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The Dietary Cation-Anion Difference and its Impact on the Milk Production in Dairy Cows

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Fodrets katjon-anjonbalans och dess påverkan på mjölkproduktion hos kor

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Key words: Acidosis, buffer, CAD, DCAD, feed efficiency, feed ration, macro minerals, milk composition, milk yield.

Sammanfattning

Katjon-anjonbalasen i fodret (DCAD) uttrycker skillnaden mellan viktiga makromineraler med hänsyn till deras laddningar i jonform. Uträkningen kan skrivas DCAD:S = Na^{+}+K^{+}-Cl^{-}-S^{2-} eller DCAD:Cl = Na^{+}+K^{+}-Cl^{-}. Syftet med denna litteraturstudie var att undersöka om det finns en optimal DCAD-nivå för lakterande kor och hur detta DCAD-värde skulle påverka foderstaten hos kor under laktation.

Torrsubstansintaget (DMI) och mjölkavkastningen påverkas positivt av ett höjt DCAD. En stagnation/topp för DMI respektive mjölkavkastning ses inom intervallet 300-600 mEq/kg torrsubstans (ts), där DMI når stagnation/topp vid ett något högre DCAD än mjölkavkastningen. DMI antas öka som en effekt av en förbättrad metabolisk balans och våmmiljö, samt en potentiellt ökad smaklighet på fodret. Det råder tvetydighet om proteinmängden i mjölken ökar eller förblir opåverkad av ett höjt DCAD. Däremot antas proteinprocenten påverkas av spädningseffekter från mjölkavkastningen. Mjölkfettmängden per dag ökar med ett höjt DCAD. Ökningen i både mjölkavkastning och fettmängd anses bland annat bero på ett ökat DMI. Ett höjt DCAD antas också ha en indirekt och positiv påverkan på foderomvandlingsförmågan hos kor. En grundfoderstat kan antas ha ett DCAD mellan cirka 250 till 350 mEq/kg ts. Om man jämför detta intervall med olika DCAD-nivåers påverkan på mjölkavkastning och DMI, kan man dra slutsatsen att mjölkkor som utfodras enligt aktuella utfodringsrekommendationer inte behöver korrigera DCAD-nivån i foderstaten för att optimera mjölkproduktionen.

Abstract

The dietary cation-anion difference (DCAD) expresses the difference between important macro minerals considering their charges. The calculation can be written DCAD:S = Na^{+}+K^{+}-Cl^{-}-S^{2-} or DCAD:Cl = Na^{+}+K^{+}-Cl^{-}. The aim of this literature review was to investigate whether there is an optimal DCAD among lactating dairy cows and how this would affect the feed ration of dairy cattle during lactation.

The dry matter intake (DMI), as well as milk yield, is shown to increase with increasing DCAD. A stagnation/top, for DMI as well as milk yield, is shown in the range of about 300-600 mEq/kg dry matter (DM), where the top of DMI is slightly above the milk yield. The DMI is thought to increase due to an improved metabolic- and ruminal balance as well as a potentially increased palatability of the feed. There is ambiguity whether the protein yield increases or remains unaffected with increasing DCAD. However, the protein percentage is thought to be affected by dilution regarding the milk yield. The total amount of fat per day is shown to increase with increasing DCAD. The increased fat yield, as well as milk yield, may originate from an increased DMI. An increased DCAD is also suggested to indirectly improve the feed efficiency. The basal feed ration has a DCAD between about 250 to 350 mEq/kg DM. When comparing this interval to the different DCAD-levels impact on DMI and milk yield, it is suggested that dairy cattle that are fed according to the current feeding recommendations, do not need to alter the DCAD-level to improve the production.

Introduction

It is generally known that plant based feedstuffs usually are contributing to an alkalizing diet. The chemical property of the diet affects parameters such as blood buffers, urinary pH and secretion of different minerals in the urine (West *et al.,* 1991; Roche *et al.,* 2005; Martins et al., 2015). In contrast to the alkalizing diet, it is well known that acidifying, anionic diets prepartum reduces the incidence of milk fever (*parturient paresis*) (Block, 1984; Ender *et al.*, 1971, Goff *et al.,* 1991). The reduction of *parturient paresis* is due to the dietary introduced weak metabolical acidosis (Vagg & Payne, 1970), which is achieved by altering the ratio of strong anions and cations in the feed (McDonald *et al.*, 2011; Iwaniuk & Erdman, 2015).

The overall metabolic impact of changes in the ratio of anions and cations in the feed (Oetzel *et al.,* 1991; Martins *et al.,* 2016) suggests that the dietary cation anion difference (DCAD) has an impact also in other stages than prepartum in the cow's production cycle. It is therefore suggested that the level of DCAD in the feed has an effect on the dairy cattle's milk production. The aim of this literature review is therefore to clarify the concept and effects of DCAD, in relation to common cattle feed and how different levels of DCAD affect the lactating cow considering milk production and feed efficiency, when the risk of milk fever, adjacent to partum, have subsided. Is there an optimal DCAD-level to optimize the milk production and feed efficiency? And if there is an optimal DCAD-level, what would it imprint in the feed ration?

Calculation of the cation-anion difference

The cation anion difference (CAD) is a measurement of the difference between important macro minerals; sodium (Na), potassium (K), chloride (Cl) and sulfur (S) (McDonald *et al.*, 2011). CAD can be calculated as milliequivalents (mEq) (Sanchez & Beede, 1996), which is a common way of measuring ions in low concentrations (Denniston *et al.*, 2008), but millimolar of charge ($mmol_c$) can also be used (Tremblay *et al.,* 2006). The CAD are applied in the feed ration by calculating the dietary cation-anion difference (DCAD). The calculations of CAD and DCAD are identical, but DCAD is expressed as mEq/kg dry matter (DM) (Hu & Murphy, 2004; Wildman *et al.*, 2007a; Iwaniuk & Erdman, 2015).

DCAD can be achieved by calculating (Hu & Murphy, 2004; Wildman *et al.*, 2007a):

DCAD:S = $Na^+ + K^+ - Cl^- - S^{2-}$/kg DM.

or

DCAD:Cl = $Na^+ + K^+ - Cl^-$ /kg DM.

Different calculations of DCAD are used in different studies (Iwaniuk *et al.,* 2015; Apper-Bossard *et al.,* 2006), but according to Hu *et al.* (2007) are cows rarely supplemented with sulfur. This contributes to a small variation in the supplements independently of the present or absence of sulphur in the calculation. However, numerical differences are notable (figure 1) (Wildman *et al.*, 2007b).

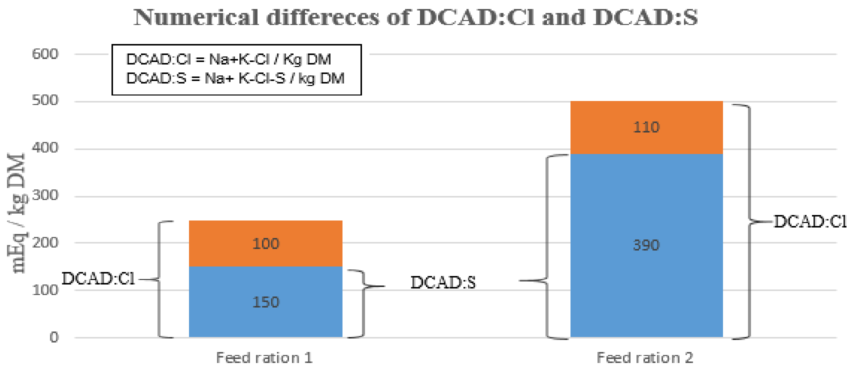

Figure 1. Two examples of the variation in DCAD depending on the presence or absence of sulfur in the calculation. The information is taken from Wildman *et al.* (2007b) where the DCAD for two feed rations was calculated by using both DCAD:Cl and DCAD:S.

The designations of DCAD:S and DCAD:Cl are created for this literature review, and are two ways of describing the same trait (DCAD). For simplicity, the reader is suggested to not pay that much attention whether DCAD:Cl or DCAD:S is used when the literature review examines changes in intervals for different traits. Nevertheless, if focusing on the numerical outcomes from any calculation, the use of DCAD:Cl or DCAD:S, may be important.

DCAD in the feed

Grasses, grains and legumes

The macro mineral that affect the DCAD of grasses the most is K, due to its high concentration in the plants (McDonald et *al.*, 2011; Tremblay *et al.*, 2006). An analysis of orchardgrass, meadow bromegrass, tall fescue, smooth bromegrass and thimothy in the Québec province in Canada showed that the K content was measured above 20 g/kg DM among the grasses, while the other contributing cationic source, Na, was measured under 0.4 g/kg DM (Tremblay *et al.*, 2006).

Thimothy is shown to have a low DCAD:S, compared to other grasses. Contrary, orchardgrass has a relatively high DCAD. The reason for the different DCAD:S for the two grasses is mostly due to variations in the above mentioned K content. Thimothy had a DCAD:S of 341 mEq/kg DM, while orchardgrass had a DCAD:S of 663 mEq/kg DM (table 1) (Tremblay *et al.*, 2006). The K impact on the DCAD:S was also shown in an analysis of 16 different legumes and grasses from pasture in Australia. A strong correlation between the K content and the DCAD:S was shown for the analysed plants. It was also shown that the average DCAD:S for all of the legumes, including plants such as white clover and subterranean clover, was more than two-fold greater than the average for the analysed grasses (Pelletier *et al.*, 2008).

Feed tables show that grains has a low DCAD compared to grasses and the above mentioned leguminous forages, due to its lower K content. The DCAD:S of oats is -32 mEq/kg DM, while wheat has a slightly higher DCAD:S of 13 mEq/kg DM (table 1) (Spörndly, 2003). The DCAD in plants can also be affected by the choice of fertilizer (Grant & MacLean, 1966;

Pelletier *et al.,* 2006; Charbonneau *et al.,* 2009), as well as season (Pelletier *et al.,* 2008) and stage of development (Pelletier *et al.,* 2006).

Table 1. Different feedstuffs showing the variation in DCAD:S and distribution of the macro minerals, Na, K, Cl and S

	DCAD:S	g/ kg DM					
Feed	mEq/kg DM	Na	K	Cl	S	Comment	Reference
Orchardgrass	663	0.051	37.6	5.08	2.52		Tremblay *et al.,* 2006.
Red clover	635	0.4	25.0	0.6	0.31	Cl and S, adapted from legume forage, NRC.	Spörndly, 2003; NRC, 2001
lusern	474	1.44	28.8	9.2	4.2	Na, K: Average whole season. Cl, S: Early blooming alfalfa hay.	Spörndly, 2003
Timothy	341	0.021	22.9	3.88	2.18		Tremblay *et al.,* 2006.
Wheat	13	0.2	4.7	-	-		Spörndly, 2003
Oat	-32	0.1	5.3	-	-		Spörndly, 2003

The DCAD of the whole feed ration can be calculated. A basal total mixed ration (TMR) diet, consisting of about 63% corn silage, 6 % alfalfa hay and 31% concentrate, with ground corn and soy bean meal as main ingredients, has a DCAD:S of about 250 mEq/kg DM (Iwaniuk *et al.,* 2015). Contrary, if the cow is fed a hypothetical diet of 10 kg DM silage (Na:1.2, K:21, Cl:5.5, S:2.1 g/kg DM), 10 kg DM concentrate (©Solid 120, Lantmännen) and 0.1 kg of minerals (Na:56, K:0, Cl: 83, S: 0,5 g/kg DM) a day (Cecilia Kronqvist, Swedish University of Agricultural Sciences, 2016-04-11) the DCAD:S would be about 300 mEq/kg DM. DCAD:Cl for the same diet would be about 324 mEq/kg DM.

Feed additives to affect DCAD

Besides the possibility to influence the dietary cation-anion difference with the basal diet, additives can be supplemented to affect the DCAD. Buffers such as potassium carbonate (K_2CO_3), sodium sesquicarbonate (NaSC) and sodium bicarbonate ($NaHCO_3$) can be added to the feed to elevate the DCAD (Iwaniuk *et al.,* 2015; Roche *et al.,* 2005). Conversely, a decreased DCAD can be achieved by the addition of anionic salts, such as ammonium chloride (NH_4Cl) (Oetzel & Barmore, 1993), magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) (West *et al.,* 1992; Roche *et al.,* 2005).

The additives can for instance be given as a part of total mixed ration (West *et al.,* 1991; Wildman *et al.,* 2007a; Iwaniuk *et al.,* 2015) or mixed with the concentrate (Stout *et al.,* 1972). It is shown that the palatability of additives vary (Stout *et al.,* 1972) and the level of toxicity might need to be considered (Oetzel *et al.,* 1988). The highest acceptance of different minerals may differ depending on the constellation of the minerals in the feed (Pherson, 1988), but it is generally said that K and S should not be fed above the level of 30 and 4.0 g/kg DM respectively, while Na and Cl, reported as NaCl, has an acceptance of 40 g/kg DM (Spörndly, 2003). This can be compared to the recommended levels of 2.2, 10, 2.6 and 2.0 g/kg DM for Na, K, Cl and S, respectively (Spörndly, 2003).

DCAD effect on feed intake

It is well known that an anionic feed can reduce the feed intake among cattle. The reduced intake may be a clinical symptom of metabolic acidosis (Hu and Murphy, 2004; Oetzel, 2002 in Gelfert *et al.*, 2006) or caused by reduced palatability of the feed (Stout *et al.*, 1972; Oetzel & Barmore, 1993; Hu & Murphy, 2004). An experiment of 29 cows, mainly crosses of Norwegian red breed and Norwegian red poll, prepartum and early postpartum, showed that the cows fed the acidifying diet had a lower feed intake than the ones fed the alkalizing diet (Ender *et al.*, 1971). Another experiment, where different anionic salts was supplemented in the concentrate, among non-lactating, pregnant cattle, showed that the intake of the concentrate-salt-mixture reduced gradually when increasing the supplementation of the anionic salts. The reduction was however not as big for magnesium sulfate ($MgSO_4$) compared to the other anionic salts in the experiment (calcium chloride ($CaC1_2$), ammonium chloride (NH_4Cl) and ammonium sulfate ($(NH_4)_2SO_4$)) (Oetzel & Barmore, 1993).

Contrary, when feeding cattle a neutral and positive DCAD:Cl in the range of 0 to 375 mEq/kg DM, the dry matter intake (DMI) increased linearly (Iwaniuk *et al.*, 2015). A positive correlation was also shown in a meta-analysis, using data from 43 different papers, with a DCAD:S ranging from -68 mEq/kg DM to 811 mEq/kg DM. A curve linear increase in DMI with increasing DCAD:S was presented, with a stagnation in the range of about 400-600 mEq/kg DM (figure 2a) (Iwaniuk & Erdman, 2015). This was not supported by a study made on Holstein-Fresian cows in early lactation, which showed that DMI decreased when DCAD:S was 520 mEq/kg DM or higher (Roche *et al.*, 2003). A reduction in DMI is also shown in Hu and Murphy’s (2004) meta-analysis of 12 different studies after a peak in dry matter intake at the DCAD:Cl of 400 mEq/kg DM (figure 2b).

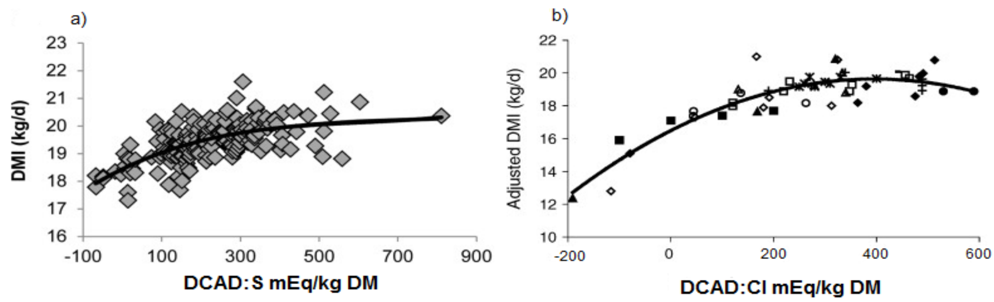

Figure 2. The x-axes of the two graphs are not comparable due to the use of DCAD:S and DCAD:Cl. **a)** Adapted from meta-analysis Iwaniuk & Erdman (2015). A curve linear increase in DMI with increasing DCAD:S is shown in the range of 0 to 500 mEq/kg DM. **b)** Adapted from meta-analysis Hu & Murphy (2004). The DMI increases quadratically with increasing DCAD:Cl with a peak at 400 mEq/kg DM. The adjustment, regarding the DMI, was made concerning the study effects in the individual 12 studies that was used for the meta-analysis.

The impact of DCAD on the milk production and milk composition

The milk yield and milk composition varies due to several physiological and environmental factors. Genetical characteristics, feeding practices, lactation number, as well as other environmental aspects, affect the milk production. The milk yield, as well as milk composition, changes throughout the lactation. After the peak in milk yield about six to eight weeks after parturition (Sjastaad *et al.*, 2010), the milk yield, as well as milk fat yield and milk protein yield reduces gradually, while the milk protein percentage and milk fat percentage increases throughout the lactation (Silvestre *et al.*, 2009). It is also shown that the DCAD has an impact on milk yield (Hu & Murphy, 2004; Iwaniuk & Erdman, 2015; Roche *et al.*, 2003) as well as milk composition (Wildman *et al.*, 2007b; Iwaniuk *et al.*, 2015; Iwaniuk & Erdman, 2015), which will be examined below.

Milk yield

The previous mentioned meta-analysis, with data from 12 different studies in the DCAD:Cl range of -193 to 636 mEq/kg DM, showed that the milk yield increased quadratically, with a peak at 340 mEq/kg DM, with increasing DCAD (figure 3b) (Hu & Murphy, 2004). However, data from another meta-analysis, indicate that the milk yield increases curve linear, rather than quadratic with increasing DCAD:S (figure 3a). The curvilinear increase of milk yield indicates a bigger impact of DCAD in the lower range of DCAD (Iwaniuk & Erdman, 2015). This is supported by Iwaniuk *et al.* (2015) that did not find any changes in milk yield when feeding the cows a DCAD:Cl in the range of approximately 300 to 875 mEq/kg DM (Iwaniuk *et al.*, 2015). The changes in milk yield are suggested to originate from improved nutrient availability, due to the earlier mentioned increased DMI (Iwaniuk & Erdman, 2015).

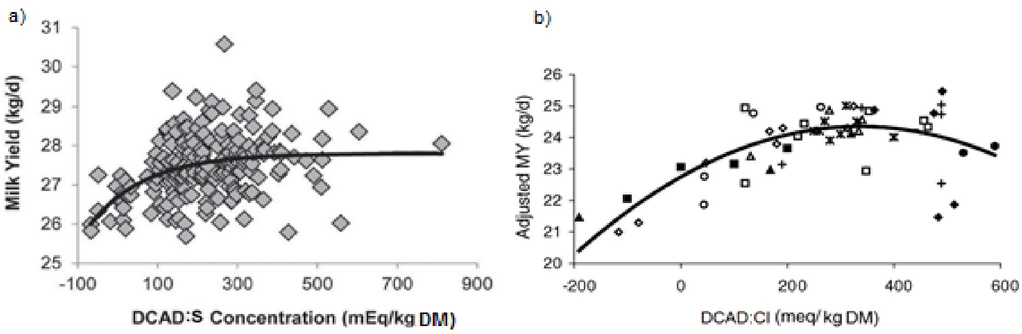

Figure 3. The x-axes of the two graphs are not comparable due to the use of DCAD:S and DCAD:Cl. **a)** Adapted from a meta-analysis by Iwaniuk & Erdman (2015). The graph shows a curve linear increase of milk yield with increasing DCAD:S. The curve stagnates at the DCAD:S level of about 300 mEq/kg DM. **b)** Adapted from a meta-analysis by Hu & Murphy (2004). The graph shows a quadratic increase of adjusted milk yield (MY) with increasing DCAD:Cl. A peak is reached at 340 mEq/kg DM. The adjustment, regarding the MY, was made concerning the study effects in the individual 12 studies that was used for the meta-analysis.

Milk protein

No difference in milk protein yield and protein percentage has been shown among 33 Holstein cows fed a DCAD:S of 200, 350 or 500 mEq/kg DM (Chan *et al.*, 2005). This is consistent to Iwaniuk and Erdmans (2015) meta-analysis with data in the DCAD:S range of -68 to 811 mEq/kg DM. However, another meta-analysis showed that the protein yield increased with increasing DCAD:Cl with a peak at 400 mEq/kg DM, followed by a decrease (Hu & Murphy, 2004). The protein percentage was unchanged and thus affected by dilution regarding the milk yield (Hu & Murphy, 2004). The unchanged protein percentage, which was shown in the above mentioned meta-analyses (Hu & Murphy, 2005; Iwaniuk & Erdman, 2015), as well as the individual experiment by Chan *et al.* (2005), is however not supported by an individual study by Wildman *et al.* (2007b) which showed an increased milk protein percentage when elevating DCAD:S from 250 to 500 mEq/kg DM.

If decreasing the DCAD by adding $(NH_4)_2SO_4$ to the feed, the concentration of κ-casein increases in the milk. The concentration of α- and β-casein is however unaffected (Martins *et al.,* 2016). It has been found that the concentration of ionised calcium (iCa), together with the altered distribution of proteins, affect the milks ability to sustain through heat treatments. The formation of coagulates in the milk was more likely to occur at 140˚C with decreasing DCAD:S (in the range of 290 to -71 mEq/kg DM). This is suggested to be due to changes in chemical properties of the micelles in the milk (Martins *et al.*, 2016; Barros *et al.* in Martins *et al.,* 2016) because of a reduced iCa and the mentioned altered concentrations of different milk proteins. Ethanol tests can also be made to analyse the stability of the milk. Milk that tolerates an ethanol mixture of 68% ethanol is said to be of good quality (FAO *et al.,* 2016). In the mentioned study, less ethanol was needed to precipitate the milk from cows fed a lower DCAD (Martins *et al.,* 2016).

Milk fat

The total amount of fat per day increases with increasing DCAD (Hu & Murphy, 2004; Iwaniuk & Erdman, 2015). Iwaniuk and Erdman (2015) describes a linear increase, while Hu and Murphy (2004) outlines a quadratic increase with a peak at 550 mEq/kg DM. Further, there is ambiguity if the milk fat percentage is affected by the DCAD-level or not. Two studies, by Chan *et al.* (2005) and the earlier mentioned meta-analysis of 12 studies, show that DCAD has no effect on milk fat percentage (Chan *et al.,* 2005; Hu & Murphy, 2004). Chan *et al.* (2005) used a DCAD:S of 200-500 mEq/kg DM, while the meta-analysis used data in the DCAD:Cl range of -191 to 636 mEg/kg DM. Contrary, an experiment of DCAD:Cl in the range of 300 to 875 mEq/kg DM, showed a positive correlation between DCAD and milk fat percentage (Iwaniuk *et al.,* 2015), and so did a recently published meta-analysis on DCAD:S with data from 43 experiments (Iwaniuk & Erdman, 2015).

Buffers has the ability to stabilize pH at (or close to) the equilibrium for different acids and bases together with their conjugates (Denniston *et al.,* 2011). Buffers has the ability to prevent the ruminal pH from being reduced by the acids that are produced by the microorganisms (McDonald *et al.,* 2011) and since a positive DCAD may be supplemented as a buffer, it will contribute to an elevated pH in the rumen (Hu & Murphy, 2004; Jenkins *et al.,* 2014). Hu and Murphy (2004) mentioned the correlation between milk fat percentage and ruminal pH as a reason for the elevated milk fat yield with increasing DCAD. The buffering effect also contributes to rearrangements of conjugated linoleic acids (CLA) in the rumen (Jenkins *et al.*, 2014) which also is suggested to contribute to the elevated fat content in the milk with increasing DCAD (Iwaniuk & Erdman, 2015). The rearrangement of CLA is supposed to affect the fat content, since the formation of some CLA, affect the lipid metabolism of the cow if reaching the duodenum. For example, CLA such as trans-10,cis-12-CLA, that increases with a reduced ruminal pH, will affect the milk fat content negatively (Maxin *et al.*, 2011; McDonald *et al.*, 2011).

The proportion of different fatty acids (FA) in the milk changes according to DCAD:S. The concentration of 16:0 fatty acid increases linearly with increasing DCAD:S in the range of 230-880 mEq/kg DM, while the concentration of fatty acids of 17:0 to 20:0 decreases (Roche *et al.,* 2005). The reason for the changed distribution of FA was supposed to be due to the enhanced DMI, which nevertheless did not increase significantly in the current experiment (Roche *et al.,* 2005).

Effect of the Na:K ratio

The Na:K ration can be modified at a constant or variable level of DCAD (Wildman *et al.,* 2007a; Iwaniuk *et al.,* 2015). No effect on milk proteins has been shown when altering the Na:K ratio (Wildman *et al.,* 2007a), but according to an experiment by Sanchez *et al.* (1994), it has an effect on DMI.

An elevated Na:K ratio is shown to increase the fat percentage and also the fat corrected milk (FCM) at a constant supplemental DCAD:Cl of 150 mEq/kg DM (Iwaniuk *et al.,* 2015). The supplemented Na:K ratio of 100:0 resulted in highest milk fat percentage and fat yield, with a total of 1.250 g of fat in the milk per day and 3.36 % fat compared to the reversed ratio of 0:100 that had 3.06 % fat and 1.132 g of fat per day in the milk (Iwaniuk *et al.,* 2015). Another experiment on 42 Holstein cows, showed no differences in milk fat percentage and milk fat yield depending on the Na:K ratio at the DCAD:Cl of 410 and 580 mEq/kg of DM, respectively. Besides, when observing the average of the whole set of experimental data, a curvilinear change was seen regarding milk yield and energy corrected milk. Both ECM and milk yield decreased significantly when decreasing the Na:K from 1:2 to 1:3, followed by an increase at a ratio of 1:4 (Wildman *et al.,* 2007a). A similar relationship was also observed on DMI when comparing the performance of 48 Holstein cows in mid lactation. A high Na:K ratio, as well as a low Na:K ratio, had the most positive effect on the DMI (Sanchez *et al.* 1994). Further, an increased Na:K ratio, is shown to elevate the amount of fat corrected milk per kg dry matter intake (FCM/DMI) in a linear manner (Iwaniuk *et al.,* 2015). Contrary, Hu and Kung (2009) could not find any changes in milk composition at different Na:K ratios at a DCAD:S of 330 mEq/kg DM.

DCAD and feed efficiency

It is suggested that an increased DCAD has positive effects on the microbe population in the rumen when increasing the DCAD by adding a cationic buffer (Wildman *et al.,* 2007b; Apper-Bossard *et al.,* 2010). At certain circumstances, the buffering effect enhances the protein utilization in the feed (Wildman *et al.,* 2007b), but Martins *et al.* (2015), further suggests that the macro minerals themselves may affect the ruminal environment.

When elevating the DCAD:S from 220 mEq/kg DM to 470 mEq/kg DM, by supplementing a cationic buffer, the amount of proteins reaching the duodenum increases (Hu *et al.,* 2007). Another experiment made on eight Holstein cows, fed two different levels of crude protein (CP), above and under the nutritional requirements, indicated an enhanced protein utilization when elevating the DCAD:Cl for the low CP diet. The protein percentage increased significantly when increasing the DCAD:Cl from 250 mEq/kg DM to 500 mEq/kg DM among cows fed the low CP diet. This was not the case in the high CP diet, where no difference in CP utilization and milk protein percentage was shown. These findings are suggested to be due to the improved ruminal environment. This may enable a reduction in protein requirements of dairy cattle, and thereby reduce the losses of nitrogen in the manure (Wildman *et al.*, 2007b).

It is further shown that the digestibility of neutral detergent fibre (NDF) increased with 6.38% when feeding dairy cattle an elevated DCAD:S in the range of -71 to 290 mEq/kg DM. The elevated DCAD was achieved by adding the buffer $NaHCO_3$. The enhanced NDF digestibility may be due the elevated pH in the rumen, which enhances the activity of cellulolytic bacteria (Martins *et al.,* 2015).

DCAD in the feed ration

The requirements of Na, K, Cl and S can all be found in the Swedish feed table for ruminants (Spörndly, 2003). The recommendations, for cows that are producing more than 30 kg of milk per day, are 2.2, 10, 2.6 and 2.0 g/kg DM of Na, K, Cl and S, respectively (Spörndly, 2003). These recommendations can be used to calculate DCAD:S, which in this case is 247 mEq/kg DM, while DCAD:Cl is 278 mEq/kg DM. However, Chan *et al.* (2005) emphasizes that the ideal level of DCAD among lactating dairy cattle is not outlined, and that the optimal level might vary depending on weather conditions.

A compilation of ten different studies at the University of Florida, suggests that that the optimal level of DCAD:Cl in the feed for dairy cows is between 250 to 500 mEq/kg of DM (Sanchez & Beede, 1996). Their suggestion is based on the fact that the milk yield, DMI, as well as FCM had a peak at 380 mEq/kg DM. Negative changes was however only seen below 250 and above 500 mEq/kg DM, which therefore was suggested to be the optimal DCAD:Cl range (Sanchez and Beede, 1996). This is supported by the fact that Roche *et al.* (2005) suggested a DCAD:S above 200 mEq/kg of DM to dairy cows on pasture. The suggestion was based on a positive, but not significant, trend in DMI as well as milk yield with increasing DCAD:S in the range of 230 to 880 mEq/kg DM (Roche *et al.*, 2005). Whether these suggestions are relevant or not, considering an optimal DCAD, will be examined in the upcoming discussion.

Discussion

The dietary cation anion difference is a wide subject with a lot of physiological effects on the dairy cow, independently of the stage of lactation or pregnancy (Oetzel *et al.,* 1991; Martins *et al.,* 2016). It is however questioned if these bodily effects are important to think about when considering milk production, milk composition as well as feed efficiency. The literature review clearly states that an increased DCAD, to a certain level, has positive effects on DMI, as well as milk yield and milk fat yield. These are some of the aspects that will be discussed below.

Considering the milk yield, the meta-analysis of Hu and Murphy (2004) shows a peak at a DCAD:Cl of 340 mEq/kg DM, followed by a decrease. This decrease was not shown in a later published meta-analysis, by Iwaniuk *et al.* (2015) that rather showed a curvelinear increase, with a stagnation at the DCAD:S of about 300 mEg/kg DM. Whether there is an distinct optimum, followed by a decrease, or if the progress of the curve should be labelled as a stagnation, is difficult to answer due to the fact that there was less data in the higher range of DCAD in the Iwaniuk and Erdman (2015) meta-analysis. There are some wonderings if the stagnated curves for milk yield, as well as DMI (figure 2a; figure 3a) would look different if the data from the highest DCAD-level would be removed. More research on dairy cattle fed different levels of DCAD above 500 mEq/kg DM is probably needed to give a clearer picture of the effects of DCAD in the higher range.

It is shown that the milk fat yield increases with increasing DCAD. Iwaniuk and Erdman (2015) showed a linear increase, with no observed stagnation. This would underline the benefit of examining the impact of DCAD in the higher range, since a higher milk fat yield might be desired by the producers for economical reasons. However, there is ambiguity considering the milk fat percentage (Chan *et al.,* 2005; Hu & Murphy, 2004; Iwaniuk *et al.,* 2015, Iwaniuk & Erdman, 2015). The reason for the different statistical outcomes is quite unclear, but one can discuss if the difference in using DCAD:Cl or DCAD:S has an impact. Additionally, it was stated that S rarely is supplemented as a mineral source (Hu *et al.,* 2006), but it has to be kept in mind that S is present in the original feed ration (Cecilia Kronqvist, Swedish University of Agricultural Sciences, 2016-04-11), which might have an impact on the credibility, as well as ability, to compare the studies using the different calculations.

The fact that not just individual experiments, but also the two earlier discussed meta-analyses showed the different alterations of DCAD and fat percentage (as well as milk yield and DMI) (Chan *et al.,* 2005; Hu & Murphy, 2007; Iwaniuk & Erdman 2015; Iwaniuk *et al.,* 2015), makes is difficult to discuss whether the reason for the increased, respectively unaffected, fat percentage is due to the approach of elevating the DCAD or if there is something else that contributes to the different statistical outcomes. The choice of cationic (or anionic) additives, as well as the basal feed ration, may affect the results of the different analyses and experiments. One can further suggest that the amount of data, as well as the choice of DCAD-interval, will affect the outcome of different studies, when it comes to all measured parameters. Further, it has to be kept in mind that the milk yield has a direct impact on the milk fat percentage through dilution. It is thus several aspects that need to be considered when discussing the percentage of fat.

There is also ambiguities regarding milk protein yield, as well as milk protein percentage. The different experimental outcomes can be deliberated in the same way as the above discussed milk fat percentage. However, the milk protein percentage was shown to mainly be affected by the milk yield by Hu & Murphy (2004), which may be the general case, since the protein yield, as well as percentage has to be compared to the milk yield, which may vary depending on DCAD-interval. This contributes to the suggestion that the eventual increased protein yield is a limited capacity, independently if the increase is obtainable or not.

Different experiments show that the Na:K ration has an impact on DMI, milk yield, ECM, milk fat percentage and milk fat yield among dairy cattle (Sanchez *et al.*, 1994; Wildman *et al.*, 2007a; Iwaniuk *et al.*, 2015). An unevenly distributed Na:K ratio seem to give the best performance. Further, it seems like an elevated Na:K ratio is preferred before a heavily reduced Na:K ratio, due to the fact that an increased amount of Na in the feed, gives a higher milk fat yield, as well as milk fat percentage, than a low Na:K ratio (Iwaniuk *et al.*, 2015). Contrary these findings, the experiment made by Hu and Kung (2009) showed no effect of the Na:K ratio at a DCAD of 350 mEq/kg DM. This may indicate that the Na:K ratio affect the performance of the dairy cow in a certain interval of DCAD, or might just affect the animal at a specific DCAD-interval regarding the amount of cationic macro minerals, since a ratio does not identify the quantity of the included parameters.

The overall positive impact of the DCAD on the productivity among dairy cattle is probably due to the increased DMI, as well as the buffering effect in the rumen when using buffering cationic additives (Ender *et al.*, 1971; Wildman *et al.*, 2007b; Iwaniuk & Erdman, 2015). The improved DMI is, for sure, partly due to the absence of metabolical acidosis (Hu & Murphy, 2004; Gelfert *et al.*, 2006) but there are probably more reasons contributing this, such as the improved ruminal environment and raised palatability of the feed (especially when reducing the anionic additives in the lower range of DCAD) (Ender *et al.*, 1971). However, the buffering effect of the rumen is thought to be independent of the macro minerals in the DCAD calculation since the buffering effect is suggested to originate from bicarbonate/carbonate part in cationic buffer and not the K^+ or Na^+. This indirect buffering effect, may contribute to misunderstandings, since the buffering effect are described in the variation of DCAD in several papers. It is considered being more correct to focus on the direct, rather than the indirect causer, which in this case is thought to be the bicarbonate/carbonate and not the macro minerals that are included in the DCAD-calculation. On the other hand, it is suggested to be relevant to highlight the buffering effect considering DCAD, since it, together with the ion source, has an impact on the cow and its productivity. It is however suggested that the cation source, supplemented as a buffer, complicates the analysis of DCAD. It is difficult to clarify whether the positive effects, by a DCAD elevated by buffers, is due to the DCAD itself, and/or the bicarbonate/carbonate. This need to be further investigated.

When it comes to feed efficiency, most of the described benefits considering an increased DCAD seem to originate from the above discussed indirect rumen buffering effects of the supplemented cation source (regarding increased CP utilization in low CP diets and NDF degradability). This is suggested since buffers were supplemented in both of the above mentioned experiments, and the fact that ruminal pH was discussed regarding the NDF degradability (Wildman *et al.*, 2007b; Martins *et al.*, 2015). The feed efficiency will, due to these aspects, not be included in the soon discussed optimal DCAD-level.

Suggestions of optimal DCAD levels has been given according to the cows enhanced DMI, milk yield, as well as impact of the milk components (Sanchez & Beede, 1996; Roche *et al.*, 2005). It seems like the final optimal DCAD agrees quite well with the earlier stated suggestion by Sanchez and Beede (1996) with an optimal DCAD:Cl of 250-500 mEq/kg DM. This is agreed since the milk yield, according to Hu and Murphy (2004) has a peak at the DCAD:Cl of 340 mEq/kg DM. Additionally, figure 3a, adapted from Iwaniuk and Erdman (2015), indicates a stagnation in milk yield at a DCAD:S about 300 mEq/kg DM, which fit in the above mentioned DCAD:Cl-interval (even though DCAD:S and DCAD:Cl are two different calculations). According to figure 2a, the DMI is shown to level out in the DCAD:S range of 400-600 mEq/kg DM, but the positive change does not seem to be remarkably greater above 275 mEq/kg DM (Iwaniuk & Erdman, 2015). Figure 2b, shows a peak in DMI at a DCAD:Cl of about 400 mEq/kg DM (Hu & Murphy, 2007), which also contributes to the suggested optimal DCAD:Cl interval. The suggested optimal DCAD interval for this literature review is therefore agreed with Sanchez and Beede (1996), but is a combination of the different optimums for both DCAD:Cl and DCAD:S. The combination of both DCAD:Cl and DCAD:S is possible since all of the optimums or stagnations for different traits fit in the DCAD interval of 250-500 mEq/kg DM. Maybe a smaller interval of 250-400 mEq/kg DM can be used to avoid the eventual reduction in production in the higher range of DCAD.

However, if the DMI, as well as milk yield and milk composition is not negatively affected by a further increased DCAD, the fat content may be further increased by elevating the DCAD-level even more (Hu & Murphy, 2004; Iwaniuk & Erdman, 2015). The elevated fat content may partly originate from the above discussed buffering effect (Iwaniuk & Erdman, 2015), but it is however discussable whether the eventual positive change in milk fat percentage is economically advantageously, since the eventual positive changes might not be big enough to give a valuable positive economic impact since the cationic source itself has to be paid for.

A lot of aspects can be discussed regarding the DCAD's positive effect on DMI, milk yield and milk composition. However, it is of greater importance to put the optimal DCAD into practice. DCAD is affected by the basic feed ration and was reported to be 250 (Iwaniuk *et al.*, 2015) or 300 mEq/kg DM for DCAD:S and 324 mEq/kg DM for DCAD:Cl for two basal feed rations. By observing these different DCADs, and including the DCAD:S and DCAD:Cl of the recommendations of mineral amounts (247 mEq/kg DM and 278 mEq/kg DM, respectively) (Spörndly, 2003), it appears like the recommendations and basal feed rations matches quite well with the above discussed optimal DCAD interval of 250-500 mEq/kg DM, or the narrower suggested range of 250-400 mEq/kg DM. This consensus would implicate that importance of adding cationic sources to increase the DCAD is unnecessary among dairy cattle that are housed under good environmental conditions and fed according to the current feeding recommendations. More research at DCAD-levels above 500 mEq/kg DM, as well as research that can distinguish between the ruminal effects of bicarbonate/carbonate and K^+ or Na^+ in DCAD-elevating buffers, is however requested.

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