Effects of Supplementation of a Combination of Palmitic and Stearic Acids on Milk and Component Production: A Meta-Analysis

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Introduction

Supplementing the lactating cow ration with a high-energy fat source is a widely adopted strategy that is commonly used to improve energy intake, milk and component production, and reproductive efficiency. A wide array of fat sources have been fed to lactating cattle in recent years including oilseeds such as cottonseed and soybeans, animal fats such as tallow, palm oil products, and various modified fat sources that have been designed to reduce or eliminate availability of unsaturated fatty acids to biohydrogenation in the rumen (Rabiee et al., 2012).

Previous authors have used meta-analytical methods as a means to determine productive and reproductive responses to specific types of supplemental fat or to dietary fat in general. Allen (2000) investigated effects of fat source, fatty acid chain length, degree of fatty acid saturation, and fatty acid esterification on dry matter intake (**DMI**) in lactating cows, and concluded that DMI is affected differently by varying fat sources, and that DMI decreases with increasing proportion of unsaturated fatty acids in the diet. Rabiee et al. (2012) used meta-analysis and meta-regression to determine the effects of supplementation with fats on milk production and components by dairy cows. Five groups of fats were evaluated including tallows, calcium salts of palm fat (Megalac; Church and Dwight Co. Inc., Princeton, NJ), oilseeds, prilled fat, and other calcium salts. The authors concluded that fat supplementation did improve milk yield (**MY**), but the results were heterogeneous across fat groups. All fat groups aside from prilled fats decreased DMI. Several fat groups were also shown to decrease milk fat (**MF**) percentage, while no fat groups influenced milk protein (**MP**) production. Rodney et al. (2015) investigated the relationship between dietary fat and fertility in dairy cattle. The authors concluded that, overall, inclusion of fat in the ration does improve fertility, with varying conclusions for oilseeds, calcium salts of fatty acids, tallow, and conjugated linoleic acid. Most recently, de Souza et al. (2016) conducted a meta-analysis and meta-regression to determine the effects of highly enriched palmitic acid supplements in late lactation dairy cows. The authors reported that MF percentage, MF yield, NDF digestibility, and fatty acid digestibility were increased with palmitic acid feeding; however, MY, DMI, body weight, and body condition score were unaffected by palmitic acid supplementation.

While the above-mentioned studies provide a thorough explanation of some general effects of dietary and supplemental fat on DMI, production, and reproduction, they do not thoroughly explore these topics in regards to supplementation with a

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combination of palmitic (C16:0) and stearic (C18:0) fatty acids. The paper by Allen (2000) limits inferences on DMI to oilseeds, unprocessed animal fat, hydrogenated triglycerides and fatty acids, and calcium salts of palm fatty acids. The paper by Rabiee et al. (2012) investigates prilled fats but gives no inference into effects of specific fatty acid profiles. Moreover, Rabiee et al. (2012) opted to exclude crossover and Latin square designs from their analysis, so only 3-4 prilled fat comparisons were included in their analysis. The paper by Rodney (2015) eschews prilled fats altogether. Finally, the de Souza et al. (2016) paper investigated the effects of highly enriched palmitic acid products alone, and did not include blended C16:0 and C18:0 supplemental fats in the analysis. With this in mind, the objective of the current analysis was to use meta-analytic methods to examine intake, milk production, milk component, and efficiency responses when lactating cows were supplemented with a prilled fat containing a blend of C16:0 and C18:0 fatty acids.

Materials and Methods

Selection Criteria

The initial selection criteria for inclusion in the primary data set were studies that reported DMI, MY, and milk component concentration and yield measurements in lactating dairy cows when a diet containing no added fat was compared to a diet containing supplemental fat in the form of prilled free fatty acids containing a blend of C16:0 and C18:0 fatty acids. Studies that did not report a measure of variability were then excluded from the data set as recommended by Borenstein et al. (2009). Although Lean et al. (2009) caution against using studies with crossover or Latin square designs due to potential carryover effects and effects of stage of lactation, the authors opted to include these studies, as the number of studies meeting criteria for analysis decreases drastically if these study types are excluded, and the goal of the current analysis was to summarize all available data. The final data set consisted of 25 studies comprising 73 treatment means published in peer-reviewed journals. The means included 39 treatments containing supplemental fat and 34 treatments that did not contain supplemental fat. Descriptive information on the individual studies and treatments included in the data set are reported in **Table 1**.

Data Extraction

Data extracted from qualifying studies included journal, year of publication, authors, trial design, length of trial feeding period, number of cows in control and treatment groups, amount of fat supplemented (g/d and % of dietary DM), DMI (kg/d), net energy (**NE**) intake (Mcal/d), MY (kg/d), MF percentage and yield (kg/d), MP percentage and yield (kg/d), milk lactose (**ML**) percentage and yield (kg/d), 3.5% fat-corrected milk (**FCM**) yield (kg/d), and ratio of 3.5% FCM to DMI (kg/kg per d). A measure of variation (**SD or SE**) was also recorded for each production variable.

Statistical Analysis

All statistical analysis was performed using R statistical software (R Core Team, 2016) and all meta-analysis was performed using the ‘metafor’ package in R

(Viechtbauer, 2010) following guidelines set forth by Lean et al. (2009) and Borenstein et al. (2009). Dry matter intake, NE intake, MY, milk component concentration and yield, 3.5% FCM, and 3.5% FCM to DMI data were analyzed via raw mean difference, which was calculated by subtracting the mean for the control group from the mean for the treatment group. Resulting positive raw mean differences favored treatment groups whereas resulting negative raw mean differences favored control groups. In cases where separate standard deviation or standard errors were reported for control and treatment cows, the appropriate designation for each was recorded for the meta-analysis. If only pooled standard deviations or standard errors were reported, then the pooled version of each was recorded. As all included studies vary in terms of days in milk, diet composition, genetics, etc., the authors opted to use random effects models utilizing the inverse of the variance for weighting as recommended by Borenstein et al. (2009). Estimates of effect size, 95% confidence intervals, and statistical significance of effect size were estimated for each production response. P-values corresponding to effect size significance were estimated using the method of Knapp and Hartung (2003), which provides more conservative estimates when number of studies is small. Mean differences and associated confidence intervals were visualized using forest plots (not shown).

Variation among studies was quantified using the I^2 statistic and assessed for statistical significance using a chi-square test of heterogeneity (Borenstein et al., 2009). The I^2 statistic estimates the proportion of total variation in effect size estimates that is due to heterogeneity. Negative I^2 values were adjusted to 0 so that all I^2 estimates were between 0 and 100 percent. An I^2 value greater than 50 percent may be indicative of substantial heterogeneity (Rabiee et al., 2012).

Publication bias was assessed visually via funnel plots (not shown). Briefly, a funnel plot is a scatter plot of effect size estimates versus their respective estimates of precision. If many large and small studies have been conducted, small, imprecise studies should be scattered around the average effect size, and studies should narrow in on the average effect size as study size and precision increase resulting in a symmetrical ‘funnel’ of data points. If publication bias exists (negative or unfavorable studies tend to not be published), the plot will appear asymmetrical with a large gap at the bottom of the plot.

Results / Discussion

Data Review and Description

All data extracted and analyzed in the meta-analysis are described in **Table 1**. As shown, multiple studies had more than one mean comparison due to multiple fat supplementation levels, changes in other dietary parameters, or similar circumstances that allowed for such. Mean comparisons were performed between control diets and treatment diets within studies that only differed in supplemental fat inclusion. Data were excluded due to non-reported estimates of variance (SE or SD) and/or differing compositions of diets in the control and treatment groups. Tests of heterogeneity, the I^2 statistic and resulting χ^2 *P* – value are reported in **Table 2**. The I^2 statistic was ≥ 44 for

all response variables except 3.5% FCM, indicating that moderate to large variation existed among mean differences for most variables, likely attributable to differences among studies in breed, stage of lactation, diet composition, reproductive status, etc. The χ^2 ($\alpha = 0.10$ due to low power) test indicated that variation among mean differences was greater than 0 for all variables. Visual analysis of funnel plots suggested minimal to no presence of publication bias.

Production Outcomes

The effects of supplementation with a combination of C16:0 and C18:0 fatty acids on DMI and NE intake, milk production, milk composition, milk component yield, and 3.5% FCM feed efficiency are shown in Table 2. The weighted average supplemental fat intake for each variable is indicated and ranged from 524 g to 645 g, well in excess of typical supplemental fat feeding rates observed on most commercial dairies. Supplementation with a combination of C16:0 and C18:0 fatty acids did not reduce DMI (-0.06 kg/d; *P* = 0.7481). Allen (2000) reported linear reductions in DMI with increasing inclusion of oilseeds, unprocessed animal fat, and calcium salts of palm fatty acids, but failed to detect a relationship between inclusion of hydrogenated fats and DMI reduction, and speculated that the observed differences in DMI reduction may be due to differences in fatty acid chain length and degree of saturation. In agreement, Rabiee et al. (2012) reported that fat supplementation, irrespective of fat source, decreased DMI by 0.875 kg/cow per day. When effects on DMI were analyzed individually by fat source, significant reductions in DMI were observed for tallow, Megalac, oilseeds, and other calcium salts but were not observed for prilled fat (-0.088 kg/d; *P* = 0.717). Rodney et al. (2015) reported that DMI was improved with oilseed supplementation (0.15 kg/d), and decreased with supplementation of calcium salts of fatty acids, tallow, and conjugated linoleic acid (**CLA**) (-0.22, -0.72, and -0.63 kg/d for calcium salts, tallow, and CLA, respectively). A significant increase in NE intake was also observed in the current study (2.13 Mcal/d; *P* = 0.0048), and is likely due to increased energy density of the ration with fat supplementation paired with little or no decrease in DMI.

Milk yield and 3.5% FCM yield increased by 1.24 (*P* = 0.0001) and 1.38 (*P* = 0.0004) kg/d, respectively. Reported effects of supplemental fat on MY are variable. Rabiee et al. (2012) reported that milk production improved by 0.244 kg/d (*P* = 0.006) with fat supplementation, but the effect was only significant for Megalac and other calcium salts and was not significant for prilled fat. Contrastingly, Rodney et al. (2015) reported a non-significant increase of 0.33 kg/d with general supplemental fat feeding, and a significant improvement only with feeding of calcium salts of fatty acids (0.73 kg/d). Purified palmitic acid fat supplements were also shown to not improve MY but did improve 3.5% FCM via an increase in milk fat percentage (de Souza et al., 2016).

Milk fat percentage (0.08%; *P* = 0.0093) and yield (0.06 kg/d; *P* = 0.0001) both increased with C16:0 and C18:0 fat supplementation. This increase is likely attributable to increased fatty acid intake and post-ruminal absorption. Moreover, feeding highly saturated fat sources such as a combination C16:0 and C18:0 has little to no negative impact on rumen VFA production or milk fatty acid synthesis in the mammary gland compared with unsaturated fatty acids. Rabiee et al. reported a similar improvement in

MF percentage (0.096%) and MF yield (0.062 kg/d) with prilled fat supplementation, while Rodney et al. (2015) found no change in MF percentage or yield with fat supplementation regardless of source.

Milk protein percentage was not different (-0.02%, $P = 0.3363$) but MP yield was increased (0.03 kg/d; $P = 0.0008$) with supplementation of a combination of C16 and C18 fatty acids. Rabiee et al. (2012) reported that prilled fat supplementation did not affect MP percentage (-0.017%; $P = 0.458$) or MP yield (0.009 kg/d; $P = 0.648$), but MP percentage was decreased by all other fat types and was decreased overall with fat supplementation (-0.077%, $P < 0.001$). Contrastingly, Rodney et al. (2015) reported no change in MP percentage or yield with fat supplementation regardless of source.

Supplementation with a combination of C16:0 and C18:0 tended to decrease ML concentration (-0.04%, $P = 0.0612$), but did not affect ML yield (0.05 kg/d; $P = 0.1553$). Other recent meta-analyses did not include ML percentage or concentration as variables of interest.

The amount of 3.5% fat-corrected milk produced per kilogram of feed intake was also improved (0.06 kg/kg per d; $P = 0.024$), an increase that again can be attributed to improved milk and component yields coupled with no change in DMI.

Conclusions

This meta-analysis is intended to summarize the production responses that have been observed when lactating dairy cows were supplemented with a combination of C16:0 and C18:0 fatty acids. The production responses observed across studies are largely heterogeneous, as indicated by moderate to large I^2 values. This analysis did not control for effects of breed, stage of lactation, diet composition, environment, etc. Nonetheless, when all studies are included in the analysis, C16:0 and C18:0 fatty acid supplementation generally had positive effects on production outcomes despite very high levels of fat supplementation. Dry matter intake and NE intake were both improved, as were MY and 3.5% FCM yield. Milk fat percentage increased by 0.08% while MP and ML percentages did not change. Yields of MF and MP were also increased. Supplementation with a combination of C16:0 and C18:0 fatty acids appears to yield significant improvement in production without harming DMI or NE intake, and may be a promising means to improving dairy cow production and energy balance that warrants further investigation.

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Table 1. Description of studies and treatments included in the analysis

Study	N cows per trt	Treatment name; treatment category (Supplemental Fat % of DM)
Grummer, 1988	4	1. Control; control (0%)
	4	2. LPF (low prilled fat); supplemental fat (3.8%)
	4	3. HPF (high prilled fat); supplemental fat (5.2%)
Schauff and Clark, 1989	4	1. Control (Experiment 1); control (0%)
	4	2. LPF (low prilled fat; Experiment 1); supplemental fat (3.6%)
	4	3. HPF (high prilled fat; Experiment 1); supplemental fat (4.9%)
	6	4. Control (Experiment 2); control (0%)
	6	5. PF (prilled fat; Experiment 2); supplemental fat (2.4%)
Skaar et al., 1989	10	1. Control; control (0%)
	9	2. Fat; supplemental fat (5%)
	10	3. Niacin; control (0%)
	10	4. Fat + niacin; supplemental fat (5%)
Wu et al., 1993	6	1. Control; control (0%)
	6	2. PF (prilled fat); supplemental fat (2.5%)
Wu et al., 1994	6	1. WCS (whole cottonseed); control (0%)
	6	2. WCSPT (whole cottonseed prilled tallow); supplemental fat (2.2%)
	6	3. WCSPT+ (whole cottonseed prilled tallow plus); supplemental fat (4.4%)
Elliott et al., 1995	16	1. High NSC (Experiment 1); control (0%)
	16	2. High NSC plus fat (Experiment 1); supplemental fat (2.5%)
	16	3. Low NSC (Experiment 1); control (0%)
	16	4. Low NSC plus fat (Experiment 1); supplemental fat (2.5%)
	8	5. High NSC (Experiment 2); control (0%)
	8	6. High NSC plus fat (Experiment 2); supplemental fat (2.5%)
	8	7. Low NSC (Experiment 2); control (0%)
	8	8. Low NSC plus fat (Experiment 2); supplemental fat (2.5%)
Elliott et al., 1996	5	1. Control; control (0%)
	5	2. Prilled FA; supplemental fat (5%)
Grum et al., 1996	8	1. Low concentrate; control (0%)
	8	2. Low concentrate plus fat; supplemental fat (3%)
	8	3. High concentrate; control (0%)
	8	4. High concentrate plus fat; supplemental fat (3%)
Chan et al., 1997a	4	1. Medium Fat plus Low Quality Protein; control (0%)
	4	2. High Fat plus Low Quality Protein; supplemental fat (2.5%)
	4	3. Medium Fat plus High Quality Protein; control (0%)
	4	4. High Fat plus High Quality Protein; supplemental fat (2.5%)

Table 1. Description of studies and treatments included in the analysis (cont.)

Study	N cows per trt	Treatment name; treatment category (Supplemental Fat % of DM)
Chan et al., 1997b	6	1. Medium Fat plus Shade; control (0%)
	6	2. High Fat plus Shade; supplemental fat (3%)
	6	3. Medium Fat plus Evaporative Cooling; control (0%)
	6	4. High Fat plus Evaporative Cooling; supplemental fat (3%)
Simas et al. 1998	8	1. DRS (dry rolled sorghum); control (0%)
	8	2. DRS + 2.5% FA; supplemental fat (2.5%)
	8	3. SFS (steam flaked sorghum); control (0%)
	8	4. SFS + 2.5% FA; supplemental fat (2.5%)
	8	5. SFS + 5% FA; supplemental fat (5%)
Harvatine and Allen, 2006a,b,c	8	1. Control, cannulated cows; control (0%)
	8	2. SFA (saturated fatty acids), cannulated cows; supplemental fat (2.5%)
	8	3. Control, non-cannulated cows; control (0%)
	8	4. SFA (saturated fatty acids), non-cannulated cows; supplemental fat (2.5%)
Moallem et al., 2007a	14	1. Control; control (0%)
	14	2. PrFA:PrFA; supplemental fat (1.25%)
Moallem et al., 2007b	14	1. Control; control (0%)
	13	2. PrFA; supplemental fat (1.9%)
Relling and Reynolds, 2007	4	1. Control; control (0%)
	4	2. SFA (saturated fatty acids); supplemental fat (3.5%)
Thering et al., 2009	5	1. Control; control (0%)
	6	2. EB100 (Energy Booster 100); supplemental fat (3.5%)
Weiss & Pinos-Rodríguez, 2009	18	1. High forage - fat; control (0%)
	18	2. High forage + fat; supplemental fat (2.25%)
	18	3. Low forage - fat; control (0%)
	18	4. Low forage + fat; supplemental fat (2.25%)
Wang et al., 2010	16	1. SFA0 (saturated fatty acids 0%); control (0%)
	16	1. SFA1.5 (saturated fatty acids 1.5%); supplemental fat (1.5%)
	16	1. SFA3 (saturated fatty acids 3%); supplemental fat (3%)
Weiss et al., 2011	8	1. Control; control (0%)
	8	2. SFA (saturated fatty acids); supplemental fat (3%)
Bernard et al., 2012	16	1. Control; control (0%)
	16	2. SAT (saturated fat); supplemental fat (1.67%)
Greco et al., 2012	10	1. CTL; control (0%)
	10	2. SFA (saturated fatty acids); supplemental fat (1.7%)
Piantoni et al., 2015a,b	12	1. 20% fNDF + 0% SFFA; control (0%)
	12	2. 20% fNDF + 2% SFFA; supplemental fat (2%)
	12	3. 26% fNDF + 0% SFFA; control (0%)
	12	4. 26% fNDF + 2% SFFA; supplemental fat (2%)

Table 2. Estimated mean difference and 95% CI for dry matter and net energy intake, milk production, milk component concentration and yield, and feed efficiency in dairy cattle supplemented with a combination of C18:0 and C16:0 free fatty acids versus a no fat control.

			Parameter				[3]Heterogeneity	
Item	[1]N	[2]Supplemental fat (g/d)	Mean Difference	SE	*P* - value	95% CI	I^2	*P* - value
Intake								
DMI (kg/d)	40	632 ± 222.4	-0.06	0.181	0.7481	(-0.40, 0.28)	92.67	0.0001
NE_L intake (Mcal/d)	13	577 ± 249.8	2.13	0.617	0.0048	(0.79, 3.48)	97.41	0.0001
Milk Production								
Milk yield (kg/d)	39	596 ± 216.3	1.24	0.260	0.0001	(0.71, 1.76)	57.00	0.0001
3.5% FCM (kg/d)	21	631 ± 258.3	1.38	0.327	0.0004	(0.70, 2.06)	27.78	0.0699
Milk Composition								
Milk fat (%)	39	628 ± 227.5	0.08	0.028	0.0093	(0.02, 0.13)	55.51	0.0001
Milk protein (%)	39	645 ± 216.6	-0.02	0.017	0.3363	(-0.05, 0.02)	82.93	0.0001
Milk lactose (%)	23	551 ± 197.7	-0.04	0.021	0.0612	(-0.09, 0.00)	76.53	0.0001
Milk Component Yield								
Milk fat yield (kg/d)	38	645 ± 232.8	0.06	0.012	0.0001	(0.04, 0.09)	73.65	0.0001
Milk protein yield (kg/d)	38	624 ± 225.7	0.03	0.007	0.0008	(0.01, 0.04)	49.60	0.0044
Milk lactose yield (kg/d)	14	613 ± 150.5	0.05	0.031	0.1553	(-0.02, 0.11)	79.23	0.0002
Efficiency								
3.5% FCM/DMI (kg/kg per d)	11	524 ± 177.7	0.06	0.024	0.0244	(0.01, 0.12)	44.60	0.0462

[1] N = number of comparisons included in analysis.
[2] Average supplemental fat feeding rate (g/d) ± SD; weighted based on inverse variance of response variable.
[3] Heterogeneity indicates how much variation exists among treatment differences; I^2 estimates what proportion of total variation in mean differences is attributable to among-means variation.

SESSION NOTES