Effects of a dairy protein product on dry matter intake and yield of milk and milk components of Holstein cows.

Final Report
Submitted to
CARGILL CORN MILLING
BLAIR, NE

By

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This study was conducted according to a protocol approved by the Institutional Animal Care and Use Committee at Michigan State University (Application #:03/10-023-00).

SUMMARY:
This study evaluated the lactational response to a dairy protein product (DPP) from the corn milling industry fed to cows varying in milk yield. The experiment utilized a crossover design with a preliminary period of 14 d followed by two treatment periods of 22 d using 32 multiparous Holstein cows past peak lactation. The treatment ration (DPPR) included DPP at ~30% of diet DM partially substituted for soybean meal, corn grain and corn silage compared to the control ration (CONR). DPPR increased DMI as well as yields of milk, fat, protein, and SNF compared to CONR ($P < 0.01$) and tended to reduce feed conversion efficiency slightly. No interactions were detected between response to treatment and milk yield of cows.

II. OBJECTIVE:
Determine the lactational response to a dairy protein product from the corn milling industry and how response varies by level of milk production.

III. STUDY DESIGN:
A) Experimental Design:
Thirty-two multiparous Holstein cows from the Michigan State University Dairy Field Lab were randomly assigned to sequence in a crossover design experiment (Table 1). Cows were selected from the herd to provide as wide of a range and uniform distribution of milk yield as possible (Table 2) to determine possible linear or quadratic effects of response to treatment diets to level of milk yield. Prior to the initial treatment period, all cows were fed a 50:50 mix of treatment diets for 14 d. The purpose of this preliminary period was to obtain baseline values for DMI, milk yield and milk composition. Following the preliminary period, half of the animals (n = 16) received DPPR for 22 d followed by CONR for 22 d. The other half of the animals (n = 16) received CONR for 22 d followed by DPPR for 22 d. Initially, each treatment period was scheduled to last 21 d but a protocol deviation necessitated the addition of one more collection day per treatment period (See *Study Protocol Deviation #: 002*).

B) Treatments:
Treatments included a ration including DPP at ~30% of diet DM (**DPPR**) and control ration (**CONR**). The DPP was provided by Cargill Corn Milling and contained ~67.4% DM, ~27.5% crude protein and ~40% NDF (Table 3). A shipment of 11.5 metric tons arrived at the MSU Dairy Field Lab on October 13, 2010 from Dayton, OH and was immediately stored in an Ag Bag.

C) Experimental Diets:
The ingredients and nutrient composition of the experimental diets are shown in Table 4. The diet ingredients for CONR consisted of corn silage, alfalfa silage, alfalfa hay, cottonseed with lint, dry ground corn grain, SoyPLUS®, soybean meal, and a vitamin-mineral mix. The DPPR included DPP at approximately 30% of the dietary DM by replacing most of the protein supplement and some of the corn silage and dry ground corn grain of the CONR. Initially, the DPP was intended to replace all protein supplement (soybean meal and SoyPLUS®) plus a portion of the corn silage and corn grain. However, the protein concentration of several feeds decreased during the preliminary period so soybean meal was added to make DPPR isonitrogenous to CONR (See *Study Protocol Deviation #: 001*).

IV. MATERIALS AND METHODS:

A) Animals:

Thirty-two multiparous Holstein cows were selected from the herd at the Michigan State University Dairy Field Lab and were in good health. Cows averaged 135 DIM with a mean milk yield of 44.6 kg/d at the start of the experiment (Table 1). The mean parity, body weight (kg) and body condition score of trial animals at the start of the study were 2.7, 682 and 2.25, respectively (Table 1).

B) Facilities and Equipment:

This study was conducted at the Michigan State University Dairy Field Lab in Lansing, Michigan. Animals were randomly assigned to stall in the North Barn and stall assignments were maintained throughout the study. Cows were fed in individual mangers during the study. The feed mangers are painted cement flooring with plastic dividers and foldable plastic extensions to prevent mixing of adjacent diets. Each feed manger is also fitted with a lock-out door. Prior to each milking, all feed lock-out doors were closed before cows were released to prohibit consumption of any other cow's diet. Lock-out doors remained closed until all cows within a given barn returned from the milking parlor and were secured in their pre-assigned stall. Every other stall divider was equipped with an automatic bowl-style water trough accessible to each cow on either side of the divider. All waterers were fitted with overflow buckets to minimize the incidence of wet feed and stalls. Stalls were bedded with sawdust over mattresses. All experimental diets were mixed daily using a Knight Auggie Reel Mixer (Model 3030) and diets were fed using feed carts with scales.

C) Animal Management:

Cows were moved to their assigned stalls on d 0 of the preliminary period. Cows were fed at 110% of expected intake once per day at 1200 h and blocked from feed at 1000 h each day of the study. Cows were milked twice per day at approximately 0400 and 1600 h. A study protocol deviation occurred on 11/15/10 when three cows were inadvertently not milked at the AM milking just prior to the start of the first treatment collection period (See *Study Protocol Deviation #: 002*). As a result, only the last three days of milk production data were analyzed for these cows from the first treatment collection period. Stalls were cleaned and new sawdust bedding was added twice per day while cows were in holding prior to each milking. Standard herd reproduction checks and breeding practices were maintained during this study for trial animals. Animals were returned to the general herd at the MSU Dairy Field Lab following the sampling and removal of orts on 12/12/10.

D) Data and Sample Collection:

Milk yield data was collected on d 11 through 14 of the preliminary period and on d 18 through d 22 of each treatment period. One milk sample was taken at each milking from d 11-14 of the preliminary period and from d 18-22 of each treatment period for the determination of milk composition including fat, protein and lactose concentrations by near infrared spectroscopy and MUN (Michigan DHIA, East Lansing, MI). A second milk sample was taken at each milking for each cow from d 18-22 of each treatment period for determination of milk fatty acid profile. Body weights were measured on two consecutive days at the end of the preliminary period and on the last two days of each treatment period between 0700 and 0800 h. Body condition scores were evaluated by three trained individuals and recorded at the end of the preliminary period and on the last day of each treatment period. The amount of feed offered and refused was recorded daily throughout the study. Samples of feed ingredients, TMRs and orts (feed refused) were collected on d 11-14 of the preliminary period and on d 18-22 of each treatment period for determination of dry matter and nutrient intakes and to qualify the preliminary and treatment diets. All samples were stored in a -20°C freezer until the end of the study.

E) Sample and Statistical Analysis:

Samples of feed ingredients, TMRs and orts were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All dried samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Ash concentration was determined after 5 h of oxidation at 500°C in a muffle furnace. Concentration of NDF was determined (Mertens, 2002) and crude protein analyzed according to Hach et al. (1987). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide; glucose concentration will be measured with a glucose oxidase method (Glucose kit #510; Sigma Chemical Co., St. Louis, MO) and absorbance will be determined with a microplate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Concentrations of all nutrients except for DM were expressed as percentages of DM. Particle size of TMR samples was determined using the three-sieve version of the Penn State Particle Size Separator (Lammers et al., 1996).

Milk samples were analyzed for fat, true protein, and lactose by mid-infrared spectroscopy (AOAC, 1990) by Michigan DHIA (East Lansing, MI) and FCM (3.5%) and solids-corrected milk yield was calculated (Tyrrell and Reid, 1965). Milk samples used for analysis of fatty acid profile were composited based on milk fat yield and centrifuged at 17,800 x g for 30 min at 8˚C. Fat cake (300-400 mg) was extracted according to Hara and Radin (1978) and methyl esters formed according to Christie (1982) as modified by Chouinard et al. (1999). Fatty acids were quantified by gas chromatography (Clarus 500, Perkins-Elmer Corp, Norwalk, CT) according to Kramer et al. (1997) using a SP-2560 capillary column (100 m x 0.20 mm id with 0.02-μm film thickness; Supelco, Bellefonte, PA). Oven temperature was 70˚C for 4 min, then ramped 13˚C/min to 175˚C and held for 27 min before being ramped again at 4˚C/min to 215˚C and held for 31 min. Helium flow was 20 cm/sec with a total run time of 80 min.

Data was analyzed using the fit model procedure of JMP (version 8.0.2, SAS Institute, Cary, NC) using the REML method according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + P_j\,T_k + pFCM_i + T_k\,pFCi_k + pFCM_i^2 + T_k\,pFCM_i^2 + e_{ijk}$$

where:
μ = overall mean,
C_i = random effect of cow (i = 1 to 32),
P_j = fixed effect of period (j = 1 to 2),
T_k = fixed effect of treatment (k = 1 to 2),
P_jT_k = period x treatment,
$pFCM_i$ = effect of preliminary 3.5% FCM,
$T_k\,pFCM_i$ = treatment x pFCM, and
$pFCM_i^2$ = quadratic effect of preliminary 3.5% FCM,
$T_k\,pFCM_i^2$ = treatment x $pFCM^2$, and
e_{ijk} = residual, which was assumed to be normally distributed.

Significance for main effects were declared at or below $P = 0.05$ and tendencies for main effects were declared at or below $P = 0.10$. The significance of the Trt x pFCM terms was also evaluated.

Particle size data was analyzed using the t-test procedure of JMP (version 9.0.0, SAS Institute, Cary, NC).

V. RESULTS AND DISCUSSION:

The dry matter percent of the DPP was lower for period 1 compared to period 2. This is because it started raining when the AgBag was being filled and the Ag Bag was opened and fed from the end that was closed first. The greater DM concentration for the toluene distillation method was likely because it retained fermentation acids volatilized by over drying.

As expected, nutrient composition of experimental diets differed in both starch and NDF concentrations (Table 3). Specifically, CONR was 7.9 percentage units higher in starch and 6.2 percentage units lower in NDF compared with DPPR as a result of replacing dry ground corn and corn silage with the DPP. Dietary crude protein concentrations were similar for DPPR and CONR. The distribution of DM of rations differed by treatment with a slightly greater fraction passing the screen with the 8mm aperture and a much greater fraction of the NDF passing this screen. However, the fraction of NDF retained and passed through this screen was much greater (Table 5).

Results for performance data are presented in Table 6. No interactions between treatment and linear or quadratic effects of preliminary fat-corrected milk yield were observed for any variable evaluated in this study. The DPPR compared with CONR, increased yields of milk, milk fat, milk protein, milk lactose, solids-not fat (**SNF**) and 3.5% fat-corrected milk as well as percentage of milk fat, milk protein and SNF in milk. No treatment differences were observed for concentrations of lactose, or somatic cell count in milk between treatments.

DPPR increased DMI compared with CONR in agreement with experiments that fed corn-milling products at greater concentrations than the present study (Kononoff et al, 2006; Mullins et al, 2010). Increased feed intake in this and other experiments might be because the corn-milling products were partially substituted for forage and cereal grain. Feed intake might be improved by replacement of forage because forage NDF is more filling than NDF from other feed sources (Allen, 2000) and by replacement of starch because propionate produced by ruminal fermentation of starch can reduce feed intake by stimulating hepatic oxidation (Allen 2000; Allen et al., 2009).

DPPR tended to decrease feed conversion efficiency (3.5% FCM/DMI) slightly compared to CONR which is consistent with previous results when corn milling products were included in a ration at 30% of the diet DM (Gehman and Kononoff, 2010). The reduction in feed conversion efficiency might be because DPPR partitioned more energy to body reserves; DPPR tended to increase body weight ($P = 0.09$). The increased energy from greater DMI for DPPR appeared to be utilized for both milk production and body energy reserves in this experiment, although no difference between treatments was detected for body condition score. Alternatively, it is possible that the reduction in feed conversion efficiency was because of lower energy concentration of DPPR compared with CONR (not measured).

Milk fatty acid profiles for each treatment are shown in Table 7. Although DPPR increased total trans C18:1 FA it decreased C18:1 trans-10 compared to CONR. While C18:1 trans-10 does not cause milk fat depression per se, it is considered to be a good marker for FA that cause milk fat depression that are more difficult to detect (Lock et al., 2007). Lower concentrations for DPPR might be related to its effect on yield and percentage of milk fat compared to CONR. However, the CLA directly linked to milk fat depression including trans-10 cis-12 CLA were below our limits of detection (0.01% of FA) for all samples in this experiment.

VI. CONCLUSIONS AND IMPLICATIONS:

The DPP evaluated in this study can effectively replace much of the protein and starch from ingredients typically utilized in dairy cattle rations when included at approximately 30% of the dietary DM. In the current study, the 2.1 kg/d increase in 3.5% FCM for multiparous cows fed the DPP diet was independent of milk production level.

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TABLES

Table 1. Mean parity, DIM, milk yield, body weight, and BCS of cows at beginning and end of the experiment.

	Beginning		End	
	Mean	SD	Mean	SD
Parity	2.7	1.1	—	—
DIM	135	51.4	179	51.4
Milk, kg	44.6	7.34	42.1	7.49
BW, kg	682	63.3	700	67.6
BCS	2.25	0.54	2.54	0.65

Table 2. Range of milk yield and DMI of cows during the covariate period.

	Range
Milk, kg/d	29.4 – 62.0
3.5% FCM, kg/d	30.7 – 69.2
DMI, kg/d	16.7 – 33.4

Table 3. Nutrient composition of the dairy protein product by period.

	Period 1	Period 2
DM, % (55° C)	66.1	68.7
DM, % (toluene distillation)	69.0	72.1
Starch, % of DM	7.5	5.8
NDF, % of DM	37.9	41.8
CP, % of DM	27.8	27.2

Table 4. Ingredients and nutrient composition of treatment rations[1]

Item	Prelim	CONR	DPPR
Ration ingredient, % of DM			
Corn silage	28.3	32.7	26.1
Dry ground shelled corn	19.4	20.5	10.2
Dairy protein product	14.5	—	29.5
Alfalfa silage	11.1	10.6	10.6
Mineral and vitamin mix[2]	8.6	8.3	8.3
Cottonseed	6.2	6.1	6.1
Alfalfa Hay	5.7	5.1	5.1
Soybean meal (48% CP)	5.0	14.3	4.0
SoyPlus[3]	1.2	2.3	—
Nutrient Composition,[4] %			
DM	51.41	55.5	55.2
Starch	27.1	27.7	19.9
NDF	33.4	30.4	36.6
CP	16.0	17.6	18.1

[1]Prelim = Preliminary period diet, CONR = control ration, DPPR = dairy protein product ration. Values other than DM are expressed as a percentage of dietary DM.

[2]Mineral and vitamin mix contained 61.0% corn grain, 11.5% limestone, 7.3% sodium bicarbonate, 6.5% dicalcium phosphate, 3.1% magnesium sulfate, 3.1% tallow, 3.0% urea, 2.8% white salt, 0.7% biotin (640 mg/lb), 0.5% trace minerals, 0.3% selenium yeast, 0.1% Rumensin 90 and 0.4% vitamin A, D and E premix.

[3]SoyPlus (West Central Soy, Ralston, IA).

[4]Values other than DM are expressed as a percentage of dietary DM.

Table 5. Fraction of treatment rations retained on screens of the Penn State Particle Separator as a percent of DM or total NDF.

Particle Size, %	CONR	DPPR	SE[2]	P value
DM, % of total DM				
> 19 mm	11.0	12.1	2.78	0.72
8 to 19 mm	28.8	27.6	1.18	0.36
1.8 to 8 mm	41.7	44.7	0.96	0.01
< 1.8 mm	18.5	15.6	1.12	0.03
NDF, % of total NDF				
> 19 mm	19.1	16.8	3.95	0.57
8 to 19 mm	46.0	35.0	2.23	<0.001
1.8 to 8 mm	28.3	40.1	1.59	<0.0001
< 1.8 mm	6.50	8.09	0.60	0.02

[1]Measured with a 3-sieve Penn State Particle Size Separator.
[2]SE = Standard Error of the Difference

Table 6. Effect of a dairy protein product on performance of lactating dairy cows

				P value	
Item	CONR	DPPR	SE	Trt[2]	Trt x pFCM[3]
Yield, kg/d					
Milk	41.6	42.8	0.64	0.02	0.52
FCM (3.5 %)	42.7	44.8	0.50	<0.0001	0.76
Milk fat	1.54	1.63	0.02	<0.0001	0.96
Milk protein	1.34	1.39	0.02	0.001	0.59
Milk lactose	1.97	2.02	0.03	0.04	0.63
SNF	3.60	3.72	0.05	0.01	0.59
Milk composition, %					
Fat	3.72	3.83	0.08	<0.01	0.73
Protein	3.24	3.27	0.04	0.05	0.94
Lactose	4.73	4.73	0.03	0.98	0.80
SNF	8.68	8.71	0.05	0.05	0.77
MUN, mg/dl	21.0	21.1	0.29	0.78	0.85
SCC (1000/ml)	65.2	70.0	22.12	0.87	0.73
DMI, kg/d	26.6	28.4	0.40	<0.0001	0.65
3.5% FCM/ DMI	1.60	1.58	0.03	<0.10	0.30
BW, kg	693.7	696.9	12.3	0.09	1.00
BCS change, /22 d	2.47	2.48	0.10	0.68	0.42

[1] Treatments were CONR = control ration; DPPR = dietary protein product ration
[2] Trt: treatment effect.
[3] Trt x pFCM: treatment by preliminary fat-corrected milk interaction effect.

Table 7. Effects of dairy protein product on milk fatty acid profile.

	Treatment LSM[1]			P[2]	
	CONR	DPPR	SE	Trt	Trt x Per
Fatty acid, % of total					
4:0	2.75	2.88	0.04	<0.001	0.22
5:0	0.033	0.032	0.002	0.90	0.16
6:0	1.52	1.56	0.19	0.009	0.02
7:0	0.024	0.023	0.002	0.38	0.13
8:0	0.86	0.87	0.01	0.06	0.04
9:0	0.028	0.027	0.002	0.51	0.10
10:0	1.98	1.99	0.05	0.70	0.08
11:0	0.22	0.21	0.01	0.14	0.49
12:0	2.61	2.58	0.06	0.45	0.19
13:0	0.17	0.17	0.007	0.63	0.60
14:0	10.1	9.9	0.10	0.008	0.11
14:1n5t	0.38	0.40	0.006	<0.001	0.34
14:1n5c	0.95	0.92	0.04	0.01	0.07
15:0	1.06	1.05	0.04	0.63	0.40
16:0	33.3	32.9	0.36	0.05	0.97
16:1n7t	0.027	0.038	0.005	0.15	0.55
16:1n7c	1.79	1.73	0.07	0.29	0.23
17:0	0.50	0.50	0.01	0.56	0.60
18:0	9.7	10.1	0.21	0.004	0.37
18:1 t6-t8	0.34	0.34	0.01	0.42	0.37
18:1 t9	0.26	0.27	0.01	0.14	0.09
18:1 t10	0.58	0.51	0.02	0.0001	0.59
18:1 t11	0.93	1.05	0.03	<0.0001	0.76
18:1 t12	0.49	0.50	0.01	0.07	0.22
18:1 c9	19.5	19.7	0.26	0.28	0.05
18:1 c11	0.45	0.36	0.01	<0.0001	0.98
18:1 c12	0.42	0.43	0.01	0.12	0.27
18:2n6c	2.64	2.67	0.05	0.21	0.14
20:0	0.127	0.138	0.002	<0.0001	0.31
18:3n3c	0.39	0.37	0.01	<0.0001	0.07
18:3n6c	0.045	0.043	0.002	0.05	0.43
CLA (c9, t11)	0.49	0.54	0.02	<0.0001	0.09
20:3n6c	0.141	0.145	0.006	0.46	0.44
20:4n6c	0.221	0.217	0.005	0.14	0.13
24:0	0.040	0.036	0.001	0.002	0.74
22:5n3c	0.56	0.53	0.002	0.17	0.83
Unknown	2.7	2.5	0.07	0.08	0.63

[1] Treatment least squares means
[2] P-values for effects of treatment (Trt), period (Per) and the interaction between them (Trt x Per).

Table 7 (cont). Effects of dairy protein product on milk fatty acid profile.

	Treatment LSM[1]		P^2	
	CONR	DPPR	SE	Trt
Fatty acid, % of total				
18:1 trans	2.60	2.67	0.07	0.05
18:1 cis	20.4	20.5	0.26	0.49
SCFA[3]	5.21	5.40	0.06	0.001
MCFA[4]	16.4	16.1	0.20	0.08
LCFA[5]	37.3	38.1	0.42	0.03
Uns % in C18	30.1	30.4	0.34	0.33

[1] Treatment least squares means
[2] *P*-values for effects of treatment
[3]Short-chained fatty acids ($C_4 – C_9$)
[4]Medium-chained fatty acids ($C_{10} – C_{14}$)
[5]Long-chained fatty acids ($> C_{16}$)

Appendix A

CARGILL CORN MILLING (BLAIR, NE)

Study Protocol Deviation

Determine the effects of a dairy protein product on dry matter intake and yield of milk and milk components of Holstein cows.

Study Location: **Michigan State University**

Deviation No.: 001 Date Deviation Occurred: 11/13/2010

Protocol Section Affected:
The DPP treatment diet will substitute DPP for all of the protein supplement as well as some high moisture corn and corn silage. The DPP content will be approximately 38% and determined by the crude protein substitution for the protein supplement. Because the CP concentration of the DPP is less than the protein supplement (~28% vs ~53%), DPP will also replace some high moisture corn and corn silage on an equal DM basis; example diets are attached.

Deviation:
The DPP treatment diet will be balanced to include DPP at 30% of the dietary DM by replacing most, or all, of the protein supplements (SBM and SoyPlus) utilized in the control diet as well as some of the dry ground corn and corn silage. Once the protein supplement are removed from the diet, additional space for the DPP will be provided by removing corn silage and dry ground corn on an equal DM basis. The diet will then be balanced to be isonitrogenous with the control diet if needed by substituting SBM for dry ground corn.

Reason for Deviation:
Changes in crude protein concentrations of diet ingredients throughout the experiment will alter the inclusion rate of DPP in the diet. This was not our original intent. After consulting with Dr. Paul Von Behren, we decided that it would be best to maintain the DPP concentration of the treatment diet at 30% of DM.

Did the Deviation Affect the Outcome of the Trial?
No, because the DPP is being included at a constant concentration throughout the study, the outcome of the study is not affected.

Study Site Investigator:

Signature Date: 11/23/10

Sponsor Representative:

Signature Date: 11-19-10

Study Protocol Deviation

Determine the effects of a dairy protein product on dry matter intake and yield of milk and milk components of Holstein cows.

Study Location: **Michigan State University**

Deviation No.: 002 Date Deviation Occurred: 11/15/2010

Protocol Section Affected:

Cows will be milked twice per day at approximately 0400 and 1600 h.

Deviation:

Three cows were inadvertently not milked during the AM milking on 11/15/10 which is considered as milking two for 11/14/10 (See Table 1). Although the first treatment diet collection period did not begin until the PM milking on 11/15/10, milk yield values were compromised two days following the incident for these cows. Two of the cows were on the DPP diet (4339 and 4434) and one was on the control diet (4450).

Reason for Deviation:

The milker inadvertently let the last three cows through the parlor having believed they were already milked.

Did the Deviation Affect the Outcome of the Trial?

No. Since this happened in the first half of the treatment sequence, an extra day was added to both collection periods, which allowed three good collection days of data for these cows in period 1, and five days in period 2. The 4 d milk yield averages pre and post deviation indicate that cows were not greatly affected by the incident (See Table 2.

Table 1: Total Daily Milk Weight by Date (lb/d)

						Sequence 1 Collection Period				
Cow	11/10/10	11/11/10	11/12/10	11/13/10	11/14/10	11/15/10	11/16/10	11/17/10	11/18/10	11/19/10
4339	86.1	86.3	87.4	85.8	*	112.2	72.5	95.1	84.9	90.2
4434	77.5	80.3	76.5	78.2	*	92.4	68.2	75.3	75.2	77.2
4450	84.4	84.9	82.6	86.9	*	109.2	77.9	83.6	78.5	85.3

* No total milk value as cow inadvertently not milked on 11/14/10 milking number two.

Table 2: Average Milk Weight (lb/d) Pre and Post Deviation[1]

Cow	Diet	Pre-deviation	Post-deviation
4339	DPP	86.4	85.7
4434	DPP	78.1	74.0
4450	Control	84.7	81.3

[1]Average daily milk weights for the four days preceding the deviation and the last four days of the collection period.

Study Site Investigator:

_______________________________ Date:__________

Signature

Sponsor Representative:

_______________________________ Date:__________

Signature