

## Statistical Analysis of Some Factors Affecting Crude Protein Balance in Lactating Dairy Cows

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### ABSTRACT

Some factors affecting crude protein balance (CPB), as a measure of efficiency, were studied using 2824 records of 501 lactating Holstein cows. The CPB ( $\text{kg d}^{-1}$ ) was calculated as crude protein (CP) intake (kg) minus CP yield (kg). Two different statistical methods including principal components (PCs) and general linear model analyses were used to study the effects of different factors. Crude protein balance had a moderate correlation with PCs 1 (-0.206), 2 (0.318) and 3 (-0.281), which accounted for 65.6% of total variations. Age, parity, lactation stage, pregnancy stage, dry matter intake (DMI), milk yield (MY), fat corrected milk yield (FCM), milk fat percentage (F%), milk lactose percentage (L%), milk fat yield (FY), milk protein yield (PY), milk lactose yield (LY), dietary levels of net energy for lactation ( $\text{NE}_L$ ), CP, ruminally undegradable protein (RUP), neutral detergent fiber (NDF) and ether extract (EE) and income over feed cost (IOFC) were correlated to CPB, at least, in one of PCs 1, 2 or 3. In general, linear model analysis CPB was significantly affected by parity and lactation stage and had significant partial linear regression coefficients on DMI ( $\text{kg d}^{-1}$ ), F%, FY ( $\text{kg d}^{-1}$ ), PY ( $\text{kg d}^{-1}$ ),  $\text{NE}_L$  ( $\text{Mcal kg}^{-1}$ ) and dietary levels of CP (%), RUP (%), NDF (%) and EE (%). The quadratic partial regression coefficients of CPB on  $\text{NE}_L$ , CP, RUP and NDF were also significant. The estimates for optimum dietary levels of  $\text{NE}_L$ , CP and RUP for minimizing CPB in the studied population were 1.49 Mcal/kg, 11.29% and 7.58%, respectively. In comparison to NRC's estimates, it seems that, more  $\text{NE}_L$ , RUP and NDF and less CP are needed to minimize protein balance in lactating dairy cows.

**Keywords:** Crude Protein Balance, Holsteins, Lactation, Nutrient use efficiency.

### INTRODUCTION

Efficiency of nutrient utilization is generally considered to be a major factor affecting farm profitability on modern dairy farms. Dairy cows excrete about 2-3 times more nitrogen (N) in manure than in milk, which contributes to increased milk production costs and environmental N pollution (Broderick, 2005). Mass N balance studies showed that, on typical dairy farms,

only 12 to 36% of the N input is retained in salable products, whereas up to about 70% is lost mainly through volatilization and leaching into the off-farm environment (Ipharraguerre and Clark, 2005).

Producers often feed high CP diets to ensure a sufficient supply of the metabolizable protein required for maximal milk and protein production of dairy cows, although some reports indicate that feeding diets with excessively high CP concentration

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and especially excess ruminally degradable CP will decrease protein efficiency in lactating dairy cows (Olmos Colmenero and Broderick, 2006; Wang *et al.*, 2007; Huhtanen *et al.*, 2008). On the other hand, recently, attention has focused on the relationship between N utilization in lactating cows and environmental pollution (Castillo *et al.*, 2000).

Crude protein balance (CPB), defined as the difference of protein intake and protein yield in milk, could be considered as a measure of protein efficiency (Baars, 1998), assuming that, at similar stages of lactation, cows with less maintenance requirements and less excretion of N in urine and manure are more efficient and have a lower CPB. Moreover, CPB may overcome some problems arising from ratio measures of efficiency (Meyer and Garrett, 1967).

The objective of this experiment was to study an empirical set of data to evaluate the effects of some environmental factors on CPB to improve protein efficiency in lactating dairy cows.

## MATERIALS AND METHODS

The data analyzed in this study were 2824 records obtained from 501 lactating Holstein cows located in two herds, from 2002 to 2003. All cows involved in this study were kept in a tie-stall housing system and milked 3 times per day. During the experimental period, the animals had ad libitum access to 172 kinds of total mixed rations, which were

different among individuals. The rations were different or occasionally changed according to factors such as herd management, availability of feed ingredients, and yield. Milk production was measured once a week and its composition was determined monthly. Feed intake was calculated weekly, as the difference of feed offered and orts, using common farm scales. For measurement of feed intake on dry matter (DM) basis, DM of feed offered and orts were determined separately.

AOAC (1990) methods were used for determination of the content of dietary dry matter (method no. 930.15), CP (method no. 984.13), crude fiber (method no. 930.10), ether extract (method no. 920.39) and acid detergent fiber excluded from residual ash (method no. 973.18). Ash-free neutral detergent fiber was determined without sodium sulfate in the neutral detergent, according to Van Soest *et al.* (1991). The amounts of NE<sub>L</sub> and RUP were calculated using NRC (2001). Table 1 presents some nutritional components of the diets offered. Crude protein balance (CPB) was calculated as the difference of CP intake and CP excreted in milk.

Descriptive statistics for yield traits in the studied population is presented in Table 2. The average milk yield and content of milk fat and milk protein of the studied cows were 25 kg, 3.5%, and 3%, respectively (Table 2). The ranges of parity and lactation stage in the studied data were 1 – 7 years and 1 – 12 months after parturition, respectively.

**Table 1.** Some nutritional components of the diets fed (On dry matter basis).

Component	Average	Standard dev.	Minimum	Maximum
DM (% of as fed) <sup>a</sup>	64.3	5.58	50.2	76.3
NE <sub>L</sub> (Mcal kg <sup>-1</sup> ) <sup>b</sup>	1.517	0.048	1.41	1.64
CP (%) <sup>c</sup>	14.31	0.861	12.47	17.78
RUP (%) <sup>d</sup>	4.73	0.364	4.08	6.34
NDF (%) <sup>e</sup>	33.89	2.111	29.94	40.84
EE (%) <sup>f</sup>	3.18	0.352	2.64	4.55

<sup>a</sup> Dry matter, <sup>b</sup> Net energy for lactation, <sup>c</sup> Crude protein, <sup>d</sup> Ruminally undegradable protein, <sup>e</sup> Neutral detergent fiber, <sup>f</sup> Ether extract.

**Table 2.** Descriptive statistics for the studied traits.

Trait	N	Average	Standard dev.	Minimum	Maximum
Milk yield (kg d <sup>-1</sup> )	2824	25.09	7.53	8	51.4
Milk fat (%)	2824	3.54	1.03	1.96	7.67
Milk protein (%)	2824	3.04	0.46	2.33	6.79
Milk lactose (%)	2824	4.87	0.32	3.33	5.52
Protein yield (kg)	2824	0.720	0.194	0.111	1.406
Income over feed costs (Rls d <sup>-1</sup> )	2824	28600	11698	-8986	75540
Crude protein balance (kg d <sup>-1</sup> )	2824	1.890	0.580	0.524	3.814

### Statistical Analysis

The data were analyzed using a multivariate principal components analysis and a general linear model. The principal components analysis was used to evaluate the variation pattern of all dependent and independent variables together and a general linear model analysis was applied to study the effects of independent variables on CPB.

The multivariate principal components analysis was applied using Minitab (release 13.20) to investigate the intrinsic variability of different variables. At this step, the relations between different variables, including dependent and independent variables, were investigated, so as a result, different variables were grouped according to their relationships. Different variables studied by multivariate methods were age, parity number, lactation stage, pregnancy stage, dry matter intake (DMI), milk yield (MY), 3.2% fat corrected milk yield (FCM), milk fat percentage (F%), milk protein percentage (P%), milk lactose percentage (L%), milk fat yield (FY), milk protein yield (PY), milk lactose yield (LY), dietary dry matter levels of net energy for lactation (NE<sub>L</sub>), crude protein (CP), ruminally undegradable protein (RUP), neutral detergent fiber (NDF) and ether extract (EE), income over feed cost (IOFC) as an economic index (Baars, 1998; Zamani *et al.*, 2005) and crude protein balance (CPB). Moreover, simple and partial correlation coefficients of CPB with IOFC were calculated for more interpretation of the results.

At the second step, the effects of some independent variables on CPB were tested using a general linear model analysis. Independent variables for general linear model analysis were herd, animals within herds, parity number, lactation stage (month), pregnancy stage (month), DMI (kg d<sup>-1</sup>), MY (kg d<sup>-1</sup>), FCM (kg d<sup>-1</sup>), F% (%), P% (%), L% (%), FY (kg d<sup>-1</sup>), PY (kg d<sup>-1</sup>), LY (kg d<sup>-1</sup>) and dietary levels of NE<sub>L</sub> (Mcal kg<sup>-1</sup>), CP (%), RUP (%), NDF (%) and EE (%). In addition to linear regression coefficients, the quadratic regression coefficients of dietary factors were added to the general linear model. The Proc GLM of SAS 9.1 (SAS, 2004) was used for general linear model analysis. Optimum levels of dietary NE<sub>L</sub>, CP, RUP and EE for minimizing CPB were estimated using partial derivation of the estimated general linear model.

### RESULTS AND DISCUSSION

The overall average of CPB in this data set was 1.89±0.580 kg d<sup>-1</sup> (Table 2). The main part of this value is due to fecal and urinary N excretions.

#### Multivariate Statistical Analysis

The results of the principal components analysis, including absolute, proportional, and cumulative proportional eigenvalues for 15 computed principal components are presented in Table 3. Each principal component (PC) has its own eigenvalue, which presents the variation that the PC

**Table 3.** Results of eigen analysis of 15 principal components (PCs).

PC	PC 1	PC 2	PC 3	PC 4	PC 5
Eigenvalue	8.5848	2.4535	2.0758	1.4129	1.1464
Proportion	0.429	0.123	0.104	0.071	0.057
Cumulative	0.429	0.552	0.656	0.726	0.784
PC	PC 6	PC 7	PC 8	PC 9	PC 10
Eigenvalue	0.9705	0.7371	0.6338	0.6106	0.5448
Proportion	0.049	0.037	0.032	0.031	0.027
Cumulative	0.832	0.869	0.901	0.931	0.959
PC	PC 11	PC 12	PC 13	PC 14	PC 15
Eigenvalue	0.304	0.2694	0.1469	0.0507	0.0328
Proportion	0.015	0.013	0.007	0.003	0.002
Cumulative	0.974	0.987	0.995	0.997	0.999

has collected. Eigenvalue 1 shows that the principal component has collected as much variation as that of one variable. As shown in Table 3, five important PCs with eigenvalues higher than 1 accounted for 78.4% of the total variations. PC1 with eigenvalue of 8.5848 accounted for 42.9% of the variations, thus, it can be considered as the most important PC for the variable investigated. PC 2 and PC 3 were also relatively important PCs, because they collected 12.3% and 10.4% of the total variations, respectively. PC 4 and PC 5 accounted for 7.1% and 5.7% of variations, respectively, and, therefore, were less important.

The matrix of correlation coefficients of different variables with PCs 1-5 is presented in Table 4, where correlation coefficients higher than 0.2 are presented as bolded numbers. CPB had noticeable correlations with PC 1 (-0.206), PC 2 (0.318) and PC 3 (-0.281).

Lactation and pregnancy stages, DMI, yield traits including MY, FCM, FY, PY, LY, dietary levels of NE<sub>L</sub> and CP, economic index of IOFC and CPB had the highest correlations with the PC 1 (Table 4), which accounted for 42.9% of the total variations of the investigated traits (Table 3). Thus, it can be concluded that lactation and pregnancy stages, DMI, MY, FCM, FY, PY, LY, dietary levels of NE<sub>L</sub> and CP, IOFC and CPB were strongly correlated.

In PC 2, the most correlated variables were age, parity, DMI, FCM, L% FY, CP, RUP, IOFC and CPB (Table 4). The correlation of CPB with PC 2 (0.318) was slightly higher than its correlation with PCs 1 and 3. This means that CPB had an intermediate correlation with age, parity, DMI, FCM, L% FY, CP, RUP and IOFC.

Crude protein balance had a negative correlation (-0.281) with PC 3, which accounted for 10.4% of the total variations (Tables 3 and 4). Age, parity, DMI, F%, L% FY, NDF, EE, IOFC and CPB were the main contributors to PC 3.

The noticeable contribution of PC 1 in the total variation (42.9%) reveals that this PC can be considered as the best PC for presenting the variation type of the studied variables. The estimated correlation coefficients of different variables with PC 1 shows that PCB, lactation and pregnancy stages, DMI, MY, FCM, FY, PY, LY, dietary levels of NE<sub>L</sub> and CP and IOFC are probably correlated. Moreover, the correlation of different variables with PCs 2 and 3 indicates that CPB is also correlated with F%, L%, RUP, NDF and EE, in addition to other variables. These results show that CPB has a very complex relationship with many other variables.

The correlation coefficients of IOFC and CPB with PCs 1, 2 and 3 were similar in PC 1 and different in PCs 2 and 3. Thus, interpretation of the relation of CPB to

**Table 4.** Matrix of correlation coefficient of different variables with five principal components\*.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5
Age	-0.019	0.418	0.457	-0.079	0.248
Parity	-0.023	0.413	0.467	-0.046	0.257
Lactation stage	0.229	-0.048	-0.098	-0.354	0.153
Pregnancy stage	0.213	-0.127	-0.126	-0.352	0.168
DMI <sup>a</sup>	-0.248	0.212	-0.229	-0.128	0.078
MY <sup>b</sup>	-0.327	-0.097	0.046	0.004	0.13
FCM <sup>c</sup>	-0.304	-0.205	0.159	-0.137	0.063
F% <sup>d</sup>	0.18	-0.149	0.258	-0.348	-0.277
P% <sup>e</sup>	0.157	-0.007	0.065	-0.529	-0.294
L% <sup>f</sup>	-0.114	-0.274	-0.245	0.036	0.209
FY <sup>g</sup>	-0.241	-0.279	0.245	-0.252	-0.01
PY <sup>h</sup>	-0.306	-0.115	0.056	-0.191	0.068
LY <sup>i</sup>	-0.325	-0.135	0.01	0.006	0.147
NE <sub>L</sub> <sup>j</sup>	-0.214	0.111	0.019	0.008	-0.381
CP <sup>k</sup>	-0.249	0.251	-0.173	-0.092	-0.215
RUP <sup>l</sup>	-0.176	0.217	-0.15	-0.217	-0.171
NDF <sup>m</sup>	0.18	-0.166	0.22	0.322	-0.087
EE <sup>n</sup>	-0.15	-0.035	0.21	0.195	-0.567
IOFC <sup>o</sup>	-0.277	-0.285	0.222	-0.031	0.106
CPB <sup>p</sup>	-0.206	0.318	-0.281	-0.082	-0.012

<sup>a</sup> Dry matter intake; <sup>b</sup> Milk yield; <sup>c</sup> Fat corrected milk yield; <sup>d</sup> Milk fat %; <sup>e</sup> Milk protein %, <sup>f</sup> Milk lactose %; <sup>g</sup> Milk fat yield; <sup>h</sup> Milk protein yield; <sup>i</sup> Milk lactose yield, <sup>j</sup> Dietary levels of net energy for lactation; <sup>k</sup> Crude protein; <sup>l</sup> Ruminally undegradable protein; <sup>m</sup> Neutral detergent fiber; <sup>n</sup> Ether extract; <sup>o</sup> income over feed cost, <sup>p</sup> Crude protein balance.

IOFC is not easily possible in PC analysis. The simple correlation coefficient of CPB with IOFC was 0.096 ( $P < 0.0001$ ), which, apparently, showed a positive relation between CPB and IOFC or a negative relation between protein efficiency and economic profit. On the other hand, the partial correlation coefficient of CPB with IOFC, adjusted for MY, was -0.832 ( $P < 0.0001$ ), indicating the net positive relation of protein efficiency with economic profit. Therefore, it could be concluded that the apparent negative relation of protein efficiency with economic profit is due to the effect of yield traits on both variables. It means that, at the same level of milk yield, more efficient animals (with less CPB) have a higher economic profit. The effect of feed efficiency on economic profit has been reported in literature (Veerkamp, 1998).

### General Linear Model Analysis

The results of the general linear model analysis are presented in Table 5. In this analysis, CPB was significantly different among herds ( $P < 0.0001$ ) and cows within herds ( $P < 0.0001$ ) and was influenced by parity ( $P < 0.0001$ ), lactation stage ( $P < 0.0001$ ), DMI ( $P < 0.0001$ ), F% ( $P = 0.0241$ ), FY ( $P < 0.0073$ ), PY ( $P < 0.0001$ ), NE<sub>L</sub> ( $P < 0.0001$ ), CP ( $P < 0.0001$ ), RUP ( $P < 0.0001$ ), NDF ( $P = 0.0002$ ) and EE ( $P = 0.0236$ ), and quadratic effects of dietary NE<sub>L</sub> ( $P < 0.0001$ ), CP ( $P < 0.0001$ ), RUP ( $P = 0.0002$ ), and NDF ( $P < 0.0001$ ).

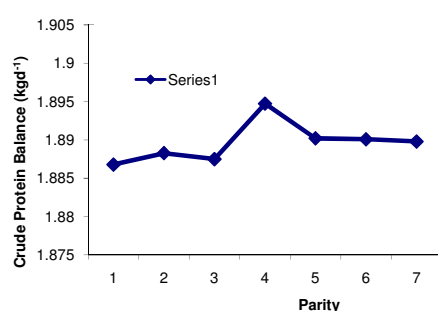
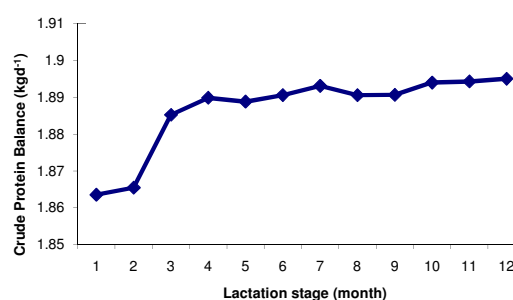
The trends of least square means of CPB over parities and lactation stages are presented in Figures 1 and 2, respectively. CPB showed a general increase at progress of parities and lactation stages. This means

**Table 5.** Result of the general linear model analysis for CPB ( $R^2=0.999$ ).

Variable	Parameter estimate	Standard error	Type III MS	F Value	P
Herd	—	—	0.03864	47.24	< 0.0001
Cow (Herd)	—	—	0.00082	1.73	< 0.0001
Parity	—	—	0.01327	28.13	< 0.0001
Lactation stage	—	—	0.00195	4.14	< 0.0001
Pregnancy stage	—	—	0.00065	1.38	0.2022
DMI <sup>a</sup>	0.14702	0.00044	53.56007	113544	< 0.0001
MY <sup>b</sup>	-0.00047	0.00182	0.00003	0.07	0.7937
F% <sup>c</sup>	0.00421	0.00187	0.00241	5.1	0.0241
P% <sup>d</sup>	-0.00543	0.00363	0.00106	2.24	0.1349
L% <sup>e</sup>	-0.00748	0.00599	0.00074	1.56	0.2119
FY <sup>f</sup>	-0.03166	0.01178	0.00341	7.22	0.0073
PY <sup>g</sup>	-0.98507	0.02441	0.76794	1627.98	< 0.0001
LY <sup>h</sup>	0.01319	0.02994	0.00009	0.19	0.6596
NE <sub>L</sub> <sup>i</sup>	-9.36124	1.27514	0.02542	53.9	< 0.0001
CP <sup>j</sup>	-0.50597	0.03711	0.08769	185.91	< 0.0001
RUP <sup>k</sup>	-0.11736	0.02786	0.00837	17.75	< 0.0001
NDF <sup>l</sup>	0.07501	0.02033	0.00642	13.61	0.0002
EE <sup>m</sup>	-0.03101	0.01368	0.00242	5.14	0.0236
NE <sub>L</sub> <sup>*</sup>	3.13790	0.41724	0.02668	56.56	< 0.0001
CP <sup>*</sup>	0.02240	0.00117	0.17431	369.53	< 0.0001
RUP <sup>*</sup>	0.00774	0.00208	0.00654	13.87	0.0002
NDF <sup>*</sup>	-0.00117	0.00030	0.00697	14.77	0.0001
EE <sup>*</sup>	0.00099	0.00131	0.00027	0.57	0.4512

<sup>a</sup> Dry matter intake; <sup>b</sup> Milk yield; <sup>c</sup> Milk fat %; <sup>d</sup> Milk protein %, <sup>e</sup> Milk lactose %; <sup>f</sup> Milk fat yield; <sup>g</sup> Milk protein yield; <sup>h</sup> Milk lactose yield; <sup>i</sup> Dietary levels of net energy for lactation; <sup>j</sup> Crude protein; <sup>k</sup> Ruminally undegradable protein; <sup>l</sup> Neutral detergent fiber, <sup>m</sup> Ether extract.

\* Quadratic regression coefficients of NE<sub>L</sub>; CP; RUP; NDF and EE, respectively.

**Figure 1.** The trend of least square means for crude protein balance over parities.**Figure 2.** The trend of least square means for crude protein balance over lactation stages.

that protein efficiency is probably decreased over different parities or lactation stages. Significant decrease of protein efficiency in later stages of lactation is in agreement with the report of Custodio *et al.* (1983). However, the effect of parity on protein efficiency in this study disagrees with Flis and Wattiaux (2005) who did not find a significant effect of parity on protein efficiency.

The significant linear relationship of CPB with DMI, F%, FY, PY,  $NE_L$ , CP, RUP, NDF and EE in the general linear model analysis (Table 5), is not in complete agreement with the results of the principal component analysis (Table 4). This is probably due to the difference of the two methods of analysis since, in GLM analysis, partial regression coefficients are adjusted for other variables in the model, while the PC analysis involves a mathematical procedure that transforms a number of possibly correlated variables into a smaller number of uncorrelated variables called principal components.

CPB had a negative partial regression coefficient on MY, P%, L%, FY and PY. However, only partial regression coefficient of PY was significant (Table 5). This indicates that high protein yielding animals probably have less CPB and, thus, are more efficient in the use of dietary CP. More protein efficiency in high producing animals is reported in other studies (Li *et al.*, 1998; Jonker *et al.*, 2002).

The partial linear and quadratic regression coefficients of  $NE_L$ , CP, RUP and NDF were significant (Table 5). This means that the relationship of CPB with dietary levels of  $NE_L$ , CP, RUP and NDF is probably nonlinear (Figures 3 to 6).

After partial derivation of the estimated general linear model, the optimum dietary levels of  $NE_L$ , CP and RUP for minimizing CPB were estimated as 1.49 Mcal  $kg^{-1}$ , 11.29%, and 7.58%, respectively. The maximum CPB was estimated at 32.1% of NDF.

The estimated requirements of  $NE_L$ , CP, RUP and NDF by NRC (2001) for large

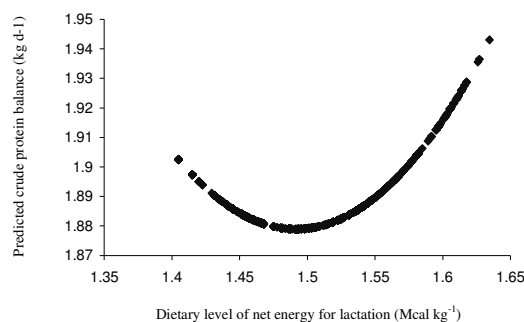


Figure 3. Crude protein balance predicted by dietary net energy for lactation.

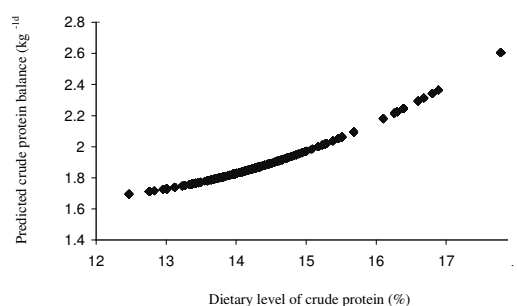


Figure 4. Crude protein balance predicted by dietary crude protein.

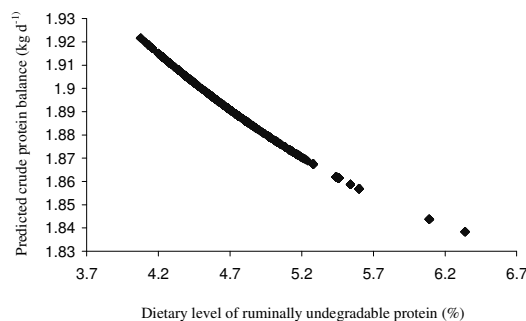


Figure 5. Crude protein balance predicted by dietary ruminally undegradable protein.

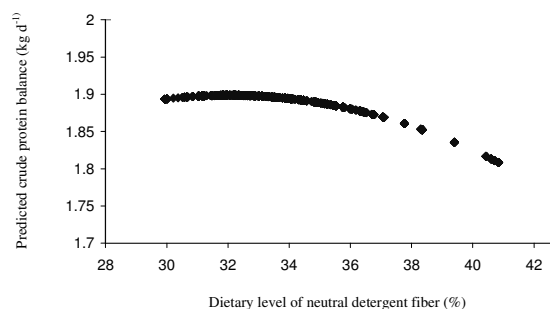


Figure 6. Crude protein balance predicted by dietary neutral detergent fiber.



breed dairy cows producing 25 kg of milk with 3.5% fat and 3% protein are 1.37 Mcal kg<sup>-1</sup>, 14.1%, 4.6% and 25-33%, on dry matter basis, respectively. The estimated dietary level of NE<sub>L</sub> for minimizing CPB in this study (1.49 Mcal kg<sup>-1</sup>) was slightly higher than the 1.37 Mcal kg<sup>-1</sup> requirement of NE<sub>L</sub> by NRC (2001). This means that a slight increase in dietary energy probably reduces CPB and prevents N loss. This finding could be attributed to an increase in fermentable metabolizable energy (FME) in most of high NE<sub>L</sub> diets, because FME provides the energy needed to supply rumen microbes for capture of N. Moreover, the fate of absorbed peptides and amino acids once inside the microbial cell will depend on the availability of energy. If energy is available, amino acids will be transaminated or used directly for microbial protein synthesis, otherwise, if energy is limiting, amino acids will be deaminated and their carbon skeleton will be fermented (Bach *et al.*, 2005). Many studies describe the effect of FME and RDP on microbial protein synthesis in the rumen (Dewhurst *et al.*, 2000; Castillo *et al.*, 2001a; NRC, 2001; Bach *et al.*, 2005).

The estimation of optimum level of dietary CP for minimizing CPB in this study (11.29%) was less than the requirement of 14.1% for CP by NRC (2001). This indicates that the requirement of CP for maximizing protein efficiency may be less than the CP requirement for maximizing production. This is in agreement with the report of Wang *et al.* (2007) who found that the animals fed higher protein diets produced more milk, but, had higher N excretion in urine than those fed lower protein diets. This finding is also supported by Olmos Colmenero and Broderick (2006) who found a significant linear decline in the apparent N efficiency (milk protein N/N intake) as dietary CP increased. However, in the study of Groff and Wu (2005), increase in dietary CP did not affect milk yield but increased N excretion and reduced the efficiency of N utilization for milk production. The decreased protein efficiency in animals fed

high protein diets is because a high CP diet generally has a higher amount of ruminally degradable protein (RDP) and, as a result, when RDP exceeds microbial needs large amounts of NH<sub>3</sub> are produced, absorbed into the blood, converted to urea in the liver, and excreted in the urine (Olmos Colmenero and Broderick, 2006). It is noteworthy that overfeeding CP reduces profit margins because of the relatively high cost of protein supplements and the poor efficiency of N use by dairy cows fed high protein diets (Broderick, 2003).

The estimated requirement of RUP for minimizing CPB in this study (7.58%) was much higher than the requirement of 4.6% for RUP by NRC (2001). Thus, it could be concluded that the increase in the RUP/RDP ratio may reduce CPB and improve protein efficiency, because at a high RDP, more N would be absorbed as ammonia or more amino acids would be deaminated, that might increase N excretion in urine (Castillo *et al.*, 2001b). The positive effect of RUP on protein efficiency is also reported by Flis and Wattiaux (2005) and Kalscheur *et al.* (2006). Moreover, Castillo *et al.* (2001b) and Reynal and Broderick (2005) found that an increase in dietary CP degradability results in more urinary N excretion. However, post-ruminal digestibility of RUP and amino acids balance could be considered as an important factor for increasing metabolizable protein flow to the intestine (Noftsker and St-Pierre, 2003) and, subsequently, optimizing dietary RUP to improve protein efficiency.

In this study, dietary NDF had a quadratic effect on CPB (Table 5). As it could be seen in Figure 6, NDF does not have a constant effect on CPB. This is probably due to the need of ruminal microorganisms for an optimal level of NDF to effectively convert dietary crude protein to metabolizable protein. After partial derivation of the general linear model, maximal CPB was estimated at 32.1% NDF. This is in the range of 25-33% as the minimal NDF requirement proposed by the NRC (2001) for large breed dairy cows producing 25 kg



of milk with 3.5% fat and 3% protein. On the other hand, CPB showed an overall decreasing trend when dietary NDF increased (Figure 6). This means that NDF requirement for improvement of protein efficiency is probably higher than the requirement proposed by the NRC (2001).

Dietary fat was another factor affecting CPB. In PC analysis, EE and CPB both had a moderate correlation with PC 3, but in opposite directions (Table 4). The opposite relation of CPB with dietary EE was supported by a significant negative partial regression coefficient of CPB on EE in the general linear model analysis (Table 5). This means that the increase in dietary lipids would decrease CPB and, thus, improve protein efficiency, probably because the protein metabolism in the rumen is altered when fat supplements interfere with fermentation. Inclusion of fat in the diet decreases protein degradation and ammonia concentration in the rumen and increases N flow to the duodenum (Jenkins, 1993). Increased efficiency of microbial protein synthesis in the rumen often accompanies those changes in protein digestion. This efficiency has been attributed to reduction of protozoa numbers in the rumen and less bacterial N recycling or to increased dilution rate of solids in the rumen because of the added fat (Jenkins, 1993). However, reductions in DM intake, milk fat percentage, and ruminal fiber digestion indicate that fermentation has been altered by dietary fat (NRC, 2001).

## CONCLUSIONS

From the results of this study, it could be concluded that protein efficiency is correlated with economic profit and affected by different factors including parity, lactation stage, yield traits and nutritional factors including dietary levels of  $NE_L$ , CP, RUP, NDF and EE. The optimum dietary levels of  $NE_L$ , CP and RUP for minimizing CPB in the studied population were estimated as 1.49 Mcal  $kg^{-1}$ , 11.29% and

7.58%, respectively. It seems that, in comparison to NRC (2001) estimates, more  $NE_L$  and RUP and less CP in the diet are needed to reduce protein balance in lactating dairy cows. Moreover, protein efficiency is probably improved by increase in NDF and decrease in EE in the diet.

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## تجزیه آماری برخی از عوامل مؤثر بر تراز پروتئین خام در گاوهای شیری شیرده

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### چکیده

برخی از عوامل مؤثر بر تراز پروتئین خام، به عنوان یک معیار بازده، با استفاده از ۲۸۲۴ رکورد ۵۰۱ گاو هلشتاین شیرده مورد مطالعه قرار گرفتند. تراز پروتئین خام (کیلوگرم در روز) به صورت پروتئین خام مصرفی (کیلوگرم) منهای پروتئین خام تولید شده (کیلوگرم) محاسبه شد. دو روش مختلف آماری، شامل تجزیه‌های مؤلفه‌های اصلی و مدل خطی تعمیم یافته برای مطالعه اثر عوامل مختلف مورد استفاده قرار گرفتند. تراز پروتئین خام دارای همبستگی متوسطی با مؤلفه‌های پایه ۱ (-۰/۲۰۶)، ۲ (۰/۳۰۸) و ۳ (-۰/۲۸۱) بود که ۶۵/۶ درصد همه پراکندگی‌ها را به خود اختصاص می‌دادند. سن، زایش، مرحله شیردهی، مرحله آبستنی، ماده خشک مصرفی، تولید شیر، تولید شیر تصحیح شده برای چربی، درصد چربی شیر، درصد لاکتوز شیر، تولید چربی شیر، تولید پروتئین شیر، تولید لاکتوز شیر، سطوح انرژی خالص شیردهی، پروتئین خام، پروتئین غیرقابل تجزیه در شکمبه، الیاف نامحلول در شوینده خنثی و عصاره اتری در جیره و درآمد مازاد بر هزینه خوراک، حداقل در یکی از مؤلفه‌های اصلی ۱، ۲ یا ۳، با تراز پروتئین خام همبستگی داشتند. در تجزیه مدل خطی تعمیم یافته تراز پروتئین خام به طور معنی‌داری تحت تأثیر دوره زایش و مرحله شیردهی قرار گرفت و ضرایب تابعیت خطی جزئی معنی‌داری از مصرف ماده خشک (کیلوگرم در روز)، درصد چربی، تولید چربی (کیلوگرم در روز)، تولید پروتئین (کیلوگرم در روز) و سطوح انرژی خالص شیردهی (مگا کالری در کیلوگرم)، پروتئین خام (درصد)، پروتئین غیرقابل تجزیه در شکمبه (درصد)، الیاف نامحلول در شوینده خنثی (درصد) و عصاره اتری (درصد) در جیره داشت. همچنین، ضرایب تابعیت درجه دوم تراز پروتئین خام از سطوح انرژی خالص شیردهی، پروتئین خام، پروتئین غیرقابل تجزیه در شکمبه و الیاف نامحلول در شوینده خنثی نیز معنی‌دار بودند. مقادیر بهینه انرژی خالص شیردهی، پروتئین خام و پروتئین غیرقابل تجزیه در شکمبه برای کمینه نمودن تراز پروتئین خام به ترتیب، ۱/۴۹ مگا کالری در کیلوگرم، ۱۱/۲۹ درصد و ۷/۵۸ درصد برآورد شدند. به نظر می‌رسد که در مقایسه با برآوردهای انجمن ملی تحقیقات آمریکا، انرژی خالص شیردهی، پروتئین غیرقابل تجزیه در شکمبه و الیاف نامحلول در شوینده خنثی بالاتر و پروتئین خام پایین‌تری برای کمینه نمودن تراز پروتئین خام در گاوهای شیری شیرده مورد نیاز باشد.