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College of Agricultural Sciences

Effect of acetate on milk fat synthesis and mammary lipogenesis

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Our long-term goal is to understand all the factors impacting milk fat yield

Nutritional Factors

Decreased by milk fat depression
- Unsaturated fat
- Fermentability
- Acidosis
- Feeding strategies

Increased by additional substrate
- Fat supplement
    - Palmitic acid
- **Acetate**
    - **Fiber digestibility**
    - **Rumen function**

Milk Fat

Non-nutritional Factors

Genetics

Season

Time of day

Stage of lactation

Parity

Heat stress

Where do the fatty acids in milk come from?

~25% are all de novo in the mammary gland (<16 carbon)
~39% are mixed source (16 carbon)
    (~50% de novo)
~35% are preformed from plasma (>16 carbon)

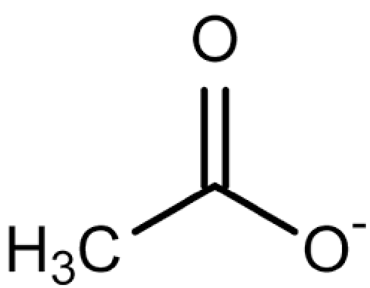
Together

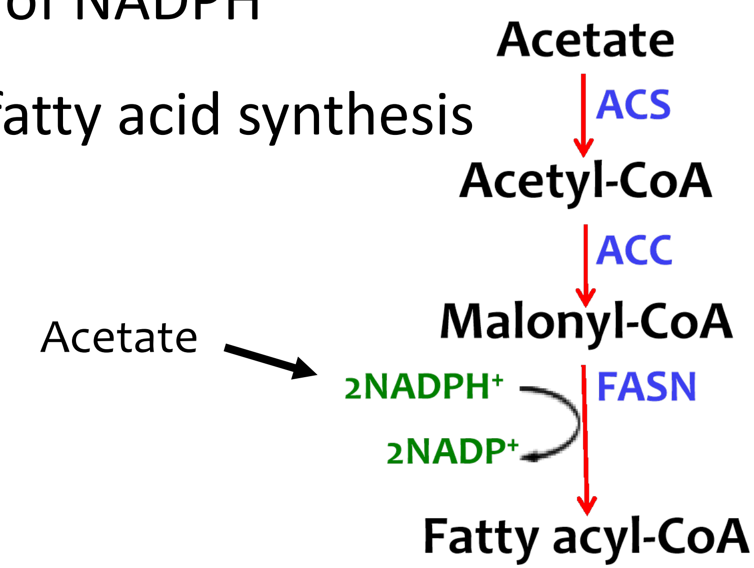
~45% are de novo
    Made from acetate, butyrate, and glucose (NADPH)

~55% Preformed FA
    85% of this directly from absorption

Acetate is a main energy and carbon substrate for milk fat synthesis in the cow

- VFA's are ~70% of total energy supply
  - 45% of this is from acetate (~30% of total energy)
- Mammary uptake is proportional to plasma concentration
- Important substrate for replenishment of NADPH
- Most important substrate for de novo fatty acid synthesis





Bauman et al, 1970; Palmquist et al, 1969, Miller et al, 1991

Where does the cow get acetate?

There is a small amount in silage

- A cow consuming 25 kg of a diet that is 65% silage would be expect to consume ~200 g of acetate from normal silage (1.25% of DM)

- ~400 g of acetate from L. buchneri silage (2.5% of DM).

How much acetate is made in the rumen per day?

- Observed in very few studies as requires labeling approaches

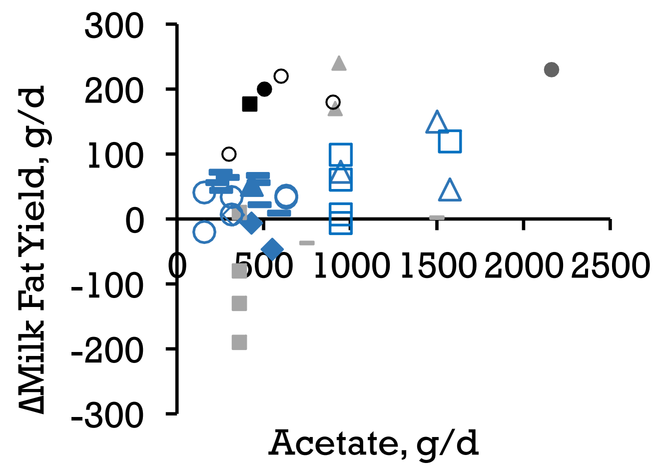
- Literature ranges from **90 to 498** g/kg digestible dry matter (**DDM**) in lactating cows, but old data with low intakes (Sutton 1985).

- Extrapolating, we would expect modern cows with an intake of 25 kg/d to produce approximately 6500 g/d of acetate.

Acetate has not been well studied considering the importance to the cow

1950s and 1960s

- Acetate deficiency proposed as the culprit of diet-induced milk fat depression
- Multiple studies feeding acetate to milk fat depressed cows, with variable success



The original list of publications was sourced from Maxin et al. (2011b) and Urrutia (2016) and updated with recent publications.

Acetate deficiency theory disproven as ruminal acetate production not changed

	Normal diet	HG/LF Diet
Rumen Production, moles/d		
Acetate	29.4	28.1[a]
Propionate	13.3	31.0[b]
B-hydroxybutyrate	7.0	9.1[c]

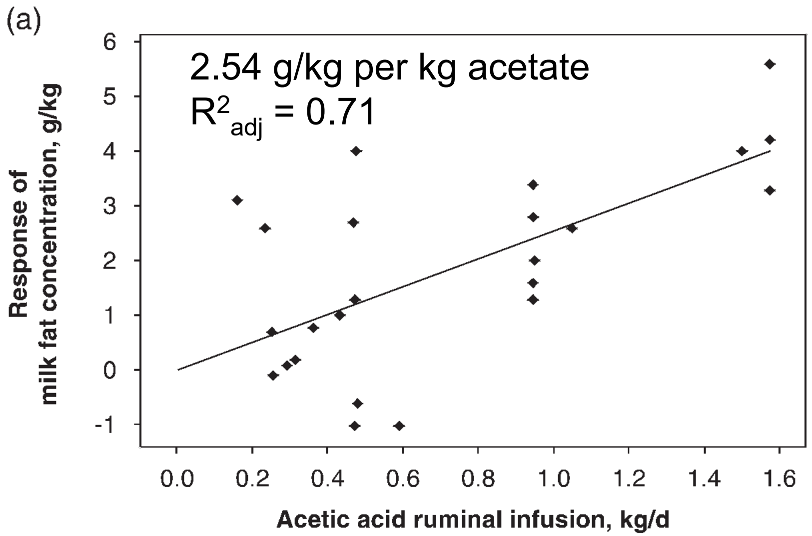
[a]Davis et al. 1967

[b]Bauman et al. 1971

[c]Palmquist et al. 1969

Glasser et al. 2011 meta-analysis of 1955 to 1978 data did find a positive relationship of acetate and milk fat

Milk Fat Concentration



Milk Fat Yield

75.5 g per kg acetate
$R^2_{adj} = 0.72$
$P < 0.001$

7.5% transfer efficiency

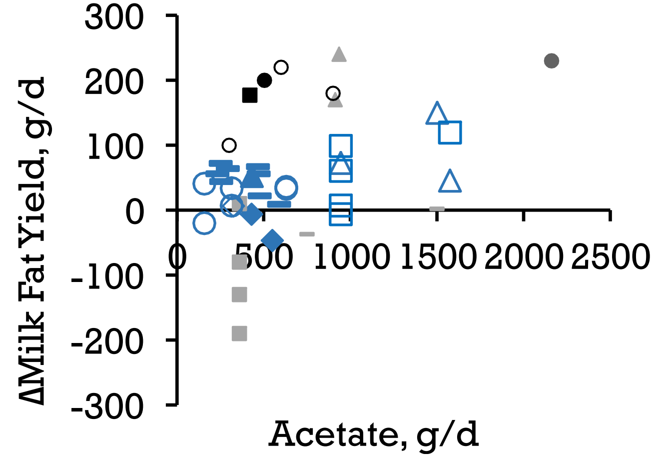
Data 1955 to 1978

Glasser et al. Animal 2011

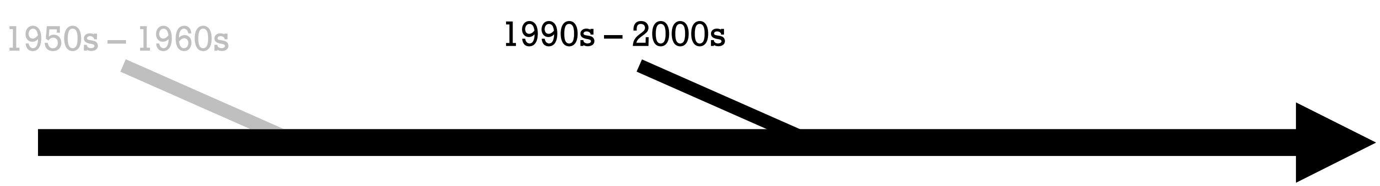
- **Sheperd and Combs (1998)** observed a 280 g increase in milk fat yield (+24%) and a 20% increase in milk fat concentration (3.41 to 4.08%) when ruminally infusing 2162 g/d of acetate for 21 d (13% net transfer).

1990s – 2000s

- Focus on intake regulation and mammary gland energy metabolism



- Most studies did not characterize responses in milk fat as treatments were short term (< 8 h).



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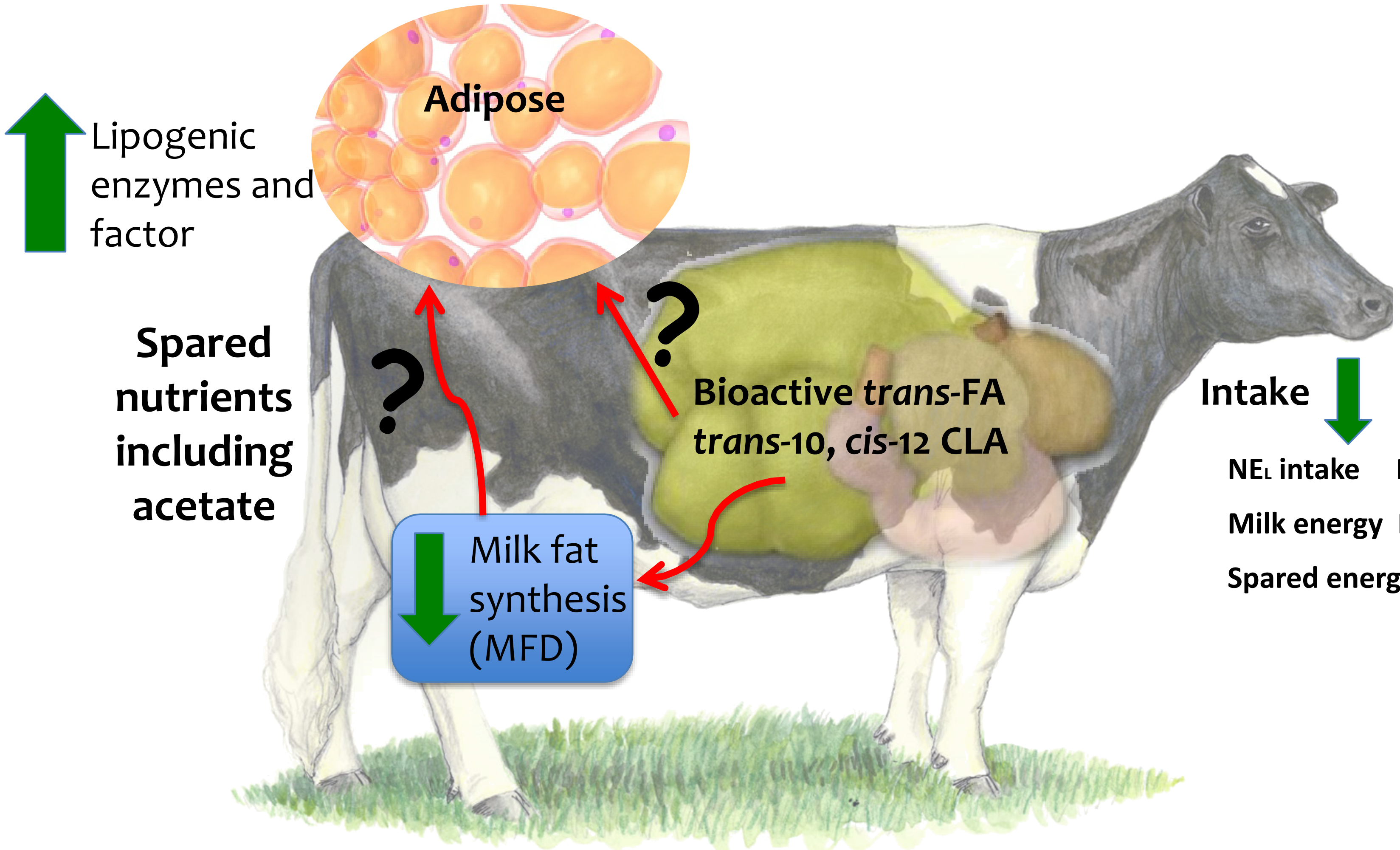
How we got into this: We were interested in where the spared energy went during MFD



NE_L intake	MJ/d	-15
Milk energy	MJ/d	-19
Spared energy		4

Harvatine et al., 2009

1st we investigated the effect of CLA and Acetate

420 g Na acetate infusion increased milk fat yield 20%

	Treatments				
Variable	Control	CLA	Acetate	SE	*P*-value
DMI, kg/d	25.6	24.8	26.4	1.19	0.20
Milk yield, kg/d	22.9	23.5	25.4	3.53	0.22
Milk fat					
%	3.87[a]	2.77[b]	4.10[a]	0.20	<0.001
g/d	864[a]	669[b]	1041[c]	138	<0.01
Milk FA source, g/day					
<16 carbons	200[a]	137[b]	244[c]	30.1	<0.001
16 carbons	223[a]	162[b]	278[c]	36.3	<0.001
>16 carbons	334[ab]	288[a]	394[b]	44.7	0.02

- Acetate increased all sources of milk fat

Urrutia et al. JDS 2017

Acetate increased plasma BHBA, but had no effect on plasma glucose, NEFA, or insulin

	Treatment		
Variable	Control	Acetate	SE
Glucose (mg/dL)	56.2	57	2.1
NEFA (μEq/L)	134	138	12
BHBA (μM)	683[a]	804[b]	56
Glucagon (pg/ml)	281	271	27
Insulin (μg/L)	1132	1063	201
RQUICKI	0.21	0.20	0.01

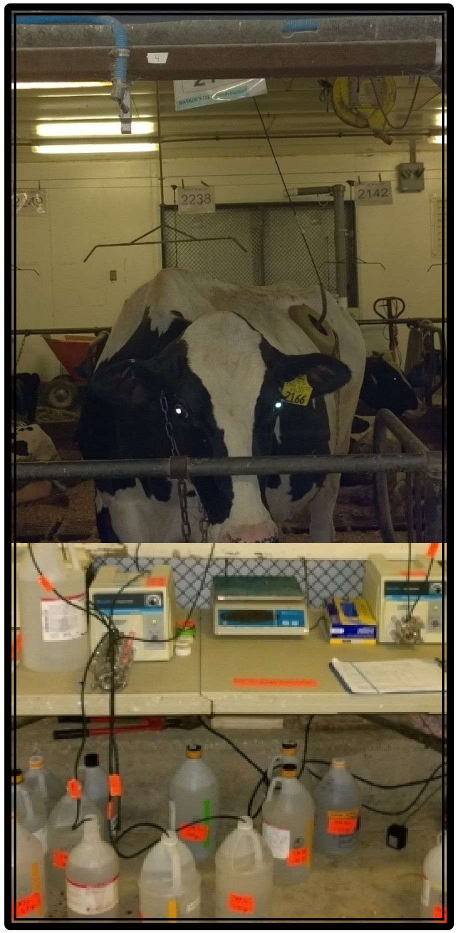
Urrutia et al. JDS 2017

What is the optimal dose of sodium acetate to increase milk fat?

- **0, 5, 10, and 15 moles/d acetate**

Which is:

0, 300, 600, and 900 g/d of acetate



Urrutia et al. J Nutr 2017

Milk fat percent was increased by up 217 g/d at 600 g/d acetate

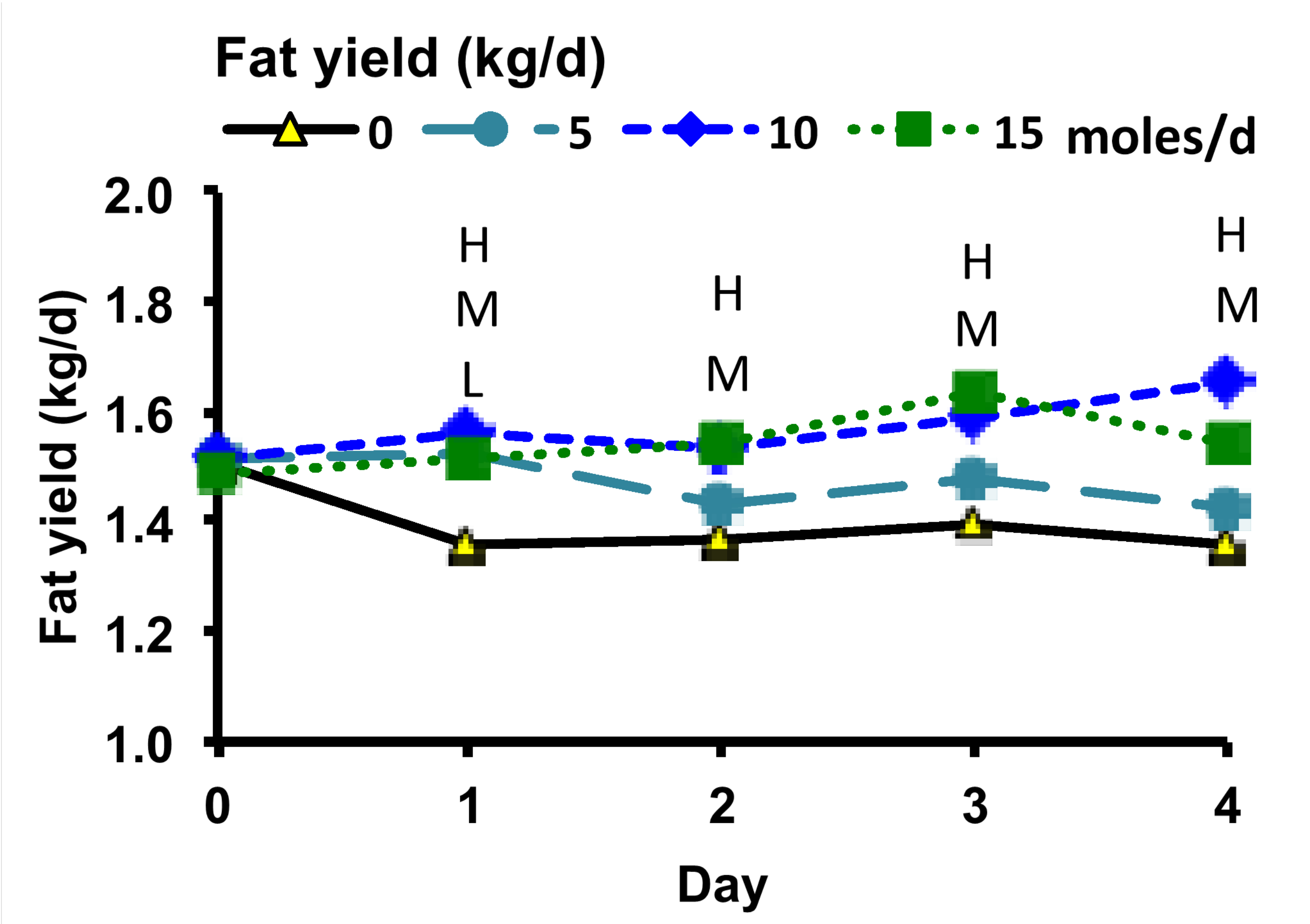
Variable	Acetate (moles/d)				SE	*P*-values				
	0	**5**	**10**	**15**	**SE**	**Trt**	**Time**	**T*T**	**L**	**Q**
DMI, kg/d	26.6	26.5	27.3	26	0.8	0.58	<0.001	0.54	-	-
Milk yield, kg/d	37.7	38.2	39.3	38.2	1.9	0.16	0.07	0.80	-	-
Milk fat										
Yield, kg/d	**1.37**	**1.47**	**1.59**	**1.55**	**0.05**	**<0.001**	**0.29**	**0.50**	**<0.001**	**<0.01**
%	**3.71**	**3.94**	**4.05**	**4.12**	**0.15**	**<0.01**	**<0.01**	**0.48**	**<0.001**	**0.15**

100 217 186 g/d increase in milk fat yield
.23 .34 0.40 % unit increase in milk fat concentration

- Acetate mass transfer >30%

Urrutia et al. J Nutr 2017

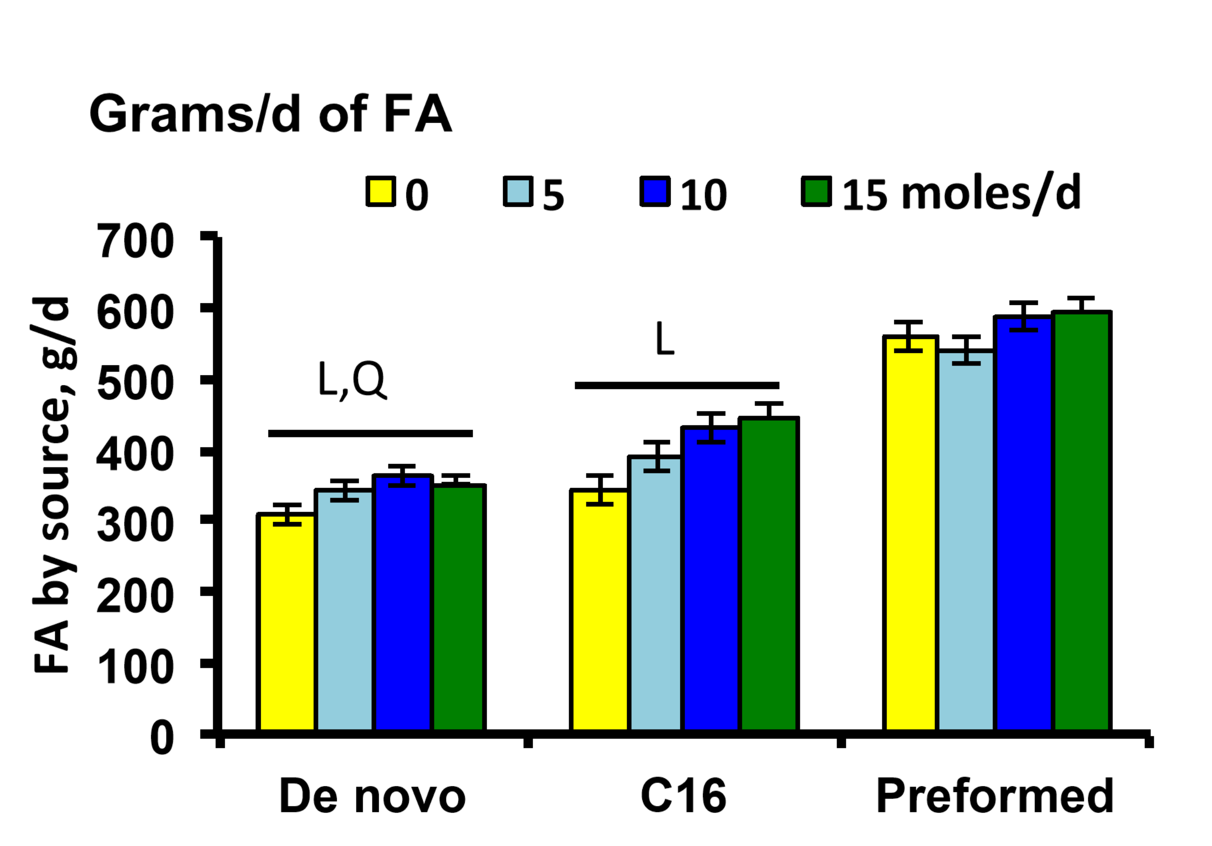
Milk fat was rapidly increased after initiation of acetate infusion



Contrast	P <0.05	P <0.10
5 vs 0	L	l
10 vs 0	M	m
15 vs 0	H	h

Urrutia et al. J Nutr 2017

Yield and concentration of de novo and 16C fatty acids increased linearly

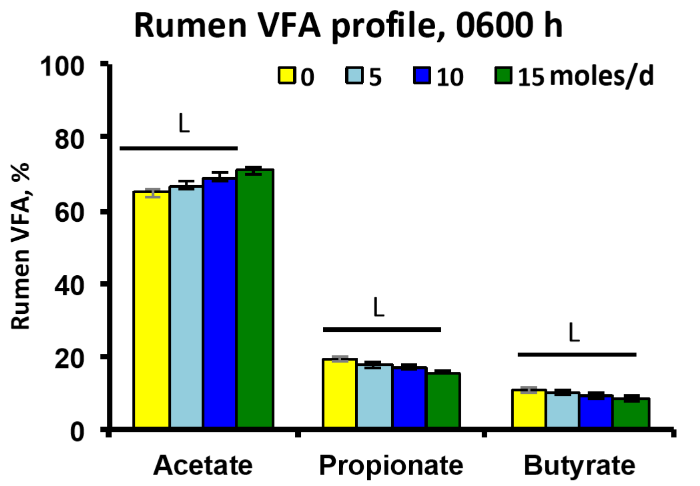


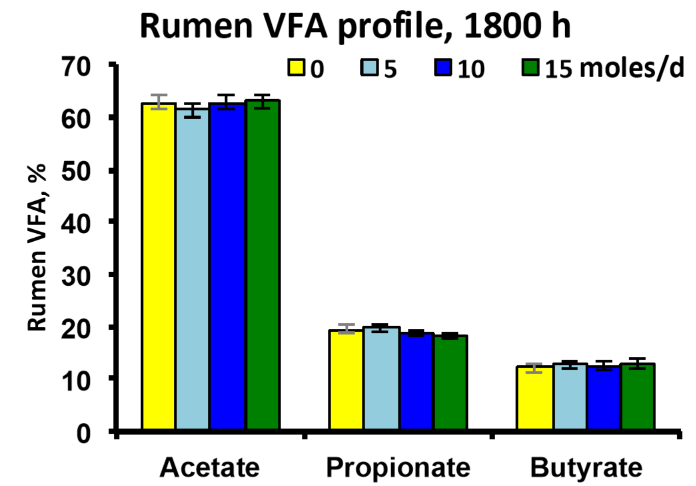
- Largest increase in 16 C fatty acids

Urrutia et al. J Nutr 2017

Rumen pH also increased linearly after feeding and rumen acetate increased before feeding

Rumen pH	Acetate (moles/d)					*P*-value		
	0	5	10	15	SE	Trt	L	Q
0600 h	6.0	6.2	6.3	6.3	0.21	0.14	-	-
1800 h	**6.0**	**6.1**	**6.2**	**6.3**	0.19	0.03	**<0.01**	0.80





Urrutia et al. J Nutr 2017

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Can we feed sodium acetate in the diet?
and…
Is the response due to the increase in buytrate?

- **Control: 3.1% Sodium Bicarbonate ($NaHCO_3$)**
- **Acetate: 2.7% Sodium Acetate (NaAc)**
- **Butyrate: 2.4% Calcium Butyrate (CaBu)**

- Treatments controlled for sodium (control vs. acetate) and mass of carbon (acetate vs. butyrate)

Butyrate decreased milk yield 1.7 kg and feed intake 2.3 kg

Urrutia et al. JDS 2019

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Dietary acetate increased milk fat, but butyrate did not

	Treatment			SE	*P-value*		
	NaHCO	NaAc	CaBu		trt	time	t*t
Milk fat, kg/d	1.50[b]	1.59[a]	1.44[c]	0.05	0.00	0.08	0.22
Milk fat, %	3.65[b]	3.77[a]	3.63[b]	0.09	0.03	0.01	0.05

- 6% and 3% increase in milk fat yield and % with acetate supply.
- 4% decrease in milk fat yield with dietary butyrate.
- 15% net transfer of dietary acetate to milk fat

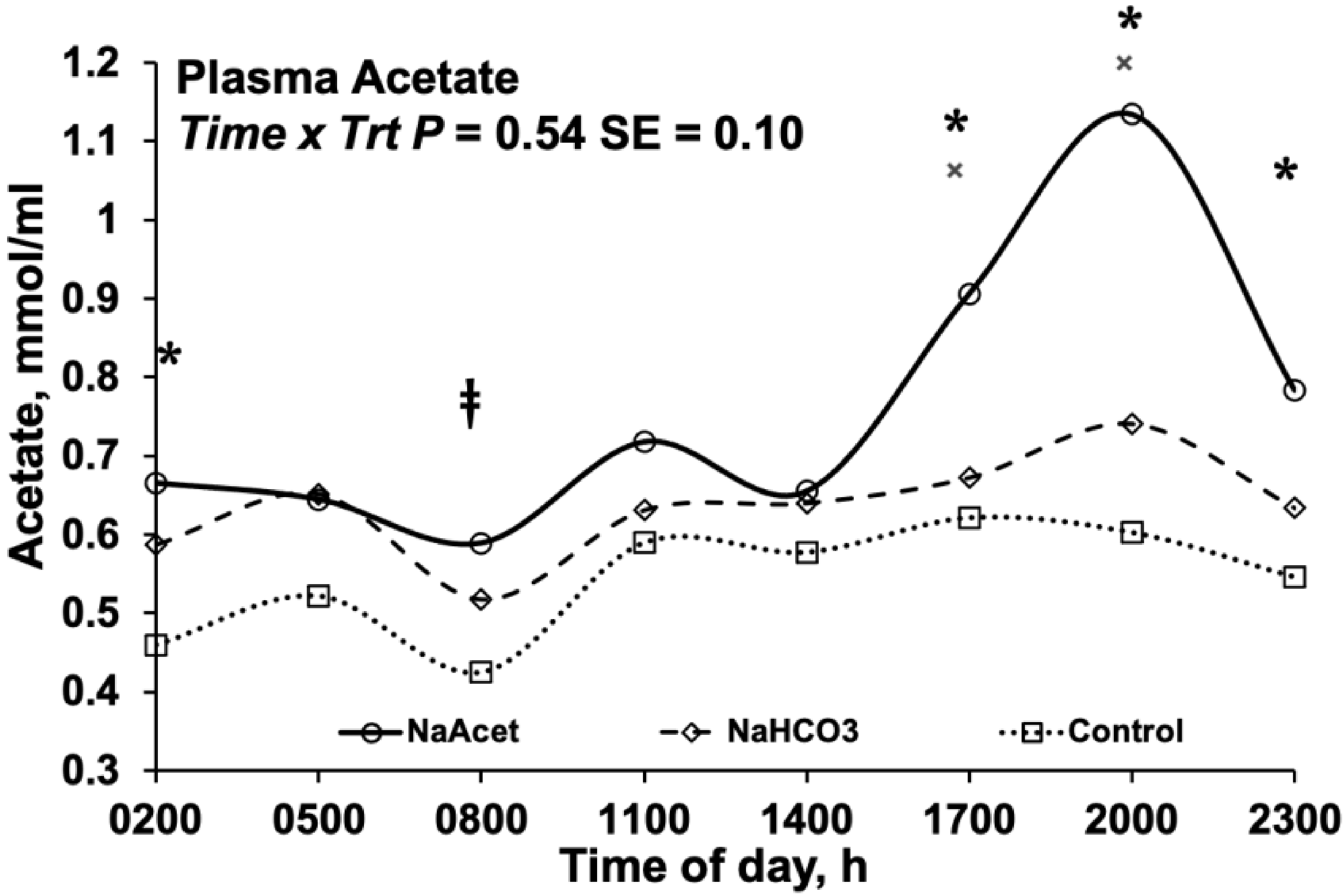
Urrutia et al. JDS 2019

Can we separate the effect of acetate from an effect of DCAD?

Variable	Treatment			SE	P-value
	Control	NaAcet	NaHCO3		trt
		Yield, kg/d			
Milk	43.5	44.2	44.7	8.23	0.48
Fat	1.46[b]	1.59--[a]	1.58[a]	0.23	0.003
Protein	1.26	1.29	1.30	0.22	0.71
		Yield, g/d			
Σ de novo	395[b]	429[a]	426[a]	28.1	0.03
Σ mixed	363[c]	441[a]	404[b]	34.5	<0.0001
Σ preformed	572[b]	582[b]	611[a]	40.0	0.03

Matamoros et al. JDS 2021

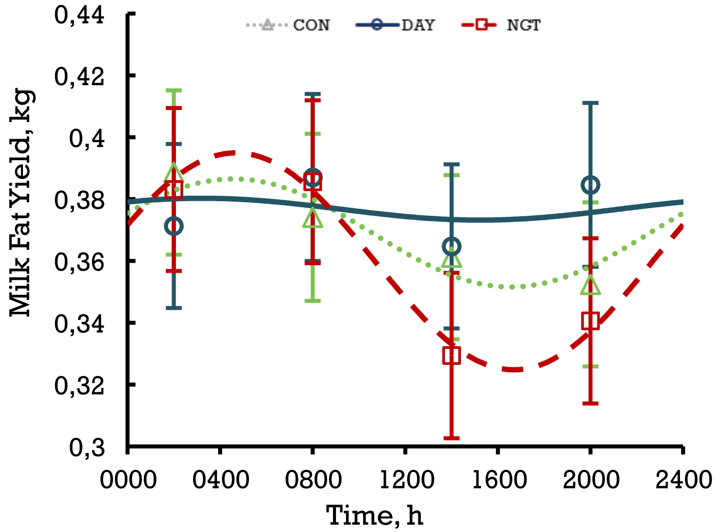
Plasma acetate was also increased during the active feeding period of the day



Matamoros et al. JDS 2021

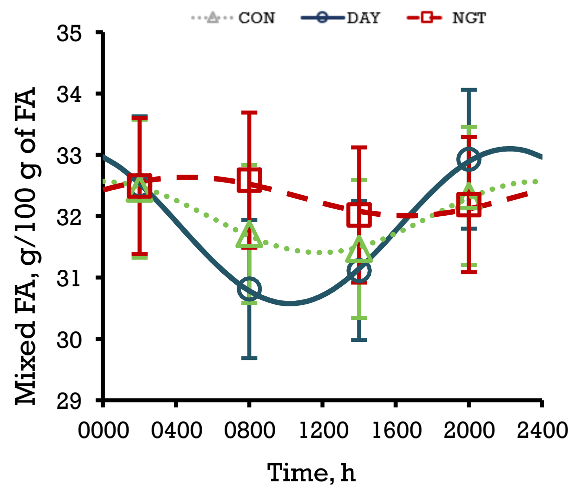
Infusion during the day vs the night also changes daily patterns

Milk Fat Yield



TRT	Mean	*P*-value
CON	0.37	0.89
DAY	0.38	0.99
NGT	0.37	0.36

16 C FA



TRT	Mean	Amp	Acro	*P*-value
CON	31.99	0.58[b]	23.85[a]	<0.01
DAY	31.84	1.26[a]	22.22[b]	<0.01
NGT	32.32	0.31[c]	4.65[c]	0.33

Matamoros et al. JDS 2022

Does acetate interact with dietary factors that are expected to change endogenous acetate supply?

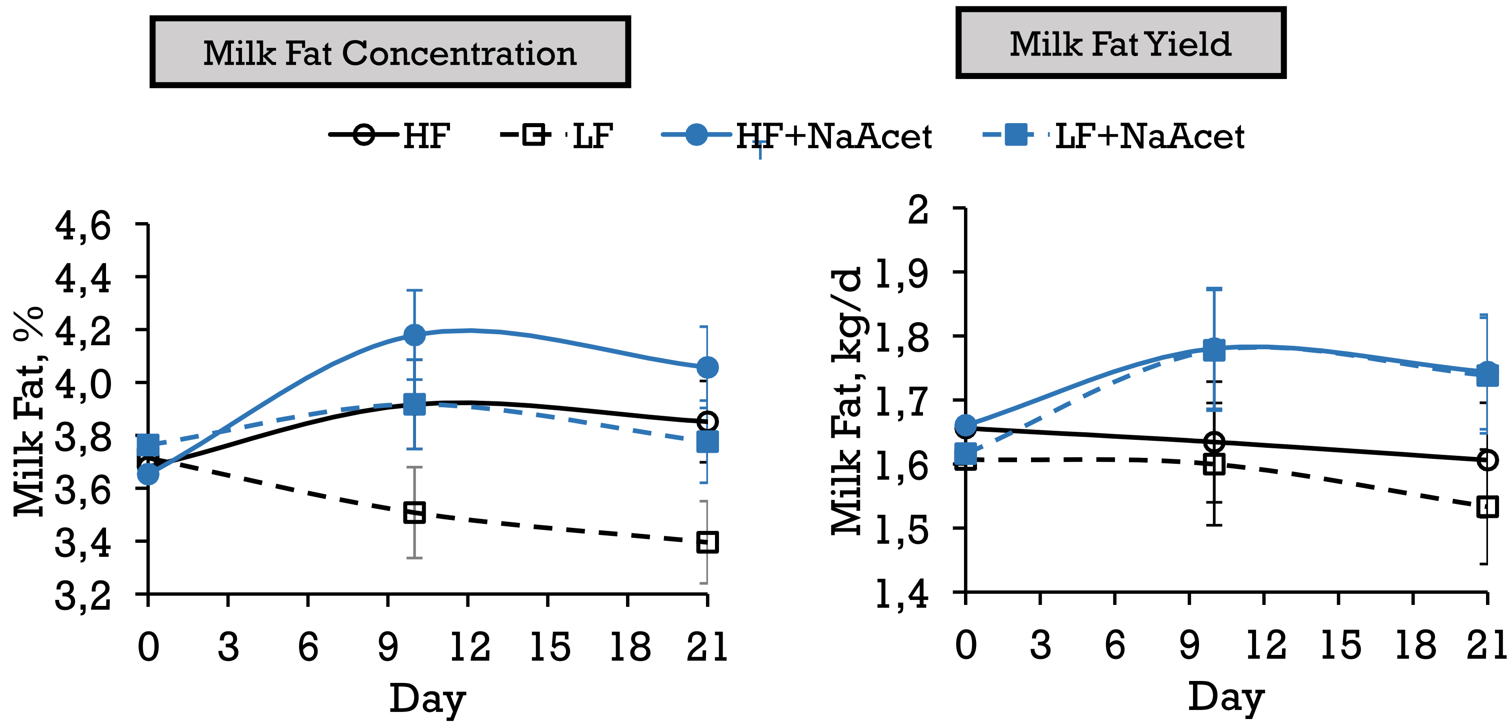
1. Effect of forage to concentrate level

2. Increasing unsaturated fatty acids

3. Increasing digestible fiber

Does acetate interact with forage:concentrate ratio?

2.5 percentage units of NDF substituted for starch



P-Value			
Fiber	<0.001	F × A	0.17
Acetate	<0.001	F×A×Day	0.91

P-Value			
Fiber	0.44	F×A	0.51
Acetate	<0.001	F×A×Day	0.74

Acetate supplementation increased milk fat synthesis, regardless of dietary fiber level

Matamoros et al. JDS 2022

Interaction with fiber digestibility?

Replacement of 7 percentage units of corn silage for soyhulls and citrus pulp

	Treatment					*P*-values		
	L Dig	LD +Acet	H Dig	HD + Acet	SEM	Dig	Acet	DxA
Milk, kg	42.7	44.6	43.7	44.0	1.91	0.82	0.22	0.36
Milk Fat								
%	**3.40**	**3.54**	**3.33**	**3.51**	0.22	0.57	**0.08**	0.79
kg	**1.45**	**1.60**	**1.48**	**1.54**	0.11	0.69	**0.02**	0.36
Milk FA								
<16 C, g	**357**	**408**	**370**	**383**	32.4	0.61	**0.01**	0.14
16 C, g	**363**	**448**	**372**	**419**	34.0	0.51	**<0.01**	0.23
> 16 C, g	561	553	553	561	46.0	0.99	0.99	0.67

Acetate supplementation increased milk fat synthesis, regardless of digestible fiber

Husnain et al. Unpublished

Interaction with unsaturated fatty acids?

1.5 percentage units of soybean oil

Variable	Treatment				SEM	*P*-value		
	Con	Acet	UFA	UFA+Acet		Fiber	Acetate	F×A
Milk, kg	45.1	45.9	47.4	48.2	2.66	0.002	0.26	0.94
Milk Fat								
%	**3.40**	**3.92**	**3.54**	**3.69**	0.20	0.61	**<0.001**	**0.03**
kg	**1.55**	**1.81**	**1.71**	**1.79**	0.14	0.11	**0.001**	**0.06**
Milk FA								
<16 C, g	**443**	**474**	**398**	**430**	35.8	<0.001	0.002	0.99
16 C, g	**418**	**486**	**369**	**425**	**34.5**	**<0.001**	**<0.001**	**0.55**
> 16 C, g	**569**	**605**	**704**	**731**	45.3	<0.001	0.03	0.73

Acetate supplementation increased milk fat synthesis slightly more in the absence of unsaturated fatty acids

Staffin et al. Unpublished

Does acetate overcome diet-induced milk fat depression caused by conjugated linoleic acid (CLA)?

Variable	Treatment				SEM	*P*-value		
	CON	Acet	CLA	CLA+Acet		CLA	Acet	C×A
Milk, kg	43.2	41.8	44.6	41.8	2.11	0.39	0.02	0.39
Fat								
kg	**1.65**	**1.71**	**1.00**	**1.04**	0.08	<0.001	0.34	0.89
%	**3.81**	**4.10**	**2.26**	**2.49**	0.10	<0.001	**0.01**	0.76
Milk FA, g/d								
< 16 C	415	429	203	216	22.9	<0.001	0.45	0.99
16 C	**369**	**439**	**215**	**232**	15.9	<0.001	**0.02**	0.12
> 16 C	619	616	447	450	32.8	<0.001	0.99	0.87

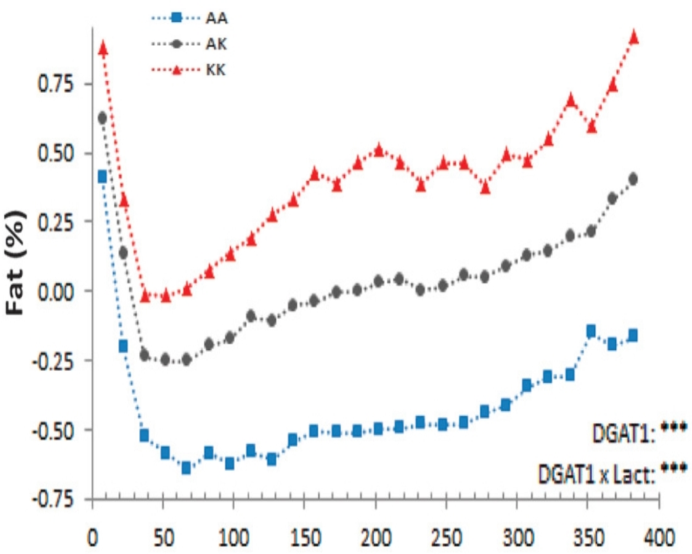
Acetate increased milk fat concentration by 8.5%. There was no difference in milk fat yield between CON and acetate treatments, suggesting that acetate stimulated milk fat synthesis, despite the decrease in milk yield.

Does acetate response depend on the genetic potential for milk fat synthesis and parity?

- Milk fat is the most heritable component of milk and genetic potential varies within animals.

- A *DGAT1* polymorphism explains ~30 to 50% of genetic variation of milk fat synthesis.

- More than 30% of the US dairy herd are primiparous cows, but parity interactions are rarely tested in research



A *DGAT1* polymorphism has a clear effect on milk fat production across lactation.

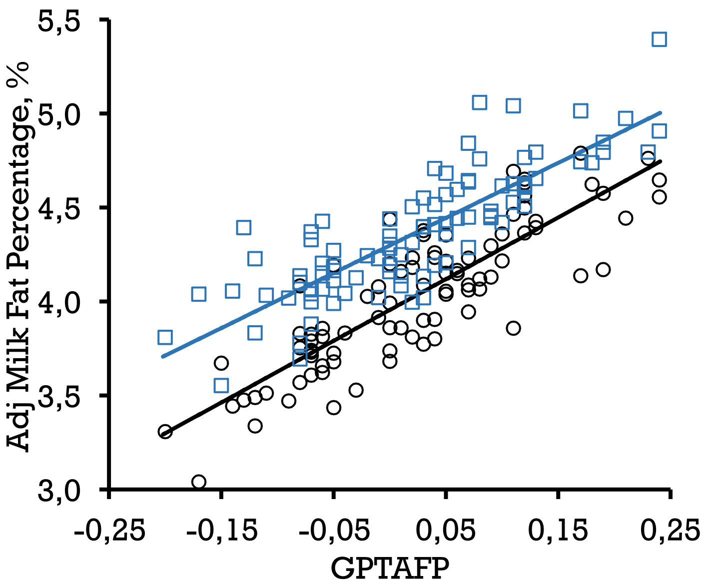
Bovenhuis et al., 2015

Acetate increased milk fat synthesis regardless of DGAT1 polymorphism or parity

Variable	KK *DGAT1*		KA *DGAT1*		SEM	*P*-value[2]					
	Con	**Acet**	**Con**	**Acet**		**Trt**	**DGAT**	**Parity**	**T×D**	**T×P**	**T×D×P**
		kg/d									
Fat yield	1.41	1.55	1.58	1.68	0.07	<0.001	0.001	<0.001	**0.25**	**0.14**	**0.41**
Primiparous	1.33	1.45	1.46	1.52	0.08						
Multiparous	1.50	1.65	1.71	1.85	0.08						

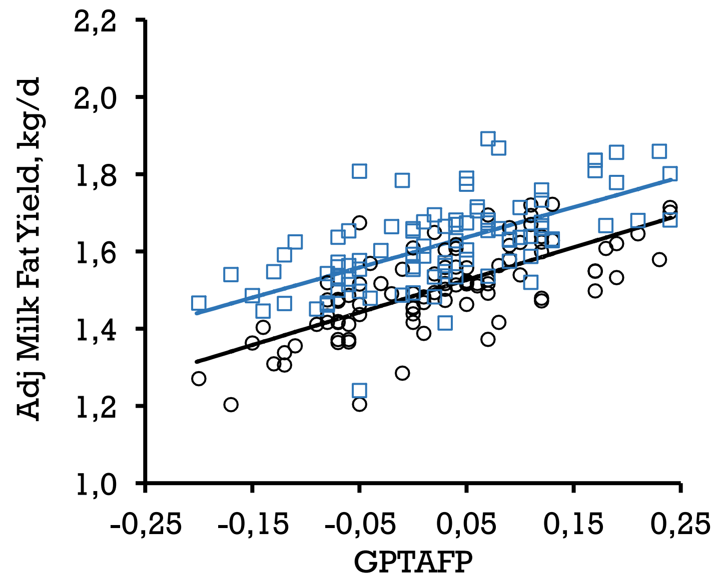
Acetate also increased milk fat yield regardless of genetic potential (GPTA) for milk fat

Milk Fat Concentration



P-Value			
Treatment	<0.001	GPTAFP	<0.001
Parity	0.72	T×G	0.39
T×P	0.03	T×G×P	0.45

Milk Fat Yield



P-Value			
Treatment	<0.001	GPTAFP	0.002
Parity	<0.001	T×G	0.73
T×P	0.34	T×G×P	0.83

Acetate increased milk fat yield regardless of GPTAFP or parity.

So, what does sodium acetate do?

Infusion and feeding consistently:

- **Rapidly increases milk fat yield mostly regardless of basal diet conditions we have tested**
  - **Not alleviation of diet-induced milk fat depression**
- **Increases 16 C fatty acids the most**
- **Increases plasma butyrate**

What is variable:

- **Small changes in intake and milk yield**
- **Magnitude of the increase and the "transfer efficiency" of acetate to milk fat**
- **Effect on >16 C FA**

It is not clear yet if the mechanism is simply substrate availability or is acting as a bioactive nutrient

Extrapolating from our data

- There may be a larger effect during winter when have higher milk fat and more de novo FA?

- Highlights importance of increasing endogenous acetate supply
  - Highly digestible forages
  - Stable rumen fermentation
    - Reduce microbial inhibitors
    - Stimulate with specific additives
    - Good rumination and rumen function

INTERNATIONAL SYMPOSIUM ON RUMINANT PHYSIOLOGY

AUGUST 26-29, 2024

ISRP 2024

CHICAGO, ILLINOIS

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- GI microbial ecology, the microbiome, and gut physiology spanning from microbial- host interactions to an update on methane production and mineral interactions
- Whole animal microbiome and integration of effects across systems including the central nervous system, lactation, reproduction, respiration, and immunity
- Post-absorptive physiological impacts of nutrients on cellular signaling
- Inflammation, metabolic endocrinology, metabolomics, and novel regulators of physiology and metabolism
- Genomics and epigenetic impacts on ruminant physiology and efficiency
- Insights from precision technology and data science and their application to ruminant physiology and management
- Integration of environment, physiology, and wellbeing in face of climate change and resource limitations

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Lab Members: **Dr. Cesar Matamoros, Alanna Staffin, Abiel Berhane, Sarah Bennett, Yusuf Adeniji, Muhammad Husnain, Muhammad Arif**

Previous Lab Members: **Beckie Bomberger. Dr. Ahmed Elzennary. Reilly Pierce, Dr. Rachel Walker, Dr. Chengmin Li, Elle Andreen, Dr. Isaac Salfer, Dr. Daniel Rico, Dr. Michel Baldin, L. Whitney Rottman, Dr. Mutian Niu, Dr. Natalie Urrutia, Richie Shepardson, Andrew Clark, Dr. Liying Ma, Elaine Brown, and Jackie Ying**

United States Department of Agriculture

National Institute of Food and Agriculture

Disclosures

- Harvatine's research in the past 10 years were partially supported by the Agriculture and Food Research Initiative Competitive Grant No. 2015-67015-23358, 2016-68008-25025, 2018-06991-1019312, 2022-67015-37089, and 2022-26800-837106 from the USDA National Institute of Food and Agriculture [PI Harvatine], Berg-Schmidt, Elanco Animal Health, BASF, Novus International, PA Soybean Board, Phode Laboratories, Kemin International, Milk Specialties Global, Adisseo, Micronutrients Inc., Organix Recylcing, Insta-Pro Intl., Cotton Inc., United Soybean Board, and Penn State University.
- Harvatine has consulted for Cotton Inc, Micronutrients, Milk Specialties Global, Axiota, and Nutriquest as a member of their science advisory boards and United Soybean Board, ELANCO, and Novus on special projects.
- Harvatine has also received speaking honorariums from Elanco Animal Health, Cargill, Virtus Nutrition, NDS, Nutreco, Mycogen, Holtz-Nelson Consulting, Renaissance Nutrition, Progressive Dairy Solutions, Intermountain Farmers Association, Diamond V, Purina, Pioneer, Adessio, Standard Nutrition, Hubbard, VitaPlus, and Milk Specialties Global in the past four years.

Thank You!