

# Effects of Inclusion of a Blended Protein Product in 35 Dairy Herds in Five Regions of the Country

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## ABSTRACT

We assessed the change in milk production in 35 dairy herds located in eight states when a blended animal-marine protein product was included in diets at 2 (22 herds) to 4% (13 herds) of dry matter. Average 305-d production across herds was 8844.1 kg (SEM = 153.7 kg) of milk, with a range of 6876.9 to 11,293.2 kg. The mean days in milk for all herds at initiation of the trial was 118.8 d (SEM = 3.7, herd mean range of 68.8 to 160.0 d), and the average daily milk production was 32.6 kg (SEM = 0.6 kg, range 24.2 to 39.6 kg). The animal-marine protein blend was included in herd rations for 30 d, beginning immediately after a DHI herd test month and ending immediately after the next DHI sample test. Cow milk records were collected for 1 to 2 mo before the protein blend was included and for 2 to 3 mo after the protein was removed. Sample days were assigned a dummy variable to indicate months off or on the animal-marine protein blend.

A total of 33,190 milk records from 7135 cows were analyzed. The numbers within herd ranged from 35 to 2012 cows. Of the 35 herds, 19 were classed as having increased milk yield, 12 herds as having no change, and 4 herds as having decreased milk yield when the animal-marine protein blend was included in the diet. The population mean for change in milk yield with the inclusion of the animal-marine protein blend was 1.24 kg/d of milk (SEM = 0.05 kg). There

was no significant effect of parity on mean response. Milk protein content was not influenced by animal-marine protein blend inclusion. Fat content was lower for the month on which the animal-marine protein blend was fed (3.51%) compared with the month prior (3.63%) and the month after (3.70%), respectively (SEM = 0.032). Stage of lactation influenced the method for calculating the production response and the actual response to the animal-marine protein blend.

(**Key words:** protein, milk production, dairy cattle)

**Abbreviation key:** **ADJTRT** = dummy coefficient for change in milk variable, **CSG** = corn silage, **CSG/LEG** = 40 to 60% of forage DM as corn silage or legumes, **DIMINT** = DIM on first test day data were collected, **LEGUM** = legume silage or hay >70% of forage in ration, **MCP** = microbial protein, **M150** = 150-d calculated adjusted milk yield, **M150DIF** = change in 150-d calculated milk yield between two sequential test days, **M305** = 305-d predicted milk yield, **M305DIF** = change in 305-d predicted yield between two sequential test days, **NAN** = nonammonia N, **SBM** = soybean meal, **TRT** = dummy coefficient for milk production change with inclusion of the animal-marine protein blend, **TRT40** = dummy coefficient for milk production change with inclusion of the animal-marine protein blend with cows less than 40 DIM on first test month deleted from the analysis.

## INTRODUCTION

As noted in a recent review (26), replacement of protein sources high in RDP, such as soybean meal (**SBM**), with protein sources high in RUP has not al-

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ways increased milk production (15, 19, 23, 26, 29). Absorbed protein may not be a limiting factor for milk production when large amounts of DM are consumed (23, 29). Furthermore, the inclusion of RUP sources in diets has not been associated with increased flow of nonammonia N (NAN) to the small intestine (6, 26) partly because microbial protein (MCP) synthesis may be reduced when SBM is replaced in diets (26). As RUP is increased in dairy rations, rumen fermentable carbohydrate and protein may be reduced, resulting in reduced MCP synthesis. The net effect is that NAN flow to the small intestine is not increased and may even be reduced. In addition, not all protein sources high in RUP provide an optimal complement of AA for milk production (15, 25, 30). Some protein sources high in RUP may reduce the proportions of lysine and methionine available for absorption, reducing the quality of absorbed AA (15, 26).

A positive response to increasing RUP in dairy rations has been associated with level of production (1, 2), stage of lactation (5, 16), age (17), and type of supplemental protein source (8, 30). Therefore, positive production responses to increasing dietary RUP may be best observed in high producing, early-lactation cows when using a high quality protein supplement in rations that maintain the supply of rumen available carbohydrate and nitrogen.

An increasing body of research (3, 25, 27, 30) suggests that protein quality influences milk volume and protein content. Methionine and lysine are the most limiting AA for milk production (15, 27). MCP is a high quality protein source. However, sufficient quantities may not be synthesized in cows producing over 30 kg of milk or in cows with decreased DMI, such as in early lactation. Therefore, some dietary protein sources high in RUP are necessary in early lactation, particularly in cows producing more than 30 kg of milk. However, the quality of AA in the sources high in RUP should provide sufficient lysine and methionine (15, 23, 27). In general, plant proteins are not good sources of lysine and methionine. Blends of animal-marine protein sources can provide a rich mixture of methionine and lysine, in addition to increasing the flow of NAN from dietary sources (19). Coefficients of rumen degradability are low, and the proteins are highly digestible (19). Therefore, a blend of animal and marine protein sources can provide a high quality, digestible RUP rich in methionine and lysine.

Because the response to protein sources high in RUP varies, producers must consider the economic benefit of including an animal-marine protein blend in diets. Forage and grain sources that vary widely in protein, fat, and carbohydrate content are fed to dairy herds. Thus, producers might find it desirable to conduct their

own product evaluation trials if simple methods were available. Such methods must control for high (10%) daily variation of production within cow and for the difference in production with time post calving. In addition, any method should measure the magnitude and variation in production response to a product, allowing for economic evaluation.

One hundred and fifty-day adjusted milk yield has been proposed as a method to evaluate monthly change in production in a herd (22). However, this method assumes a uniform distribution of cows by DIM in the herd month to month and does not allow a calculation of variance (10). Galligan et al. (11) proposed that a change in projected 305-d milk yield be used to assess production changes in a herd. In modeling changes with an impulse input of production change, Ferguson et al. (10) found the regression method within cow to be sensitive and specific for assessing production changes and could provide a useful tool for producers.

The purpose of this project was to investigate production responses to an animal-marine protein blend (Prolak, HJ Baker and Sons, Inc., Stamford, CT) across a variety of dairy herds in several regions of the United States. In addition, several methods of assessing production change were compared, such as change in 305-d milk yield (11), change in 150-d milk production (22), and a regression estimate of milk change (10).

## MATERIALS AND METHODS

### Farm Selection

This study involved 35 dairy herds in eight states (Florida, North Carolina, Virginia, Pennsylvania, Michigan, Iowa, Wisconsin, and Arizona). The cooperation of dairy producers and herd nutritionists were obtained to examine the effects of a blended animal-marine protein source at 2 to 4% of ration DM on milk production. The study was conducted during a stable a feeding period as possible to minimize changes in other dietary components, particularly forages. When the protein blend was included in the diet, a protein equivalent amount of SBM, or other RDP source, was removed. Diet composition on the dairy farms in this study varied widely (Table 1). For the trial, selected farms were all Holstein herds and subscribed to a production testing system with either monthly DHIA tests or daily milk weights recorded automatically on a farm computer system. All herds were milked twice a day. The start date for the trial ranged from September 1992, to March 1993, for the 35 herds. The last test date for data collection across all herds ranged from January 5, 1993, to June 14, 1993.

**Table 1.** Demographics of the herds included in the study.<sup>1</sup>

State	Herd	Cows (n)	Forage base	Protein type	Protein blend inclusion, %	M305 kg	SEM kg
23	1	56	CSG/LEG	Vegetable	4%	9378	218
23	2	87	CSG/LEG	Vegetable	4%	9644	174
23	3	85	CSG/LEG	Vegetable	4%	9433	176
23	4	41	LEGUM	Vegetable	2%	10,105	253
23	5	43	LEGUM	Vegetable	2%	8419	247
23	6	56	CSG	Animal	2%	8368	216
23	7	35	CSG/LEG	Vegetable	4%	7990	274
42	8	59	CSG/LEG	Animal	2%	9332	214
48	9	64	LEGUM	Mixed	4%	9253	202
48	10	75	CSG/LEG	Animal	2%	8756	187
48	11	75	CSG/LEG	Animal	2%	9637	187
48	12	77	CSG/LEG	Mixed	2%	9380	184
48	13	176	CSG/LEG	Mixed	4%	8600	122
48	14	83	CSG/LEG	Mixed	4%	11,213	178
48	15	186	CSG	Animal	4%	9622	119
48	16	95	LEGUM	Animal	4%	10,765	166
48	17	52	LEGUM	Mixed	4%	8870	225
48	18	62	LEGUM	Mixed	4%	10,849	207
48	19	65	LEGUM	Mixed	4%	8666	201
52	20	151	LEGUM	Vegetable	4%	9196	132
52	21	76	LEGUM	Vegetable	4%	7721	186
52	22	109	CSG/LEG	Vegetable	2%	8659	155
52	23	69	CSG	Vegetable	4%	6633	195
52	24	98	CSG	Vegetable	2%	8334	164
52	25	127	CSG	Vegetable	2%	9359	144
52	26	66	CSG	Mixed	4%	9405	199
52	27	73	CSG	Vegetable	2%	7809	189
52	28	107	CSG/LEG	Vegetable	4%	8138	157
52	29	163	CSG	Vegetable	2%	8490	128
55	30	131	CSG	Mixed	2%	9004	145
55	31	238	CSG	Mixed	4%	8044	105
58	32	1151	CSG	Vegetable	4%	8123	48
58	33	2012	CSG	Mixed	4%	9084	36
86	34	721	CSG/LEG	Vegetable	4%	9046	62
86	35	371	CSG/LEG	Vegetable	4%	8651	84
Total		Cows 7135	Herds				
			CSG	12	Vegetable	18	2%
			CSG/LEG	14	Mixed	11	4%
			LEGUM	9	Animal	6	22

<sup>1</sup>Forage = primary forage type n ration: CSG = corn silage >70% of forage DM; CSG/LEG = 40–60% of forage DM as corn silage or legume silage or hay; LEGUM = legume silage or hay >70% of forage DM as legume silage or hay. Protein source = Vegetable—only vegetable protein sources in ration; Mixed—up to 2% of ration DM as an animal protein blend; Animal—>2% of ration DM as an animal protein blend. Protein blend inclusion: 2% = percent of ration DM as animal-marine protein blend (Prolak); 4% = percent of ration DM as animal-marine protein blend (Prolak). State = National Dairy Herd Improvement State Code. Number of cows within each herd included in the study. For inclusion cows had to be between 0 to 300 DIM on first test month and have production tested on successive test days. M305 = mean 305 projected milk yield for cows on the study for each herd with SEM.

**Data Collection**

Production data were collected monthly from each herd for each cow from the DHIA system or other production databases. Information collected included cow identification, parity, milk volume, milk fat and protein content (if available), SCC (if available), and DIM. Information for each sample day was stored in a file that included date of sample day and herd index. In the data file, sample days were coded as 0 for months data were collected with no inclusion, and 1 for the month when the protein blend was in the diet. In addition,

each test day was categorized relative to the month of inclusion, -2 for 2 mo prior to inclusion, -1 for one sample month prior to inclusion, 0 for sample month of protein blend inclusion, 1, 2, or 3 for sequential monthly samples after protein blend removal.

For individual cow records to be included in the data for final analysis, cows had to be on the test at least three sequential sample days, with at least one record before and one record after the protein blend was included. An individual cow could have as few as three to as many as six test-day observations for data analysis.

## Diets and Feeding Management

The forage base of rations employed by the 35 herds was categorized into three groups: primarily corn silage-based rations (1, >70% forage DM as corn silage, **CSG**), mixed forage rations consisting of legume silage or hay and corn silage (2, 40 to 60% forage DM as corn silage or legume, **CSG/LEG**); and primarily legume silage or hay based rations (3, >70% of forage DM as legume forage, **LEGUM**) (Table 1).

Concentrated sources of energy were primarily based on corn in combination with other grains and byproduct feeds. Protein sources included SBM plus other protein sources or blends. Herds were categorized as those with no animal or marine protein in the existing diet (1, Vegetable, Table 1), herds with 1 to 2% of the ration DM as an animal or marine proteins (2, Mixed, Table 1); and herds with more than 2% of the ration DM in the form of an animal or marine proteins (3, Animal, Table 1).

Feeding protocols included herds that used a TMR, those that used a basal TMR to which a supplemental top dress of concentrate and protein meals was added, and those that were fed forages and grains separately. For herds on TMR, cows were grouped based on production and were offered a ration of blended ingredients accordingly. A few TMR-fed herds utilized one ration for all cows regardless of production. Herds in which the ingredients of the TMR varied depending on production of the group may or may not have included the animal-marine protein blend in the TMR fed to groups with lower production. Data from these groups were not included in the analysis. Herds that were fed supplemental concentrate and or protein meals typically were offered these based on level of production. In these herds, the animal-marine protein blend was included in the protein supplement to supply at least 2% of ration DM.

Production data were collected from the farms for 1 to 2 mo before the protein was included in the ration blend. Immediately after the -1 sample day, the animal-marine protein blend was substituted into the ration as part of the protein source at 2 to 4% of ration DM (Table 1). An equivalent amount of protein was removed from the ration, so that diets might remain isonitrogenous and isocaloric to the previous ration. It was the intent of the study to vary only the protein source.

An outline of the experimental feeding regime is shown in Table 2. After a test day (test code = -1) the animal-marine protein blend (Prolak) was included in the ration until the next test period (test code = 0), approximately 30 d. After this sample day, the animal-marine protein blend was removed from the ration.

Milk production records were collected from the herd for the next 1 to 3 mo.

## Statistical Methods

Regression analysis was used to analyze milk production (10). Records were constructed which included data for herd, cow, parity (LACT = 1, 2, 3+), DIM, milk volume, test-day category (-2 through 3), and TRT (0 or 1). Data were sorted by herd. The data set was analyzed by cow within herd with SAS software using the following linear model (28):

$$\text{Milk}_{ijk} = \text{Intercept}_i + \text{beta}_i * \text{DIM}_k + \text{TRT}_{ij} + \varepsilon_{ijk}$$

where

Milk<sub>ijk</sub> = the milk production on the kth test day for the ith cow on the jth treatment;

Intercept<sub>i</sub> = the intercept for the ith cow;

beta<sub>i</sub> = the DIM coefficient for the ith cow;

DIM<sub>k</sub> = the kth day of test for the ith cow;

TRT<sub>ij</sub> = the jth test day dummy variable for treatment for the ith cow; and

ε<sub>ijk</sub> = the residual error for the ith cow.

The coefficient of TRT represented the change in milk on test month code 0 from the prediction of milk based on the intercept and slope across DIM for each test day. If the slope of the milk curve exactly fit the lactation change over test day, the coefficient of TRT would be zero. If milk volume changed on test day code 0 from the typical pattern of production, then the coefficient for TRT would differ from zero. Individual, random variation of milk production across test months was controlled by performing a linear regression by each cow for milk yield on DIM and the dummy variable for inclusion of the animal-marine protein product. Regression outputs for cows were aggregated by herd to calculate a TRT mean and variance.

Parameter estimates for Intercept<sub>i</sub>, beta<sub>i</sub>, and TRT<sub>ij</sub> were submitted to PROC GLM in SAS, with herd and parity as class variables. The least square means of TRT for herd was tested for difference from zero based on a t statistic to determine if the predicted change in milk was significant. Herd responses were then classed as negative, not different from zero, or greater than zero based on  $P < 0.10$ .

This study represented an experiment with identically replicated treatments in 35 herds with differing number of cows. The estimate of TRT is better known for herds over 1000 cows than in herds with less than 50 cows on the study. To control for differing herd

**Table 2.** Outline of experimental design with the animal-marine protein blend.<sup>1</sup>

	Sequences represented							Animal marine-protein
Test-day code								
-2	-2	-2	-2	... <sup>2</sup>	...	...	...	No supplement
-1	-1	-1	-1	-1	-1	-1	-1	No supplement
0	0	0	0	0	0	0	0	Supplement added after sample day
								Supplement in ration
								Supplement removed after test day
								milk measured
1	1	1	1	1	1	1	1	No supplement
2	2	...	...	2	...	2	...	No supplement
3	...	...	3	3	3	...	...	No supplement
Number of cows								
470	1576	810	1134	799	313	1566	467	Total cows 7135
Percent								
6.6	22.1	11.4	15.9	11.2	4.4	21.9	6.5	

<sup>1</sup>Prolak, HJ Baker and Sons, Inc. Supplement included ration immediately after test day -1 at 2 to 4% of ration DM. Supplement was removed from the ration immediately after test day 0. A total of 7135 cows from 35 herds were used in the study.

<sup>2</sup>Indicates milk volume was not recorded on that test day.

size, the population mean was calculated based on the estimation of population parameters from identically replicated experiments using the following formula:

$$\text{Mean TRT} = \frac{\sum(\mu_i/\delta_i^2/\eta_i)}{\sum(\eta_i/\delta_i^2)}$$

where

- $\mu_i$  = mean TRT of the ith herd,
- $\delta_i^2$  = variance of the ith herd,
- $\eta_i$  = number of cows in the ith herd.

The variance for TRT across all herds was calculated as follows:

$$\delta_i^2 = \frac{\sum(\nu_i \delta_i^2)}{\sum(\nu_i)}$$

where

- $\delta_i^2$  = variance for TRT in the ith herd and
- $\nu_i$  = degrees of freedom in the ith herd.

Simulation work had suggested the utility of a regression method to analyze milk production responses (10). However, this method is also biased in estimating response when cows under 40 DIM on the first sample day were included in the analysis. The treatment effect could be estimated by eliminating these cows from the analysis or by adjusting the estimate of TRT response with the following formula

Bias in response:

$$\text{kg of milk} = 4.064 * e^{(-0.0497 * \text{DIMINT})}$$

where

**DIMINT** = DIM on first test day.

In addition to analyzing production responses by the regression method, the change in predicted 305-d milk yield for test day -1 was subtracted from predicted 305-d for test day 0 for each cow (11). Then the herd mean change in 305-d yield (P305) was calculated along with the variance of the change in 305-d yield. This was tested for difference from zero using a t statistic after a method outlined by Galligan et al. (11). In addition, the change in 305-d yield was ranked above and below zero and a nonparametric sign test was used to assess any significant change in production. Likewise the 150-d adjusted milk (**M150**) was calculated for test day -1 and test day 0 using the formula of Nordlund (22) for each cow. The difference in M150 day yield was calculated as the change from test day -1 to test day 0. The herd mean change in M150 day yield was calculated along with the variance of the change in M150. We tested this change for difference from zero by using a t statistic. The predictions of herd increases or decreases in milk with the P305 or the M150 were compared with the TRT predictions.

Herd level factors that may have influenced response to the inclusion of the animal-marine protein blend were analyzed with the following model:

$$\text{TRT}_i = \text{Herd}_i(\text{Factor}_j) + \text{Factor}_j + \epsilon_{ijk}$$

where:

$\text{TRT}_i$  = regression estimate of TRT for n cows in the ith herd

$\text{Herd}_i(\text{Factor}_j)$  = jth factor nested in the ith herd

Factor<sub>j</sub> = jth factor  
 $\varepsilon_{ijk}$  = residual error

Factors tested in the above model for responses to Prolak included forage base of the ration CSG, CSG/LEG, LEGUM inclusion rate of Prolak (2% of DM or 4% of DM), prior feeding of animal-marine blends (Vegetable, Mixed, Animal), age, and production level. Age effects were examined by categorizing cows based on lactation number (LACT1 = 1, LACT2 = 2, LACT3 = 3 and greater). Within each herd, cows were placed into quartiles based on their projected 305-d milk production relative to the herd mean 305-d milk yield and standard deviation in the herd (MGRP = 1, lowest quartile, MGRP = 2, next lowest, MGRP = 3, next to highest, and MGRP = 4 the highest quartile). The error term used to calculate F test statistic for these factors was Herd<sub>i</sub>(Factor<sub>j</sub>).

## RESULTS AND DISCUSSION

A total of 42,452 production records were collected across the study period for all 35 herds. For the final analysis a total of 33,190 records for 7135 cows from 35 herds were used in the analysis. Distribution of cows on test date was as follows: test days -1, 0, 1 (470 cows), test days -1, 0, 1, 2 (1576 cows); test days -1, 0, 1, 3 (313 cows), -1, 0, 1, 2, 3 (467 cows); test days -2, -1, 0, 1 (810 cows); test days -2, -1, 0, 1, 2 (1134 of cows); test days -2, -1, 0, 1, 2, 3 (799 cows), and test days -2, -1, 0, 1, 3 (1566 cows) (Table 2). Average 305-d milk yield for the herds on the initial test day was 8844.1 kg (SEM = 153.7 kg), with a range from 6876.9 to 11,293.2 kg of milk. At the first test day, average DIM for all herds was 118.8 d (SEM = 3.65 d), with a range from 68.8 to 160.0 d. Mean DIM on test day with the animal-marine protein inclusion was 160.3 d (SEM = 3.5 d) (range for DIM at inclusion 117.8 to 202.7 d). Milk production on first test day was 32.6 kg (SEM = 0.6 kg; range of 24.2 kg to 39.6 kg) fat content was 3.63% (SEM = 0.01) and protein content was 3.21% (SEM = 0.01).

Mean results by herd for the regression parameters are presented in Table 3. The overall population mean response for TRT was 1.24 kg/d (SEM = 0.05). Nineteen herds had a mean TRT coefficient significantly greater than zero, indicating an increase in milk production with inclusion of the animal-marine protein blend (TRT = 1, Table 3). Twelve herds had a mean TRT coefficient not different from zero, indicating no significant change in milk production (TRT = 0) with the inclusion of the animal-marine protein blend in the ration. Four herds had a mean TRT coefficient, which

was significantly less than zero, indicating a decrease in milk production (TRT = -1) (Table 3).

The mean TRT response was not significantly different across forage classes, level of protein inclusion, or level of prior animal-marine protein feeding (Table 4). Most herds had a forage base of a blend of CSG/LEG (14 herds) or primarily CSG (12 herds), whereas only nine herds had a predominant forage base of legumes (LEGUM = 9) (Table 4). The mean TRT estimates for herds classed as CSG and CSG/LEG were 0.99 kg (SEM = 0.12 kg) and 0.93 kg (SEM = 0.13 kg), respectively, compared with 0.48 kg (SEM = 0.18 kg) for the LEGUM class herds (Table 4). These means were not significantly different. Nine of 12 CSG herds increased milk production, compared with 7 of 14 CSG/LEG and 3 of 9 LEGUM herds, proportions that are not different based on a chi-square distribution ( $\chi^2 = 3.77$ , df = 2).

The majority of herds had added Prolak at 4% of DM compared with 2% of DM (22 herds at 4% of DM vs. 13 herds at 2% of DM, respectively, Table 4). The mean TRT estimate for herds that included Prolak at 4% of DM was 0.57 kg (SEM = 0.10 kg) compared with 1.28 kg (SEM = 0.14 kg) for herds that included Prolak at 2% of DM, but these means were not significantly different. However, 10 of 13 herds which fed the 2% inclusion rate had increased milk production compared with 9 of 22 herds fed the 4% inclusion rate ( $\chi^2 = 4.27$ ,  $P < 0.05$ ). The magnitude of the difference in TRT estimates and the higher proportion of herds that had increased milk production with the lower inclusion rate suggests that optimal inclusion rates of animal-marine protein blends should be more closely examined. Inclusion rates at 4% of DM of Prolak possibly resulted in a drastic reduction in RDP protein sources in order to maintain isonitrogenous diets, which may have had negative consequences on rumen microbial synthesis and resulted in fewer herds increasing in production (26). We were not able to separate the potential interaction between inclusion rate and previous feeding of animal-marine protein sources.

Eighteen herds were not fed any animal-marine protein blend prior to the trial (Vegetable, Table 4), diets of 11 herds included animal-marine protein blend at less than 2% of the DMI (Mixed, Table 4), and diets of 6 herds included animal-marine protein blend greater than 2% of DMI (Animal, Table 4). Prior animal-marine protein feeding did not influence the response to the inclusion of Prolak in the ration (Table 4). Although not significantly different, the lowest absolute mean TRT was in herds fed no prior animal-marine protein blend, 0.58 kg (SEM = 0.11 kg) versus 0.86 kg (SEM = 0.14 kg) and 1.58 kg (SEM = 0.20 kg) for Vegetable, Mixed, and Animal factors, respectively

**Table 3.** Mean TRT value by herd in response to the animal-marine protein blend in ratios of dairy cows for a 30-d period.<sup>1</sup>

State	Herd	No. of records	Inter kg	SEM, inter	Dimbeta	SEM, DIM	TRT kg	SEM, TRT	P<	Resp.
23	1	56	44.0	1.8	-0.0631	-0.0094	0.8	0.6	0.1600	0
23	2	87	48.8	1.4	-0.0801	-0.0073	0.5	0.5	0.3200	0
23	3	85	43.2	1.4	-0.0585	-0.0076	0.6	0.5	0.2200	0
23	4	41	50.3	2.0	-0.0876	-0.0107	1.9	0.7	0.0050	1
23	5	43	49.8	2.0	-0.1214	-0.0104	-2.6	0.7	0.0001	-1
23	6	56	45.8	1.7	-0.0974	-0.0092	0.1	0.6	0.9200	0
23	7	35	37.0	2.1	-0.0456	-0.0115	-2.1	0.7	0.0050	-1
42	8	59	37.1	1.7	-0.0398	-0.0091	2.2	0.6	0.0001	1
48	9	64	42.1	1.6	-0.0680	-0.0085	1.4	0.5	0.0100	1
48	10	75	41.7	1.4	-0.0679	-0.0077	2.5	0.5	0.0001	1
48	11	75	41.2	1.5	-0.0308	-0.0079	1.6	0.5	0.0010	1
48	12	77	34.3	1.5	-0.0214	-0.0078	2.4	0.5	0.0001	1
48	13	176	41.8	1.0	-0.0722	-0.0052	1.3	0.3	0.0001	1
48	14	83	58.8	1.4	-0.0947	-0.0075	-0.8	0.5	0.0750	-1
48	15	186	50.0	1.0	-0.1047	-0.0051	2.9	0.3	0.0001	1
48	16	95	48.7	1.3	-0.0669	-0.0068	0.1	0.4	0.8000	0
48	17	52	39.0	1.8	-0.0328	-0.0097	-0.5	0.6	0.4100	0
48	18	62	46.8	1.6	-0.0436	-0.0088	-0.1	0.6	0.9300	0
48	19	65	43.8	1.6	-0.0902	-0.0084	0.4	0.5	0.4800	0
52	20	151	39.7	1.0	-0.0486	-0.0055	3.7	0.3	0.0001	1
52	21	76	34.2	1.5	-0.0333	-0.0080	0.0	0.5	0.9800	0
52	22	109	39.6	1.2	-0.0532	-0.0066	1.4	0.4	0.0007	1
52	23	69	24.6	1.6	0.0026	-0.0084	-3.5	0.5	0.0001	-1
52	24	98	35.9	1.3	-0.0354	-0.0070	0.5	0.4	0.2530	0
52	25	127	43.7	1.1	-0.0695	-0.0061	1.9	0.4	0.0001	1
52	26	66	43.2	1.6	-0.0539	-0.0084	1.0	0.5	0.0518	1
52	27	73	34.3	1.5	-0.0336	-0.0082	2.1	0.5	0.0001	1
52	28	107	32.3	1.3	-0.0220	-0.0067	0.6	0.4	0.1599	0
52	29	163	40.1	1.0	-0.0650	-0.0053	1.1	0.3	0.0008	1
55	30	131	39.4	1.1	-0.0526	-0.0060	1.5	0.4	0.0001	1
55	31	238	34.3	0.8	-0.0413	-0.0045	1.7	0.3	0.0001	1
58	32	1151	38.3	0.4	-0.0619	-0.0021	1.4	0.1	0.0001	1
58	33	2012	41.1	0.3	-0.0541	-0.0015	1.2	0.1	0.0001	1
86	34	721	36.8	0.5	-0.0373	-0.0026	2.3	0.2	0.0001	1
86	35	371	36.5	0.7	-0.0375	-0.0035	-0.2	0.2	0.3800	0

<sup>1</sup>Inter = Mean intercept for the regression model across cows in the herd; Dimbeta = slope of milk decline as a function of days in lactation across cows in the herd; TRT = estimate of the effect of inclusion of an animal-marine protein blend on milk production; Resp. = Response = -1, TRT < 0, production went down, 0, TRT = 0, production did not change, 1, TRT > 0, production increased.

(Table 4). The proportion of herds that increased in milk—8 of 18 Vegetable, 7 of 11 Mixed herds, and 4 of 6 Animal herds—was not different.

The mean TRT estimate was not different across lactation groups [0.96 kg (SEM = 0.13 kg) for LACT1, 0.52 kg (SEM = 0.17 kg) for LACT2, and 0.88 kg (SEM = 0.14 kg) for LACT3 (Table 4)]. However, TRT estimates were different across milk production classes (Table 4). Mean TRT estimates declined from 1.51 kg (SEM = 0.22 kg), to 1.09 kg (SEM = 0.13 kg), to 0.61 kg (SEM = 0.15 kg) to -0.01 kg (SEM = 0.24 kg) as milk production quartile increased from lowest to highest within each herd (Table 4). Ten of 18 herds ranked below the average 305-d milk production (8844.1 kg) and 9 of 17 herds ranked above the average 305-d milk production increased in milk yield with the inclusion of the animal-marine protein blend. The decrease in TRT response with increasing milk production group does not appear to be associated with herd level milk production but is an effect in higher producing cows

within herd. In simulation models, milk production was not a significant confounder influencing estimates of known changes in milk production using the regression method (10). Within herd factors, such as feed consumption in higher producing cows may be influencing the response to inclusion of animal-marine protein sources in this study. However, we do not have sufficient within-herd observations to account for this effect.

In a previous study (10), we identified stage of lactation as a significant confounder when using the regression method to estimate production responses. Stage of lactation influenced whether cows were pre- or post-lactation peak at time of data collection. Milk yield increases following calving and typically reaches apogee between 30 to 50 d postcalving (18). Following peak production, lactation decline is linear (18). Cows that are prepeak on the initial test month before the animal-marine protein blend is included in the diet will have a positive coefficient for TRT associated with

the shape of the lactation curve (10). Previous work (10) using the regression method to analyze production responses identified cows less than 40 DIM on the initial test day as having a positive TRT coefficient even with no actual change in production because cows rise, plateau, and decline in milk across sequential sample months (10). The shape of the lactation curve in these cows is an inverted “U” and thus is not amenable to linear analysis without correcting for the bias. Including these cows in the analysis will bias the estimate of TRT in a positive direction. Therefore, the milk production response to the inclusion of the animal-marine protein blend may be overestimated depending on the proportion of cows less than 40 DIMINT at time of the study. One method to reduce the bias is to

eliminate cows less than 40 DIMINT from the analysis (TRT40). Alternatively, the regression estimate may be adjusted for the bias associated with DIMINT with a formula developed in a companion paper ( $ADJTRT = TRT - 4.064 * e^{(-0.0497 * DIMINT)}$ ), where ADJTRT is dummy coefficient for change in milk production, with inclusion of an animal-marine protein blend in a herd ration adjusted for initial DIM on first test month of data collection (10).

Table 5 presents herd summary data for TRT40 and ADJTRT. Eliminating cows less than 40 DIMINT from the analysis reduced number of animals to 5362 from the initial 7135 (Table 5). The TRT40 estimate across all herds was 0.78 kg (SEM = 0.05 kg) and not significantly different from the ADJTRT estimate of 0.82 kg

**Table 4.** Summary of factors influencing response to an animal-marine protein blend in 35 dairy herds.

Level of factor <sup>1</sup>	Herds (n)	Cows (n)	Mean TRT	SEM
<b>Forage<sup>2</sup></b>				
CSG	12	4370	0.99	0.12
CSG/LEG	14	2116	0.93	0.13
LEGUM	9	649	0.48	0.18
<b>Protein sources<sup>3</sup></b>				
VEGETABLE	18	3563	0.58	0.11
MIXED	11	3026	0.86	0.14
ANIMAL	6	546	1.58	0.20
<b>Concentration of animal-marine blend (Prolak)</b>				
2% of DM	13	1127	1.28	0.14
4% of DM	22	6008	0.57	0.10
<b>Production<sup>4</sup></b>				
Lowest quartile, MGRP1	35	1063	1.53 <sup>a</sup>	0.22
Second quartile, MGRP2	35	2555	1.10 <sup>b</sup>	0.13
Third quartile, MGRP3	35	2391	0.61 <sup>c</sup>	0.15
Fourth quartile, MGRP4	35	1079	-0.05 <sup>d</sup>	0.24
Missing		47		
<b>Parity number<sup>5</sup></b>				
		n, cows		
First, LACT1	35	2614	0.96	0.13
Second, LACT2	35	2172	0.52	0.17
Third and greater, LACT3	35	2346	0.88	0.14
Missing		3		

<sup>1</sup>Means within factor with different superscript differ by  $P < 0.10$  (a,b,c,d). Factor indicates classification of herd factors for examining TRT (mean regression coefficient calculated to estimate a mean response to an animal-marine protein blend for a 30-d period in rations fed to dairy cows. Milk production data was collected over three to six sequential test periods, with protein inclusion in the middle test period).

<sup>2</sup>Forage = primary forage in the ration: CSG = primarily corn silage, over 70% of forage DM as corn silage; CSG/LEG = 40 to 60% of forage DM as corn silage or legume silage or hay; LEGUM = primarily legume silage or hay, over 70% of forage DM as legume.

<sup>3</sup>Protein sources = Source of protein supplements in control ration period. VEGETABLE, only plant and plant by-products in ration prior to inclusion of Prolak; MIXED, up to 2% of ration DM as animal-marine protein prior to Prolak inclusion; ANIMAL, > 2% of ration DM as animal-marine protein prior to Prolak inclusion.

<sup>4</sup>Level of production: ranking of production within herd based on 305-d predicted milk yield on the first test month. Each quartile was defined based on the mean 305-d production within each herd. The overall mean 305-d yield across all herds was: lowest quartile, 6708 kg (the minimum mean to the maximum mean for the lowest quartile across all herds, 4662 to 8946 kg); second quartile, 8199 kg (the minimum mean to the maximum mean for the second quartile across all herds, 6204 to 10,689 kg); third quartile, 9539 kg (the minimum mean to the maximum mean for the third quartile across all herds, 7434 to 12,188 kg); fourth quartile, 11,208 kg (the minimum mean to the maximum mean for the fourth quartile across all herds, 9384 to 13,548 kg).

<sup>5</sup>LACT1 = First parity animals; LACT2 = second parity animals; LACT3 = third and greater parity animals.

**Table 5.** Mean herd responses when only cows greater than 40 DIM at the start of data collection (DIMINT) are included in the analysis (TRT40) or when TRT is adjusted for the initial DIM (ADJTRT).<sup>1</sup>

State	Herd	N40 <sup>2</sup>	TRT40	SEM	P<	Resp <sup>3</sup>	NADJ	ADJTRT <sup>4</sup>	SEM	P<	Resp
23	1	32	0.1	-0.7	0.8107	0	56	0.2	0.6	0.7642	0
23	2	71	-0.3	-0.5	0.5480	0	87	0.2	0.5	0.7019	0
23	3	74	0.2	-0.5	0.6330	0	85	0.3	0.5	0.4490	0
23	4	38	1.5	-0.6	0.0160	1	41	1.7	0.7	0.0100	1
23	5	35	-2.8	-0.7	0.0001	-1	43	-2.9	0.6	0.0001	-1
23	6	51	-0.2	-0.6	0.6901	0	56	-0.1	0.6	0.8161	0
23	7	26	-1.5	-0.8	0.0591	-1	35	-2.3	0.7	0.0011	-1
42	8	46	2.9	-0.6	0.0001	1	59	1.9	0.6	0.0004	1
48	9	54	1.0	-0.5	0.0555	1	64	1.2	0.5	0.0224	1
48	10	70	2.2	-0.5	0.0001	1	75	2.4	0.5	0.0001	1
48	11	55	0.7	-0.5	0.1734	0	75	1.3	0.5	0.0061	1
48	12	69	2.3	-0.5	0.0001	1	77	2.1	0.5	0.0001	1
48	13	147	0.8	-0.3	0.0192	1	176	1.0	0.3	0.0022	1
48	14	74	-1.3	-0.5	0.0041	-1	83	-1.1	0.5	0.0163	-1
48	14	186	2.9	-0.3	0.0001	1	186	2.9	0.3	0.0001	1
48	16	79	-0.5	-0.4	0.2596	0	95	-0.1	0.4	0.7966	0
48	17	40	-0.7	-0.6	0.2564	0	52	-0.9	0.6	0.1340	0
48	18	54	-0.3	-0.5	0.5647	0	62	-0.3	0.5	0.5518	0
48	19	59	0.0	-0.5	0.9307	0	65	0.2	0.5	0.6967	0
52	20	116	2.6	-0.4	0.0001	1	151	3.2	0.3	0.0001	1
52	21	70	-0.1	-0.5	0.9089	0	76	-0.2	0.5	0.6760	0
52	22	85	1.1	-0.4	0.0100	1	109	1.0	0.4	0.0125	1
52	23	50	-3.4	-0.6	0.0001	-1	69	-4.0	0.5	0.0001	-1
52	24	72	-0.3	-0.5	0.4456	0	98	-0.0	0.4	0.9398	0
52	25	107	1.6	-0.4	0.0001	1	127	1.6	0.4	0.0001	1
52	26	48	0.3	-0.6	0.6616	0	66	0.5	0.5	0.3078	0
52	27	53	1.7	-0.5	0.0017	1	73	1.6	0.5	0.0013	1
52	28	93	0.1	-0.4	0.8020	0	107	0.4	0.4	0.3549	0
52	29	147	0.9	-0.3	0.0135	1	163	0.9	0.3	0.0047	1
55	30	107	1.2	-0.4	0.0013	1	131	1.1	0.4	0.0024	1
55	31	207	1.5	-0.3	0.0001	1	238	1.5	0.3	0.0001	1
58	32	870	0.7	-0.1	0.0001	1	1151	1.0	0.1	0.0001	1
58	33	1454	0.9	-0.1	0.0001	1	2012	0.7	0.1	0.0001	1
86	34	352	1.7	-0.2	0.0040	1	721	1.5	0.2	0.0001	1
86	35	271	-0.8	-0.240	0.0001	-1	371	-0.7	0.2	0.0009	-1

<sup>1</sup>TRT40 = Estimate of the effect of inclusion of an animal-marine protein blend (Prolak) on milk production when only cows >40 DIM on the first test month are included in the analysis.

<sup>2</sup>N40 = Number of cows in each herd used in the analysis when DIMINT < 40 was deleted.

<sup>3</sup>Resp. = Response, -1, TRT < 0, production went down; 0, TRT = 0, production did not change; 1, TRT > 0, production increased.

<sup>4</sup>ADJTRT = Estimate of the effect of inclusion of an animal-marine protein blend (Prolak) on milk production when estimate of TRT coefficient adjusted using the following ADJTRT = TRT - 4.064 \* e<sup>(-0.0497 \* DIMINT)</sup> (10).

(SEM = 0.05 kg). The TRT40 and ADJTRT estimates are lower than the TRT estimate, 0.78 and 0.82 kg compared with 1.24 kg, respectively, and represent the response to the animal-marine protein blend corrected for the stage of lactation bias. The overall response to inclusion of the animal-marine protein blend across all herds was around 0.8 kg of milk.

With the ADJTRT, 18 herds were categorized as increasing in production with inclusion of the animal-marine protein blend, 12 herds did not change, and 5 herds decreased in milk (Table 5). With the TRT40, 17 herds were categorized as increasing in production with inclusion of the animal-marine protein blend, 13 herds did not change, and 5 herds decreased in milk (Table 5). The classification of the herd responses and mean response estimates were very similar using each

method (Table 5). Milk production response was classified differently in three herds (herds 11, 26, and 35) between the TRT, TRT40, and ADJTRT estimates. Only one herd was classified differently between the TRT40 and ADJTRT methods (herd 11).

Herd 26 was estimated to have increased milk production from the TRT estimate (1.0 kg, SEM = 0.5 kg, Table 3) but did not change in milk production with the ADJTRT (0.5 kg, SEM = 0.5 kg, Table 5) and TRT40 (0.2 kg, SEM = 0.6 kg, Table 5) estimates. Likewise, herd 35 did not change in production with inclusion of the animal-marine protein blend (-0.2 kg, SEM 0.2 kg, Table 3) but had decreased milk production with the ADJTRT (-0.7 kg, SEM 0.2 kg, Table 5) and TRT40 coefficients (-0.8 kg, SEM 0.2 kg, Table 5). The sign of the TRT estimates was the same in herds 26 and 35

for all three methods; however, the coefficients became less positive with correction for stage in lactation bias. Including early-lactation cows or not adjusting for the bias in estimating TRT with these cows in the analysis influenced the classification of milk production response in these herds.

Herd 11 was the only herd in which the TRT40 and ADJTRT estimates of milk response differed. Herd 11 was classified as having increased in milk using TRT (1.7 kg, SEM = 0.5 kg, Table 3) and ADJTRT (1.3 kg, SEM = 0.5 kg, Table 5) but as not having changed in milk using TRT40 (0.07 kg, SEM = 0.5 kg, Table 5). The TRT40 adjustment removes cows from the analysis, which may result in a loss in power to detect changes, whereas the ADJTRT method uses all cows in the analysis. In addition, there may be a higher response to RUP in early lactation (5, 16), which would not be observed if early-lactation cows are removed from the analysis. Possibly, early-lactation cows in herd 11 were more responsive to the animal-marine blend than later-lactation cows, resulting in a higher ADJTRT estimate than a TRT40 estimate. By adjusting for the stage of lactation bias with the mathematical formula, we can estimate the early-lactation response to the animal-marine protein blend.

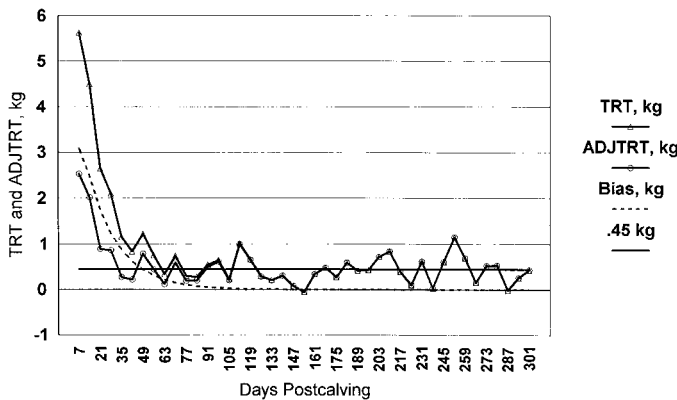
Figure 1 presents the shape of the TRT and ADJTRT coefficients as a function of week post calving, classed based on DIMINT (Week 1 = DIMINT<7, Week 2 = 6

< DIMINT<14,...). The unadjusted estimate of TRT is initially 5.6 kg of milk and then follows an exponential decline to a mean of 0.45 kg. The bias in estimating TRT as a function of DIM at initial test month is also presented in Figure 1. The bias has an initial value of 3.1 kg of milk and asymptotes to 0 kg of milk. The ADJTRT curve is the difference between the bias in estimating TRT and the TRT estimate. Response is 2.5 kg in the first week post calving and declines to a mean of 0.45 kg with week post calving (Figure 1). Cows from 1 to 6 wk post calving have a mean TRT response of 1.13 kg of milk, compared with a mean TRT response of 0.47 kg for cows after 6 wk postcalving. Response to the inclusion of the animal-marine protein blend appears to be higher in early lactation, which is consistent with other observations (5, 16).

The DIM at initial test day represents the stage of lactation when cows went on the study. However, due to experimental design, the actual stage of lactation when the animal-marine protein blend was fed was 30 to 60 d later. No cow that received the protein blend during the first 30 d of lactation was included in the study analysis. Therefore, the adjusted estimate of the production response to the animal-marine protein blend may represent a conservative estimate, because inclusion of the product earlier in lactation may have had a larger impact on milk yield.

The calculation of the TRT coefficient suggests that a majority of the herds responded positively to the animal-marine protein blend. However, this method cannot rule out that other factors in the herds may have influenced milk production the month the protein blend was included in the diet. Because the analysis only included cows in the herd for sequential tests, the change in milk was not due to cow removal and entry. The production change associated with TRT was due to change in milk within those cows in the herd on sequential test days that included the inclusion of the animal-marine protein blend.

Other methods proposed for assessing change in milk production include the change in M150 milk and change in M305 (305-d predicted milk yield) (11, 22). The M150 classified 27 of the 35 herds as having a significant increase in production when the animal-marine product was included in the ration (Tables 6 and 7). Eighteen of the 19 herds that were classified as having a positive response in milk using the regression analysis were also classified as positive by the M150 (Table 6). The M150 however also classified 9 of the 12 herds that were classified as having no response by the regression method as having had a positive response in production with inclusion of the animal-marine protein blend in the ration. Of the four herds classified as having a negative response using the re-



**Figure 1.** The magnitude of the dummy variable estimating the change in milk with the animal-marine protein blend (TRT) plotted against DIM on the first test day of data collection. TRT is the estimate of the change in milk production associated with the inclusion of an animal-marine protein blend at 2 or 4% of ration DM for 30 d. TRT is estimated by the following regression model (Milk = DIM + TRT, by cow). The graph presents the mean TRT grouped for cows by DIM on initial test day. Days in milk on initial test day influences the prediction of TRT due to the shape of the lactation curve, and the bias has the form: milk, kg = 4.064(0.098)\*(-0.0497(0.0021)\*DIMINT) presented as the --- line in the figure. The meant TRT (kg) by the initial days in milk is, -Δ-. The ADJTRT (kg) is the estimate of the response using the above equation to adjust the TRT estimate, -○-. The constant solid line - represents a mean of 0.45 kg.

**Table 6.** Classification of herd responses using the change in the M150 adjusted milk and the change in the predicted M305 compared with the regression method.

	Method		
	TRT <sup>1</sup>	M150DIF <sup>2</sup>	M305DIF <sup>3</sup>
Herd production response			
Negative	4	3	2
No change	12	5	15
Increase	19	27	18
Overall mean change, kg	1.24	1.90	148.7
SEM	0.05	0.06	8.1

<sup>1</sup>TRT = Mean coefficient for the dummy variable indicating the month the animal-marine protein blend was included in the diet calculated using a regression model for milk production as the dependent variable and DIM and TRT as the independent variables for 3 to 6 sequential production tests.

<sup>2</sup>M150DIF = Calculated M150 milk for test code 0 – the calculated M150 for test code -1. M150 milk = Sample day milk + ((DIM-150)\*0.0029\*sample day milk) (22).

<sup>3</sup>M305DIF = Projected M350 milk for test code 0 minus the projected M150 for test code -1.

gression method, the M150 predicted that three herds had decreased production and one herd had no significant change.

The change in M150 (1.90 kg/d) overestimated the production response with the inclusion of the animal-marine protein blend, (Table 6). Regressing TRT on M150 gave a coefficient of 0.637 kg of TRT/kg of M150 change (SEM = 0.006, r<sup>2</sup> = 0.60). The change in M150 overpredicted the number of positive herds and overestimated the magnitude of the production response.

Using the change in M305 and testing whether the change was different from zero resulted in classifying 16 herds as having increased milk production and two herds as having decreased production (Tables 6 and 7). Of the 19 herds that showed increased milk production with the regression method, the change in M305 agreed in 13 of the cases (Table 7). Three of the 12 herds with no production change were predicted to have increased in milk using the change in M305 yield (Table 7). The overall change in M305 was 142.21 kg of milk (SEM = 8.21, Table 6). This represents the increase in milk that would accumulate over the time from the DIM at code 0 test to 305 d of production due to the change in production with the inclusion of the animal-marine protein blend. The mean DIM for test code 0 were 168; the remaining days to 305 were 137 d. Dividing 142.21 by 137 d gives an average yield of 1.04 kg of milk/d, an estimate of the increase in milk with inclusion of the animal-marine protein blend (11). Regressing TRT on M305 change suggested that a 1 kg change in milk was associated with a 114.63 kg (SEM = 1.31 kg) change in M305.

Cows less than 100 d post calving will have a bias in change in 305-d prediction due to underprediction

of M305 yield. The mean estimate of the change in M305 will be more positive in herds with more cows less than 100 d post calving. To remove the bias in magnitude of M305 prediction, a nonparametric test may be used to assess directionality of change in M305. If the herd difference in milk is random from one test month to the next, half the cows should experience an increase in M305 and half the cows should experience a decrease in M305 prediction. A sign test statistic may be calculated based on the proportion of cows with an increase or decrease in the change in M305 to test if a significant change in production has occurred in a herd.

A sign test statistic was calculated by using the change in M305 to classify a herd as having a positive, negative, or no change in production based on the number of cows with a positive or negative change in M305. The sign test statistic was calculated based on the proportion of cows with a positive or negative difference in M305 milk production on test code 0 minus the M305 production on test code -1. Fifteen of the 19 herds classified as having a positive change in milk by the regression method also were classified as having a positive change in milk using the sign test based on

**Table 7.** Herd classification using the regression method, the change in M150 predicted yield, the change in the M305 predicted yield, and the change in the M305 predicted yield assessed by a 'z' statistic.

Total	Positive	No Change	Decrease
TRT <sup>1</sup> > 40 DIMINT			
Regression classification			
Positive	19	2	...
No change	12	...	11
Decrease	4	...	...
M150 DIF <sup>2</sup>			
Regression classification			
Positive	19	18	1
No change	12	9	3
Decrease	4	...	1
M305-Sign test			
Regression classification			
Positive	19	15	4
No change	12	4	8
Decrease	4	...	...
M305 DIF			
Regression classification			
Positive	19	15	4
No change	12	3	9
Decrease	4	...	2

<sup>1</sup>TRT = Mean coefficient for the dummy variable indicating the month the animal-marine protein blend was included in the diet calculated using a regression model for milk production as the dependent variable and DIM and TRT as the independent variables for three to six sequential production tests.

<sup>2</sup>Calculated M150 milk for test code 0 – the calculated M150 for test code -1. M150 milk = Sample day milk + ((DIM-150)\*0.0029\*sample day milk) (22).

<sup>3</sup>Projected M350 milk for test code 0 minus the projected M150 for test code -1.

the change in M305 (Table 7). Four of the positive herds were identified as not significantly changing in milk using the change in M305 and the sign test. The M305 agreed in 10 of the 12 herds that had no significant change in milk production. Two herds that were classified as having had no significant change in production using the regression method were classified as having increased in milk by using the change in M305. All four herds classified as having decreased in milk using the regression method were also negative by using the change in M305 and the sign test.

Overestimation of response to the inclusion of the animal-marine protein blend in the ration when using the change in M150 or M305 may be related to inclusion of cows less than 100 DIM in the analysis. The changes in both M150 and M305 across sequential months are positively biased for cows less than 100 DIM. The decline in milk used in the M150 equation would not apply to cows less than 100 DIM. Likewise, cows less than 100 d post calving typically increase in predicted 305-d yield from one test day to a sequential test day. The increase in M305 with each sequential test day is less as DIM approach 100 to 120. The change in M150 and M305 may be improved as a method to assess production change by using only cows over 100 DIM. To assess this effect, the data were reanalyzed with cows under 100 DIM on test code zero deleted from the analysis.

Table 8 presents summary responses for TRT, ADJTRT, **M150DIF** (change in 150-d calculated milk yield between two sequential test days), and **M305DIF** (change in 305-d calculated milk yield between two sequential test days) with records for cows less than 100 DIM on the test day with inclusion of the animal-marine protein blend deleted from the analysis. There is 100% agreement on herd classification for TRT and ADJTRT. Mean estimates of TRT and ADJTRT are the same, 0.7 kg. Only 17 herds were classified as increasing in milk, compared with 19 herds for TRT when all data were included (Table 3) and 18 herds when ADJTRT was used to classify herds using all original data (Table 5). The M150DIF classified 16 herds as increasing in milk and estimated the overall change in milk production as 0.9 kg/d, significantly higher than the regression estimates (Table 8). Regressing TRT on M150DIF resulted in a coefficient of 0.544 kg M150DIF/TRT (SEM = 0.007), suggesting the M150DIF was 1.84 times higher than the TRT estimate for change in milk. Overall the change in M150DIF agreed with TRT and ADJTRT response classes in 74.3% of the herds (26 of 35 herds, Table 8). The M305DIF agreed with the change in TRT and ADJTRT in 65.7% of herds (23 of 35 herds, Table 8). The mean change in M305DIF was 61.8 kg (SEM =

8.2 kg). With cows less than 100 DIM deleted from the analysis, the M150DIF improved in agreement with the TRT response classes from 68.6 to 74.3% (Tables 7 and 8), but the M305DIF decreased in agreement from 74.3 to 65.7% (Tables 7 and 8). Deleting records of cows less than 100 DIM improved the performance of the M150DIF and decreased the performance of the M305DIF when compared with the TRT or ADJTRT estimates for change in milk production.

The increase in milk production in herds with the animal-marine protein blend inclusion suggests that absorbed protein limited production in a majority of the herds. More than half the herds experienced an increase in milk with the inclusion of animal-marine blend. The increase in milk may have been related to an increase in absorbed protein or may have been related to provision of a limiting AA. The response to undegraded protein from animal-marine sources has been linked to forage base in the ration (4), stage of lactation (5, 16, 30), parity (17), level of production (1, 2), and DMI (23, 29).

Broderick et al. (4) found the response to dietary fish meal was greater when legume silage was the forage base compared with legume hay. Polan et al. (23) speculated that the lack of response to inclusion of animal-marine proteins in dairy rations may be related to increased DM consumption and corn silage-based rations, which provide more rumen fermentable carbohydrate than legume rations and may stimulate more microbial protein synthesis. Responses to animal-marine blends may be more apparent in rations that contain legume forages since these forages contain increased amounts of RDP and decreased amounts of rumen degradable carbohydrate than does corn silage. However, forage base (Table 4) did not significantly influence the mean TRT response across the 35 herds. This may be because forage source alone does not account for the total RDP or rumen available carbohydrate in dairy rations. Complementary feed sources may mask any deficiency of haylage-based diets in rumen available carbohydrate. In fact, Polan et al. (23) observed a lack of response in primarily corn silage based rations, whereas Wattiaux et al. (29) observed a lack of response to animal proteins in legume based rations. Both papers (23, 29) attributed the lack of response to high DM consumption, which provided sufficient amounts of absorbed protein on basal rations.

Dry matter intake may have been a significant factor influencing responses across herds. Two farm managers commented that measurements of feed intake declined with inclusion of the animal-marine protein blend. These farms had a negative TRT response. However, not all negative treatment herds reported con-

**Table 8.** Classification of herd responses for TRT<sup>1</sup>, ADJTRT<sup>2</sup>, M150 difference, and M305 difference when only cows greater than 100 DIM on the test month of the animal-marine inclusion are included in the analysis.

State	Herd	N100 <sup>3</sup>	TRT RESP	ADJTRT RESP	M150DIF RESP <sup>4</sup>	M305DIF RESP <sup>5</sup>
23	1	23	0	0	0	0
23	2	62	0	0	0	-1
23	3	58	0	0	1	1
23	4	28	1	0	0	0
23	5	28	-1	-1	-1	-1
23	6	42	0	0	-1	0
23	7	23	-1	-1	0	0
42	8	37	1	1	1	1
48	9	46	1	1	1	1
48	10	60	1	1	1	1
48	11	47	0	1	1	1
48	12	57	1	1	1	1
48	13	131	1	1	0	0
48	14	70	-1	-1	-1	-1
48	15	183	1	1	1	1
48	16	59	0	0	0	-1
48	17	34	0	0	0	0
48	18	45	0	0	0	0
48	19	52	0	0	0	-1
52	20	88	1	1	1	1
52	21	60	0	0	0	0
52	22	74	1	1	0	0
52	23	45	-1	-1	-1	-1
52	24	60	0	0	0	0
52	25	99	1	1	1	0
52	26	37	0	0	0	0
52	27	52	1	1	1	1
52	28	86	0	0	1	0
52	29	134	1	1	-1	0
55	30	74	1	1	1	1
55	31	184	1	1	1	1
58	32	696	1	1	0	0
58	33	1128	1	1	1	1
86	34	234	1	1	1	1
86	35	189	-1	-1	1	1
Mean, kg/d			0.7	0.7	0.9	61.8
SEM, kg/d			0.1	0.1	0.1	8.2

<sup>1</sup>TRT = Estimate of the effect of inclusion of an animal-marine protein blend (Prolak) on milk production when only cows > 100 DIM on the first test month are included in the analysis.

<sup>2</sup>ADJTRT = Estimate of the effect of inclusion of an animal-marine protein blend (Prolak®) on milk production when estimate of TRT coefficient adjusted using the following ADJTRT = TRT - 4.064 \* e<sup>(-0.0497 \* DIMINT)</sup> (10).

<sup>3</sup>Number of cows in each herd in the analysis when DIMINT (DIM on first test day data were collected) < 40 was deleted.

<sup>4</sup>Classification of change in milk production based on the difference in 150-d adjusted milk yield for test day with inclusion of Prolak minus the 150-d adjusted milk yield for the test day prior to inclusion of Prolak. Formula for adjusted 150-d milk based on (22): M150 milk = Sample day milk + ((DIM-150)\*0.0029\*sample day milk).

<sup>5</sup>Classification of change in milk production based on the difference in 305-d predicted yield for test day with inclusion of Prolak minus the 305-d predicted yield for the test day prior to inclusion of Prolak. RESP = Response, -1, TRT < 0, production went down; 0, TRT = 0, production did not change; 1, TRT > 0, production increased.

sumption problems. Production within herd significantly influenced TRT (Table 4). Lower producing cows had greater responses, and response decreased as milk production increased across quartiles of production within a herd. Typically lower producing cows consume less DM than higher producing cows. At a given concentration of CP in a ration, feed intake is a major

factor influencing supply of absorbed protein. Rations are typically balanced to average production levels with lead factors included to support higher producers. Despite CP concentrations over 16% CP, low producing cows may be deficient in absorbed AA due to low DMI. The animal-marine blend helped these cows more than it did higher producing herd mates. Herd level produc-

tion was not associated with the positive or no response to the animal-marine blend inclusion in the diet. The production effect was a within-herd effect since the quartile ranking was based on production within each herd. This suggests that farm managers should not consider that low producing cows do not require RUP in rations. Aharoni et al. (1) observed greater responses in lower producing cows when fish meal was included in commercial rations. In contrast, Armentano et al. (2) found production responses greater in high producing cows. Dry matter intake and content of rumen degradable carbohydrate, RDP, and RUP may influence the response that occurs at varying production levels as RUP sources are added to dairy rations.

Khorasani et al. (17) observed higher responses in multiparous cows to increases in RUP. These responses may be related to higher production in older cows when fed diets with moderate amounts of CP. Age, estimated from parity, did not influence response to animal-marine protein blend in this study (Table 4).

Christensen et al. (6, 7) and Henson et al. (15) observed no response to added RUP from animal sources. Christensen et al. (6, 7) found that microbial crude protein synthesis was reduced when more RUP was included in the ration, resulting in no net effect on AA flow to the duodenum. Henson et al. (15) observed similar effects with varying RUP sources and identified that methionine and lysine were still limiting for milk production across the various rations they used. Santos et al. (26) identified reduction in microbial synthesis with inclusion of increased RUP in rations as a major problem limiting supply of NAN to the duodenum and a potential factor limiting responses to RUP. It was anticipated that amount of the animal-marine protein blend or the type of protein source(s) in the ration prior to inclusion might have influenced the response across herds. However, these factors were not identified as significantly influencing the herd level response in this study (Table 4). No ration component on the farm was associated with predicting response. It may be that more careful delineation of diets and feed intake would have been necessary to more accurately characterize herds with a high probability of responding to the protein blend. It was not possible to do that from the data collected in this study.

Herds with no change in production when the animal-marine protein blend was included were not apparently limited by absorbed protein at the time of the experiment. No adaptation period was used prior to the inclusion of the animal-marine protein blend other than that it was included in the ration for 30 d before the production test. The blend was incorporated at the planned inclusion rate immediately after the test code -1. Most data suggest that cows should adapt to a new

feed ingredient with a 3- to 5-d period. Thus, we felt that adaptation to the protein blend should have occurred by 30 d to the next test date. However, several farms reported feed intake problems through the sample period. In general, consumption of the animal-marine protein blend was not reported as a problem in the majority of herds in the study.

Some research (1, 2, 4, 5, 8, 12, 13, 14, 17, 24) has reported that milk production increased with increased RUP; other research (3, 6, 7, 9, 15, 16, 23, 25, 29, 30) found that production did not clearly change with more RUP (Table 9). Mean increase in milk production was 0.086 kg/%RUP, as a fraction of total CP in the ration (SEM = 0.028,  $P < 0.005$ ), for the positive studies using a model with milk production as the dependent variable and independent variables of study, as a class variable, and continuous independent variables of time post calving of the study, DMI, CP, NDF, and NSC content of the ration in addition to RUP expressed as a percentage of the CP. The regression coefficient for the studies with no significant change was 0.053 kg/%RUP (SEM = 0.047,  $P < 0.269$ ) (Table 8). Studies with no significant response to added RUP had a reduction in MCP synthesis, calculated from NRC equations (21). The reduction in microbial protein synthesis was associated with lower rumen degradable N, 356 g compared to a rumen degradable N of 422 g in studies with positive response to dietary RUP (Table 8). Therefore, an important factor that may limit the milk yield response to added RUP may be a reduction in MCP synthesis. Furthermore, studies with a positive response to added RUP also had a significant increase in DMI, 0.70 kg/d; whereas studies with no significant response to added RUP had a decrease in DMI with added RUP, -0.75 kg/d.

Therefore, it is important to ensure that RDP is adequate when RUP sources are added to dairy rations and DMI is at least maintained at preinclusion levels. Reduction in DMI with added RUP in the studies with no positive response to RUP may have been associated with lower MCP synthesis and not palatability problems. Reduction in RDP in herds with negative or no response to added animal-marine protein blend may explain the lack of positive response.

Two effects may account for a positive response to added RUP. First, increasing RUP may increase flow of NAN to the small intestine by providing more escape feed protein. Secondly, DMI may increase with added RUP in diets. This will also increase the flow of rumen escape protein to the small intestine, because of higher passage rates of feed protein. Milk production may increase from both effects. Using regression coefficients from the positive studies (1, 2, 4, 5, 8, 12, 13, 14, 17, 24), and estimating DMI increases of 0.7 kg,

**Table 9.** Meta analysis of studies with a positive or no response to added RUP. Positive studies (1, 2, 4, 5, 8, 12, 13, 14, 17, 24); studies with no consistent response (3, 6, 7, 9, 15, 16, 23, 25, 29, 30).<sup>1</sup>

Item	No response		Positive response	
Degradable N intake	356 <sup>a</sup>	6.9	422 <sup>b</sup>	7.9
Microbial protein synthesis	359 <sup>a</sup>	3.7	408 <sup>b</sup>	4.3
Degradable N intake				
Basal ration	373.8	11.1	415.3	10.2
Ration with more RUP	338.3	6.4	394.8	9.3
Difference	35.5		20.5	
Regression				
Classification factors controlled for in the model				
Study	$P < 0.0001$		$P < 0.0001$	
Time	$P < 0.0001$		$P < 0.0001$	
Regression coefficient for continuous variables				
DMI	1.377	0.238	0.920	0.054
CP	0.789	0.276	0.323	0.111
UIPIP <sup>2</sup>	0.053	0.047	0.086	0.028
NSC	0.574	0.160	0.139	0.062
NDF	-0.048	0.197	0.177	0.066
R <sup>2</sup>	0.952		0.975	

<sup>1</sup>Regression model was milk = study + time postcalving + DMI + CP + UIPIP + NSC + NDF. Study and time postcalving were class variables. All other variables were treated as continuous variables.

<sup>2</sup>UIPIP = Rumens undegradable protein of the ration as a percentage of the CP.

the inclusion of the animal-marine protein blend at 3% of DM for an equivalent amount of protein from SBM would have increased the absolute RUP content of the ration approximately 4% units of RUP as a percentage of ration DM. Based on the coefficient of response from the positive studies, milk production would have been expected to increase by 0.34 kg associated with increasing RUP. However, an increase in DMI of 0.7 kg would have increased milk production an additional 0.96 kg of milk. Combined, the production response would be predicted to be 1.30 kg of milk. The mean response for the positive herds was 1.9 kg (SEM = 0.10 kg) of milk for all cows and 1.6 kg (SEM = 0.1 kg) of milk for cows >40 d in milk on initial test day. These values are similar to that which may have been expected based on analysis of the positive response studies for inclusion of RUP in rations that maintain RDP.

## CONCLUSIONS

Including an animal-marine protein blend in rations in 35 herds increased milk production significantly in 19 of the herds. The response did not differ by forage type, protein source, or level of inclusion in the ration. Parity did not influence response, although milk production within herd did, cows of the higher quartile in milk production within a herd did not respond to inclusion of the protein blend. The results of this study suggest that inclusion of animal-marine proteins in rations should be made on a herd-by-herd basis with no blanket recommendations about potential herd re-

sponses based on forage type or protein source. When RUP is increased in rations, care should be taken to ensure RDP is adequate based on forage type or protein source. The method of analysis used in this study is a sensitive technique that can be used to estimate production responses to many management inputs on dairy farms.

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