Protein, Fats and Fertility in Dairy Cows

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Summary

Levels of dietary protein that optimize milk production may depress fertility in dairy cows. While fertility responses of dairy cows fed diets with increased levels of crude protein have not been consistent, trends towards increased services/conception and days open have been reported. New concepts in ruminant protein nutriture partition crude protein into rumen undegradable and degradable fractions. Few studies investigating protein effects on fertility have accounted for partitioning of dietary protein. Dairy cows in their fourth or greater lactation are more likely to become pregnant by 120 days postpartum when fed diets balanced for rumen degradable and undegradable protein. Superovulation studies suggest fertilization failure occurs in older cows fed diets with high levels of rumen degradable protein.

Increases in milk yield has been reported in dairy cows receiving fat supplemented diets. Fats protected from rumen degradation have been advocated over vegetable oils and fat mixtures of grease and tallow. Dairy cows fed protected fats have a 2.052 risk odds ratio for pregnancy compared to cows not receiving fats. Conception rate in cows fed protected fats is 59.3% versus 41.5% in control cows. Fat included in dairy cattle rations may not only benefit production, but also reproduction.

Introduction

There have been concerns that levels of crude protein (CP) fed to optimize milk production in dairy cows may depress fertility. Information from seven studies is presented on a comparative basis in Table 1. Diets are divided into low (12-13%), medium (15-16%) and high (17-20%) levels of CP. Data are presented for services/conception and days open standardized to the medium CP level. In most, but not all, studies, increasing levels of CP protein were associated with increases in services per conception and days open.

Factors that confound or interact with CP level to alter responses in fertility are not clear. Folman *et al.* (1981) demonstrated that protecting soy bean meal from rumen degradation improved reproduction at 16% CP levels. Kaim *et al.* (1983) reported that only cows entering their fourth or greater lactations had decreased fertility when fed 19% versus 16% CP rations. Thus, rumen degradability of protein sources and age of cow may modify effects of CP on fertility.

TABLE 1. Comparative effects of dietary protein on fertility[1].

Measurement and reference		No. of cows per treatment	Dietary protein (%) 12-13	15-16	17-20
Services/Conception					
Edwards et al.	(22)	9-9-9	0.88	1.00(2.6)	1.04
Folman et al.	(26)	19-20	—	1.00(1.8)	1.25
Jordan & Swanson	(34)	15-15-15	0.79	1.00(1.9)	1.37
Kaim et al.	(37)	98-107	—	1.00(1.8)	1.31
Piatowski et al.	(51)	17-18	—	1.00(2.0)	1.40
Huber	(32)	418-237-223	1.12	1.00(1.9)	1.01
Aalseth et al.	(1)	32-31	—	1.00(1.5)	1.14
Chandler et al.	(14)	67-69	1.13	1.00(2.1)	—
Carroll et al.	(11)	57	1.00(1.5)	—	1.20
Days Open					
Edwards et al.	(22)	9-9-9	0.87	1.00(141)	0.99
Folman et al.	(26)	19-20	—	1.00(96)	1.04
Jordan & Swanson	(34)	15-15-15	0.72	1.00(98)	1.10
Piatowski et al.	(51)	17-18	—	1.00(82)	1.55
Huber	(32)	418-237-223	1.16	1.00(107)	1.13
Aalseth et al.	(1)	32-31	—	1.00(82)	0.98
Chandler et al.	(14)	67-69	1.08	1.00(130)	—
Carroll et al.	(11)	57	1.00(72)	—	1.13

1. Diets containing 15-16% CP were assigned a value of 1.00. Actual values are in parentheses.

We have been involved in research into aspects of protein and its effects upon fertility for the last two years. This paper presents some of our findings.

Case Report

In September, 1984, we were called to investigate a fertility problem of six months duration in a herd of 50 Holstein cows. Conception rates in the herd had fallen suddenly in the spring of the year from 50% to 20%. Cows were cycling normally and had no obvious signs of infectious disease as the cause of the problems. Serologic samples for IBR, BVD, and PI3 viruses and Leptospirosis, Brucellosis and Hemophilus bacteria had been non-remarkable. The herd was on a twice yearly 5-way Leptospirosis bacterin and yearly intranasal IBR and PI3 virus vaccination program. Postbreeding infusions of 500 to 1,000 mg of oxytetracycline and injections of GnRH at time of insemination had failed to improve conception rates.

We visited the farm in October, 1984 to analyze management practices and examine the cows. Cows were in good body condition and environment was good. Average daily milk production per cow was over 30 kg. No obvious infectious cause could be found on the basis of rectal and vaginal speculum examinations, blood serology and aerobic uterine swab cultures. No semen was stored on the farm, and all inseminations were performed by trained technicians from three different bull studs. Heat detection efficiency was good with 70% of available heats being serviced. Breeding intervals calculated from farm records indicated that cows were cycling normally, but failing to conceive.

Fertility problems began in April, 1984, shortly after corn silage was not available and a new ration was formulated for use with legume hay and haylage. Computer assessment (Galligan *et al.* 1986) of feeding programs in January and April, 1984 are presented in Table 2. The ration fed from January through March, 1984 was well balanced for energy, CP and degradable intake protein. Fertility during this time had been good. The ration changes in April resulted in high crude protein, high rumen degradable intake protein and marginal net energy levels. Elevations in degradable intake protein preceded and were coincident to the decline in fertility.

TABLE 2. Evaluation of rations for January and April 1984 and recommended ration adjustment in October 1984.[a]

	Jan. 1984	Apr. 1984	Oct. 1984
Ingredients (percent of DM)			
Legume haylage/hay	32.8	48.5	31.2
Corn silage	11.2	0	9.0
Corn/shelled, ground ear	38.0	30.5	35.1
Oats	2.6	2.1	2.5
Roasted soy beans	0	9.1	8.2
38% CP Premix	14.4	8.8	0
Dist. dry grains	0	0	12.9
Mineral and vitamins	1.0	1.0	1.0
Calculated composition (DM basis)			
Protein (%)			
Crude	16.0	18.4	16.0
Degradable intake protein	61	67	62
Undegradable intake protein	39	33	38
Net energy (Mcal/kg)	1.60	1.54	1.64
Acid detergent fiber (%)	21.4	24.9	21.3
Percent of NRC requirements for Milk and maintenance			
Dry matter intake	100	100	100
Net energy (Mcal/kg)	99	94	100
Acid detergent fiber	102	117	102
Protein (%)			
Crude protein	100	115	100
Degradable intake protein	100	120	100
Undegradable intake protein	100	104	100

a. 727 kg cow producing 36 kg milk. NRC (1978, 1985).

To document the over feeding of protein, cows milking between 30 to 140 days postpartum were bled for determination of serum urea nitrogen (SUN). These values were compared to cows bled at similar stages postpartum in our university herd. Our university herd was used as a control because these cows were fed diets balanced for rumen degradable and undegradable protein. Cows in the case herd had significantly higher levels of SUN than cows in the university herd (23.6 ±2.8 mg/dl versus 15.7 ±2.4 mg/dl, $p<.01$).

We reformulated the feeding program (Table 2) based on NRC (1978, 1985) recommendations for energy, crude protein and rumen degradable and undegradable protein. We bled the cows one month later in November, 1984. SUN levels declined significantly from the previous month to 18.2 ±2.1 mg/dl ($p<.01$ compared to earlier levels). Conception rates improved in accordance with the dietary changes (Table 3).

TABLE 3. Analysis of fertility by season for 1984 and 1985.

	Year 1984	Year 1985	Chi Square	Level of Significance
Jan., Feb., Mar.				
No. Animals				
Total	16	35		
Pregnant	9	16		
% Pregnant	56	46	.49	.49
Apr., May, June, July, Aug., Sept.				
No. Animals				
Total	44	59		
Pregnant	10	28		
% Pregnant	23	47	6.62	.01

Presented in Table 3, are comparisons of fertility for similar time periods for 1984 and 1985. In January through March, 1984 and 1985, when diets were balanced for energy, CP and rumen degradable protein, there were no significant differences in conception rates. However, from April through September, conception rates were significantly better ($p<.01$) in 1985 than in 1984.

Presented in Figure 1 is the relationship between SUN and probability of pregnancy as described by logistic regression analysis (Kleinbaum *et al.* 1982). Cows were bled at breeding, and conception rates were recorded for ranges of SUN. Conception rate declined significantly in cows with SUN above 20 mg/dl ($X^2 = 89.4$ $p<.01$).

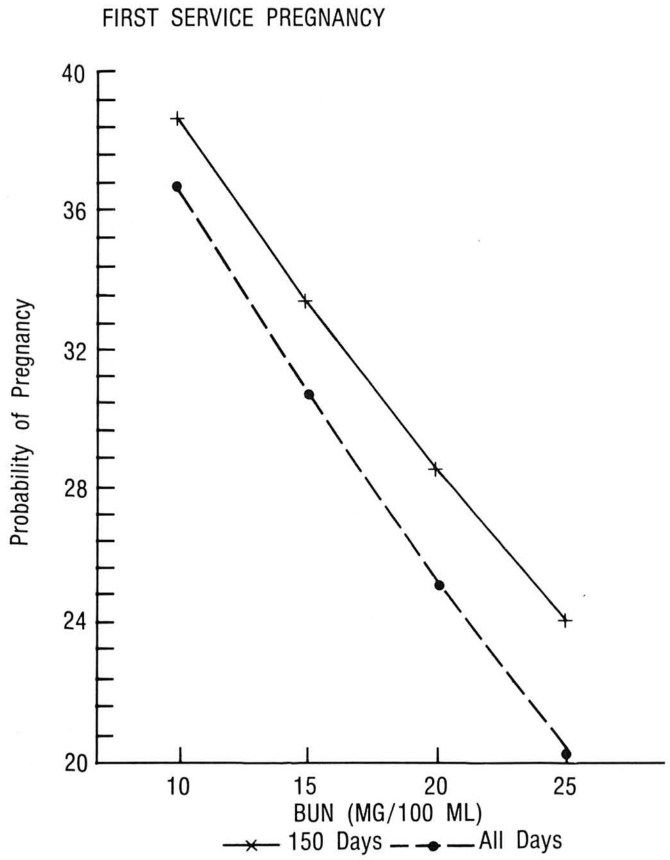
Research Herd

To more closely investigate relationships between fertility and protein degradability, an on farm study was conducted in a herd of 120 Holstein cows. Cows were matched on the basis of age, previous milk production and breeding history and assigned to receive one of two diets (Table 4). Amounts of soy bean meal (48% CP), raw soy bean seeds, and

FIGURE 1. Relationship between serum urea nitrogen and pregnancy rate. Serum was collected on the day of breeding or within two weeks of breeding. Cows were placed into five groups on the basis of SUN (mg/dl): 10.5 (9-12, n=5), 14.5 (13-16, n=14), 18.5 (17-20, n=18), 22.5 (21-24, n=17) and 26.5 (25-28, n=11). Asteriks denote observed values. The solid line is pregnancy predicted by the following equation:

$$Preg = \frac{1}{(-.978+.21773\ (SUN)-.023189\ (SUN)^{\wedge}2+7\times10-4\ (SUN)^{\wedge}3\ (i+e\ \ \ )}$$

The dashed lines are standard errors of predicted values.



distillers' dried grains with solubles were varied to provide equicaloric and isonitrogenous diets with 72% (diet 1) and 62% (diet 2) rumen degradable protein. Protein and energy sources were fed individually twice a day to each cow based on milk production. Corn silage and legume hay were fed free choice from bunk feeders. Diet 2 provided optimal balances of rumen degradable and undegradable protein, whereas diet 1 contained excessive amounts of rumen degradable protein and was deficient in undegradable protein as established by the NRC publication (1985).

Cows were fed the diets for 120 days postpartum. Every two weeks they were examined rectally to monitor uterine involution, weigh taped, body scored and blood sampled for SUN. Pregnancy data was analyzed using logistic regression (Kleinbaum *et al.* 1982). Logistic regression is used to

TABLE 4. Diets used to evaluate the effects of protein degradability on dairy cow fertility.

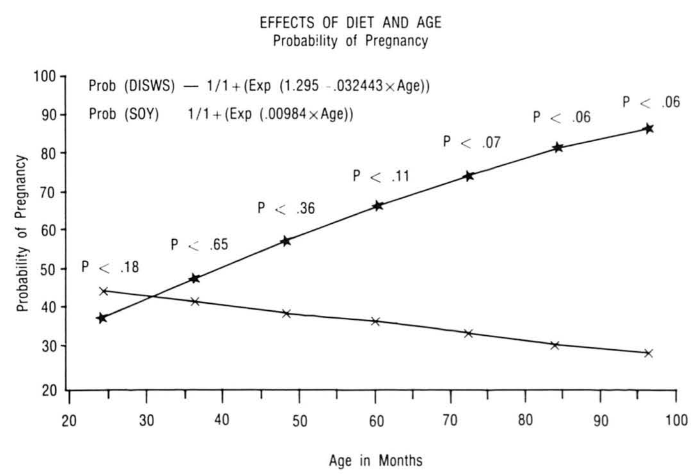
	1	2	3
Ingredients (% DM)			
Corn silage	34.7	34.7	34.7
Alfalfa hay	11.0	11.0	11.0
Ground ear corn	29.2	24.8	20.0
Raw soy beans	13.8	6.9	14.2
Soy bean meal (48% CP)	8.1	6.3	18.2
Distillers dried grains with solubles	0	13.7	0
Minerals/vitamins	3.2	2.6	2.1
Estimated ration composition (DM basis)a			
Protein (%)			
Crude protein	16.5	16.4	21.6
Degradable intake protein	71	63	71
Undegradable intake protein	29	37	29
Net energy (Mcal/kg)	1.66	1.66	1.66
Ether extract (%)	5.8	5.8	5.8

a. Based on NRC (1978, 1985).

analyze factors which influence the expression of a dichotomous variable, in this case pregnancy. The statistical measure of association is the risk odds ratio. In this experiment this is the risk of pregnancy, given exposure to diet 2 versus diet 1. Use of the logistic model allows for control of factors which may influence pregnancy through confounding or interaction. From the logistic model, probabilities of the event occurring can be calculated (Fig. 2).

Presented in Figure 2 is the relationship calculated from the logistic model for the development of pregnancy in cows in both dietary groups. Age is recorded in months at time of calving. Figure 2 computes the probability of pregnancy for cows on diet 1 and 2 as a function of age. There was a trend that cows less than 30 months of age at calving were more likely to become pregnant by 120 days on diet 1 than on diet 2 ($p<.18$). However with increasing age, this relationship changed and cows fed diet 2 were more likely to become pregnant by 120 days. This effect did not approach signifi-

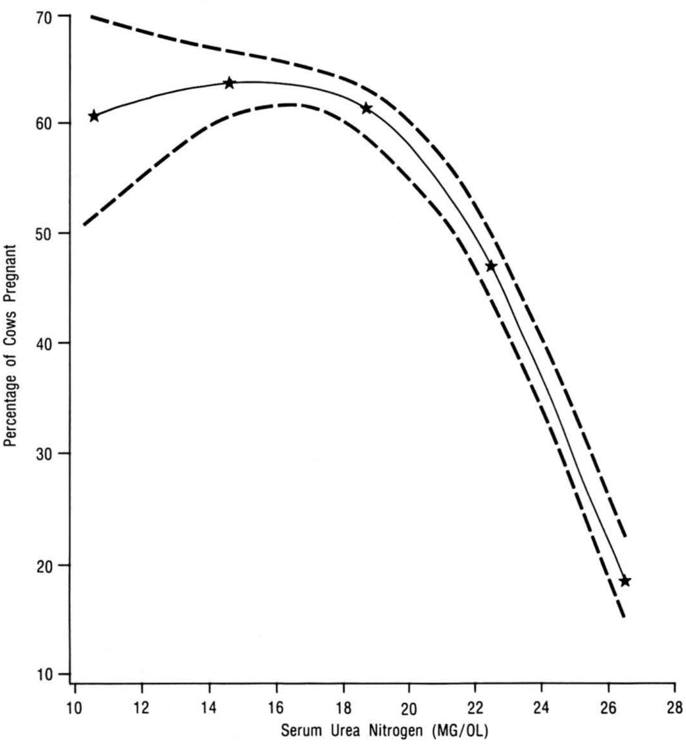
FIGURE 2. Probability of pregnancy for Diet 1 and Diet 2 presented in Table 4. Calculated from the logistic equations in the legend on the graph.



cance a the 5% level of probability until cows were above 60 months of age, or in their fourth lactation. This is similar to results of Kaim et al. (16) where cows were not affected by CP level in the diet until they were in their fourth or greater lactation. However the response we observed is not to CP level but to degradability of protein sources. It is difficult to explain the modifying influence of age upon the effect of rumen degradability of protein on pregnancy. Perhaps older cows develop subtle changes in their physiology or subclinical pathologies to cause alterations in requirements for amino acids or responses to metabolic end products of rumen degradable protein.

To investigate the relationship of SUN and conception rate, 210 cows in 8 herds were used in a field trial investigating diets 1, 2 and 3 in Table 4. Each herd was divided into two groups. Cows were assigned to one of two diets within the herd, either diet 2 or 1 or diet 2 or 3. Diets were fed for 150 days postpartum. Blood was collected every 2 weeks to monitor SUN. Logistic regression of first service conception rate versus SUN concentration is presented in figure 3. Increasing SUN was associated with a ROR of 0.95 for pregnancy ($p<.04$). This is about a 1% decline in conception rate for every 1 mg/dl increase in SUN. These results must be interpreted with caution as all confounding factors, such as level of milk production, have not been investigated in this data set as yet. Cows with low SUN may have higher conception rates because they are giving less milk.

FIGURE 3. Probability of pregnancy for serum urea nitrogen values for 210 cows from eight herds. Each herd was divided into two groups and cows assigned to diets 1 or 2 or 2 or 3 in Table 4. Model was calculated by logistic regression controlling for herd.



Embryo Data

To investigate potential mechanisms of infertility with high levels of rumen degradable protein, cows in two herds were fed diets similar to those in Table 4. Additionally, cows in a third herd were fed a diet with 21% CP that provided 28% of the CP as rumen undegradable protein and 72% as rumen degradable protein (diet 3). This diet provided adequate absorbable protein and excessive degradable protein. Cows which had fully involuted, had palpably normal uteri and showed no signs of uterine pathology were super ovulated between 60 and 120 days postpartum. Cows were inseminated with two straws of frozen semen every 12 hours for three breedings from the time they were observed in heat. Frozen semen from the same bull was used for all inseminations and the same technician inseminated all the cows in each of the herds.

Embryos were collected by the same person by intrauterine lavage using standard embryo collection techniques. Embryos were then graded and categorized by an experienced embryologist who was blind to the dietary treatments.

Failure to superovulation was defined as harvest of all unfertilized embryos or no harvest of embryos from cows with palpable corpus luteum on both or either ovary. There were no differences in total embryos or in quality of embryos harvested on each diet. However, significantly more cows failed to respond to superovulation on diets 1 and 3 than on diet 2 (Table 5). Eighty percent of cows which failed to respond with a successful flush were in their fourth or greater lactation. Regardless of concentration of dietary protein (16 vs 21%), cows fed diets with high levels of rumen degradable protein had superovulation failure rates of about 33% compared to 4% for cows fed the diet containing recommended levels of rumen degradable protein.

Fertilization failure or changes in oviductal transport of early fertilized embryos may be the mechanism affecting fertility in cows fed diets not balanced to provide optimal amounts of rumen degradable protein.

Sperm Motility and Urea

To further investigate possible mechanisms for fertilization failure in cows fed diets with excessive rumen degradable protein, we examined the effect of urea on progressive motility of bull spermatozoa. We chose to examine urea as a potential toxicant to sperm, since urea has been reported to affect sperm function (Dasgupta *et al.* 1970, Umezaki and Fordney-Stettlage 1975), and uterine levels of urea in the case herd were elevated. Fresh ejaculates were collected from a young Holstein sire with excellent semen parameters for motility and morphology. Aliquots were taken and placed in extender to provide 20 million sperm per ml. Urea was added to extender to provide 20, 50 and 200 mg mg/dl final concentration. Two observers, blind to sample source, graded progressive motility on samples prepared for them by a third person, who selected samples at set time intervals in a random pattern. Motility declined in all samples over time. However, there appeared to be no effect of urea concentration on progressive motility of bull sperm in extender.

TABLE 5. Summary of results of super ovulation to the three diets.

	Diet			
	1	2	3	level of significance 1 vs 2, 2 vs 3
Number cows	22	23	12	
Number failures	7	1	4	0.2 .025
Percent failure (%)	31.8	4.4	33.3	
Mean Number Embryos	7.6	8.0	7.6	
Unfertilized (% total)	36	28	7	
Fertilized (% total)	64	72	93	
Grade (% fertilized)[1]				
1	51	38	70	
2	20	29	12	
3	13	17	8	
4	5	12	4	
5	13	9	6	

1. Embryo grades for quality are presented as a percentage of fertilized embryos. 1 = superior to 5 = poor.

Fat

Fat has been advocated for dairy cattle rations to increase energy density without decreasing fiber (Palmquist and Jenkins, 1980). Cows consuming fat supplemented diets may be more metabolically efficient due to optimum ratios of lipogenic, glucogenic and aminogenic nutrients (Kronfeld, 1976). Fats above 3 to 5% of the total ration dry matter depress rumen function, therefore fats protected from rumen degradation have been advocated for dairy cattle rations. Two types of protected fats are calcium salts of palm oil, which are insoluble in rumen liquor at normal rumen pH, and hard fats, saturated fatty acids which have a high melting point and therefore resist degradation in the rumen. We investigated the effects of dairy fat prills, a hard fat composed of palmitic (47%), stearic (36%) and oleic (14%) acids on reproduction in dairy cows.

Cows in four herds, three in southeast Pennsylvania and one in Israel were used in a field study to investigate the effects of fat supplemented diets on production and reproduction. Diets are presented in Table 6. Diets were similar except prills replaced some of the grain components in the experimental diet. Cows in each herd were divided into two treatment groups and diets were fed for 120 to 150 days postpartum. Two hundred and fifty-three cows were involved in the experiment.

To summarize the reproductive effects, cows receiving the fat supplemented diets had an overall conception rate of 59.3% versus 41.5% in the control cows ($p < .001$). First service conception rates were similar, 59.1% versus 42.6% for fat supplemented versus control cows ($p < .01$). There were no differences in days to first service between treatment groups, but the improved conception rate in cows fed the fat supplemented rations meant days open was significantly less in the fat supplemented cows, 103 ± 4.8 SEM compared to 118 ± 4.5 SEM in the control cows.

TABLE 6. Dietary ingredients and nutrient composition of diets used to test the effect of dietary prills on reproduction in dairy cows.

	LCFA	
	No	Yes
Ingredients		
Alfalfa Haylage	20.0	20.9
Alfalfa Hay	8.6	9.0
Corn Silage	25.4	26.4
HMEC	23.2	16.6
Roasted Soybeans	7.4	7.7
SBM48	4.5	5.3
Distillers Dry Grains	8.2	9.2
Minerals/Vitamins	2.7	2.8
Added Fat	0	2.1
Calculated Composition		
NEL (Mcal/kg)	1.64	1.70
Crude Protein (%)	16.3	16.9
UIPIP (%)	34	35
ADF (%)	19.6	19.8
Fat	4.0	6.1

UIPIP = Undegraded intake protein as a percent of CP.

Conclusion

Our observations indicate that high levels of rumen degradable protein may depress fertility of lactating dairy cows. Older cows appear to be affected to a greater extent. The decreased fertility may be due to fertilization failure. Important interactions with energy may occur in diets high in crude protein and/or rumen degradable protein to cause infertility on a herd basis. Although not likely to be a toxicant, herds with SUN above 20 mg/dl and experiencing low conception rates, should have their ration evaluated for energy, protein level and degradability of protein. More work is needed to identify factors which modify responses of fertility to protein level and type.

Protected fats supplemented in dairy cattle rations may not only benefit production, but also reproduction. Cost break-even points need to be determined not only based on milk yields but also in relation to savings in days open and improved conception rates.

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