



# Effects of Quality and Amounts of Dietary Protein on Dairy Cattle Reproduction and the Environment



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## Abstract

The objective of this paper is to review the effects of quality and amounts of dietary protein on dairy cattle reproduction and the environment. Protein supplementation is one of important nutrient in dairy production. But, an excess amount of protein beyond the requirements of dairy cow affects reproduction as well as the environment. Protein is needed to meet nitrogen requirements of rumen microorganisms as well as used as a direct source of protein for milking cows. An excess amount of rumen degradable protein can be excreted into the environments and impose an impact on it. Excess rumen degradable protein also results in high amounts of ammonia in the blood of dairy cow when there is no sufficient amount of energy to convert ammonia into microbial protein. In addition, conversion of excess ammonia is energy demanding which can result in negative energy balance. This excess ammonia and negative energy balance result in reduced reproductive performance in dairy cows. Therefore, it is important to optimize rumen degradable and rumen undegradable protein content of diets and synchronization of protein with energy is best strategies to overawed ammonia pollution and reproductive problem in the dairy cows.

**Keywords:** Protein level; Protein quality; Reproduction; Environmental impact; Dairy cattle; Undegradable protein; Dietary protein; Nutrient management; Cattle reproduction; Urease; Faecal N; Ammonia; Luteal phase; Oestrous cycle; Detoxification process; Degradable protein; Methionine; Amino acid

**Abbreviations:** UDN: Undegradable Dietary Nitrogen; RDN: Rumen Degradable Nitrogen; MUN: Milk Urea Nitrogen; NEB: Negative Energy Balance; AA: Amino Acid

## Introduction

Protein is an important limiting nutrient in ruminants [1]. To produce milk, and more importantly, to achieve high yields, and thereby exploiting the whole production potential of cows, high inputs of feed with high protein contents have become a common practice in dairy farming [2,3]. However, many studies have shown that high inclusion of protein in the diets of dairy cows leads to a decreased nitrogen (N) efficiency (the ratio between N content in the diet and N in produced milk) and increased excretion of N via urine and milk [4-6]. A significant part of the dietary N is thereby lost, in both environmental and economic terms, leading to decreased production efficiency of dairy systems.

Dietary protein intake is the most important factor determining milk nitrogen efficiency, reduction of urinary nitrogen losses, and consequently, ammonia emissions from dairy cow manure. Feeding cows less protein can dramatically decrease urinary N excretion and increase the efficiency of N use. Excess feed N is deaminated and excreted as urea, an N waste compound, in urine and milk, while undigested ruminal undegradable protein and metabolic N (sloughed intestinal

cells and hindgut fermentation products) are excreted in the faeces [7]. The route and amount of N excretion are of primary environmental concern; urinary N is more volatile than faecal N and is rapidly converted to ammonia. However, reducing the amount of protein fed can have negative impacts on productivity if the diet is not correctly balanced. Therefore, both nutrient intake and nutrient excretion (nutrient management) must be carefully considered.

High protein diets are frequently fed to cows to increase milk production. However, many studies have reported that increasing the percentage of crude protein in the diet results in reduced fertility [8,9]. Other authors have reported effects in some circumstances but not in others [10,11] and some have reported no effect [12,13]. The process by which this happens is still unclear, but recent research has shown that cows fed excess protein (more than 10-15% above requirements) required more services per conception and had longer calving intervals [14]. Therefore, the objective of this paper is to review effects of quality and amounts of dietary protein on dairy cattle reproduction and the environment.

### Effect of Protein on Milk Yield

Protein is an important limiting nutrient in ruminants [1]. It contains two fractions: rumen degradable nitrogen (RDN) and undegradable dietary nitrogen (UDN). Thus, sufficient supply with RDN and UDN or AA is important to satisfy animal's requirements. Kalscheur [15] reported that ruminal undegradable protein needs to be supplement when microbial protein synthesis alone is insufficient to meet the metabolizable protein requirements in dairy animals especially during early lactation. On supplementation of an increased dietary rumen undegradable protein milk yield was increased [16-19]. Similarly, Marghazani found higher milk yield from 40% RUP on supplementation of (30,40,50 and 60%) rumen undegradable protein. Garduza-Arias [20] found that the average milk production was not affected by the amount of RUP (30 and 40%) in the supplement. In the study of Zhai [21] no significant difference in milk yield was observed on supplementation of different levels of RUP (30.8,36.2, and 41.6%). The higher RDP than requirements of rumen microbes results in wastage of expensive part of a diet and also decreases the RUP for efficient utilisation in the small intestine for more yields [22]. On supplementation of 11.3,10.1, 8.8 and 7.6% RDP on DM basis and on RUP (7.1%), there was a loss of production as dietary RDP decreased. The reduction may be due to reduced DMI [15] which is associated with a reduction in energy supply to the animal and lead to the reduction in milk yield. Higher dietary RDP concentrations are proposed to increase ruminal microbial growth and digestion, which leads to an increase in milk yield [23].

### Effect of Protein on Milk Composition

Milk composition is a trait of animal species, which can be altered under normal production systems. Changes in a composition are attributed to the altered genetic makeup and by the dietary modifications. Nutrition has direct impacts on milk composition [24]. On supplementation of RUP (30 and 40%) milk fat was not affected by the amount of supplement [20]. Similarly, on supplementation of 11.3 10.1, 8.8 and 7.6% RDP on DM basis and RUP (7.1%), milk protein yields were not significantly affected. However, milk fat yield decreased linearly from 1.43 to 1.15 kg/d as RDP in the diet decreased from 11.3 to 7.6% of DM. Titi [25] observed no significant difference in milk protein percentage for cow fed 14 and 16% CP, while milk fat content was higher in 16% CP. Kanjanaputhipong and Buatong [16] also found significantly higher butter fat, protein, solids-not-fat and total solids from 38.5% on supplementation of 24.1 and 38.5% RUP of CP for dairy cows. A major factor is the low transfer efficiency (25 to 30%) of dietary protein to milk is accounting for the inability of diet to alter milk protein content [24]. Castillo [26] found supplementation of extra CP (210g/kg and 290g/kg DM) did not affect milk fat, protein, and lactose yield or composition. Protein degradability also had no significant effect on milk composition. In the study of Zhai [21] no significant difference in milk composition was observed

on supplementation of different levels of RUP (30.8,36.2, and 41.6%).

### Ammonia Emissions from Dairy Cattle

Dietary protein is the most important factor determining milk N efficiency, urinary N losses, and consequently, ammonia emissions from dairy cow manure [27]. The main source of nutrient pollution from cattle is excess N excretion. Ammonia is an important environmental pollutant that impacts the quality of human and animal life [28]. Ammonia emissions from dairy operations are an important source of N pollution [29]. Ruminants excrete excess dietary N mainly through urine [30]. Urea, the major form of urinary N is rapidly converted to ammonia after excretion [31]. Dairy farming is also controlled by various regulations to control emissions into the environment [32]. Thus, decreasing N excretion from dairy cows will help reduce ammonia pollution by dairy operations.

Ammonia can cause serious environmental problems and health problems in gaseous or particulate phases. Nitrogen excretion in the ruminant animal is basically composed of undegradable protein, endogenous material, a microbial protein that escapes digestion and urinary N. Urea is produced mainly in the liver to eliminate excess ammonia present in the blood. Ammonia in blood is a result of domination of amino acids in animal tissues. In ruminants, ammonia in circulation can also come from microbial degradation of N compounds [33]. Urea in contact with urease, an enzyme produced by microorganisms found in both faeces and in soil is converted to ammonia and then volatilized. The process of ammonia volatilization occurs by mass transfer from the top layer of the manure slurry to the air surrounding the manure surface. The mass transfer is dependent on temperature and air velocity at the manure top layer [34].

### Dietary Protein Level on Nitrogen Excretion in Manure

Dietary protein intake is the most important factor determining ammonia emissions from dairy cow manure. Overall intake of N affects the total amount of N excreted via manure, but the type of carbohydrate and forage provided in the diet have greater impacts on the route (faecal or urinary) of excretion [35]. Dietary protein excess can increase N excretion in manure, especially in urine, increasing ammonia volatilization. A number of studies have shown that the amount of ammonia produced from cattle manure is correlated with N intake [36]. Protein in the diet directly affects the amount excreted, as shown in a recent study by Lee [37] where manure from cows consuming a 16.7% CP diet had an increased ammonia emission rate, and urinary N contribution to nitrate-N was 100% greater than manure from cows consuming 14.8% CP diet. Similarly, in the study by Burgos [36] ammonia emissions were measured for cows consuming diets ranging from 15% to 21% CP. The results showed that the amount of urea in manure increased linearly with dietary CP and was almost 3 times higher in manure from cows consuming a

21% CP diet when compared to the 15% CP cows. However, on supplementation of different level of dietary CP (10.2%,11.9%, and 13.5%) faecal excretion of N was not affected by CP level [38].

Between 57 and 78% of urinary N is in the form of urea (De Boer et al., 2002) which is rapidly converted to ammonia (NH<sub>3</sub>) during manure collection and storage as compared to faecal N [31]. Therefore, a sizeable reduction in NH<sub>3</sub> emissions would be achieved by decreasing dietary protein considering the relatively rapid rate of NH<sub>3</sub> volatilization from urine [39]. James [40] have demonstrated that reducing dietary N intake by Holstein heifers resulted in decreased NH<sub>3</sub> emissions from their manure. In addition, Frank & Swensson [41] noticed that manure ammonia emissions from cows fed a 19% CP diet were three times higher than those fed a 13% CP diet. Similarly, Burgos & Jackson [42,43] found that manure ammonia emissions decreased linearly when the CP in the feed was decreased from 18% to 12% of DM.

### Dietary Protein Level on Nitrogen Excretion in Urine

The route and amount of N excretion are of primary environmental concern; urinary N is more volatile than faecal N and is rapidly converted to ammonia by ureases present in soil and on pen floors [37]. Excess dietary N (from excess feed CP as well as AA from cell turnover and enzyme production) is converted to urea, which is a soluble compound that will diffuse into various body fluids, such as blood, milk, and urine. About 80% of N consumed in excess of 500 g/d is believed to be excreted in urine in dairy cows [44]. Naves et al. confirmed that high urea content had higher nitrogen excretion in faeces (g/100g of N intake) and urine (g N/d and g/100g of N intake). In Zhang [38] study, urinary urea N excretion was increased with increasing dietary CP on supplementation of 10.2, 11.9 and 13.5% CP level. Castillo also showed that urinary N excretion was increased by 74 g N/d with the increase in dietary CP level from 210 g/kg DM to 290 g/kg DM. The Urinary N excretion is mostly related to increased degradability of protein in the rumen [45]. Total N excretion, as well as urinary N excretion, can be decreased if overall dietary CP concentration can be reduced. Cantalapiedra-Hijar [46] showed that decreasing dietary CP content from 16.5% to 12.0% in Jersey cows in late lactation decreased the urinary N excretion by more than 50% and increased the N-efficiency from 26.4 to 31.3%, but this came at the expense of decrease in both milk yield by 2kg/day and milk protein yield by 18%. Similarly, Hymøller, et al. [47-49] showed an increased N-efficiency and decreased N excretion with lowered CP levels at the expense of decreased milk yields. Cyriac [50] also concluded that feeding less RDP (11.3,10.1,8.8 and 7.6%) improved apparent N efficiencies from 27.7 to 38.6% with a trend for lost milk production. Such improvements in N efficiency will have positive environmental impacts. An increased N-efficiency is achieved by lowering the CP level in the diet, ranging from 36.5 to 30.4% with CP contents of 12-17.2% whereas at the higher CP levels (16.5-18.8%) the N-efficiency ranged from 33.0 to 26.4%. Thus, lower MUN and

UUN concentrations indicate a more efficient N-utilization leading to a higher N-efficiency, which is a higher proportion of dietary N, is converted to N in the product [51].

However, more moderate decreases in dietary CP levels allows increased N-efficiency and decreased N excretion without any significant losses on production levels [52]. This might be achieved by decreasing dietary CP with a simultaneous increase in RUP or starch content. Interestingly, maximum microbial protein yield did not give the optimal N-efficiency, but the optimum was achieved by balancing the dietary protein degradation and microbial protein synthesis [52]. Indeed, Nadeu [53] showed that energy availability from carbohydrates as well as synchronization of protein and carbohydrate metabolisms are important for efficient nitrogen utilization by dairy cows. Reynal & Broderick [52] suggested that the optimal dietary CP level to be 17.7% (11.7% RDP) when striving for an optimal N-efficiency, that is balancing the need for a high profitability of the production system and the need for minimizing the negative environmental effects of excessive N-excretion.

### Dietary Protein Level on Reproduction

The effect of dietary protein on reproduction is complex. Prolonged inadequate protein intake has been reported to reduce reproductive performance. More recently it has been found that reproductive performance may be impaired if a protein is fed in amounts that greatly exceed the cow's requirements. Studies have reported negative associations between high dietary CP and a range of fertility parameters, many studies have found little or no effect [54]. Excessive protein can have negative effects on reproduction [55]. Overfeeding protein during the breeding season and early gestation, particularly the rumen receive an inadequate supply of energy, may be associated with decreased fertility [9]. This decrease in fertility may result from decreased uterine pH during the luteal phase of the oestrous cycle in cattle fed high levels of degradable protein. Meza-Herrera [56] reported that high concentrations of pre-conception protein resulted in reduced uterine pH and reduced fertility rate but did not affect luteal function at 15 days post-insemination. Research result indicated that cows fed excess protein (more than 10-15% above requirements) required more services per conception and had longer calving intervals [25,57]. Similarly, on supplementation of 15-19% CP lower conception rate from 65 to 53% was observed [58]. The negative effects of protein supplementation are associated with an increase in blood urea-N, which affects ovarian follicular and embryo development [59]. On supplementation of two levels CP (14 and 16%) with different levels of rumen bypass methionine (0,15 and 25%) more services per conception (1.8vs1.5) and longer calving intervals (358 vs 351) were observed in cow receiving 16% CP [25].

### Dietary Protein Quality on Reproduction

While limited studies have directly addressed the influence of the type of dietary protein on fertility parameters, a recent study

by Waterman et al. [60-62] reported improvements in a range of reproductive traits in cows fed high levels of rumen undegradable protein. On supplementation of two levels of RUP (32.78 and 27.47%) the shortest postpartum mating (81.33±19.83 vs. 91.83±31.16 days), shortest service per conception (1.17±0.41 vs. 1.50±0.55 times) and the shortest days to first estrus (97.33±41.52 vs. 142.33±66.87 days) was observed in 32.7% supplemented group [63]. Similarly, on supplementation of 24.1 and 38.5% RUP of CP for dairy cows higher incidence of cystic ovaries and shorter days to first estrus were observed in dairy cows fed 38.5% RUP indicated that increasing the level of RUP in the diet can have a beneficial effect on fertility when associated with reduced plasma urea concentrations. In this study higher milk urea nitrogen (MUN) is observed in dairy cows fed 24.1% CP due to excess RDP which can affect fertility. Recent data suggest that MUN concentrations above 15.4 mg/dL may result in a reduced probability of pregnancy success in dairy cows [64]. Studies of Sawa & Jankowska [65,66] also clearly suggested a deterioration of the reproduction rate in dairy cows with increasing milk urea concentrations. However, Řehák [67] found, the cows with the lowest MU had the longest calving to first service interval.

An increase in RUP may increase the supply of amino acid (AA) for intestinal absorption, which may improve the glucogenic potential of the supplement [60] or contribute an essential AA such as methionine, which can improve ovarian function [68]. Ardalan & Titi [25,69] also reported that supplementation of rumen bypass methionine can improve reproductive performance of dairy cows. Since AA and peptides from degraded RUP are absorbed in the intestine and is readily available to the ruminant, excess RUP has shown to stimulate the pancreas to increase insulin production [70]. Insulin affects ovarian tissues by enhancing LH receptor synthesis and actions of the pituitary through these receptors. Kane [71] suggested that undegraded protein works to improve reproduction by mediating luteinizing hormone and follicle stimulating hormone production.

Effects of supplementing feedstuffs high in undegradable intake protein (UIP) on reproduction are appearing to be dependent on the energy density of the diet [72]. Research by Kane [71] demonstrated negative effects on reproductive hormones when high (0.71 lb/d) levels of UIP were supplemented, but not at low (0.25 lb/d) or moderate (0.48 lb/d) levels. Heifers fed additional UIP (0.55 lb/d) during development reached puberty at a later age and heavier weight and fewer were serviced in the first 21 days of the breeding season. Pregnancy rate was not affected [73]. However, on supplementation of RUP (30 and 45%) the interval from calving to first oestrus and the period to first luteal activity was not different in dual-purpose cow, which may be due to similar MUN. But, the interval from parturition to first normal luteal activity and the percentage of animals with luteal activity tended to be improved in the 45% RUP treated group. The negative effects of protein supplementation are associated with an increase in blood urea nitrogen (BUN), which affects

ovarian follicular and embryo development [59]. According to Hammond [74], increased degradability of dietary protein can lead to increased ruminal ammonia concentrations resulting in increased BUN concentrations. Rajala-Schultz [64] and Sawa [65] stated that monitoring the concentration of urea in cow's milk may enable identification of the causes of health problems in cows which mainly affect fertility (Table 1).

**Table 1:** Reproductive performance in double purpose cows which received two different amounts of rumen undegradable protein.

	Treatment			
	30% RUP (N=10)	45% RUP (N=10)	SEM	p-value
First oestrous postpartum (days)	42	45	18.1	0.86
First normal luteal activity (days)	99	82	8.02	0.15
Duration of first normal luteal phase (days)	12	12.8	1.28	0.72
Percentage of cows with luteal activity	10	40	-	0.14

There is a report, which indicates that feeding excess RDP has a negative effect on fertility and delays the first ovulation or oestrus, reduces the conception rate to first insemination, increases the number of days opens and lowers the overall conception rate [75]. There are several proposed mechanisms for this effect including an exacerbated negative energy balance for cows fed diets high in RDP in comparison to diets high in RUP [76] and proven deleterious effects of both ammonia and urea on both oocyte and embryo development [77,78]. An excessive intake of degradable protein and a relative shortage of energy to synthesize bacterial proteins will result in the accumulation of excessive ammonia in the rumen [79], which is absorbed through the ruminal wall and converted into urea in the liver. This detoxification process consumes energy and thus may exacerbate negative energy balance (NEB) in early post-partum [80]. NEB is associated with a high incidence of irregular cycles that can both increase the interval to the first service and reduce conception rates [81]. Similarly, Řehák [67] found that NEB had a greater effect on the length of calving to first service interval. Rochijan [63] suggested that synchronizing the rate of dietary energy and nitrogen release is a possible way to avoid excess blood urea nitrogen and excessively high levels of plasma ammonia, leading to improved reproductive efficiency [82-86].

## Conclusion

Protein supplementation for a dairy cow is common practice to improve reproductive activity and increase milk production in the dairy farm. However, excess supplementation of protein above requirements of cow increase excretion of nitrogen in faeces and urine. This excreted nitrogen can cause environmental pollution in the forms of ammonia. Ammonia is one of cause for global warming now a day. Beyond this, excess protein supplementation mostly in the forms of rumen degradable protein can impair the reproductive function of dairy cows.

This is mostly related to the availability of energy to convert ammonia into microbial protein. Ammonia conversion is energy demanding and results in negative energy balance in the dairy cow. Additionally, availability of excess ammonia in the blood and milk results in reduced reproductive performance through disturbing reproductive hormones in the dairy cows. Therefore, optimizing protein content of diets and synchronization of protein with energy is best strategies to overawed ammonia pollution and reproductive problem in the dairy cows.

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