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Decreasing Risk of Rumen Upset and Low Milk Fat by Feeding High Oleic Soybeans

FINAL REPORT

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BACKGROUND

Low milk fat or milk fat depression (**MFD**) is characterized by up to a 50% reduction in milk fat yield in response to diets high in starch or unsaturated oils (Harvatine et al., 2009). These diets are commonly fed to increase dietary energy intake, but they simultaneously alter rumen fermentation and induce the production of unique bioactive fatty acids (**FA**) from metabolism of dietary polyunsaturated fatty acids (**PUFA**). These bioactive FA leave the rumen and are transported to the mammary gland, where they act as inhibitors of milk fat synthesis. Over the past two decades great strides have been made in the identification of the mechanism of milk fat depression and dietary risk factors that contribute to its occurrence. However, milk fat depression still commonly occurs on dairy farms, principally because of the need to feed energy dense diets to meet the energy demand of high producing cows and the complex interaction of dietary factors making anticipation difficult. Milk fat has been one of the most valuable milk components making this an economically important issue.

Importantly, oil sources differ in their impact on rumen fermentation. Oils higher in C18:2 (conventional soybeans) and rapidly available oils have a greater impact (He et al., 2012). Recent work with high oleic soybeans in dairy cows supports this, but the experiments were not designed to specifically test the effect of milk fat (Lopes et al., 2017;Weld and Armentano, 2018).

Objective

Demonstrate that high oleic soybeans reduce the risk of diet induced milk fat depression allowing higher inclusion rates in dairy diets.

Hypothesis

High oleic soybeans will result in higher milk fat yield due to a lower amount of the bioactive *trans*-10 C18:1 that decreases milk fat compared to conventional soybeans.

MATERIALS AND METHODS

Experimental Design and Treatments

The experiment was conducted at the Pennsylvania State University Dairy Production Research and Teaching Center and all procedures approved by the Penn State Institutional

Animal Care and Use Committee. Eighteen non-cannulated multiparous Holstein cows (2.7 ± 0.8 parities; 153 ± 64 DIM; 100.6 ± 15.4 lb milk; 3.47 ± 0.58% milk fat; Mean ± SD at end of pretrial period) were housed in a tie-stall barn with mattresses and sawdust bedding and randomly assigned to treatment sequences in a crossover design with 24 d periods (Table 1). One cow was removed in Period 1 because of mastitis. Cows were individually fed a TMR at 110% of expected daily intake. Barn light was manually controlled on an 18 h light and 6 h dark schedule (dark ~2300 to 0500 h). Each period consisted of two dietary phases. The first 14 d a diet with 5% roasted soybean was fed and the last 10 d roasted soybean was increased to 10%. Treatments were conventional and high oleic soybeans (Plenish soybeans provided by Perdue Agribusiness Inc.). A common base ration was mixed in a stationary Rissler mixer and roasted soybeans were added in a mobile Rissler cart. A common diet was fed for 7 d before the trial and during a 7 d washout between periods.

Data and Sample Collection and Analysis

Cows were milked twice daily at 0600 and 1800 h and milk yield determined by an integrated milk meter (AfiMilk; SAE Afikim, Israel) and analyzed as the average of d 13 and 14 and 23 and 24. The parlor stalls were calibrated weekly using data from the entire herd (>200 cows) over 7 d. Stall adjustments were determined by modeling the effect of day, milking (AM/PM), cow, and stall, excluding observations of experimental cows during treatment periods. Milk was sampled at both milkings on d 10, 21, 27, and 31 of each period and analyzed by FTIR (Dairy One Lab, Ithaca, PA). Milk fatty acid profile was determined by gas chromatograph by our previously published methods (Rico and Harvatine, 2013).

Statistical Analysis

Data were statistically analyzed using the MIXED procedure of SAS with repeated measures (version 9.3, SAS Institute Inc., Cary, NC). The model included the random effects of period and cow and the fixed effect of soybean type (Conventional and High C18:1), soybean level (5 and 10% of diet), and the interaction of soybean type and level. The TOEP, AR(1), or VC covariance structure was used, soybean level was the repeated variable, and cow by treatment was the subject. The preplanned contrasts tested the effect of treatment at each time point and denominator degrees of freedom were adjusted by the Kenward-Rogers method. The preplanned contrast tested the effect of treatment within each dietary phase. Significance was declared at $P < 0.05$ and $P < 0.10$ for main effects and interactions, respectively, and tendencies at $P < 0.10$ and $P < 0.15$ for main effects and interactions, respectively.

RESULTS

Diet Ingredients and Composition

The diets were based on a high corn silage diet commonly fed in the dairy industry (Table 2). During the pretrial and washout period the diet was balanced to ~31% neutral detergent fiber (NDF) to reduce risk of milk fat depression before initiation of the treatments and contained approximately 44% corn silage, 15% alfalfa haylage, and 2.6% chopped grass hay and straw on a dry matter basis. The pretrial diet also contained conventional roasted soybeans. In the first dietary phase cows were fed a similar diet that substituted conventional soybeans for high oleic soybeans. During the second dietary phase, the risk for milk fat depression was increased by reducing dietary forage and increasing roasted soybeans to 10.5% of the diet. This decreased dietary NDF 2.7 percentage points and increased starch 1.7 percentage points. The amount of canola meal in the diet was decreased to reduce the increase in dietary protein to 0.7 percentage points.

Effect on Feed Intake and Milk Production

There was no effect of high oleic acid soybeans on feed intake, but intake increased when feeding the lower forage and higher soybean diet (1.4 and 1.5 kg/d in conventional and high oleic; Table 3). This was likely due to the decrease in dietary fiber reducing the filling effect of the diet and allowing cows to increase feed intake.

There was no effect of high oleic soybeans or soybean level of milk yield (Table 3). Cows averaged over 43 kg/d in the experiment (>94 lb/d), which is representative of the high group on a dairy farm. Overall, high oleic soybeans increased milk fat percent 0.17 percentage units (3.37 vs. 3.54%) and tended to increase milk fat yield 89 g/d and there was no interaction with the lower forage and higher soybean diet. This increase in milk fat yield with high oleic soybeans is worth approximately $0.45/cow/d based on average milk fat value over the past two years.

We expected the lower fiber and higher soybean diet to cause milk fat depression, but it increased milk fat concentration 0.2 percentage units and increased milk fat yield 92 g/d. It appears that decrease in fiber and increase in starch and unsaturated fatty acids was not sufficient to cause the shift in rumen fermentation required to cause milk fat depression, but that the increase in absorbed fat allowed increased synthesis of milk fat. The increase in milk fat yield with this higher level of soybeans is worth approximately $0.51/cow/d based on average milk fat value over the past two years.

There was no effect of high oleic soybeans or dietary fiber and soybean level on milk protein concentration and yield (Table 3). Milk urea nitrogen (MUN) is an indicator of nitrogen efficiency and was not effected by high oleic soybeans, but was increased with the low fiber and high soybean diet as expected as this diet increase dietary protein level.

Effect on Milk Fatty Acid Profile

Milk fatty acids are either made "de novo" in the mammary gland from acetate and ketone bodies or taken up as preformed fatty acids from the plasma. Fatty acids less than 16 carbons are entirely made by the de novo pathway, 16 carbon fatty acids are both made de novo and taken up from the plasma, and fatty acids greater than 16 carbon are entirely taken up from the plasma. There was no effect of high oleic soybeans on the concentration of de novo, 16 carbon, or preformed fatty acids in milk fat (Table 3). The lower fiber and higher soybean diet decreased de novo synthesized FA and mixed source 16 C fatty acids by almost 3 and 9%, respectively, and increased preformed fatty acids over 10%. This is expected as the soybeans increase absorption of fat that the mammary gland uses for milk fat and subsequently decreases de novo synthesis.

Rumen microbes metabolize unsaturated fatty acids to saturated fatty acids and create *trans* fatty acids as intermediates. *Trans*-11 C18:1 is the main intermediate of the normal biohydrogenation pathway that is predominant under normal fermentation conditions. *Trans*-10

C18:1 is produced by alternate biohydrogenation pathways that occur during diet induced milk fat depression and are associated with up to a 50% decrease in milk fat yield.

High oleic acid soybeans decreased *trans*-11 C18:1 and had a larger effect when soybeans were fed at the higher rate and *trans*-11 C18:1 was also increased with the lower fiber and higher soybean diet as expected. The response is likely attributable to two factors. First, the lower amount of C18:2 in high oleic soybeans reduces that amount of unsaturated fatty acid being biohydrogenated through the *trans*-11 C18:1 intermediate. Secondly, it is likely that the high oleic soybeans have less of an impact on the microbial populations resulting in increased capacity for *trans*-11 C18:1 biohydrogenation.

High oleic acid soybeans decreased *trans*-10 C18:1 and there was no effect of the low fiber and high soybean diet (Table 3). This indicates that the high oleic acid soybeans reduced the occurrence of altered rumen biohydrogenation associated with milk fat depression.

Lastly, the odd and branch chain fatty acids (OBCFA) originate either from microbial synthesis in the rumen or elongation of odd and branch chain volatile fatty acids in the mammary gland. Previous work has observed an association between OBCFA and microbial fermentation as the OBCFA of different microbial species differ. We have also observed a decrease in OBCFA during diet induced milk fat depression. Total OBCFA were increased overall over 7% by high oleic soybeans and were decreased overall over 12% by the low fiber and high soybean diet. There was not interaction of high oleic soybeans and the basal diet. We expect this indicates that the high oleic soybeans had less of a negative effect on fibrolytic bacteria in the rumen as these have been previously associated with higher OBCFA levels.

OVERALL CONCLUSIONS AND IMPLICATIONS

The study demonstrated that high oleic soybeans increase milk fat through decreased biohydrogenation induced milk fat depression and increased rumen health as indicated by *trans*-10 C18:1 and odd and branch chain fatty acids in milk. Additionally, it demonstrated the ability of soybeans to increase milk fat through providing preformed fatty acids for the mammary gland to use for milk fat synthesis.

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Table 1: Overview of the experimental design testing the effect of conventional (Conv.) and high oleic (C18:1) roasted soybeans fed to dairy cows at either 5 or 10% of the diet.

		Period 1			Period 2	
	Pretrial	**Phase 1**	**Phase 2**	**Washout**	**Phase 1**	**Phase 2**
Sequence	7 d	14 d	10 d	7 d	14 d	10 d
1	Control	5% Conv.	10% Conv.	Control	5% High 18:1	10% High 18:1
2	Control	5% High 18:1	10% High 18:1	Control	5% Conv.	10% Conv.

Table 2. Ingredient and nutrition composition of diets comparing conventional (Conv.) to high oleic (C18:1) roasted soybeans.

Item	Pretrial & Washout	Conv. Soybean		High 18:1 Soybean	
		5%	10%	5%	10%
Feed Ingredient, % of DM					
Corn Silage	44.1	45.0	42.4	45.0	42.4
Alfalfa Haylage	14.7	15.0	14.1	15.0	14.1
Grass Hay/Straw	2.6	2.6	2.6	2.6	2.6
Ground Corn	13.2	11.8	12.3	11.8	12.3
Canola Meal	12.3	12.3	10.1	12.3	10.1
Heat treated SBM	0.88	0.88	0.88	0.88	0.88
Conv. Roasted Soybeans	5.3	5.3	10.5	-	-
Plenish Roasted Soybeans	-	-	-	5.3	10.5
Molasses	3.5	3.5	3.5	3.5	3.5
Vit/Min Suppl.	1.8	1.8	1.8	1.8	1.8
Soybean meal	1.3	1.3	1.3	1.3	1.3
Heat treated soy meal[1]	0.88	0.88	0.88	0.88	0.88
NPN[2]	0.44	0.44	0.44	0.44	0.44
Nutrient, % of DM[3]					
NDF	30.9	31.2	28.5	31.2	28.5
ADF	20.8	20.9	19.1	20.9	19.1
CP	17.0	17.1	17.8	17.1	17.8
Starch	25.0	24.3	26.0	24.3	26.0
Sugar	5.7	5.7	6.4	5.7	6.4

[1]Fed as AminoPlus (Ag Processing Inc.; Donated by ADM Alliance Animal Nutrition).

[2]Coated urea (Optigen, Alltech Inc.).

[3]Balanced for composition based on forage analysis before experimental periods.

Table 3. Effect of conventional (Conv.) and high oleic (C18:1) Plenish roasted soybean when fed at 5 and 10% of the diet on milk production and milk fatty acid profile.

	Treatment Means[1]							
	Conv. Soybean		High 18:1 Soybean			*P*-Values[2]		
Item	Low	High	Low	High	SEM	Type	Level	Type* Level
Milk, kg/d	43.8	43.7	43.4	44.8	1.28	0.69	0.28	0.18
Milk Fat								
%	3.28	3.46	3.42	3.66	0.12	<0.05	0.01	0.69
g/d	1393	1464	1461	1574	108	0.08	0.01	0.55
Milk Fatty acids, % FA								
<16C[3]	27.2	26.2	27.1	26.5	0.39	0.60	0.01	0.47
16C[4]	29.8	26.9	29.4	27.0	0.56	0.52	<0.001	0.54
>16C[5]	37.4	41.5	37.8	41.5	0.70	0.42	<0.001	0.57
trans-10 C18:1	0.79	0.89	0.62	0.63	0.13	0.01	0.96	0.67
trans-11 C18:1	0.97	1.17	0.82	0.94	0.07	<0.001	<0.001	0.27
cis-9 C18:1	18.4	19.5	20.4*	22.2*	0.5	<0.001	<0.001	0.35
OBCFA[6]	3.88	3.37	4.13*	3.66*	0.09	<0.001	<0.001	0.76
Milk Protein								
%	3.04	3.05	3.06	3.08	0.06	0.24	0.34	0.85
g/d	1291	1321	1309	1330	41	0.71	0.34	0.88
MUN, mg/dL[7]	11.56	12.94	11.98	12.74	0.45	0.74	0.06	0.57
Feed intake, kg/d	27.5	28.9	27.9	29.4	1.12	0.20	<0.001	0.98

[1]Treatments were conventional (Conv.) and high oleic (C18:1) soybeans fed in at 5% in a higher forage diet (Low) or 10% in a lower forage and higher starch diet (High).

[2]Main effect of type of high oleic soybeans (type) and soybean feeding level (level) and their interaction.

[3]Fatty acids <16 C originate from de novo synthesis in the mammary gland.

[4]16 carbon fatty acids originate both from de novo synthesis and uptake of preformed fatty acids.

[5]Fatty acids >16 C orginate from update of preformed fatty acids from the plasma.

[6]Odd and branch chain fatty acids (OBCFA) that originate either from microbial synthesis or de novo synthesis from odd and branch chain fatty acids.

[7]Milk urea nitrogen (MUN).