratio on health (Russo, 2009); how-

Table 2. Nutrient composition of ingredients (mean \pm SD) used in experimental diets

Item	BMR corn silage	Grass haylage	Concentrate mix ¹ (Control)	Concentrate mix ² (Barley)	Concentrate mix ³ (Wheat)	Sprouted barley ⁴	Sprouted wheat ⁵
No. of samples	3	3	3	3	3	3	3
DM, % fresh matter	32.2 \pm 3.01	42.8 \pm 1.95	88.8 \pm 0.17	88.3 \pm 0.22	88.2 \pm 0.15	14.6 \pm 2.69	17.5 \pm 3.22
CP, % DM	7.70 \pm 0.20	16.3 \pm 1.32	22.4 \pm 0.89	24.1 \pm 1.02	23.8 \pm 0.71	12.0 \pm 0.55	14.9 \pm 0.23
SP, % CP	72.0 \pm 2.76	61.8 \pm 3.17	21.1 \pm 3.21	20.3 \pm 2.15	20.4 \pm 0.87	57.1 \pm 1.61	64.3 \pm 0.74
NDICP, % CP	11.7 \pm 0.40	15.3 \pm 2.23	8.60 \pm 0.78	7.13 \pm 0.68	7.57 \pm 0.60	11.7 \pm 0.90	8.33 \pm 0.81
ADICP, % CP	9.63 \pm 0.55	7.53 \pm 0.64	5.40 \pm 2.52	3.53 \pm 0.21	3.63 \pm 0.40	6.77 \pm 0.31	3.93 \pm 0.64
aNDFom, % DM	40.3 \pm 1.11	47.5 \pm 2.60	19.6 \pm 2.12	17.7 \pm 0.85	18.9 \pm 0.64	29.7 \pm 0.44	19.9 \pm 2.21
ADF, % DM	24.3 \pm 0.45	30.6 \pm 0.99	11.7 \pm 3.67	11.3 \pm 2.15	12.3 \pm 1.04	14.1 \pm 0.98	7.67 \pm 2.05
Lignin, % DM	2.42 \pm 0.34	4.15 \pm 1.45	2.52 \pm 0.17	2.14 \pm 0.32	1.87 \pm 0.28	2.61 \pm 0.04	1.75 \pm 0.45
Starch, % DM	28.7 \pm 0.57	0.57 \pm 0.55	19.6 \pm 1.31	18.9 \pm 1.39	18.8 \pm 1.27	26.0 \pm 2.94	33.4 \pm 4.29
ESC, % DM	1.07 \pm 0.15	5.47 \pm 0.42	12.7 \pm 0.85	9.23 \pm 1.02	8.83 \pm 1.25	25.3 \pm 2.69	25.5 \pm 2.95
Ether extract, % DM	2.80 \pm 0.32	4.16 \pm 0.45	3.51 \pm 0.38	3.34 \pm 0.52	2.58 \pm 0.55	3.21 \pm 0.17	2.63 \pm 0.25
Ash, % DM	5.98 \pm 4.03	8.42 \pm 0.84	8.91 \pm 1.31	15.9 \pm 4.09	12.2 \pm 0.75	3.70 \pm 1.01	3.66 \pm 0.91
Ca, % DM	0.21 \pm 0.04	0.73 \pm 0.08	1.89 \pm 0.20	2.44 \pm 0.13	2.66 \pm 0.11	0.12 \pm 0.01	0.10 \pm 0.01
P, % DM	0.28 \pm 0.02	0.37 \pm 0.04	0.50 \pm 0.07	0.55 \pm 0.02	0.53 \pm 0.06	0.45 \pm 0.01	0.48 \pm 0.02
Mg, % DM	0.15 \pm 0.01	0.24 \pm 0.01	0.42 \pm 0.02	0.49 \pm 0.02	0.48 \pm 0.02	0.16 \pm 0.01	0.19 \pm 0.01
K, % DM	1.20 \pm 0.05	3.03 \pm 0.54	1.27 \pm 0.08	1.40 \pm 0.01	1.39 \pm 0.02	0.69 \pm 0.03	0.52 \pm 0.04
S, % DM	0.10 \pm 0.01	0.20 \pm 0.01	0.32 \pm 0.02	0.34 \pm 0.03	0.35 \pm 0.02	0.13 \pm 0.01	0.16 \pm 0.01
Na, % DM	0.03 \pm 0.01	0.04 \pm 0.01	0.92 \pm 0.18	1.18 \pm 0.22	1.27 \pm 0.14	0.04 \pm 0.02	0.02 \pm 0.00
Cl, % DM	0.09 \pm 0.00	0.33 \pm 0.02	0.60 \pm 0.15	0.70 \pm 0.14	0.71 \pm 0.05	0.13 \pm 0.01	0.10 \pm 0.01
Fe, mg/kg of DM	109 \pm 29.1	273 \pm 54.3	432 \pm 301	388 \pm 90.0	398 \pm 63.1	63.7 \pm 7.57	53.0 \pm 2.65
Mn, mg/kg of DM	15.0 \pm 1.00	60.7 \pm 8.96	84.3 \pm 7.64	113 \pm 6.24	119 \pm 8.08	24.3 \pm 1.53	74.0 \pm 2.65
Zn, mg/kg of DM	20.7 \pm 1.15	31.3 \pm 2.08	122.7 \pm 12.7	148 \pm 12.2	155 \pm 21.0	49.0 \pm 13.9	63.0 \pm 4.00
Cu, mg/kg of DM	5.67 \pm 0.58	10.0 \pm 1.00	30.0 \pm 4.00	36.7 \pm 3.79	42.3 \pm 4.04	8.67 \pm 0.58	6.33 \pm 1.15
ME, Mcal/kg of DM	2.69 \pm 0.15	2.45 \pm 0.11	2.86 \pm 0.06	2.62 \pm 0.17	2.73 \pm 0.04	2.94 \pm 0.05	3.11 \pm 0.08

¹⁻³Concentrate mixes (i.e., Control, Barley, Wheat) contained ground corn, soybean meal, soybean hulls, wheat middlings, citrus pulp, canola meal, dextrose, blood meal, calcium carbonate, palmitic acid-enriched supplement (BergaFat F100, Berg+Schmidt), sodium bicarbonate, salt white, PAN dairy VTm, rumen-protected Met and Lys products (Smartamine ML, Adisseo), magnesium OX, magnesium sulfate, rumen-protected Met (Smartamine M, Adisseo), live yeast (Levucell SC2, Lallemand Animal Nutrition), Selenium 06.

⁴⁻⁵Sprouted grains (i.e., barley and wheat) were harvested after 6 d of growth using a semi-automatic hydroponic system (Renaissance Ag, Vineyard, Utah).

Table 3. Particle size distribution (% as-fed, mean \pm SD) of experimental diets and refusals

Distribution, % as-fed	Diet ¹			Refusals ¹		
	Control	Barley	Wheat	Control	Barley	Wheat
Samples, n	9	9	9	9	9	9
>19.0 mm	3.47 \pm 1.30	5.15 \pm 1.59	5.85 \pm 2.88	4.87 \pm 1.96	5.47 \pm 1.55	5.35 \pm 1.00
8.0 to 19.0 mm	38.1 \pm 3.81	47.8 \pm 3.04	46.4 \pm 2.73	42.1 \pm 1.82	51.2 \pm 2.63	48.6 \pm 1.13
3.18 to 8.0 mm	12.6 \pm 1.49	17.3 \pm 1.61	17.8 \pm 1.26	13.0 \pm 1.21	18.2 \pm 1.60	17.6 \pm 1.11
<3.18 mm	45.9 \pm 4.10	29.8 \pm 3.36	30.0 \pm 2.45	40.0 \pm 1.91	25.2 \pm 1.79	28.4 \pm 1.98
pef ² , %	54.1 \pm 4.10	70.2 \pm 3.36	70.0 \pm 2.45	60.0 \pm 1.91	74.8 \pm 1.79	71.6 \pm 1.98

¹Control = diet without sprouted grains; Barley = diet with 10% sprouted barley substituted for conventional concentrates; Wheat = diet with 10% sprouted wheat substituted for conventional concentrates.

²pef = physically effectiveness factor.

ever, others have argued not to consider the ratio but rather the concentration of n-3 FA (Russo, 2009).

Challenges and Opportunities for Hydroponically Grown Fodder

Feeding sprouted grains in place of conventional concentrates including soybean meal, soybean hulls, canola meal, and dextrose is a viable strategy to maintain production performance in modern high-producing cows. Through this study, we learned that there are several challenges in terms of using hydroponics to produce fodder for dairy cows. First, the large DM% variability of sprouted grains will negatively impact the consistency

in diet composition. The watering system needs to accurately distribute the same amount of water over time. The method to collect representative samples should be determined to accurately estimate DM concentration of sprouted grains. Second, sprouted grains at different germination stages are in the same hydroponic fodder system, meaning any issues in the hydroponic system can negatively impact all sprouted grains at any stage of growth, and the consequence will likely last for the remainder of sprout growth before harvest. Thus, it is critical to develop ways to closely monitor the hydroponic fodder system and alert farmers in a timely manner when problems develop. Third, the hydroponic fodder system we used was semi-automatic, and it took

Table 4. Effects of replacing conventional concentrates with sprouted barley or wheat on dry matter intake, milk yield and composition, MUN, and BW in dairy cows

Item	Diet ¹			SEM	P-value ²
	Control	Barley	Wheat		Treatment
Water intake, L/d	151	133	133	14.2	0.39
DMI, kg/d	32.7 ^a	31.1 ^b	31.8 ^{ab}	1.40	<0.001
Milk yield, kg/d	46.1	45.8	45.7	0.64	0.59
Milk yield/DMI, kg/kg	1.43 ^b	1.49 ^a	1.45 ^{ab}	0.03	0.01
ECM, ³ kg/d	56.3	56.6	55.6	0.77	0.39
ECM/DMI, kg/kg	1.73 ^b	1.85 ^a	1.75 ^b	0.04	<0.01
Milk fat, %	4.92	5.09	4.91	0.11	0.25
Milk fat, kg/d	2.27	2.32	2.24	0.05	0.32
Milk true protein, %	3.37	3.34	3.36	0.02	0.16
Milk true protein, kg/d	1.55	1.52	1.53	0.02	0.22
Milk lactose, %	4.90	4.88	4.91	0.01	0.30
Milk lactose, kg/d	2.26	2.23	2.24	0.04	0.54
Milk TS, %	14.2	14.3	14.2	0.12	0.39
Milk TS, kg/d	6.53	6.54	6.46	0.08	0.46
Milk SCC, \times 1,000 cells/mL	20.0	24.8	23.6	1.12	0.10
Milk N, % of N intake	28.8 ^b	30.5 ^a	29.5 ^{ab}	0.68	<0.01
MUN, mg/dL	10.2 ^b	10.2 ^b	10.8 ^a	0.24	0.03

^{a-b}Within a row, least squares means without a common superscript letter differ ($P < 0.05$).

¹Control = diet without sprouted grains; Barley = diet with 10% sprouted barley substituted for conventional concentrates; Wheat = diet with 10% sprouted wheat substituted for conventional concentrates.

²Trends were observed for DMI ($P = 0.07$ for Wheat vs. Control; $P = 0.09$ for Wheat vs. Barley) and somatic cell count (SCC, $P = 0.10$ for Barley vs. Control).

³Energy-corrected milk (ECM) = (0.327 \times kg of milk) + (12.95 \times kg of milk fat) + (7.65 \times kg of milk protein); Tyrrell and Reid (1965).

Table 5. Effects of replacing conventional concentrates with sprouted barley or wheat on BW, ruminal pH, rumination, physical activity, plasma glucose, ruminal ammonia N, and apparent total-tract digestibility of nutrients in dairy cows

Item	Diet ¹			SEM	P-value ²
	Control	Barley	Wheat		Treatment
BW, kg	742 ^b	747 ^{ab}	752 ^a	3.39	0.02
BW change, kg/21 d	-9.33 ^b	1.08 ^a	6.53 ^a	2.99	<0.01
Ruminal pH	6.54	6.45	6.49	0.07	0.58
Rumination, min/d	550	563	550	8.35	0.34
Physical activity	410	417	415	5.27	0.47
Plasma glucose, mg/dL	48.6	48.5	46.8	1.91	0.75
Ruminal NH ₃ -N, mg/dL	4.81	6.69	6.16	0.72	0.11
Apparent total-tract digestibility, %					
DM	76.2 ^b	74.2 ^c	77.7 ^a	0.44	<0.001
OM	77.4 ^{ab}	76.0 ^b	79.1 ^a	0.52	<0.001
CP	71.7 ^b	71.1 ^b	74.6 ^a	0.58	<0.001
aNDFom ³	62.5 ^a	59.7 ^b	63.2 ^a	0.86	<0.01
ADF	56.0 ^{ab}	51.9 ^b	59.3 ^a	1.80	0.01
Starch	99.3 ^a	98.0 ^b	99.2 ^a	0.10	<0.001

^{a-c}Within a row, LSM without a common superscript letter differ ($P < 0.05$).

¹Control = diet without sprouted grains; Barley = diet with 10% sprouted barley substituted for conventional concentrates; Wheat = diet with 10% sprouted wheat substituted for conventional concentrates.

²Trends were observed for OM ($P = 0.08$ for Wheat vs. Control) and rumen ammonia N ($P = 0.10$ for Barley vs. Control).

³aNDFom = amylase and sodium sulfite treated NDF.

one person approximately 2 to 3 h/d to complete the work. The more advanced fully-automatic system may motivate or discourage farmers to adopt this technique. The decision would largely depend on the ultimate profitability of using this hydroponic production system. Fourth, the germination process causes DM loss of seeds. Exploring ways to improve the ratio of fodder to seed on a DM basis is needed. Further research should focus on the inclusion level of sprouted grains and the use of sprouted grains as alternative forages. A life cycle assessment should also be conducted to examine the utilization of natural resources, greenhouse gas emissions, and manure excretion of nutrients. Lastly, hydroponics is not a panacea but can offer an alternate way to produce feeds for livestock.

CONCLUSIONS

Although the replacement of conventional concentrates with sprouted grains decreased DMI, feeding sprouted grains sustained production performance in high-producing Holstein cows. Compared with the concentrates, sprouted barley improved feed efficiencies expressed as milk yield/DMI and ECM yield/DMI, while sprouted wheat enhanced BW gain. The apparent total-tract digestibility of nutrients was elevated for sprouted wheat versus barley. The differences in milk FA profile indicated that ruminal fermentation may have been modified by feeding different diets (i.e., conventional concentrates vs. sprouted barley vs. sprouted

wheat). Based on our results, there were not beneficial effects of feeding hydroponically grown fodder on milk FA profile in dairy cows.

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ORCIDS

Yu Zang  <https://orcid.org/0000-0002-7349-3980>

Fabian Andres Gutierrez Oviedo  <https://orcid.org/0000-0001-7585-0606>

Rob Harding  <https://orcid.org/0009-0004-5740-1953>

RECENT ARTICLE Regarding McDonald Beef Cattle

<https://www.businesswire.com/news/home/20241114754025/en/McDonald%E2%80%99s-USA-Syngenta-and-Lopez-Foods-Collaborate-to-Help-Produce-Beef-More-Sustainably-in-the-US>

The term "sustainable" is thrown around so loosely in agriculture as if it were saltwater taffy from a parade float. To me, something sustainable must also translate to profitability. In agriculture or any business sector, unless you are profitable you cannot "sustain" being in business. This article shares some cool feel-good stats about Enogen corn and how it will save the planet, so in a way, it sustains a natural resource in water, but it doesn't talk about how this translates into finishing beef in cost per pound of gain or for dairy in Income Over Feed Cost. What Syngenta should be looking at is NovaGreen and NovaJuice. I know from experience that when NovaGreen is fed to lactating dairy cattle, feed efficiency is improved by at least 5%, and water intake in the cattle is decreased by 4 gallons per head/day, while water that is used to grow the feed is estimated at 95% less than traditional forage production. In beef cattle that are being fed for processing, like what McDonalds is doing, the feed efficiency improvement is greater than 17% and we had a lower cost per pound of gain - NovaGreen is sustainable!!!

The values quoted in the article as benefits are a fraction of those from feeding NovaGreens.