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Research Article

# Statistical evaluation of some environmental factors affecting the gross efficiency of crude protein in high producing dairy cows

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**Abstract:** Some environmental factors affecting the gross efficiency of crude protein (GECP) were studied using 2824 records of 501 lactating Holstein cows. The effects of some independent variables on the GECP were tested using a general linear model analysis. The GECP was significantly affected by parity and pregnancy stage and linearly related to dry matter intake, milk yield, milk fat, protein and lactose percentages, protein and lactose yields, dietary levels of net energy for lactation ($NE_L$), crude protein (CP), ruminally undegradable protein (RUP), and ether extract (EE). Quadratic relation to the GECP with dietary levels of $NE_L$, CP, RUP, and EE were also significant. This study showed that high producing animals, especially high protein yielding cows are more efficient users of dietary CP. Dietary requirements of $NE_L$, RUP, and EE to maximize the GECP were estimated as 1.61 Mcal/kg, 7.14%, and 5.42%, respectively. In comparison to NRC estimations, it seems that more $NE_L$, less CP, and more RUP are needed to maximize the protein efficiency of lactating dairy cows.

**Key words:** Gross efficiency of crude protein, lactation, Holsteins, requirements

## Introduction

Efficiency of nutrient utilization is a major factor affecting profitability in modern dairy farms. Dairy cows are one of the most efficient farm animals for utilization of dietary protein (1). However, they still excrete about 2 to 3 times more N in manure than in milk, which contributes to increased costs of milk production and environmental N pollution (2). Thus, recently, attention has focused on the relationship between nitrogen utilization in lactating cows and environmental pollution (3).

To ensure a sufficient supply of the protein required for maximal milk production, dairy cows are often fed with high dietary crude protein. Feeding excess protein has a high energetic cost; the energetic cost of feeding 1 kg of extra CP is equivalent to 0.72 Mcal of $NE_L$ (4). If a cow producing 45 kg of milk/day and eating 25 kg of DM/day required 17% CP in its diet, then feeding an extra 2% CP (a diet with 19% CP) would cost 0.36 Mcal $NE_L$/day, decrease milk yield by 0.5 kg/day, and gross efficiency by 0.3 percentage points (5). Moreover, overfeeding CP reduces profit margins, because of the relatively high cost of protein supplements and poor efficiency of dairy cows fed high protein diets to utilize N (6). The literature states that higher levels of CP intake will decrease CP efficiency (3,7,8).

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In addition to dietary CP, there are many other factors affecting an animal's ability to efficiently utilize CP. The aim of this study was to assay and determine the optimum levels of the main dietary components to improve gross efficiency of crude protein utilization in high producing dairy cows.

## Materials and methods

### Data

A dataset of 2824 monthly records of 501 lactating Holstein cows, located in 2 herds (the Ravansar dairy farm in Kermanshah province and the research dairy farm of the Animal Science Department, University of Tehran, Iran), from 2002 to 2003 was used for this study. Descriptive statistics for productive traits of the population studied are presented in Table 1. Average values of milk yield, milk fat, and milk protein in the studied population were 25 kg, 3.5%, and 3%, respectively. All cows involved in this study were kept in a tie-stall housing system and milked 3 times in a day. All animals had ad libitum access to different total mixed rations, which were different among individuals, according to milk yield. The diets were sometimes changed, according to herd management and availability of feed ingredients. Milk production was measured once a week and its composition determined monthly. Feed intake was measured as the difference of feed offered and orts. DM of feed offered and orts were measured separately.

AOAC (9) methods were used for determination of dietary dry matter, crude protein, crude fiber, ether extract, and acid detergent fiber. Neutral detergent fiber was determined without sodium sulfite in the neutral detergent, according to Van Soest et al. (10). The amounts of $NE_L$ and RUP were calculated using NRC models (11) as follows:

$$NE_L(\text{Mcal/kg}) = [0.703 \times ME(\text{Mcal/kg})] - 0.19$$

For feeds with more than 3% EE, this equation was as follows:

$$NE_L(\text{Mcal/kg}) = 0.703 \times ME - 0.19 + ([0.097 \times ME + 0.19)/97] \times [EE - 3])$$

where EE is a percentage of ether extract, and ME is metabolizable energy and could be estimated as $ME(\text{Mcal/kg}) = 1.01 \times DE - 0.45$. For feeds with more than 3% EE, this equation will be changed as follows:

$ME(\text{Mcal/kg}) = [1.01 \times DE - 0.45] + 0.0046 \times (EE - 3)$, where DE is digestible energy (Mcal/kg) and its estimation is dependent on type of feed.

For most feeds:

$$DE(\text{Mcal/kg}) = (tdNFC/100) \times 4.2 + (tdNDF/100) \times 4.2 + (tdCP/100) \times 5.6 + (FA/100) \times 9.4 - 0.3$$

where, tdNFC, tdNDF, and tdCP are truly digestible nonfiber carbohydrate (NFC), NDF, and CP, respectively, and FA is fatty acids, all as a percent of DM.

Dietary levels of tdNFC, tdNDF, and tdCP could be calculated by the following equations:

$$tdNFC = 0.98(100 - [NDICP) + CP + EE + Ash] \times PAF$$

$$tdNFC = 0.75 \times (NDFn - L) \times [1 - (L/NDFn)^{0.667}]$$

$$tdCP(\text{forages}) = CP \times \exp[-1.2 \times (ADICP/CP)]$$

$$tdCP(\text{concentrates}) = [1 - (0.4 \times (EDICP/CP))] \times CP$$

In these equations, NDICP is neutral detergent insoluble N × 6.25, PAF is processing adjustment factor (11), NDFn = NDF – NDICP, L is acid detergent lignin, and ADICP is acid detergent insoluble N × 6.25. All values are expressed as a percent of dry matter.

Nutritional components of the fed diets are presented in Table 2. The different components of milk, including protein, lactose, fat, and total solids were measured using an infrared milk analyzer (Milko-Scan 133B, Foss Electric, Denmark). The gross efficiency of crude protein (GECP) was calculated as milk CP/CP intake.

### Analysis

The effects of some independent variables on the GECP were tested using a general linear model analysis. The independent variables were: herd, animals within herd, parity number, lactation stage as month, pregnancy stage as month, dry matter intake (DMI) as kg/day, milk yield (MY) as kg/day, 3.2% fat corrected milk yield (FCM) as kg/day, milk fat percentage (F%), milk protein percentage (P%), milk lactose percentage (L%), milk fat yield (FY) as kg/day, milk protein yield (PY) as kg/day, milk lactose yield (LY) as kg/day, dietary levels of net energy for lactation ($NE_L$) as Mcal/kg, crude protein

Table 1. Descriptive statistics for productive traits in the studied population.

| Trait | N | Average | SD | 5% quantile | 95% quantile |
|---|---|---|---|---|---|
| Milk yield (kg/day) | 2824 | 25.09 | 7.53 | 13 | 36 |
| Milk fat (%) | 2824 | 3.54 | 1.03 | 2.37 | 5.62 |
| Milk protein (%) | 2824 | 3.04 | 0.46 | 2.48 | 3.77 |
| Milk lactose (%) | 2824 | 4.87 | 0.32 | 4.30 | 5.29 |
| Protein yield (kg) | 2824 | 0.720 | 0.194 | 0.364 | 1.014 |
| Gross efficiency of crude protein | 2824 | 0.273 | 0.071 | 0.172 | 0.399 |

Table 2. Descriptive statistics for some nutritional components of fed diets on dry matter basis.

| Item* | Average | SD | Minimum | Maximum |
|---|---|---|---|---|
| DM (%) | 64.30 | 5.58 | 50.20 | 76.30 |
| $NE_L$ (Mcal/kg) | 1.52 | 0.048 | 1.41 | 1.64 |
| CP (%) | 14.31 | 0.861 | 12.47 | 17.78 |
| RUP (%) | 4.73 | 0.364 | 4.08 | 6.34 |
| NDF (%) | 33.89 | 2.111 | 29.94 | 40.84 |
| ADF (%) | 22.04 | 1.71 | 16.97 | 29.40 |
| EE (%) | 3.18 | 0.352 | 2.64 | 4.55 |

* DM: dry matter, $NE_L$: net energy for lactation, CP: crude protein, RUP: ruminally undegradable protein, NDF: neutral detergent fiber, EE: ether extract.

(CP), ruminally undegradable protein (RUP), neutral detergent fiber (NDF), and ether extract (EE) as dry matter basis. In addition to linear regression coefficients, quadratic regression coefficients of dietary factors were added to the general linear model. ADF was not considered in the general linear model analysis, because concentrations of dietary NDF and ADF are highly correlated in most of diets and expressing the fiber requirement as NDF is superior to ADF for many reasons (11). Proc GLM of SAS (12) was used in the analysis.

Optimum levels of dietary $NE_L$, CP, RUP, NDF, and EE for maximizing the GECP were estimated using partial derivation of estimated regression model.

## Results

The overall average of the gross efficiency of crude protein (GECP) in the studied population was 0.273 ± 0.071 (Table 1).

The result of the general linear model analysis is presented in Table 3. The GECP was significantly affected by most of the studied variables, including parity ($P < 0.0001$), pregnancy stage ($P = 0.0229$), DMI ($P < 0.0001$), MY ($P < 0.0001$), F% ($P < 0.0001$), P% ($P < 0.0001$), L% ($P < 0.0001$), FY ($P < 0.0001$), PY ($P < 0.0001$), LY ($P < 0.0001$), $NE_L$ ($P < 0.0001$), CP ($P < 0.0001$), RUP ($P < 0.0001$), and EE ($P < 0.0001$). The quadratic relation of $NE_L$ ($P < 0.0001$), CP ($P < 0.0001$), RUP ($P < 0.0001$), and EE ($P < 0.0001$) with

Table 3. The effect of independent factors on gross efficiency of crude protein ($R^2 = 0.9351$).

| Variable | Parameter estimate | Standard error | Type III MS | F value | P |
|---|---|---|---|---|---|
| Herd | – | – | 0.00211 | 3.78 | 0.0525 |
| Cow (Herd) | – | – | 0.00056 | 2.22 | <0.0001 |
| Parity | – | – | 0.00140 | 5.58 | <0.0001 |
| Lactation | – | – | 0.00028 | 1.12 | 0.3391 |
| Pregnancy | – | – | 0.00056 | 2.23 | 0.0229 |
| DMI | −0.0146 | 0.00032 | 0.52747 | 2099.86 | <0.0001 |
| MY | 0.0082 | 0.00133 | 0.00969 | 38.59 | <0.0001 |
| F% | −0.0123 | 0.00137 | 0.02037 | 81.09 | <0.0001 |
| P% | 0.0204 | 0.00266 | 0.01481 | 58.97 | <0.0001 |
| L% | 0.0261 | 0.00438 | 0.00895 | 35.61 | <0.0001 |
| FY | 0.0591 | 0.00862 | 0.01180 | 46.98 | <0.0001 |
| PY | 0.3198 | 0.01783 | 0.08083 | 321.79 | <0.0001 |
| LY | −0.1511 | 0.02186 | 0.01199 | 47.75 | <0.0001 |
| $NE_L$ | 5.6347 | 0.93195 | 0.00918 | 36.56 | <0.0001 |
| CP | −0.5003 | 0.02709 | 0.08565 | 340.98 | <0.0001 |
| RUP | 0.1285 | 0.02035 | 0.01002 | 39.89 | <0.0001 |
| NDF | 0.0205 | 0.01485 | 0.00048 | 1.90 | 0.1682 |
| EE | 0.0510 | 0.00999 | 0.00654 | 26.05 | <0.0001 |
| $NE_L^2$ | −1.7531 | 0.30495 | 0.00830 | 33.05 | <0.0001 |
| $CP^2$ | 0.0150 | 0.00085 | 0.07834 | 311.85 | <0.0001 |
| $RUP^2$ | −0.0090 | 0.00152 | 0.00891 | 35.47 | <0.0001 |
| $NDF^2$ | −0.0004 | 0.00022 | 0.00065 | 2.58 | 0.1084 |
| $EE^2$ | −0.0047 | 0.00096 | 0.00612 | 24.38 | <0.0001 |

* DMI: dry matter intake, MY: milk yield, F%: milk fat%, P%: milk protein%, L%: milk lactose%, FY: milk fat yield, PY: milk protein yield, LY: milk lactose yield, $NE_L$, CP, RUP, NDF, and EE: dietary levels of net energy for lactation, crude protein, ruminally undegradable protein, neutral detergent fiber and ether extract, respectively. $NE_L^2$, $CP^2$, $RUP^2$, $NDF^2$, and $EE^2$: quadratic regression coefficients of $NE_L$, CP, RUP, NDF, and EE, respectively.

the GECP were also significant. The GECP was not significantly affected by herd (P = 0.0525), lactation stage (P = 0.3391), and dietary NDF, neither linear (P = 0.1682) nor quadratic (P = 0.1084).

By partial derivation of estimated regression model (Table 3), dietary requirements of $NE_L$, RUP, and EE to maximize the GECP were estimated as 1.61 Mcal/kg, 7.14% and 5.42%, respectively. The least GECP was estimated at level of 16.67% CP. The relationships of predicted GECP with dietary levels of $NE_L$, CP, RUP, and EE are presented in Figures 1 to 4, respectively.

## Discussion

The average GECP in the studied population (0.273) was similar to the values of 0.27 and 0.277, reported by Arieli et al. (13) and Huhtanen (14), respectively, and slightly lower than the 0.29 reported by VandeHaar (5) for the US dairy industry.

Positive partial regression coefficients, estimated for MY, P%, L%, FY, and PY indicate that the GECP would be automatically increased with an increase in these production traits. However, PY had the highest F value and the least P value among production traits (Table 3). Thus, PY could be considered as the most important production trait affecting the GECP. Therefore, it would be expected that higher protein producing animals are more efficient users of dietary CP. The positive correlation of yield traits with the GECP has been proved by Li et al. (15), who found a high positive genetic correlation between crude protein efficiency and production. Similarly, Jonker et al. (16) reported that an increase of FCM through management factors increases N efficiency.

The GECP had a positive partial regression to dietary levels of $NE_L$, RUP, and EE. Other variables including DMI, F%, LY, and CP had negative partial regression coefficients in the regression model. The negative partial regression coefficient of CP (Table 3) reveals that an increase of dietary CP will result in a decrease of the GECP. However, the effect of CP on the GECP is not completely linear (Figure 2). This finding is in agreement with Olmos Colmenero and Broderick (7), who found a significant linear decline in the apparent N efficiency (milk protein N/N intake) as dietary CP increased. In their study, CP efficiency decreased from 36.5% at 13.5% CP to 25.4% at 19.4% CP (7). Wang et al. (8) found that animals fed high protein diets produce more milk and milk protein, but have a higher N excretion in their urine than those fed low protein diets, indicating decreased efficiency of nitrogen utilization. Similarly, in a study by Arieli et al. (17), milk protein efficiency in heat-stressed cows was negatively related to dietary CP concentration. It has been well established that an increase of dietary CP will increase the amount of protein degraded in the rumen; thus as a result, when ruminally degradable protein (RDP) exceeds microbial needs, large amounts of $NH_3$ are produced, absorbed into the blood, converted to urea in the liver, and excreted in the urine. This urea, in the manure, can be rapidly hydrolyzed to $NH_3$ and lost by volatilization to the environment (18).

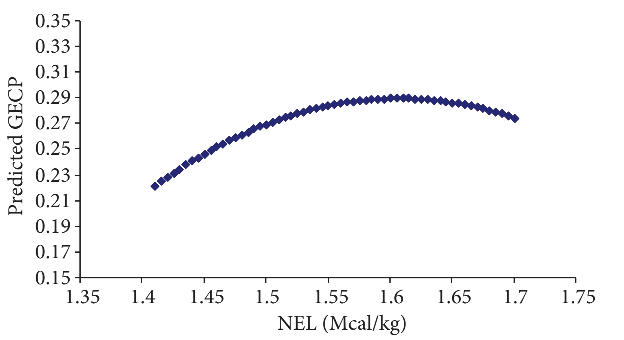


Figure 1. Quadratic relation of the gross efficiency of crude protein (GECP) predicted by dietary net energy for lactation ($NE_L$).

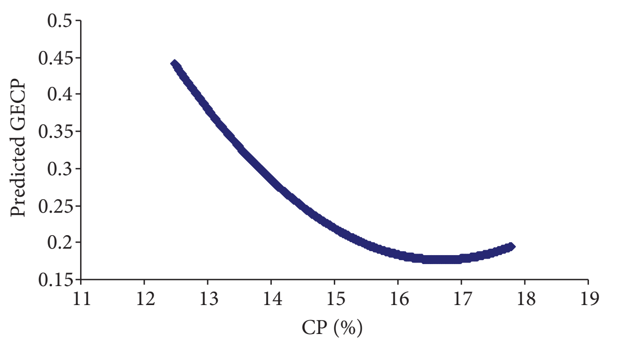


Figure 2. Quadratic relation of the gross efficiency of crude protein (GECP) predicted by dietary crude protein (CP).

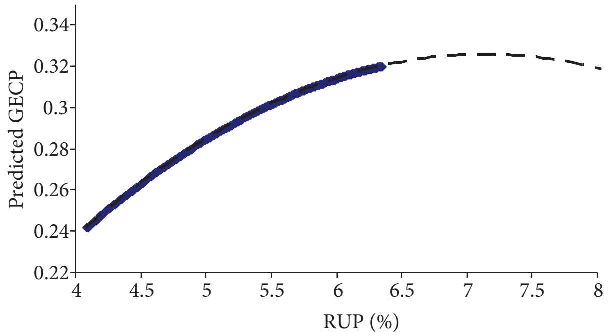


Figure 3. Quadratic relation of the gross efficiency of crude protein (GECP) predicted by dietary ruminally undegradable protein (RUP) and the estimated trend line for out of range values.

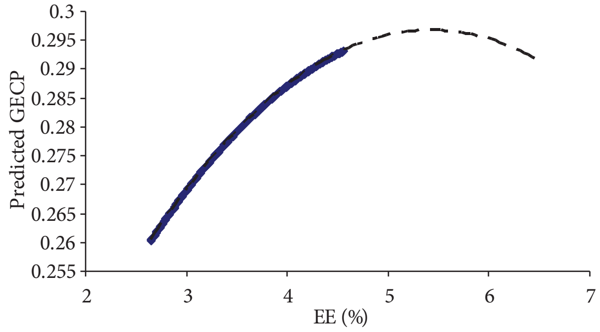


Figure 4. Quadratic relation of the gross efficiency of crude protein (GECP) predicted by dietary ether extract (EE) and the estimated trend line for out of range values.

The GECP had significant linear and quadratic regression coefficients on dietary RUP (Table 3) which reveals a nonlinear relationship between the RUP and the GECP (Figure 3). It means that dietary RUP has a variable effect on the GECP. There are many opposite results in literature about the effect of RUP on N efficiency. For example, many attempts have been made to substitute high RUP sources in dairy diets to increase the flow of metabolizable protein to the small intestine. On the other hand, Santos et al. (19), after reviewing reports from 15 in vivo studies, concluded that replacement of soybean meal with high RUP sources did not increase the duodenal flows of total nonammonia nitrogen (NAN), essential AA, Lys, or Met. Instead, supplementation with high RUP sources decreased microbial NAN flow to the duodenum in 76% of the studies (20). Moreover, Brito and Broderick (21) compared some isoprotein diets with different sources of protein and found that cows fed solvent soybean meal (less RUP) have a higher apparent N efficiency (GECP) than those fed cotton seed meal (high RUP).

Dietary fat is another factor affecting protein efficiency. In the present study, the GECP had significant linear and quadratic regression coefficients on EE (Table 3, Figure 4). This might be because metabolism of protein in the rumen is also altered when fat supplements interfere with fermentation. In many studies, inclusion of fat in the diet decreased protein digestion and ammonia concentration in the rumen and increased N flow to the duodenum (22). Increased efficiency of microbial protein synthesis in the rumen is often accompanied by a change of protein digestion. This efficiency is attributed to the 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 (22). However, reductions in DM intake, milk fat percentage, and ruminal fiber digestion indicate that fermentation is altered by dietary fat (11). On the other hand, milk yield response to supplemental fat is curvilinear and the response diminishes as supplemental fat in the diet increases (11,23). This is in agreement with the effect of dietary EE on the GECP in the present study.

The estimated requirements of $NE_L$ and RUP by the National Research Council (11) for large breed dairy cows producing 25 kg of milk, with 3.5% fat and 3% protein, are 1.37 Mcal/kg and 4.6%, respectively, which are slightly less than the estimated levels of $NE_L$ (1.61 Mcal/kg) and RUP (7.14%) for maximizing the GECP in the present study.

The higher estimate of $NE_L$ requirement to maximize the GECP means that an increase in dietary energy probably increases protein efficiency. This finding could be attributed to an increase of fermentable metabolizable energy (FME) in most of the high $NE_L$ diets, because FME provides the energy supply needed for rumen microbes to capture N. Moreover, the fate of absorbed peptides and amino acids, once inside the microbial cell, depends 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 limited, amino acids will be deaminated and their carbon skeleton will be fermented (24).

The estimated requirement of RUP for maximizing the GECP in the current study (7.14%) was much higher than the RUP requirement (4.6%) reported by the National Research Council (11). Thus, it could be concluded that a decrease of protein degradability in the rumen may improve protein efficiency, because, with high levels of RDP, more amino acids would be deaminated, more N would be absorbed as ammonia, and more N would be excreted in urine (25). The positive effect of RUP on protein efficiency is also reported by Flis and Wattiaux (26) and Kalscheur et al. (27). However, post-ruminal digestibility of RUP and amino acid balance could be considered as important factors for increasing metabolizable protein flow to the intestines (28), thus optimizing dietary RUP to improve protein efficiency.

The general trend of the GECP over dietary CP in the present study (Figure 2) shows that the need of dietary CP for maximizing the GECP is less than the requirement of CP (14.1%), reported by the NRC (11). Thus, it could be concluded that the requirement of CP for maximizing protein efficiency may be less than the CP requirement for maximum production.

According to the results of this study, it could be concluded that many environmental factors may affect the protein efficiency of lactation. Generally, high producing animals, especially high protein yielding ones, are more efficient at utilizing dietary CP. To avoid N loss and improve protein efficiency, it is necessary to optimize different dietary components, such as $NE_L$, CP, RUP, and EE. The estimates of optimum levels for dietary $NE_L$, RUP, and EE to maximize the GECP were 1.61 Mcal/kg, 7.14%, and 5.42%, respectively. In comparison to the nutrient requirements of the NRC (11), it seems that more $NE_L$, less CP, and more RUP are needed to maximize protein efficiency in lactating dairy cows.

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