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REVIEW

NUTRITIONAL STRATEGIES TO IMPROVE NITROGEN USE EFFICIENCY BY GRAZING DAIRY COWS

Juan Pablo Keim[1*], and René Anrique[1]

Grazing dairy cows are characterized by converting a low proportion of dietary N into milk N. This low N use efficiency (NUE) is mainly due to an excessive supply of crude protein (CP) of pasture with regard to animal requirements. Excess N in the diet has negative effects for the animal; it alters the organoleptic characteristics of milk and pollutes the environment through N excretions. The aim of this review was to analyze the available information on nutritional strategies to improve NUE by grazing dairy cows, such as diluting the dietary N through supplementation, synchronizing ruminal carbohydrate (CHO), and N fermentation rates, and using pastures with a high water soluble carbohydrates (WSC) content. There is a beneficial effect of carbohydrate supplementation on NUE, with an increase in milk yield (MY) of 0.83 ± 0.34 kg milk kg^{-1} supplement and a reduction of ammonia N (NH_3-N) in the rumen. A better synchrony between CHO and rumen N does not improve MY and NUE. However, a positive effect on NUE was identified as a result of increasing the WSC/CP ratio. The use of cultivars with high WSC content increases the dry matter intake (DMI), MY, and milk N production, with no consistent effect on NUE.

Key words: Concentrate supplementation, ruminal synchrony, high sugar ryegrass, crude protein, milk production.

One of the objectives of animal nutrition is to provide the amount of nutrients that the animal needs to achieve a certain level of production. Any deficiency will translate into a lower production, while an excessive nutrient supply involves losses that will increase production costs and can also affect the animal and the environment (Hristov and Jouany, 2005; Pacheco *et al*., 2008).

Grazing systems, compared to confinement systems which provide a balanced ration, reach lower levels of production and nutritional efficiency. An example of this situation is the low conversion of dietary N into milk N, ranging between 13 and 31% in grazing systems and 40 to 45% under confinement systems with balanced rations (Delagarde *et al*., 1997; Verité and Delaby, 2000). A low N use efficiency (NUE) is basically due to the incapacity of animals to build significant amounts of protein reserves, being necessary to adjust protein supply according to protein requirements (Hoekstra *et al*., 2007). Energy is the main limiting nutrient for productivity under grazing conditions, while amino acids supply usually exceeds animal requirements (Kolver *et al*., 1998).

Dietary N which is not converted into milk N has negative effects on the animal, reducing energy availability due to the energy spent in the conversion of ammonia (NH_3) into urea (Pacheco *et al*., 2008); besides excess dietary N it is associated with reproductive problems (Butler, 1998), lower dry matter intake (DMI; Cosgrove *et al*., 1999), risk of intoxication due to excess nitrates (Bolan and Kemp, 2003), undesirable odors or flavors in milk (Bendall, 2001) and environmental pollution, mainly through excretion of urinary N (UN; Pacheco *et al*., 2008). Due to the negative effects related to low NUE, it is necessary and challenging to develop strategies that allow increasing NUE in dairy systems. Under confinement conditions there are several approaches to optimize the proportion of dietary N converted into milk N (Verité and Delaby, 2000; Castillo *et al*., 2001; Hristov and Jouany, 2005; Flachowsky and Lebzien, 2006; Hoekstra *et al*., 2007), unfortunately there is still uncertainty about the pathways that may improve NUE under grazing dairy systems.

This review is focused on analyzing factors that explain the low NUE of grazing systems in temperate regions, and the nutritional strategies that have been evaluated to improve the conversion of dietary N into milk N, such as reducing N content of pastures, lowering N content of the diet through supplementation, improving ruminal synchrony between energy and protein, and the use of *Lolium perenne* L. cultivars with a high sugar content.

Nitrogen metabolism

Nitrogen utilization in ruminants occurs through a series of processes that take place in the rumen and

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[1] Universidad Austral de Chile, Facultad de Ciencias Agrarias, Independencia 641, Valdivia, Chile.
[*] Corresponding author (juan.keim@uach.cl).
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the animal. While rumen bacteria can metabolize a variety of N sources, animals need amino acids for their metabolism (Pacheco and Waghorn, 2008). Therefore, the requirements of both microorganisms and host must be taken into consideration when formulating diets.

In ruminants, N arrives to the small intestine as microbial protein, ruminal undegraded protein (RUP) and as endogenous protein, and collectively contribute to metabolizable protein (MP; Schwab *et al.*, 2005). Microbial protein is highly digestible and is characterized by a balanced amino acid profile, while in RUP the amino acid composition resembles that of the precursor feed (NRC, 2001). Total digestible protein in the small intestine corresponds to MP composed by amino acids and peptides that are absorbed and made available for animal metabolism (Lapierre *et al.*, 2005), while excess MP is deaminated by the liver and excreted as urea through the urine (Bohnert *et al.*, 2002). Therefore, as N intake increases urinary N increases considerably with no effect on fecal N, being urinary N the main determinant of the negative relationship between N intake and NUE (Figure 1).

Microbial protein is the result of microbial metabolism and in most cases represents the main source of the amino acids absorbed by the intestine (70 to 100%) of the host animal (Schwab *et al.*, 2005). Microbial protein is synthesized mainly from NH_3 (38 to 80%) generated from protein degradation (Pacheco and Waghorn, 2008) and peptides, when protein is rapidly degraded in the rumen (Hristov and Jouany, 2005; Nolan and Dobos, 2005).

The amount of microbial protein synthesized depends on the nutritional quality of the feed, intake level and retention time of solids and liquids in the rumen (NRC, 2001; Hristov and Jouany, 2005; Nolan and Dobos, 2005) and may potentially reach 50 g per 100 g CP ingested (Pacheco *et al.*, 2008). In terms of nutritional factors influencing microbial protein synthesis it is important to consider the nature of energy and N sources, among them; microbial growth and the efficiency of NH_3 utilization are highly determined by the availability of carbohydrates (CHO) in the rumen (Newbold and Rust, 1992; Heldt *et al.*, 1999). Therefore, the control of factors associated to CHO availability in the rumen, such as the amount supplemented, its source and degradability, and the synchronization between the rate of release of energy and N in the rumen could help improving the efficiency of ruminal NH_3 and dietary N utilization (Hristov and Jouany, 2005).

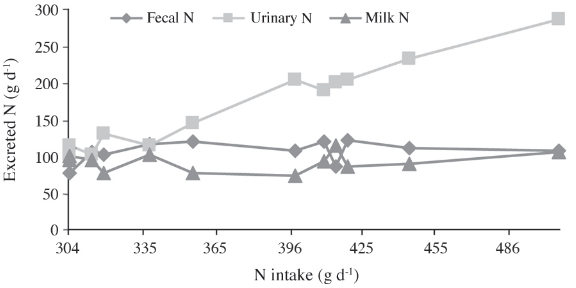

Figure 1. Relationship between N intake and N excreted in milk, feces and urine (adapted from Steinshamn *et al.*, 2006).

The concentration of ruminal NH_3 increases when intake of rumen degradable protein (RDP) is high compared to fermentable energy, favoring NH_3 absorption and transport to the liver for urea synthesis (Castillo *et al.*, 2001; Kulling *et al.*, 2001). Urea is released into the blood stream and excreted through the urine, normally allowing toxic effects associated to excessive ammonia to be avoided (Lapierre *et al.*, 2005). The detoxification process has an energy cost of 4 moles of ATP per mol of urea synthesized (McBride and Kelly, 1990), which is equivalent to 7.17 kcal ME g^{-1} N as urea (Tyrell, 1970; cited by Pacheco and Waghorn, 2008) or to 3.3 kcal ME g^{-1} urea.

In lactating dairy cows, between a 60 and 90% of ingested N is eliminated through feces and urine depending on N intake and production level (Flachowsky and Lebzien, 2006). Urinary N excretions up to 500 g d^{-1} have been measured with high N intake (Steinshamn *et al.,* 2006; Pacheco and Waghorn, 2008).

Pasture as a source of nitrogen

In dairy systems based on high quality pastures (2.75-2.9 Mcal ME kg^{-1} DM), energy supply is regarded as the main limiting factor for milk production, allowing to sustain production levels of up to 27 L cow^{-1} d^{-1}, while protein is not limiting with production levels up to 35 L cow^{-1} d^{-1} (Kolver *et al.*, 1998).

The N contained in forages is found as proteins, peptides, free amino acids, nucleic acids, nitrates, and secondary metabolites (Goswami and Willcox, 1969). True protein (TP) normally ranges between 70 and 90% of total CP (Tamminga, 1986) and RDP (Pacheco and Waghorn, 2008). The RUP content in fresh pasture is generally low and with values ranging between 11.7, 8 and 3% CP for a permanent pasture with 27% CP, adjusted for rumen passage rate of 8, 5 and 2% h^{-1}, respectively (Anrique *et al.*, 2008). Also RUP increases slightly with longer grazing intervals (Hoekstra *et al.*, 2007).

CP content and N fractions of pastures are variable and influenced by factors such as botanical composition and season of the year (Fulkerson *et al.*, 1998; Aufrere *et al.*, 2003), also by physiological stage and agronomical managements (Peyraud and Astigarraga, 1998). Increasing N fertilization tends to increase CP and non protein N (NPN) content in the plant, and also degradability of N fractions in rumen, therefore decreasing the contribution of RUP (Nolan and Dobos, 2005). Pastures undergoing intensive N fertilization have reached CP levels higher than 300 g kg^{-1} DM (Pacheco *et al.*, 2008) with NPN concentrations from 240 to 420 g kg^{-1} total N (Goswami and Willcox, 1969), thus increasing the risk of negative effects on the animal and the environment and achieving a low NUE (Cosgrove *et al*., 1999; Bendall, 2001; Bolan and Kemp, 2003; Pacheco *et al*., 2008). The low NUE obtained under grazing conditions is basically due to the high dietary N intake, coupled with a high concentration of soluble protein and an imbalance and asynchrony between protein and energy supplies to the rumen (Nolan and Dobos, 2005; Hoekstra *et al*., 2007; Pacheco and Waghorn, 2008), and represents a loss of N and energy potentially available for productive purposes (Pacheco *et al*., 2008).

NUE of grazing dairy cows ranges between 13 and 31% (Verité and Delaby, 2000), with higher efficiency being observed with low CP content in the ration and high milk productions (Bach *et al*., 2000; Pacheco *et al*., 2008). As it has been possible to store in the milk up to 45% of the ingested N under experimental conditions (Delagarde *et al*., 1997), we believe that it may be possible to increase NUE in grazing dairy systems by developing nutritional strategies to reduce N intake and optimize microbial protein synthesis.

Strategies to improve N use efficiency in grazing systems

Several authors have suggested strategies to improve NUE (Verité and Delaby, 2000; Castillo *et al*., 2001; Hristov and Jouany, 2005; Flachowsky and Lebzien, 2006; Hoekstra *et al*., 2007; Pacheco *et al*., 2008; Pacheco and Waghorn, 2008), that under grazing conditions are restricted to the following: i) reducing N intake from the pasture, ii) synchronizing CHO and N supply in the rumen through supplements, iii) by using forage species with high WSC and of lower CP contents.

Reducing N intake from pasture based diets. A reduction of N content in pastures may be achieved through agronomic management such as selecting forage species with lower N content, which is a long term process, or by reducing the amount of N supplied as fertilizer or by increasing grazing intervals (Peyraud and Astigarraga, 1998). However, reducing N fertilization can decrease forage yield and longer grazing intervals should reduce the nutritional quality of the pasture. On the other hand, providing supplements with a low CP content is an alternative that allows diluting dietary N concentration (Pacheco *et al*., 2008), may not affect milk production and may increase stocking rate on the farm.

The purpose of supplementation to improve NUE should be focused on decreasing dietary CP content, avoiding an excess of CP available in the rumen, and increasing the energy supply to help converting dietary N into microbial protein (Hristov and Jouany, 2005; Pacheco *et al*., 2008).

A higher synthesis of microbial protein would reduce N excretions only if there is enough energy to allow the differential of absorbed amino acids to be retained in milk (Flachowsky and Lebzien, 2006; Pacheco *et al*., 2008) and if the animal has the genetic potential to produce a higher amount of milk protein (Chagunda *et al*., 2009).

The use of concentrates with low CP and high NSC contents has improved NUE (Keady *et al*., 1998), while using protein supplements increased considerably the N excretion through the urine and reduced NUE (Mulligan *et al*., 2004). Reducing CP content in the diet from 180 to 160 g kg^{-1}, through ration formulation, made possible to maintain milk production, reduce N intake by 10 to 15 percentage units and N excretion by 13 to 20 percentage units (Satter *et al*., 2002), indicating the possibility to increase NUE by the reduction of CP in the diet without affecting milk production.

Table 1 summarizes the results from studies that analyzed the effect of using different CHO sources on productive and metabolic responses of grazing dairy cows. Pasture was the main component of the diet except for treatments in which the cows received up to 10 kg of concentrates. The CP content of the supplements ranged between 121 and 260 g kg^{-1}, and an average NUE of 24 ± 4.8% (minimum 15.4 and maximum 32.9%: n = 52) was observed. Based on 91 diets evaluated in different countries, Castillo *et al*. (2000) observed an average NUE of 28%.

Cows consuming only pasture had a NUE of 21.0 ± 6.1% while supplemented grazing cows improved their NUE, storing in the milk 24.6 ± 3.8% of the ingested N, showing a positive effect of CHO supplementation on NUE (Van Vuuren *et al*., 1993; Berzaghi *et al*., 1996; Carruthers and Neil, 1997; Robaina *et al*., 1998; Reis and Combs, 2000; Walker *et al*., 2001; Bargo *et al*., 2002; Steinshamn *et al*., 2006). This improvement may be due to a decrease in CP content of the diet and to an increase of microbial protein synthesis. However, it is important to highlight that the improvement of NUE as a response to CHO supplementation occurs mainly when the pasture has a CP content higher than 200 g kg^{-1} (Van Vuuren *et al*., 1993; Berzaghi *et al*., 1996; Reis and Combs, 2000; Bargo *et al*., 2002); whereas with a lower CP content the effects are less important (Carruthers and Neil, 1997; O'Mara *et al*., 1997; Robaina *et al*., 1998; Walker *et al*., 2001; Steinshamn *et al*., 2006). Nevertheless, the highest reported values of NUE under grazing conditions are still lower than the ones obtained with cows fed total mixed rations (TMR), indicating that despite CHO supplementation there is still the possibility to develop other strategies that could improve NUE even more under grazing conditions.

The results summarized in Table 1 also show that the ruminal NH_3-N decreases with energy supplementation (Van Vuuren *et al*., 1993; Berzaghi *et al*., 1996; Carruthers and Neil, 1997; O'Mara *et al*., 1997; Jones-Endsley *et al*., 1997; Reis and Combs, 2000; Bargo *et al*., 2002), which suggests a higher NH_3-N utilization for microbial protein synthesis (Berzaghi *et al*., 1996; Carruthers and Neil, 1997; O'Mara, 1997).

Table 1. Effects of reducing the CP content of the diet by carbohydrate supplementation on NUE and milk production in grazing dairy cows.

PI	SI	CP	NH_3-N	MN	EN	MY	NUE	Authors
— kg d^{-1} —		g kg^{-1}	mg dL^{-1}	— g d^{-1} —		kg d^{-1}	%	
13.0	0.0	201	22.0	NA	NA	NA	-	Van Vuuren *et al.*, 1993
8.3	5.0	185	16.0	NA	NA	NA	29.0	
8.3	4.9	182	14.5	NA	NA	NA	29.0	
10.5	6.6	201	20.3	NA	NA	NA	26.0	
9.1	6.7	198	20.1	NA	NA	NA	27.0	
9.5	7.1	196	13.5	NA	NA	NA	28.0	
10.2	7.3	204	16.7	NA	NA	NA	27.0	
11.4	0.0	260	32.5	NA	NA	NA	16.0	
9.3	4.5	215	21.3	NA	NA	NA	22.0	
9.5	3.8	222	21.6	NA	NA	NA	20.0	
11.6	0.0	260	33.4	NA	NA	NA	16.0	
9.3	4.0	218	28.3	NA	NA	NA	21.0	
9.9	4.3	214	27.1	NA	NA	NA	21.0	
13.0	0.0	251	22.4	88	434	19.5	16.9	Berzaghi *et al.*, 1996
9.8	5.4	194	17.1	109	362	23.7	23.1	
14.5	0.0	176	17.6	100	301	19.9	24.9	Carruthers and Neil, 1997
14.3	1.3	161	13.2	106	286	20.3	27.0	
14.3	0.0	132	7.5	96	215	18.7	30.8	
14.0	1.3	121	5.4	98	210	18.8	31.8	
13.6	0.0	152	7.2	109	221	21.4	32.9	O'Mara *et al.*, 1997
11.5	2.7	143	5.6	103	223	19.8	31.6	
11.7	6.4	214	19.1	110	510	24.8	17.8	Jones-Endsley *et al.*, 1997
10.5	9.6	204	17.6	113	543	25.4	17.2	
14.9	0.0	179	NA	68	359	14.0	16.0	Robaina *et al.*, 1998
12.3	4.4	170	NA	85	369	16.4	18.7	
12.1	0.0	179	NA	53	293	10.6	15.4	
10.7	4.3	170	NA	74	334	14.8	18.2	
13.9	0.0	195	22.5	99	334	21.8	22.9	Reis and Combs, 2000
12.7	5.0	172	17.7	127	361	26.8	26.0	
9.7	10.0	155	13.8	148	343	30.4	30.2	
12.1	0.0	153	NA	63	234	12.4	21.2	Walker *et al.*, 2001
12.0	3.0	152	NA	83	282	15.6	22.8	
11.2	5.0	143	NA	98	273	18.3	26.3	
10.6	7.0	145	NA	105	303	19.9	25.7	
10.4	9.0	145	NA	111	339	20.7	24.7	
9.2	10.4	140	NA	119	320	21.9	27.2	
9.9	0.0	121	NA	49	143	10.0	25.4	
9.8	3.0	124	NA	60	194	11.8	23.5	
9.8	5.0	125	NA	74	222	14.8	24.9	
9.8	7.0	126	NA	83	256	16.9	24.4	
15.0	0.0	135	NA	78	246	15.5	24.2	
14.3	3.0	131	NA	94	269	18.4	25.8	
13.1	4.9	131	NA	96	281	18.3	25.4	
12.2	5.9	132	NA	98	285	18.2	25.5	
17.5	0.8	202	15.2	91	500	19.1	15.4	Bargo *et al.*, 2002
15.2	8.6	172	9.1	146	508	29.7	22.4	
20.5	0.7	194	15.3	104	554	22.2	15.8	
16.1	8.7	169	8.7	149	522	29.9	22.2	
13.4	5.0	164	NA	141	341	29.6	29.3	Mulligan *et al.*, 2004
15.4	0.8	193	NA	144	355	27.6	28.4	
13.9	5.0	203	NA	152	467	32.3	24.6	
15.9	0.0	157	NA	114	286	24.9	28.5	Steinshamn *et al.*, 2006
14.7	1.8	156	NA	126	285	26.9	30.6	

PI: pasture intake; SI: supplement intake; CP: dietary crude protein content; NH_3-N: ruminal ammonia N; MN: milk N; EN: N excreted through urine and feces; MY: milk yield; NUE: N use efficiency; NA: not available.

Since animals do not have the ability to store the excess of ingested N (Hoekstra *et al.*, 2007), as N intake increases, milk and fecal N concentrations remain relatively constant, while the amount of urinary N excreted increases proportionally (Steinshamn *et al.*, 2006) reaching levels closer to 50% of total ingested N (Hristov and Jouany, 2005). In the reviewed studies, the amounts of urinary and fecal N vary with CHO supplementation, with positive (Jones-Endsley *et al.*, 1997; Robaina *et al.*, 1998; Reis and Combs, 2000; Walker *et al.*, 2001), neutral (O'Mara, 1997; Bargo *et al.*, 2002) or negative responses (Berzaghi *et al.*, 1996; Bargo *et al.*, 2002). This may be caused by a number of factors determining the N losses, such as the CP concentration of the supplement and the ration, a possible increase of the total DMI caused by the supplementation, an increase of the amount of available energy for microbial protein synthesis, and a possible decrease of DM digestibility as a consequence of a lower ruminal pH. If concentrate supplementation does not dilute CP concentration of the diet, it may not decrease the urinary N excretions, because the surplus of ingested N regarding N requirements would not be reduced. Furthermore, if DMI increases and CP concentration of the diet remains constant, higher amounts of daily urinary N excretions may be expected.

It has been observed that in supplemented dairy cows there was an increase of the proportion of fecal N compared to urinary N (Van Vuuren *et al.*, 1993; Carruthers and Neil, 1997; Bargo *et al.*, 2002; Mulligan *et al.*, 2004; Steinshamn *et al.*, 2006). As mentioned above, fecal N should remain relatively constant, but could increase due to a higher DMI and a lower N digestibility. When the MP meets animal requirements, increasing microbial protein synthesis or RUP supply may have no effect on NUE, but may produce an increase in the proportion of fecal N in relation to urinary N (Flachowsky and Lebzien, 2006; Pacheco *et al.*, 2008), which corresponds to the indigestible fraction of MP and RUP at intestinal level. When higher amounts of circulating N are converted into fecal microbial N, via fermentation in the posterior digestive tract, a transference of potential urinary N to fecal N, an environmentally less labile source of N, could be achieved (Gressley and Armentano, 2007) and therefore the potential impact on the environment may be reduced.

The effect of CHO supplementation on pasture diets has been also analyzed using continuous culture fermenters, evaluating ruminal digestion and N metabolism (Bach *et al.*, 1999; Bargo *et al.*, 2003; Wales *et al.*, 2009). Ruminal NH_3-N decreased as a consequence of supplementation. The amount of microbial N produced (g d^{-1}) kept constant, however, with low and medium substitution rates (kg pasture kg^{-1} concentrate) there was an increase in the proportion of microbial N in relation to total N flow (Bargo *et al.*, 2003). In other CHO supplementation experiments, it has been found a significant increase in bacterial N synthesis (Bach *et al.*, 1999), or no response (Wales *et al.*, 2009) compared with a pasture only diet. In order to avoid a negative effect on the OM digestibility and efficiency of microbial protein synthesis (g N kg^{-1} digested OM), it has been suggested that grain inclusion should not exceed 24% of total diet (Wales *et al.*, 2009). At higher levels the efficiency of microbial N synthesis tends to decrease with supplementation (Bach *et al.*, 1999; Bargo *et al.*, 2003).

From a productive point of view, concentrate supplementation of grazing dairy cows causes an increase in milk production (Berzaghi *et al*., 1996; Carruthers and Neil, 1997; Robaina *et al*., 1998; Ries and Combs, 2000; Walker *et al*., 2001; Bargo *et al*., 2002; Mulligan *et al*., 2004; Steinshamn *et al*., 2006), however, the response observed has been variable with a media of 0.83 ± 0.34 (n = 23) kg milk kg^{-1} supplement and lower than in confinement, limiting the use of this alternative to the relationship between price paid per liter of milk, the cost per kilogram of supplement and the animal response. The highest milk production in supplemented grazing dairy cows usually occurs due to an increase in total DMI and digestibility of the diet. However, there are several factors affecting the milk response (MR) like herbage allowance, amount and type of supplement, and production level and stage of lactation of the cow. Higher herbage allowances are related to higher substitution rates (SR; SR = (pasture DMI in unsupplemented treatment – pasture DMI in supplemented treatment)/supplement DMI), a small increase in total DMI and therefore a lower MR to concentrate supplementation. The milk response per kilogram of concentrate decreases as the amount of concentrate increases, being lower above 3-4 kg concentrate d^{-1} when pasture quality and quantity are not limiting. Finally, cows in early lactation have a higher MR than cows in late lactation.

In summary, supplementation with energy sources and low protein content increases NUE in grazing dairy cows, due to a decrease in the CP content of the diet and a possible higher microbial protein synthesis. Another beneficial effect is that it decreases the proportion of urinary N in relation to fecal N, since the last one has a lower impact on the environment. Supplementation decreases pasture intake per cow, allowing increasing the stocking rate, and a higher milk yield per cow. So it would allow producing more milk protein per surface unit with lower losses to the environment (Verité and Delaby, 2000). However, the milk response to supplementation, the price paid per liter of milk and the cost per kilogram of supplement must be considered to avoid a negative effect on the profitability of the dairy farm.

Improving ruminal synchrony between CHO and N. The concept of ruminal synchrony proposed by Johnson (1976) establishes that ruminal NH_3 utilization and microbial protein synthesis would be maximized if there is a synchrony between the availability of energy and N in the rumen (balanced amounts at the same time). This could be achieved by changing the CHO or N sources, changing the feeding patterns (time of supplementation regarding grazing) or the feeding frequency (Cabrita *et al*., 2006). A better ruminal synchrony could be achieved under grazing systems, when the CHO sources have a degradation rate of 13 to 14% h^{-1}, because this is similar to the RDP degradation rate of the pasture (Van Vuuren *et al*., 1990), although degradation rate of the pasture will vary with different circumstances (Aufrere *et al*., 2003).

Table 2 shows the results obtained in experiments aiming at improving ruminal synchrony between CHO and N, either by changing the fermentation rates of CHO and N through modification of the diet ingredients (Shabi *et al*., 1998; Casper *et al*., 1999; Gehman *et al*., 2006) or changing the feeding patterns (Kolver *et al*., 1998; Trevaskys *et al*., 2004; Lund *et al*., 2008).

Casper *et al*. (1999) evaluated the supplementation with corn (*Zea mays* L.) or barley (*Hordeum vulgare* L.) plus soybean (*Glycine max* Merr.) meal or extruded soybean meal to improve ruminal synchrony between CHO and N, while Gehman *et al*. (2006) determined the effect of supplementing grazing cows with corn, barley or citrus pulp. None of these studies could determine any clear effects on NUE when modifying the degradation rate of CHO and CP, however, Casper *et al*. (1999) observed a tendency towards a higher NUE when supplementing with barley and soybean meal (synchrony due to the fast degradation of CHO and CP) and corn with extruded soybean meal (synchrony due to the lower degradation rate of CHO and CP), and determined lower amounts of ruminal NH_3-N (barley plus soybean meal), which indicates a better utilization of N for the synthesis of microbial protein. Meanwhile, milk production was higher in diets supplemented with corn as a consequence of a higher DMI. Total intake can decrease when supplementing with large amounts of rapidly degradable CHO sources, due to a decrease of OM digestibility and a lower ruminal passage rate (Chamberlain and Wilkinson, 1996). Gehman *et al*. (2006) determined a higher NUE when supplementing with corn (C) or barley (B) rather than when using citrus pulp (CIP), even though considering the synchrony the supplementation with B or CIP should increase NUE, since their degradation rate (13 a 14% h^{-1}) would be expected to allow an optimum ruminal NH_3 utilization by grazing dairy cows. Experimentally, a disadvantage of modifying the ingredients of the diet is that there are effects that could be attributed to ruminal synchrony, when they would actually be a consequence of factors linked to the supplemented ingredient (Hristov and Jouany, 2005). In order to identify effects that are a consequence of better ruminal synchronization, it is recommended to change the feeding patterns (Newbold and Rust, 1992), specifically, the moment when the supplement is offered to the animals in relation to the grazing event.

From the studies of Kolver *et al*. (1998), Trevaskys *et al*. (2004), and Lund *et al*. (2008) we define a synchronized diet as the one where the highest proportion of rapidly degradable CHO was supplemented before grazing to match the energy supply (from the degradation of the CHO in the supplement) and NH_3 content at the rumen (from the degradation of CP of the pasture). In the three studies there was a decrease in N intake for diets with a better synchrony, but only Trevaskys *et al*. (2004) found

Table 2. Effects of applying supplementation strategies, to optimize carbohydrate and nitrogen synchronization, on NUE and milk production of grazing dairy cows.

Supplementation criteria	DMI	NI	MY	NH_3-N	UN	MN	NUE	Authors
	kg d^{-1}	g d^{-1}	kg d^{-1}	mg dL^{-1}		g d^{-1}	%	
BG	18.8	491.3	29.1	16.3	21.8*	121.3	24.7	Kolver *et al*., 1998
AG	19.3	510.0	29.9	18.4	22.3*	125.0	24.5	
75%BG, 25%PM	14.7	791.0	25.1	NA	NA*	124.4	16.0	Trevaskys *et al*., 2004
25%BG, 75%PM	14.7	836.0	24.3	NA	NA*	126.8	14.9	
SMAM, BPM P1	19.2	555.2	31.1	NA	7.4**	157.1	28.3	Lund *et al*., 2008
BAM, SMPM P1	18.5	524.8	30.6	NA	6.6**	154.1	29.4	
SMAM, BPM P2	18.6	520.0	25.8	NA	7.7**	144.0	27.7	
BAM, SMPM P2	18.0	497.6	25.1	NA	7.2**	140.0	28.1	
ROM/RCP	16.7	432.0	18.5	16.6	17.6**	101.5	23.5	Shabi *et al*., 1998
ROM/SCP	16.4	432.0	18.2	14.4	17.0**	99.8	23.1	
SOM/RCP	15.5	400.0	17.5	18.2	19.9**	93.3	23.3	
SOM/SCP	16.1	416.0	18.2	15.3	19.8**	97.0	23.3	
C	25.0	592.0	30.6	NA	10.1*	134.8	22.8	Gehman *et al*., 2006
B + M	22.8	560.0	29.9	NA	11.4*	129.8	23.2	
CP + M	25.2	592.0	30.0	NA	9.9*	127.0	21.4	
C + S	20.7	549.8	25.1	15.0	19.1**	127.5	23.2	Casper *et al*., 1999
C + ES	21.8	593.0	27.5	10.4	18.4**	143.1	24.1	
B + S	19.9	509.4	23.8	9.0	18.1**	123.8	24.3	
B + ES	20.0	537.6	23.5	11.3	20.0**	119.3	22.2	

DMI: dry matter intake; NI: nitrogen intake; MY: milk yield; NH_3-N: ruminal ammonia N; UN: urea N; MN: milk N; NUE: N use efficiency; NA: not available; *: milk urea N; **: plasma urea N; BG: supplementation at beginning of grazing; AG: supplementation 4 h after grazing: 75%BG, 25%PM: 75% of the supplement before grazing, 25% during afternoon milking; 25%BG, 75%PM: 25% of the supplement before grazing, 75% during afternoon milking; SMAM, BPM: soybean meal supplied in the morning and barley in the afternoon; BAM, SMPM: barley supplied in the morning and soybean meal in the afternoon; ROM/RCP: rapidly degradable organic matter, rapidly degradable crude protein; HOM/SCP: rapidly degradable organic matter, slowly degradable crude protein; SOM/RCP: slowly degradable organic matter, rapidly degradable crude protein; SOM/SCP: slowly degradable organic matter, slowly degradable crude protein; C: corn; B + M: barley plus molasses; CP + M: citric pulp plus molasses; C + S: corn plus soybean; C + ES: corn plus extruded soybean; B + S: barley plus soybean; B + ES: barley plus extruded soybean.

a slight increase of NUE (+ 1.1%), as a consequence of a lower CP content in the pasture of the treatment with better synchrony (308 vs. 328 g kg^{-1}). There were no differences in MY and urea N levels. The effects of synchrony in MY and N excretion are not clear (Newbold and Rust, 1992; Hristov and Jouany, 2005) and less important than expected (Cabrita *et al*., 2006). A proof of this is that Kaswari *et al*. (2007) did not obtain significant differences between isoenergetic and isoproteic diets with different synchrony indexes for ruminal N-NH_3 content, microbial protein synthesis, and metabolizable energy flow. Factors explaining the lack of response with synchronizing degradation of CHO and N, may be that ruminants have the capacity to recycle the excess N into urea and supply it to the rumen during periods of deficit, remaining available for microbial protein synthesis when slowly degradable CHO are fermented and release energy (Marini *et al*., 2004; Lapierre *et al*., 2005), also ruminal bacteria can recover rapidly from N shortages (Newbold and Rust, 1992), so that their metabolic functions are not affected.

The lack of response to a higher synchrony in cows fed pastures with high CP content (more than 200 g kg^{-1} on DM basis) would be caused by an excess of MP in relation to the requirements of dairy cows (for a milk production of around 30 L d^{-1}), therefore the animal would not be able to convert the higher MP supply, generated by a better synchrony, into milk N (Trevaskys *et al*., 2004). Cows with a higher genetic merit for protein synthesis could store a higher amount of consumed N in milk (Chagunda *et al*., 2009). Therefore selecting cows with higher milk protein production would allow depositing the excess of MP at the intestinal level into milk N.

Increase of water soluble carbohydrate concentration of the pasture

Effect of agronomic management. A higher WSC content in the forage allows to decrease N intake and urinary N, because there is an inverse relationship between sugar content and CP of the forages (Tas *et al*., 2006b). Therefore, the beneficial effects of forages with a high WSC content should be a consequence of a higher energy supply at the rumen and a lower N intake (Taweel, 2006).

It is possible to improve the WSC/CP ratio by reducing N fertilization (Taweel, 2006), increasing grazing intervals (Peyraud and Astigarraga, 1998), using selected cultivars with high sugar content (Miller *et al*., 2001), or adjusting the pasture intake according to the daily patterns of WSC and CP contents of the plant (Trevaskys *et al*., 2004). WSC concentration in grasses is higher at sunset than during the morning (Rutter *et al*., 2004; Hristov and Jouany, 2005) as a consequence of sugar accumulation (especially sucrose) due to photosynthesis, finding higher levels at the moment when photosynthesis and respiration rates are equal. Meanwhile CP content is higher during the morning than at sunset, because there is a negative relationship between WSC and CP content (Cosgrove *et al*., 2007).

In theory, maximizing animal DMI at sunset would allow to improve NUE. Forage samples that were collected at sunset have a wider WSC/CP ratio than those collected at the morning (Trevaskys *et al*., 2004). Trevaskys *et al*. (2004) determined the effect of offering a daily strip of pasture to dairy cows in the morning or the afternoon, observing an increase in DMI, MY, milk N content and NUE when the cattle entered the pasture in the afternoon (Table 3). Nevertheless, due to the high CP content of the pasture (328 and 268 g kg^{-1} DM, in the morning and afternoon, respectively), NUE remained within low standards (13.9 and 16.3% for cows entering the field in the morning and afternoon, respectively).

Use of high sugar varieties. Miller *et al*. (2001) evaluated milk production in late lactating dairy cows fed a perennial ryegrass pasture, selected for a high WSC content (HS), or a standard cultivar. They observed a higher digestible DMI (+ 1.5 kg d^{-1}), greater MY (+2.7 kg d^{-1}) and NUE (+ 7%), and a lower proportion of urinary N (10%) in cows fed the HS cultivar. Two years later in a similar study with early lactation cows, Moorby *et al*. (2006) determined a higher WSC content (243 vs. 161 g kg^{-1} DM), higher digestibility and a lower NDF content for the HS cultivar as compared to the control, resulting in a higher pasture (15.3 vs. 13.1 kg DM d^{-1}) and total DMI (18.8 vs. 16.6) and a non significant tendency to a higher milk yield (+ 2.3 kg d^{-1}). However there were no differences in NUE, but values (average of 36.7%) are very high for grazing cows, which was attributed to the low CP content of the pasture (average 101.5 g kg^{-1} DM). In this experiment urinary N decreased 7% and fecal N increased 14% for cows fed HS, which would lessen the environmental impact associated to N excretions for cows fed HS cultivars.

Taweel *et al*. (2005) evaluated an HS perennial ryegrass with lactating dairy cows (126 ± 21 DIM). The HS cultivars had 20% more WSC than standard cultivars and, as a different from Miller *et al*. (2001) and Moorby *et al*. (2006) findings, their NDF and CP contents were similar, so the observed responses could be attributed to the increase in WSC content. There were no significant differences for pasture DMI, total DMI, digestible DMI, MY, and NUE, while MUN and ruminal NH_3-N tended to be lower in cows fed the HS cultivar. In a subsequent study Taweel *et al*. (2006) did not observe differences in DMI, MY, and NUE in early lactation cows (75 ± 7 d) fed with HS and standard cultivars. The reason behind the lack of response with feeding HS cultivars, observed in these two studies, could be that an increase in WSC content of around 20% would not be enough to increase palatability and forage digestibility (Taweel *et al*., 2005; 2006). Although there was a difference in WSC content between HS and standard cultivars, the energy and CP content were similar, so the only difference between HS and standard cultivars was the ruminal CHO rate of degradation. As discussed above, the effects off modifying CHO and N degradation i.e. ruminal synchrony does not increase milk yield neither NUE. Even though MUN was lower for HS varieties, indicating a possible higher microbial protein synthesis, the CP levels of the diets

Table 3. Effect of water soluble carbohydrate content in the pasture on NUE and milk production of grazing dairy cows.

	WSP	CP	DMI	MY	MUN	EN	MN	NUE	Authors
	g kg^{-1}		kg d^{-1}		mg dL^{-1}	g d^{-1}		%	
P AM	74	328	14.7	24.3	NA	720	116	13.9	Trevaskys *et al*., 2004
P PM	124	268	16.4	26.8	NA	699	136	16.3	
HS	165	92	15.1	15.3	NA	197	83	29.8	Miller *et al*., 2001
Control	126	106	14.2	12.6	NA	223	68	23.3	
HS	243	104	18.8	32.7	NA	240	136	36.2	Moorby *et al*., 2006
Control	161	99	16.6	30.4	NA	201	119	37.2	
HS	181	159	20.2	23.8	18.3	361	129	26.1	Taweel *et al*., 2005
Control	157	162	20.6	24.7	20.5	377	131	25.6	
HS	180	151	20.3	25.1	16.7	340	132	27.8	
Control	149	157	21.1	26.6	19.9	367	141	27.5	
HS	129	184	18.4	27.5	NA	404	135	24.9	Taweel *et al*., 2006
Control	105	186	17.6	26.9	NA	389	131	25.0	
HS Y 1	149	183	17.5	28.1	14.0	433	137	24.1	Tas *et al*., 2006a
LS Y 1	132	175	17.5	27.0	14.5	411	128	23.8	
LS Y 1	110	182	15.6	26.0	15.6	382	124	24.4	
HS Y 1	144	179	18.0	28.8	14.4	435	139	24.2	
HS Y 2	114	210	17.0	25.8	16.0	499	124	19.9	
LS Y 2	107	195	17.3	25.8	15.0	482	123	20.3	
LS Y 2	87	207	17.4	25.2	16.5	515	117	18.5	
HS Y 2	131	194	18.4	25.7	14.9	499	124	19.9	
c1 Y1	192	160	20.2	26.9	8.8	373	144	27.8	Tas *et al*., 2006b
c2 Y1	170	157	21.5	28.1	9.4	393	147	27.3	
c3 Y1	158	166	21.4	26.3	9.6	420	141	25.1	
c4 Y1	195	150	20.1	26.8	7.3	348	139	28.5	
c5 Y1	172	156	20.9	27.9	8.8	376	146	28.0	
c6 Y1	152	159	20.7	28.3	8.7	378	146	27.8	
c1 Y2	131	204	17.1	24.6	17.4	412	122	22.9	
c2 Y2	110	198	17.3	24.7	17.4	403	120	22.9	
c3 Y2	93	194	16.8	23.8	16.4	385	116	23.1	
c4 Y2	113	190	16.1	22.5	16.3	361	107	22.9	
c5 Y2	100	193	16.5	23.3	16.7	376	113	23.1	
c6 Y2	98	203	17.1	23.9	17.2	415	117	22.0	

WSC: water soluble carbohydrates; CP: crude protein; DMI: dry matter intake; MY: milk yield; MUN: milk urea N; EN: excreted N; MN: milk N; NUE: N use efficiency; NA: not available; P AM: grazing started in the morning; P PM: grazing started in the afternoon; HS: cultivar with high WSC content; LS: standard cultivar.

were sufficient to satisfy animal requirements, so that the difference of microbial protein would not be used for productive purposes.

Tas *et al*. (2006a) determined a higher WSC content for HS cultivars (210 to 260 g kg^{-1} for the first and second season of evaluation, respectively). WSC content decreased and the CP concentration increased during the second season, attributed to a higher N fertilization of the pasture. During 2002, DM and N intake, milk and protein production in early lactation cows was higher for HS cultivars, however, during 2003 there were no differences between HS and standard cultivars. MUN content was lower for the HS treatment during the two seasons. The increase in WSC content at the expense of the CP and NDF concentrations did not show the expected differences between cultivars for NUE during the two seasons (24.1 and 24.6% during 2002; 19 and 20.9% in 2003). Tas *et al*. (2006b) evaluated the productive response in early lactation dairy cows fed with six cultivars of perennial ryegrass that had different WSC content. The cultivars with higher WSC content showed a lower CP and NDF content, however, there were no differences in DMI and MY. There were no significant differences in NUE between cultivars.

The information in Table 3 allowed us to determine a high correlation ($r^2 = 0.75$) between the WSC/CP ratio and NUE (Figure 2), which tends to increase linearly (NUE = 9.329 * WSC/CP + 16.57) as the WSC/CP ratio increases, in other words, to improve NUE, it would be necessary to increase the WSC concentration and, simultaneously, decrease the CP content.

In summary, the use of HS cultivars increases DM intake (Miller *et al*., 2001; Moorby *et al*., 2006; Taweel *et al*., 2006), milk production (Miller *et al*., 2001; Moorby *et al*., 2006; Tas *et al*., 2006a; Taweel *et al*., 2006) and milk N (g d^{-1}). However, NUE improved consistently only in one study (Miller *et al*., 2001), while MUN decreased only in one experiment (Taweel *et al*., 2005). A higher milk production was observed when cows were fed with HS pasture, this was associated with a higher DMI but not to a better nutrient utilization (Miller *et al*., 2001; Trevaskys *et al*., 2004; Moorby *et al*., 2006; Tas *et al*., 2006a; Taweel *et al*., 2006). A higher, but not significant, NUE average was observed when cows consumed HS cultivars, when comparing with values obtained with cows fed conventional cultivars (24.7 ± 4.8% vs. 20.9 ± 5.9%), however, this happened mainly because of the low CP content of the pastures evaluated, without an important effect of the increase in WSC content of the plant. Therefore, it would be expected that with permanent pastures such as those present in Southern Chile, characterized by a high CP content during autumn and spring (Anrique *et al*., 2008), NUE might be lower to those values described, despite the use of HS cultivars and close to those observed by Trevaskys *et al*. (2004).

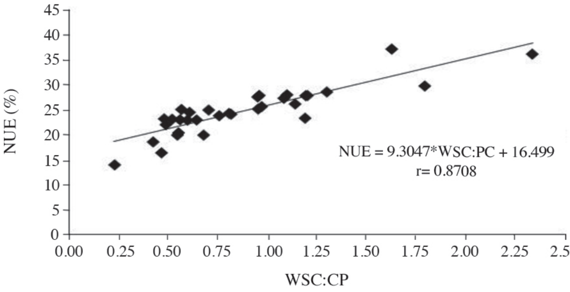

Figure 2. NUE as affected by the WSC:CP ratio of the pasture (data taken from Miller *et al*., 2001; Trevaskis *et al*., 2004; Taweel *et al*., 2005; 2006; Moorby *et al*., 2006; Tas *et al*., 2006a; 2006b).

CONCLUSIONS

A low NUE is observed in grazing dairy cows, which is attributed to the high CP content of most pastures in temperate areas during autumn and spring. The most efficient strategy to improve NUE and reduce urinary N under these conditions seems to be to reduce dietary CP content through supplementation with low-protein concentrate feeds. The supplementation allows increasing animal stocking rate and milk protein production per surface unit and decreases the loss per product unit. A better ruminal synchrony has not meant a higher NUE under grazing conditions, while the use of HS cultivars shows inconsistent results.

In order to improve NUE it is necessary to increase the WSC/CP ratio of the pasture plants. Therefore, a viable alternative would be to combine practices such as carbohydrate supplementation and to offer pasture during the afternoon. Additionally, genetic selection of dairy cows, orientated towards a higher concentration of milk protein, is important for cows to increase milk N retention.

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Estrategias nutricionales para mejorar la eficiencia de uso del nitrógeno en vacas lecheras en pastoreo. Las vacas lecheras en pastoreo se caracterizan por convertir una baja cantidad del N dietario en N lácteo. Esta baja eficiencia en el uso del N (NUE) se debe principalmente al excesivo suministro de proteína cruda (CP) de la pradera en relación a los requerimientos del animal. El exceso de N en la dieta tiene efectos negativos para el animal, altera las características organolépticas de la leche y contamina el medio ambiente a través de las excreciones de N. El objetivo de este trabajo fue analizar la información disponible sobre estrategias nutricionales para mejorar

EUN en vacas lecheras a pastoreo, tales como diluir la concentración de N dietario a través de la suplementación, sincronizar la tasa de fermentación de carbohidratos (CHO) y N en el rumen, y utilizar praderas con alto contenido de carbohidratos solubles (WSC). Se observa una relación beneficiosa entre la suplementación con CHO y la NUE, con un aumento en producción de leche (MY) de 0.83 ± 0.34 kg leche kg^{-1} suplemento y menor cantidad de N amoniacal ($N-NH_3$) en el rumen. La mejora en la sincronía entre CHO y N a nivel ruminal, no genera los efectos esperados en PL y EUN. Se observó un efecto positivo sobre la NUE al mejorar la relación WSC/CP. La utilización de cultivares con un mayor contenido de WSC incrementa el consumo de MS, MY y la cantidad de N lácteo, mientras que la NUE no mejora consistentemente.

Palabras clave: Suplementación con concentrados, sincronía ruminal, ballicas altas en azúcar, proteína cruda, producción de leche.

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