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Increasing Prepartum Dietary Crude Protein using Poultry By-Product Meal Dose Not Influence Performance of Multiparous Holstein Dairy Cows

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Abstract: The aim of this study was to compare the effects of two levels of Crude Protein (CP) using Poultry by-Product Meal (PBPM) fed during late gestation on the performance, blood metabolites and colostrum composition of Holstein cows. Twenty multiparous cows 26±6 days before expected calving were assigned randomly to two treatments containing 1) 140 g kg^{-1} DM CP (34 g kg^{-1} DM PBPM) 2) 160 g kg^{-1} DM CP (75 g kg^{-1} DM PBPM). The cow's BCS was 3.56±0.5 on average, at the beginning of the trial. Yields of milk, protein, lactose and fat were not affected by prepartum dietary CP level. Colostrum composition (fat, CP and total solids percents), blood metabolites (Ca, glucose, total protein, albumin, globulin, urea N and cholesterol) and metabolic diseases incidence were not influenced by prepartum dietary CP level. There was no significant difference between treatments in body weight and BCS changes. As expected, blood urea N before calving was higher in the cows fed 160 g kg^{-1} DM CP diets ($p<0.002$). Serum cholesterol during prepartum ($p<0.03$) and postpartum ($p<0.01$) periods was significantly lower in 160 g kg^{-1} DM CP treatment. In general, although postpartum glucose level increased in cows which received 160 g kg^{-1} DM CP in the diet, it seems that there is no other obvious advantages over feeding the 140 g kg^{-1} DM CP diet. So feeding this level of CP diet to close up cows is recommended.

Key words: Close up, crude protein level, performance, holstein cow, BCS

INTRODUCTION

The periparturient period (i.e., 21 days prepartum to 21 days postpartum) may be the most critical time in a dairy cow's production cycle as, generally, most of the health problems of dairy cows occur during this period (Park *et al*., 2002). Prepartum nutrition of dairy cows can have a dramatic impact on health during the early postpartum period and on subsequent lactation performance (Santos *et al*., 2001). During the last few weeks of gestation, dairy cows experience a dramatic decline in Dry Matter (DM) intake (Greenfield *et al*., 2000; Hayirli *et al*., 1999) which may be the cause of compromised nutrient balance and postpartum performance. Because of the lower DM intake during the last weeks of the dry period, dietary concentrations of nutrients must be adjusted upwards to ensure adequate nutrient intake and minimize potential metabolic disorders during the transition period that occur immediately before, to several weeks after, calving (Santos *et al*., 2001).

Since, the publication of National Research Council (1989), a considerable amount of research has been conducted to determine the amount of CP and RUP required in the diet of the late-gestation dairy cow (Greenfield *et al*., 2000; Hook *et al*., 1989). The mid-1990s ushered in a period of active research in the area of nutrition and management of dry dairy cows that focused on strategies to reduce the incidence of metabolic disease associated with calving and enhance productivity of cows in their subsequent lactation (Robinson *et al*., 2004).

During the prepartum period maternal nutrient requirements progressively increase as a result of udder tissue synthesis and colostrum production, as well as uterine development prior to calving. All of these processes require protein and, due to declining DM intake, problem intake can be compromised unless dietary Crude Protein (CP) levels are increased. However, results on the impacts of increasing the dietary CP level in prepartum dairy cows are contradictory. In this case Greenfield *et al.* (2000) and Hartwell *et al.* (2000) reported that increasing prepartum CP had negative effects on cow's performance, while Robinson *et al.* (2004) and Santos *et al*. (2001) did not observe any effects on mature cows with elevating dietary CP. However, Van Saun *et al.* (1993) observed positive effects from increasing prepartum CP on cow's performance.

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The animal industry uses various source of protein and essential lipids in monogastric and ruminant feeding. The bone and meat meal are forbidden ingredients for ruminant feeding and, thus, consumption of co-products from poultry industry is in development, especially in Iran. PBPM has a more constant nutritive value compared to meat meal (National Research Council, 2001; Mustafa *et al*., 2000). The rumen escape CP content of PBPM is similar to that of soybean meal. In Iran dairy farms use protein levels more than National Research Council (2001) recommendations in prepartum diets. Considering crescive use of PBPM in the world (and Iran) and CP levels more than National Research Council recommendations in prepartum diet in Iran, so a practical study in Iran conditions for increasing CP along with PBPM is crucial.

MATERIALS AND METHODS

Animal management: Moultiparous Holstein cows (n = 20; 766±117 kg body weight (BW); 3.56±0.5 Body Condition Score (BCS), 26±6 days until expected calving) were used in a completely randomized block design. The prepartum period of the study was conducted during the autumn and winter of 2007 (December to January). Cows were paired by parity and randomly assigned to one of two treatments: 1) 14% crude protein (3.4% PBPM) and 2) 16% crude protein (7.5% PBPM). Diets were fed as TMR (once and twice daily for the pre- and postpartum periods, respectively) for approximately 5% refusal and cows were group fed. During the postpartum period, cows were housed in the same fresh barn and were fed group. The amount of feed offered and refused was measured daily. Diets formulating was done using the National Research Council (2001) model (Table 1). Experimental diets composition is shown in Table 2. Because the trial was done on a commercial dairy farm, there were constraints on the experimental design as dictated by the farm manager during the prepartum period. Milk production data were collected only for the three weeks lactation, but other data (such as the amount of colostrum, reproductive parameters, body weight and BCS) were collected until 120 days after calving.

Sampling and analysis: Samples of all TMR and diet ingredients were analyzed for DM, CP (Kjeldahl N×6.25), ether extract, ash, NDF and ADF according with AOAC (1990). Cows were milked four times daily at 0600, 10000, 1400 and 2200 h in the milking parlor. Milk yield was recorded daily. Milk samples were taken weekly from consecutive am and pm milkings, composited and analyzed by Milk-O-Scan minor (78110; Foss, Denmark) for fat, protein, lactose and SNF. Colostrum samples were collected during the first milking postpartum and analyzed for CP, fat, total solids and ash. Cows were scored for body condition (BCS; Wildman *et al*., 1982) by two skilled individuals at the beginning of experiment, 1 days before parturition and at 21-days postpartum.

Blood samples (10 mL) were collected at 7-days before calving and on 1 day postpartum of the coccygeal vein or artery using vacutainer tubes 2.5 h after

Table 1: Ingredient composition of prepartum and postpartum diets (dry matter basis)

	Prepartum diets		Postpartum diet
Ingredients	Treatment 14%	Treatment 16%	21 DIM
Alfalfa hay	34.0	34.0	33.84
Corn silage	33.0	33.0	7.30
Barley, ground	6.9	5.5	6.50
Corn grain, ground	6.9	5.5	12.02
Megalac[a]	1.4	0.1	1.04
Canola meal	2.2	2.2	1.55
Whole cottenseed	2.2	2.2	5.48
Cottonseed meal	-	-	0.25
Soybean meal	5.2	5.2	5.56
PBPM[b]	3.4	7.5	10.06
Beet pulp	-	-	8.60
Molasses, beet	-	-	4.92
Dicalcium phosphate	-	-	0.78
Calcium carbonate	0.5	0.5	0.21
Sodium bicarbonate	-	-	0.69
Ferrous sulfate	-	-	0.06
Monensin	-	-	0.02
Toxiban	-	-	0.09
Mineral[c] and vitamin[d] mix	0.6	0.6	0.44
Salt	-	-	0.15
Anionic salt[e]	3.3	3.3	-
Multi vitamin	-	-	0.02
Vitamin A[f]	0.2	0.2	0.09
Vitamin E[g]	0.2	0.2	0.10

[a]Behparvaran Company, Iran. [b]PBPM contained 3.55% Ca, 22.73% Fat, 0.93% Salt, 53.81% Protein and 92.64% DM. [c]Mineral mix contained 200000 mg kg^{-1} of Ca, 90000 mg kg^{-1} of Mg, 13500 mg kg^{-1} of Mn, 17500 mg kg^{-1} of Fe, 3500 mg kg^{-1} of Cu, 14300 mg kg^{-1} of Zn, 35 mg kg^{-1} of Co, 90 mg kg^{-1} of Se and 210 mg kg^{-1} of I. [d]Vitamin mix contained vitamin A 1500000 IU, vitamin D3 400000 IU and vitamin E 6000 mg kg^{-1}. [e]Anionic premix contains: 0.1% Ca, 1% Mg, 15.46% Cl, 3.81% S, 0.02% Na, 0.77% K, 8.6% CP (% DM) and NE_L 1.03 Mcal kg^{-1}. [f]Vitamin A contained 5000000 IU kg^{-1}. [g]Vitamin E contained 550 IU kg^{-1}

Table 2: Nutrient composition of experimental diets (dry matter basis)

	Prepartum diets		Postpartum diet
Nutrients	Treatment 14%	Treatment 16%	21 DIM
NE_L[a] (Mcal kg^{-1})	1.62	1.62	1.64
CP (%)	14.00	16.00	18.00
RDP (%)	10.20	11.40	11.90
RUP (%)	3.80	4.60	6.10
NDF (%)	26.80	26.40	21.10
Forage NDF (%)	22.20	22.20	20.50
ADF (%)	25.20	25.10	21.40
NFC (%)	28.40	26.90	27.40
DCAD[b] (mEq kg^{-1})	-33.00	-32.00	+320.00
Ca (%)	1.10	1.10	1.90
P (%)	0.50	0.60	0.90
Vitamin A (IU kg^{-1})	10000.00	10000.00	8200.00
Vitamin E[c] (IU kg^{-1})	11.00	11.00	1000.00

[a]NE_L prepartum 1.6x maintenance and postpartum 3.1x maintenance. [b]DCAD: Dietary cation-anion difference; (Na+K)-(S+Cl). [c]Vitamin E shortage elevated by IM injection at 21, 7 days before calving

feeding (Dann *et al*., 1999). After collection, the samples were centrifuged (Hettich; D-78532 T tuttlingan, Germmany) at 3,000×g for 10 min for serum separation. Serum was harvested and frozen at -20°C for later analyses. Blood glucose measured (Glucotrend, England) while taking blood sample. Samples of serum were analyzed for Ca (O-Kresolphta leine-Komplexon method), urea (Berthelot enzymatic method), albumin (Bromcresol Green method), globulin, total protein (Biuret method) and cholesterol (CHOD-PAR enzymatic method) by Pars Azmun kites (Pars Azmun Laboratory, Tehran, Iran). The absorbance was read using spectrophotometer (Perkin-Elmer, Colemen Instruments Division, Oak Brook, IL).

Newborn calves were weighted within 1 h of birth. Parturition difficulty was determined based on a 5-point scale with a score of 1 needing no assistance and the score of 5 needing cesarean or leading to calf death. Placenta weight and the time interval between calving and placenta expulsion were recorded for individual cows. Postpartum rectal temperature was recorded after morning milking. A case was diagnosed as retained placenta if the placenta was not expelled within 24 h of calving. A case was diagnosed as milk fever if the cow was recumbent, blood calcium dropped to <5.5 mg dL^{-1} and the rectal temperature decreased drastically. Subclinical hypocalcaemia was defined as blood calcium concentrations of less than 7.5 mg dL^{-1} (Goff and Horst, 1997).

The state of cows uterus investigated by an expert with two methods (transrectal palpation and manual vaginal examination) at 21th day after calving for endometrit. Reproductive parameters such as pregnancy duration, days open, services per conception, first artificial insemination, pregnancy rate and first service conception rate during first 120 days of lactation were recorded. Estrus was detected twice daily (each time 30 min) by visual observation and cows were artificially inseminated after a postpartum voluntary waiting period of 45 to 60 days after calving. Of course due to few number of cow in current experiment, we analyzed the reproduction data but we do not emphasis on them.

Statistical analysis: Data were analyzed using MIXED procedure of SAS (2003). The randomized complete block model included the fixed effects of treatment, block (parity and projected calving date) and their interaction. The effect of cow within block by treatment was considered random. Group based DMI data were compared between treatments using day as the replication.

Data of the amount of milk yield were covariate corrected at the base of previous calving records. Treatment differences with $p<0.05$ were considered significant and $p<0.1$ were considered a tendency.

RESULTS

Feed intake, productivity and body parameters: Treatments didn't influence DM intake (Table 3). Nutrients composition and colostrum production at first milking immediately after calving was not affected by prepartum diets (Table 3). Also, milk yield, fat corrected milk yield (FCM), energy corrected milk (ECM) and milk composition did not change significantly among treatment groups (Table 3). As shown in Table 3, there was no significant differences in BW and BCS changes between treatments.

Blood metabolites: Concentrations of BUN in prepartum cows were significantly affected by dietary treatment (Table 4). Prepartum intake of CP did not have a significant effect on postpartum concentrations of BUN in cows (Table 4).

Table 3: Effect of prepartum protein level on dry matter intake, productivity and body parameters of dairy cows during the pre- and postpartum periods

	Treatment			
Items	14%	16%	SEM[a]	p-value
Prepartum DMI (kg)	12.20	12.40	1.03	0.54
Postpartum DMI (kg)	14.80	14.70	1.45	0.68
Colostrum yield and component				
Raw colosrum	5.78	5.35	1.60	0.79
Fat (%)	5.49	4.88	0.75	0.20
Fat (g day^{-1})	303.00	235.00	0.06	0.31
Protein (%)	15.79	16.51	0.71	0.33
Protein (g day^{-1})	929.00	885.00	0.29	0.88
Lactose (%)	2.76	2.60	0.20	0.44
Lactose (kg day^{-1})	0.16	0.14	0.04	0.60
SNF (%)	18.62	19.09	0.72	0.53
SNF (kg day^{-1})	1.10	1.03	0.34	0.84
Total solids (%)	24.08	23.87	1.14	0.86
Milk yield and component				
Raw milk	38.13	38.97	1.52	0.59
4% FCM[b] (kg day^{-1})	40.88	41.31	1.47	0.78
3.5% FCM[c] (kg day^{-1})	44.35	44.80	1.60	0.79
ECM[d] (kg day^{-1})	43.33	43.97	1.37	0.65
Fat (%)	4.56	4.51	0.18	0.78
Fat (kg day^{-1})	1.71	1.71	0.07	0.94
Protein (%)	3.23	3.28	0.13	0.72
Protein (kg day^{-1})	1.21	1.25	0.05	0.42
Lactose (%)	4.37	4.45	0.10	0.42
Lactose (kg day^{-1})	1.67	1.73	0.07	0.40
SNF (%)	8.59	8.75	0.19	0.45
SNF (kg day^{-1})	3.26	3.38	0.12	0.37
Change in BW[b] (kg)	-65.40	-56.03	9.77	0.36
Change in BW[c] (kg)	-76.80	-69.93	14.34	0.64
Change in BCS[d]	0.11	0.05	0.07	0.40
Change in BCS[e]	-0.31	-0.31	0.06	0.80

[a]SEM, Standard error of the mean. [b]Beginning of experiment to immediately after calving. [c]Immediately after calving to end of experiment. [d]Beginning of experiment to last week of gestation. [e]Last week of gestation to end of experiment

Table 4: Effect of prepartum protein level on blood metabolites of dairy cows

Items	Prepartum 14%	16%	SEM[a]	p-value
Glucose (mg dL^{-1})	62.12	69.24	8.05	0.40
BUN (mg dL^{-1})	17.33	20.66	0.82	0.002
Ca (mg dL^{-1})	7.02	7.19	0.55	0.76
Total protein (g dL^{-1})	7.12	7.14	0.28	0.96
Albumin (g dL^{-1})	3.90	4.09	0.11	0.12
Globulin (g dL^{-1})	3.22	3.05	0.29	0.57
Cholesterol (mg dL^{-1})	102.20	78.33	9.57	0.03
Glucose (mg dL^{-1})	86.22	94.47	14.41	0.58
BUN (mg dL^{-1})	16.54	17.23	0.80	0.41
Ca (mg dL^{-1})	6.53	6.58	0.55	0.94
Total protein (g dL^{-1})	7.03	7.17	0.25	0.59
Albumin (g dL^{-1})	3.89	3.97	0.18	0.67
Globulin (g dL^{-1})	3.13	3.19	0.22	0.77
Cholesterol (mg dL^{-1})	78.95	57.58	6.45	0.01

[a]SEM: Standard error of the mean

Table 5: Effect of prepartum protein level on parturitional characteristics

Items	Treatment 14%	16%	SEM[a]	p-value
Parturition difficulty[b]	1.97	1.47	0.34	0.17
Calf birth wt. (kg)	49.21	46.59	2.06	0.23
Placenta wt. (kg)	5.84	5.48	0.38	0.37
Body temp. (°C)	38.58	38.56	0.06	0.66

[a]SEM: Standard error of the mean. [b]Parturition difficulty score-five point scale: 1 = No problem, 2 = Slight problem, 3 = Needed assistance, 4 = Considerable force, 5 = Caesarian)

Plasma glucose was not affected by prepartum diet neither prepartum nor postpartum, but was lowest in cows fed the 14% CP diet (Table 4). Significant differences for cholesterol were observed between treatments in last week of gestation and immediately after calving (Table 4). As shown in Table 5 other blood metabolites (Ca, total protein, albumin and globulin) were not significantly affected by experimental rations.

Parturitional characteristics: There were no differences between treatments for parturition difficulty as measured on a five-point scale where 1 = no difficulty (Table 5). No significant differences for calf birth weight were noted between treatments. Body placenta and body temperature were not affected by prepartum diets (Table 5).

Metabolic diseases: Metabolic diseases were recorded daily and reported as the number of incidents observed (Table 6). Incidences of retained fetal membranes, dystocia, displaced abomasum, milk fever, hypocalcimia, mastitis, endometritis and clinical ketosis were similar between treatment groups. One case of displaced abomasum was observed in 16% ration. Hypocalcemia was observed in 87 and 75% of the cows fed 14 and 16% CP diets, respectively. Milk fever and endometrit were not seen in none of the treatments.

Table 6: Effect of prepartum protein level on incidence of metabolic diseases in dairy cows

Items	Treatment 14%	16%
Dystocia	0	0
Retained fetal membranes	0	1
Hypocalcimia	7	6
Milk fever	0	0
Clinical ketosis	0	0
Displaced abomasum	0	1
Mastitis	1	1
Endometritis	0	0

Table 7: Effect of prepartum protein level on reproductive parameters of dairy cows

Items	Treatment 14%	16%	SEM[a]	p-value
Pregnancy duration (d)	282.5	281.7	1.78	0.63
Days open (d)	90.4	90.7	14.8	0.98
First AI (d)	70.6	79.8	7.51	0.25
SPC[b]	1.8	1.3	0.46	0.30
First service CR[c] (%)	56.1	75	…..	…..
Pregnancy rate (%)	76.9	87.5	…..	…..

[a]SEM: Standard error of the mean. [b]SPC: Services per conception. [c]CR: Conception rate

Reproductive parameters: Reproductive parameters were not affected by prepartum protein level (Table 7). The average first AI was not significantly altered by prepartum diets. Dietary treatments had no influence significant on average days open. Conception rate at first service and pregnancy rate at 120 DIM were 56.15, 75 and 76.92, 87.5%, respectively (Table 7). The average pregnancy duration was not affected by rations.

DISCUSSION

Productivity and body parameters: Prepartum Feed intake were not different among the two treatment groups (Table 3) which agrees with others (Greenfield *et al*., 2000; Grummer *et al*., 1995; Hartwell *et al*., 2000; Huyler *et al*., 1999; Johnson and Otterby, 1981; Van Saun *et al*., 1993). In general, CP level of prepartum diet doesn't significantly influence DMI. Colostrum production and its component was not affected by prepartum diets which agrees with the results of Santos *et al*. (2001). Only colostrum fat percent numerically was lower for 16% treatments (Table 3). Researches studies show that decreasing fat percent of milk or colostrum of fresh cow is useful for animal health (Griinari and Bauman, 2006).

Putnam and Varga (1998) showed that mature Holstein cows fed diets with 10.6, 12.7, or 14.5% CP were all considered to be in positive protein balance. The few studies that observed improvement on lactational performance when cows were fed diets with higher prepartum CP either used primigravid cows (Hook *et al*., 1989; Van Saun *et al*., 1993) that have lower DMI as a ercentage of body weight and higher protein requirements for growth, or fed control diets with less than 12% CP (Chew *et al*., 1984; Hook *et al*., 1989). Others (Greenfield *et al*., 2000; Hartwell *et al*., 2000) also observed that increasing prepartum protein above 12% of diet DM for multiparous Holstein cows was detrimental to postpartum performance.

In lactating animals, live weight change alone does not provide a good measure of growth because it also reflects mobilization of body reserves in early lactation and conceptus growth in the late lactation (Friggens *et al*., 2007). In agreement with others (Greenfield *et al*., 2000; Hartwell *et al*., 2000; Huyler *et al*., 1999; Park *et al*., 2002; Putnam and Varga, 1998; Santos *et al*., 2001; Van Saun, 1993). BCS changes in comparison with BCS of last week of gestation ending in calving and BCS changes in comparison to BCS of the end of experiment were not significant statistically between treatments. Since BCS reflects energy resources of cows, these data propose that feeding prepartum rations with increasing CP level had no effect on body resources loss during late 3 weeks of gestation and first 3 weeks of lactation.

Blood metabolites: The concentration of BUN is known to be a function of dietary CP content, rumen degradability of CP, energy intake (Jordan *et al*., 1983; Oltner *et al*., 1985), time of sampling relative to feeding and level rumen ammonia (Gustafsson and Palmquist, 1993). By increasing CP level and the following RDP, ammonia level in rumen increased and as a result blood urea N concentration increased. In fact, blood urea N is the index of protein intake.

Higher glucose concentration in cows fed diet with 16% CP probably is due to increase in gluconeogenesis or decrease in glucose intake by lateral tissues. Danfær *et al.* (1995) suggested that AA can account for up to 40% of glucose synthesis, although the range can be considerable (2 to 40%). Putnam and Varga (1998) observed that increasing prepartum CP from 10.6 to 12.7 and then to 14.5% had a linear positive effect on plasma glucose concentrations.

Significant decrease in cholesterol in treatment two in last week of gestation and immediately after calving probably is due to amino acid availability (particularly, positive effect of amino acids on gluconeogenesis) in transition cow's liver. As a result, glucose availability inhibited fatty acids β-oxidation and adipose brake down decreased and reduced triglycerides accumulation. So, acetyl-coA resulted from β-oxidation, which is roleing in esteroids synthesis and particularly cholesterol, will not available. Other blood metabolites (calcium, total protein, albumin and globulin) were not significantly affected by experimental rations that these findings were in accordance to others (Murphy, 1999; Ryan *et al*., 2003).

Parturitional characteristics: In case of parturitional characteristics, our results are in agreement with Putnam *et al.* (1999) and Butler *et al.* (2002) but are not in accordance with Park *et al.* (2002). Evidence from sheep (McNeill *et al*., 1997) suggests that, although markedly underfeeding protein to cows can inhibit fetal growth in late gestation, no further increase in fetal growth appears to occur at high levels of protein supplementation.

Reproductive parameters: As mentioned earlier, we don't emphasis on reproduction results due to a few number of cows. Nevertheless, these data may be useful for some ones.

CONCLUSIONS

Increasing dietary CP level with a PBPM during the last 3 week of gestation did not change the lactational performance of dairy cows during the first 21 days in milk. Milk and colostrum composition, metabolic and reproductive parameters, BW and BCS change were not affected by higher prepartum dietary protein. Only serum cholesterol showed significant difference between treatments. Thus although present results for some data for 16% treatment were better than results of 14% treatment, but these results do not support proper reasons for feeding high protein diets and we need more study and more experiments with much more stocks. In general this study and data recommends feeding ration with 14% CP in comparison to 16% for close up cows.

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