Effects of diet, feeding and daily variation on acid–base balance in horses

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Abstract
This paper reviews the acute and chronic effects of feeding, diet composition and daily variation on equine acid–base status. The dietary cation–anion difference (DCAD) has a marked effect on blood acid–base balance in both the short term and long term. In general, diets with a low DCAD generate an acute acidosis that develops into a persistent though mild systemic, strong ion acidosis with long-term feeding. In contrast, high-DCAD diets result in a mild, persistent strong ion alkalosis characterized by elevated plasma pH, $\frac{\text{HCO}_3^-}{\text{Cl}^-}$ and total CO$_2$ (TCO$_2$). The acute blood acid–base responses to feeding include alterations in plasma pH and plasma concentrations of $\text{HCO}_3^-$, TCO$_2$, $\text{Cl}^-$, Na$^+$, K$^+$ and protein. The forage component of the diet appears to be primarily responsible for the acute responses. There is limited research examining abrupt alterations in DCAD and the influence of daily variation on equine plasma acid–base balance. It is concluded that the composition of the diet, duration of adaptation to the diet, timing of feeding and time of day need to be considered when examining acid–base balance in horses.

Keywords: review; strong ion difference; nutrition; dietary cation–anion difference; dietary cation–anion balance

Introduction
The regulation of blood acid–base balance is affected by a complex interplay among: (1) intestinal absorption of nutrients; (2) gas, metabolite and ion transfer processes between cells and the extracellular fluid compartment; (3) renal excretion of water, electrolytes and metabolic end-products; (4) respiratory elimination of CO$_2$; (5) losses of water and electrolytes via sweat; and (6) hydration status. While acid–base status has traditionally been defined in terms of plasma partial pressure of CO$_2$ (PCO$_2$), $\text{HCO}_3^-$ and pH$^1$, it is increasingly recognized that this definition does not allow for the determination of specific factors that generate or contribute to acid–base disturbances$^{2-4}$. The physicochemical approach to acid–base chemistry described by Stewart$^{5,6}$ recognizes that plasma pH, $\text{HCO}_3^-$ and total CO$_2$ (TCO$_2$) are dependent on the concentrations of strong and weak ions in solution, as well as on PCO$_2$$^{2-4}$. Because of these relationships, it has been recognized that the dietary balance of ionic minerals, or electrolytes, has direct effects on plasma acid–base balance in both the short and long term.

The purpose of this review is to summarize the equine literature with respect to the effects of diet, feeding and daily variation on blood acid–base balance in the horse at rest, with a minor emphasis on the impact of diet on acid–base responses during exercise. We start with a discussion of the dietary cation–anion difference (DCAD), followed by the acute effects of feeding on acid–base balance and the chronic effects of feeding different types of diet, and then summarize what is known about the influence of daily variation on plasma acid–base balance. In general, these effects have been studied for a greater period of time in domestic food animals (cattle, swine and sheep) and the responses in these animals are better understood$^{7–11}$. Thus this review also considers the non-equine literature, with the aims of providing a basis for the interpretation of results obtained in equids and to suggest avenues for further research.

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**Physicochemical approach to acid–base chemistry**

The physicochemical approach allows for a complete analysis of how independent acid–base variables affect the dependent variables, and thereby increases our ability to understand the origins of acid–base disturbances and how these are corrected. The independent acid–base variables defined by Stewart\(^5,6\) include the strong ion difference ([SID]), the concentration of weak ions ([ATOT]) and PCO\(_2\) (see Lindinger\(^4\) for review). The main component of plasma [ATOT] is the anionic proton-binding charge of albumin and globulin\(^12\), while inorganic phosphorus is a minor component\(^13\). Strong ions are those that are nearly completely dissociated in aqueous solutions at physiological pH in the range 6.0–7.6. Plasma [SID] is calculated as the sum of the concentrations of the primary strong basic cations ([Na\(^+\)] + [K\(^+\)]) minus the sum of the concentrations of the strong acidic anions ([Cl\(^–\)]).

With exercise, the organic acid lactate, a strong acidic anion with a pK\(_a\) of 3.86, also needs to be included with [Cl\(^–\)]. Other strong ions such as Ca\(^{2+}\), Mg\(^{2+}\) and SO\(_4^{2–}\) are low in concentration and essentially neutralize one another. They can therefore be neglected in most situations. Increases in [SID] contribute to alkalinization, while decreases in [SID] (i.e. increased [Cl\(^–\)] or [lactate\(^–\)]) contribute to acidification (Table 1).

Plasma [ATOT] comprises the anionic charge carried by all weak ions in plasma, including albumin, inorganic phosphate (Pi) and some amino acids. In most studies, plasma [ATOT] is calculated as a function of total plasma protein concentration ([PP]), with the assumption that [Pi] does not change appreciably. This assumption may not hold in feeding and exercise studies and increased accuracy of plasma [ATOT] determinations should consider [Pi]\(^15\). Because the pK\(_a\) of most weak acids is on the acid side of neutral, increases in [ATOT] contribute to acidification of the plasma compartment (Table 1).

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**Table 1** How changes in independent acid–base variables in plasma contribute to changes in some dependent acid–base variables

<table>
<thead>
<tr>
<th>Change in independent variable</th>
<th>Associated change in dependent variable</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Contributes to plasma alkalinization</strong></td>
<td></td>
</tr>
<tr>
<td>Increase in [SID]</td>
<td>Increase in pH</td>
</tr>
<tr>
<td>Decrease in [ATOT]</td>
<td>Increase in [HCO(_3^–)]</td>
</tr>
<tr>
<td>Decrease in PCO(_2)</td>
<td>Increase in TCO(_2)</td>
</tr>
</tbody>
</table>

| **Contributes to plasma acidification** | |
| Increase in [SID] | Decrease in pH |
| Decrease in [ATOT] | Decrease in [HCO\(_3^–\)] |
| Increase in PCO\(_2\) | Decrease in TCO\(_2\) |

[SID] = strong ion difference; [ATOT] = concentration of weak ions; PCO\(_2\) = partial pressure of CO\(_2\); TCO\(_2\) = total CO\(_2\).

Carbon dioxide is a strong acid and increases in plasma PCO\(_2\) therefore contribute to acidification, while decreases in PCO\(_2\) contribute to alkalinization of the plasma compartment (Table 1). From amongst the independent acid–base variables, changes in PCO\(_2\) tend to be the most rapid due to both production of CO\(_2\) by the tissues and elimination of CO\(_2\) by the lungs.

The physicochemical approach to acid–base balance recognizes that the concentrations of the dependent variables are determined by the concentrations of the independent variables. However, it must simultaneously be recognized that the independent variables are affected by endogenous and extrinsic influences. These include feeding or oral/intravenous administration of electrolyte solutions, environmental influences, exercise or other stressor conditions, and disease (for reviews see references 3, 4 and 14–16).

**Dietary cation–anion difference**

The effects of forage, grains or concentrates on blood acid–base balance depend on the amount and types of carbohydrate, protein and fat, as well as on the DCAD. The effects of organic nutrients have been the topic of recent reviews\(^14–17\) and are not dealt with in detail in the present review. The major cations and anions in the diet directly affect plasma acid–base balance through effects on plasma [SID] (Fig. 1). These effects on plasma [SID] depend on the rates and efficiency of intestinal absorption of ions, the transport of ions into cells and the excretion of ions by the kidneys and sweat glands. With respect to intestinal absorption, Kronfeld\(^14\) assumed 100% efficiency for monovalent ions and 50% efficiency for divalent ions. While monovalent ion absorption within the small intestine ranges from 50 to 70%\(^18\), there appears to be nearly complete absorption as digesta transit continues through the gastrointestinal (GI) tract\(^19\). Coenen and Vervuert\(^20\) report that 100%, 77% and 96% of dietary Na\(^+\), K\(^+\) and Cl\(^–\) are absorbed during transit of digesta through the GI tract, with 90%, 80% and 100% of the absorbed Na\(^+\), K\(^+\) and Cl\(^–\), respectively, utilized within the body. In cattle, dietary calcium availability ranges from 35% for some forages, to 43% for grains and 51% for mineral supplements, with sulphur availability somewhat lower than for calcium\(^21\). In swine, about 65% of dietary calcium is bioavailable\(^22\).

The strong cations and anions, together with the weak acid inorganic phosphate, comprise the DCAD, which has units of meq kg\(^–1\) dry matter (DM)\(^14\). Hence, Kronfeld and co-workers\(^14,23\) defined DCAD = (Ca\(^{2+}\) + Mg\(^{2+}\) + Na\(^+\) + K\(^+\)) – (Cl\(^–\) + SO\(_4^{2–}\) + P\(^–\)). However, most studies do not include inorganic phosphate (P\(^–\)) because it is not a strong ion and hence, by definition, does not belong in the equation.
Feeding and daily variation on acid–base balance

Accordingly, and consistent with the concept of [SID], the DCAD determines the dietary acid, or H^+, load; the dietary ‘load’ of H^+ is directly and inversely proportional to the DCAD\(^{14,16}\). The DCAD (also often termed dietary cation–anion balance, DCAB, or the dietary electrolyte balance, DEB) of feeds is often defined as \((Na^+ + K^+) - Cl^-\). This definition accounts for only the monovalent ions since they are the most prevalent ions within forage and concentrates and appear to have the greatest metabolic impact. Others include the strong divalent anion sulphate (SO\(_4^{2-}\)) in the equation so that DCAD = \((Na^+ + K^+) - (Cl^- + SO_4^{2-})\). The basis for the computation of DCAD is provided in the Appendix.

The lack of a standardized definition for DCAD that considers the bioavailability of the ingested ions has resulted in flawed experimental designs and interpretation of data within the literature on horses, cattle, swine, and sheep. In particular, studies that included SO\(_4^{2-}\), but not the divalent cations, in experimental DCADs have an erroneously low DCAD, leading to the interpretation that low-DCAD diets are not very acidogenic. Indeed, Tucker et al.\(^{24}\) and Baker et al.\(^{25}\) have suggested that if sulphur is to be included in the DCAD equation, it needs a modifying coefficient.

Some coefficients have been provided for cattle that ‘correct’ for intestinal absorption rates of the divalent ions, but these coefficients vary with the type of feed and the form of the electrolyte within the feed\(^{16}\). There is a need for research in horses that better addresses the dietary bioavailability of strong inorganic ions, so that a more accurate, standardized DCAD may be applied to the feeding of horses. This will lead to improved interpretation of the effects of DCAD on plasma acid–base status, performance, health status and growth. In the meantime, there is justification for use of the simplified formula \((Na + K - Cl)\) since phosphate is a weak acid of low concentration (and thus should not be included in the equation) and has a minimal effect on blood acid–base balance, and feed contents of Ca\(^{2+}\), Mg\(^{2+}\) and SO\(_4^{2-}\) generally neutralize each other and are less readily absorbed from the upper GI tract. The formulae used for calculating the DCAD of diets by studies cited in this review are provided in Table 2.

Both the type of carbohydrate in the diet and the DCAD may independently affect post-feeding and chronic acid–base balance. Forages are lower in carbohydrate but have a high DCAD (primarily due to higher K\(^+\) content with negative charge balance provided by weak acids) while grains tends to be high in carbohydrates but have low DCAD due to a high content of Cl\(^-\) required to balance strong cations. The DCAD (Na + K – Cl) of various grains has been reported as ~58 meq kg\(^{-1}\) DM for corn and ~73 meq kg\(^{-1}\) DM for oats, while alfalfa and Bermuda grass hay have DCAD of ~329 and ~427 meq kg\(^{-1}\) DM, respectively\(^{21}\). The DCAD of grain concentrates can be manipulated at the mill to produce a feed with any desired DCAD. Additionally, most feeds are eaten dry, resulting in transient shifts of water and ions from the extracellular fluid compartment, i.e. plasma and interstitial fluids, into the upper GI tract\(^{24,25}\). This fluid shift results in increased plasma [protein] and decreased plasma [SID] elicited through changes in plasma [Na\(^+\)], [Cl\(^-\)] and [K\(^+\)] (Fig. 2). From changes in measured plasma strong ion concentrations, it appears that this fluid contains K\(^+\) (decrease in plasma [K\(^+\)]) and Na\(^+\) (no appreciable change in plasma [Na\(^+\)]) and Cl\(^-\) (but proportionately less Cl\(^-\) since plasma [Cl\(^-\)] increases). It is known from other studies that these immediate post-feeding changes are associated with plasma acidification (see below) and that this is due primarily to the

**Table 2** Dietary cation–anion difference (DCAD) used by various equine researchers

<table>
<thead>
<tr>
<th>Formula for calculating DCAD</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+) + K(^+) – Cl(^-)</td>
<td>Baker et al.(^{26}), Ralston et al.(^{28}), Ralston(^{29}), Stutz et al.(^{30}), Wall et al.(^{31})</td>
</tr>
<tr>
<td>Na(^+) + K(^+) – Cl(^-) – SO(_4^{2-})</td>
<td>Baker et al.(^{26}), Ralston et al.(^{28}), Ralston(^{29}), Stutz et al.(^{30}), Wall et al.(^{31})</td>
</tr>
<tr>
<td>Na(^+) + K(^+) + Ca(^{2+}) + Mg(^{2+}) – Cl(^-) – SO(_4^{2-}) – HPO(_4^{2-})</td>
<td>Graham-Thiers et al.(^{23})</td>
</tr>
</tbody>
</table>
Manipulation of DCAD

The DCAD of feeds is easily manipulated by adding weak salts of strong basic cations (Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\)) and strong acidic anions (Cl\(^-\), lactate\(^-\), SO\(_4^{2-}\)). Lactate, although a strong acid, is sometimes included in feed to enhance palatability\(^{36}\). The DCAD reported in some studies that used SO\(_4^{2-}\) from MgSO\(_4\) to ‘lower’ DCAD\(^{25}\)

is probably a misrepresentation because the strong basic cation Mg\(^{2+}\) negates the SO\(_4^{2-}\) and both of these ions are absorbed similarly by the small intestine\(^{18,37}\).

Baker et al.\(^{25}\) compared the alkalogenic power of Na\(^+\) and K\(^+\) (strong basic cations) and the acidogenic power of SO\(_4^{2-}\) and Cl\(^-\) (strong acidic anions). DCAD was calculated as

\[ (\text{Na} + \text{K}) - (\text{Cl} + \text{S}) \text{ meq kg}^{-1} \text{ DM} \]

The high-DCAD diets ranged between 360 and 409 meq kg\(^{-1}\) DM. The dietary quantity of Mg was similar in all treatments (0.12–0.16% DM). Horses were fed the diets for a 12-day period, followed by a 3-day sample collection period. Horses consuming low-DCAD diets (0–53 meq kg\(^{-1}\) DM) had lower plasma pH and [HCO\(_3^-\)] than those consuming the high-DCAD diets, and there was a tendency for lowered [HCO\(_3^-\)] when

Increase in plasma [A\(_{TOT}\)] and secondarily to the decrease in measured [SID] (Fig. 3).
SO\textsubscript{2}\textsuperscript{2-} was substituted for Cl\textsuperscript{-} (Fig. 4). It was concluded that dietary Na\textsuperscript{+} and K\textsuperscript{+} were similarly alkalogenic and that SO\textsubscript{2}\textsuperscript{2-} has less acidogenic power than Cl\textsuperscript{-}. Above a certain DCAD, there appears to be little effect of a 12- to 15-day feeding regimen on plasma acid–base balance (Fig. 4); however, there is a need for further studies with longer time courses. In general, these results support the theory that plasma [H\textsuperscript{+}] and [HCO\textsubscript{3}\textsuperscript{-}] are dependent upon the DCAD of the feed.

**Acute responses to feeding**

This section separately considers the effects of forage-only diets, grain/concentrate-only diets and mixed forage and concentrate diets, and then addresses the effects of high-protein and high-fat diets. In the majority of studies, the acute responses to feeding are for horses that have been adapted to a particular diet for a minimum of 2 weeks. The acute responses to abrupt changes in diet, and particularly DCAD, do not appear to have been systematically studied in horses. Insight will be provided from studies performed on cattle (ruminant) and swine (non-ruminant).

**Forage-only**

Little is known regarding the acute and chronic effects of forage-only diets on plasma constituents. When 5.5 kg of hay was fed (requiring 2–3.4 h to consume), the first hour of feeding saw increases in packed cell volume (PCV) and [PP] while plasma [K\textsuperscript{+}] decreased\textsuperscript{54}. This was followed by returns towards baseline during the next two hours, while plasma [Na\textsuperscript{+}] and [Cl\textsuperscript{-}] increased slightly. While plasma acid–base status was not measured, the increase in [PP], and hence [ATOT]), would contribute to acidification (Figs 2 and 3). However, Ralston et al.\textsuperscript{29} reported that the feeding of hay alone had a minimal effect on plasma pH (increased from 7.40 to 7.42) during the first hour after feeding. The subsequent time course of response remains to be quantified, as do the effects of different forage sources on acid–base responses. When comparing alfalfa and non-alfalfa forage in a mixed ration, Southwood et al.\textsuperscript{38} found no effect of forage source on plasma acid–base state.

In general, as horses consume forage, there is an initial movement of water and K\textsuperscript{+} from the plasma compartment (probably into the GI tract due to the high osmolality of the dry material) sufficient to increase PCV, [PP] and decrease plasma [K\textsuperscript{+}]. Because equine saliva has a high [K\textsuperscript{+}] (12–18 meqL\textsuperscript{-1}) and low [Na\textsuperscript{+}] (21–29 meqL\textsuperscript{-1}) and [Cl\textsuperscript{-}] (18–26 meqL\textsuperscript{-1}) compared with plasma\textsuperscript{59}, it has been suggested that salivary and pancreatic secretions may have contributed to the alterations in blood composition\textsuperscript{33,35}. However, it remains unknown for the horse if these secretions can fully account for the magnitude of change in [PP] and [K\textsuperscript{+}]. The bidirectional movement of water and ions into and out of the upper GI tract is probably responsible for at least a significant portion of the observed changes.

**Grain/concentrate-only**

In the second part of their study, Kerr and Snow\textsuperscript{34} fed horses 1.8 kg of concentrate at 09:15 and 12:15, and at 16:30 a mixed ration of 2.7 kg of concentrate plus 5.5 kg of hay was given. At 15:00, there was a significant increase in both PCV and [PP] in response to the second feeding which was absent after the first feeding. The increase in [PP] (and hence [ATOT]) is consistent with a mild plasma acidification (pH 7.41–7.39) reported by Ralston\textsuperscript{29} during the first hour after feeding a single meal of sweet feed. To further examine the potential for high-carbohydrate diets to be acidogenic, glucose, sucrose, fructose or molasses were mixed into a basal ration of 1.7 kg of rolled barley. All combinations resulted in small reductions in plasma pH; the sucrose and fructose combinations decreased pH significantly during the first hour of feeding, but the molasses and glucose combination did not reduce pH until 4–6 h later. Unfortunately, a barley-only control does not appear to have been performed, thus making interpretation of these results difficult. Ralston\textsuperscript{29} suggested that the source of acidity during the first hour of feeding is from volatile fatty acids (VFAs) produced by bacteria within the small intestine. While there is some fermentation in the small intestine when feed is highly fermentable\textsuperscript{40}, the nearly complete and rapid absorption of these carbohydrates in this region of the gut minimizes this site as a VFA source\textsuperscript{21}. It is more likely that the rapid acid–base responses are associated with fluid shifts and differential rates of carbohydrate and electrolyte absorption by the upper GI tract, and the effects of these on tissue metabolite and ion transport rates. The contributions of VFAs to the longer-term (4–6 h post-feeding) pH decrease may be estimated from increases in the strong ion ‘gap’ ([SIG])\textsuperscript{42} pre- and post-feeding, where plasma [Na\textsuperscript{+}] + [K\textsuperscript{+}] – [Cl\textsuperscript{-}] – [HCO\textsubscript{3}\textsuperscript{-}] – [SIG] = 0. Stewart\textsuperscript{5} refers to [SIG] as ‘unmeasured strong anions’.

Additional insight is provided from studies on swine and cattle. Budde and Crenshaw\textsuperscript{11} fed growing swine an acidogenic, a control or an alkaligenic diet (DCAD = Na\textsuperscript{+} + K\textsuperscript{+} – Cl\textsuperscript{-}; –35, 112 and 212 meqkg\textsuperscript{-1} DM, respectively) for 13–17 days. There was no difference amongst diets with respect to feed intake and growth performance, suggesting that growing swine have the ability to compensate for dietary loads of acids or bases. After 2 weeks on the diets, there was a linear relationship between DCAD and plasma acid–base variables. The acidogenic
diet was associated with decreased pH, PCO₂, [HCO₃⁻] and base excess, with elevated [Cl⁻] and [Ca²⁺]. The elevated plasma [Ca²⁺] with the acidogenic diet appeared to be a result of an increased dietary calcium load with this treatment, compared with the other two, and there was no evidence of impaired bone growth and strength in this group¹¹. Similar conclusions were drawn for dairy calves when DCAD (Na⁺ + K⁺ − Cl⁻ − SO₄²⁻) of 0 and 200 meq kg⁻¹ DM were compared¹⁰, but this result may be specific to the inclusion of SO₂⁻ in the calculation of DCAD.

In contrast to the conclusions of short-term studies, longer-term studies (28–84 days) showed beneficial effects of increased DCAD up to about 250 meq kg⁻¹ DM on feed intake, growth and nutrient digestibility in growing swine¹⁵, growing steers⁴⁴, finishing steers⁴⁵ and growing dairy calves⁴⁶,⁴⁷. These studies also showed a similar plasma acid–base profile to that described by Budde and Crenshaw¹¹. DCADs greater than 250 meq kg⁻¹ DM were associated with reductions in feed intake and weight gain compared with diets with DCAD of ~250 meq kg⁻¹ DM¹³,⁴⁴,⁴⁷.

The results obtained from research on prepartum and lactating dairy cows show a requirement for diets with reduced DCAD (near 0 meq kg⁻¹ DM) to prevent milk fever and excessive reductions in feed intake³⁸,⁸,²⁴. It remains unknown whether similarly beneficial effects of lowered DCAD occur in other lactating ruminants, or in horses.

**Forage with grain or concentrate**

In their study, Kerr and Snow³⁴ also fed horses a mixed ration at 16:30 that consisted of 2.7 kg of concentrate plus 5.5 kg of hay; this was after the earlier feedings of 1.8 kg of concentrate at 09:15 and 12:15. In response to this third feeding there occurred an increased [PP] and PCV while plasma [K⁺] decreased. These responses were similar to those seen when horses were fed only hay, while the feeding of concentrate appeared to attenuate these responses. Some of these differences may be caused by varying DCAD or due to inherent daily influences. Also using a mixed ration, Ralston *et al.*²⁸ controlled for DCAD (>200 meq kg⁻¹ DM), protein and caloric content in the provision of two meals, one consisting of 60% grain and 40% forage, and the other 10% grain and 90% forage. Within 2 h of being fed the high-grain meal, horses exhibited a lower plasma pH, with a more rapid onset, than when fed the low-grain meal. In contrast, hay alone resulted in an apparent increase in venous pH (7.40 – 7.42) during the same post-feed time period. The reasons for the increased acidosis with the high-grain meal were not determined, but may be associated with increased fluid shifts, elevated plasma [protein] and increased VFA production.

Stutz *et al.*⁵⁰ fed horses a diet of 60% concentrate/40% Bermuda grass hay at four different DCADs (see Table 3) for 22 days. On the 22nd day, jugular venous blood was sampled hourly before feeding and for 17 h, with feedings occurring at time 0 and 12 h. This study is unique in providing a detailed time course of responses of plasma pH, PCO₂ and [HCO₃⁻] to four different DCAD diets. Independent of the effects of DCAD, for all diets there occurred a maximum decrease in pH and a peak in PCO₂ at 1 h after feeding, with these parameters rapidly returning to pre-feeding values by 3 h. In contrast, plasma [HCO₃⁻] reached a minimum 2–3 h post-feeding and was normalized 6–9 h post-feeding. These responses occurred subsequent to both feedings but were most pronounced after the morning feeding, suggesting a possible daily variation in the responses.

In the studies described above, the feeding frequency through the day had not yet been considered as a variable affecting acid–base status. The results of some of these studies indicated that at least a portion of the responses might have been due to the ingestion of one to three large meals, raising the hypothesis that the magnitude of the responses might be attenuated if the same amount of food were provided in smaller amounts but more frequently. As a control, Clarke *et al.*⁴⁹ fed horses their entire 24-h ration of forage and concentrate at 09:00, to demonstrate the influence of a single, large feeding on the measured variables. They reported significant increases in serum [PP] and [Na⁺] and decreases in [K⁺] between 0.5 and 2 h post-feeding, followed by gradual decreases in [PP] and [Na⁺] and increased

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**Table 3** Duration of feed adjustment and dietary cation–anion difference (DCAD; meq kg⁻¹ dry matter) of feeds studied. Refer to Table 2 for the method used by various researchers to calculate DCAD.

<table>
<thead>
<tr>
<th>Duration of feed conditioning (days)</th>
<th>Low DCAD</th>
<th>Medium–low DCAD</th>
<th>Medium–high DCAD</th>
<th>High DCAD</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>21</td>
<td>125</td>
<td>231</td>
<td>360</td>
<td>Baker *et al.*²⁶</td>
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<tr>
<td>24</td>
<td>24</td>
<td>127</td>
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<td>352</td>
<td>Baker *et al.*²⁷</td>
</tr>
<tr>
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<td>53</td>
<td>360</td>
<td>405</td>
<td>Baker *et al.*²⁵</td>
</tr>
<tr>
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<td>none</td>
<td>86</td>
<td>110</td>
<td>307</td>
<td>Cooper *et al.*³²</td>
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<td>14</td>
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<td>190</td>
<td>380</td>
<td>McKenzie *et al.*⁴⁸</td>
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<td>95</td>
<td>165</td>
<td>295</td>
<td>Popplewell *et al.*³³</td>
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<td>22</td>
<td>−50</td>
<td>50</td>
<td>150</td>
<td>250</td>
<td>Stutz *et al.*⁵⁰</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>107</td>
<td>201</td>
<td>327</td>
<td>Wall *et al.*³¹</td>
</tr>
</tbody>
</table>
Feeding and daily variation on acid–base balance

[\text{K}^+]$. For their control, Jansson and Dahlborn$^{50}$ fed their horses twice during the day and found that only plasma [PP] and [\text{K}^+] varied with feeding; the absence of [\text{Na}^+] response may have been due to a lower Na$^+$ content of the diet, or to the amount of time it took the horses to consume their entire meal. Similar results have also been reported by Yashiki et al.$^{51}$, but this study suffers from infrequent blood sampling, different amounts and timings of feed administration and superimposition of exercise. In the first two studies, plasma [\text{Cl}^−] was not measured so the plasma [SID] responses remain unknown. In Yashiki et al.'s$^{51}$ study, plasma [\text{Cl}^−] varied only with exercise and showed within-day changes of less than 10%. The abrupt increase in [PP], with decrease in [\text{K}^+], probably reflects net movements of water and K$^+$ from the vascular compartment into the upper GI tract as a result of feeding. The subsequent increase in plasma [\text{K}^+] and decrease in [PP] probably represent net intestinal absorption of the water and K$^+$, with the diets having a high K$^+$ content. Next, Clarke et al.$^{49}$ and Jansson and Dahlborn$^{50}$ divided the same total daily ration into six equal portions and fed one portion every 4 h for a 24-h period. Clarke et al.$^{49}$ reported that serum [\text{Na}^+] was the only variable that showed some variation, while Jansson and Dahlborn$^{50}$ found that only plasma [\text{K}^+] exhibited significant variation. No significant changes in [PP] were found, showing that frequent feeding of small meals attenuates fluid shifts between the vascular compartment and upper GI tract. From these two studies, it can be inferred that horses that have constant access to feed (i.e. grazing horses) do not exhibit pronounced, acute changes in acid–base balance. However, commonly used episodic feeding practices will result in acute changes in blood acid–base balance that are determined by the content and amount of feed eaten.

**Dietary protein**

Dietary protein is acidogenic because the oxidation of amino acids derived from the diet releases the strong acid divalent anion \text{SO}_4^{2−} and the weak acid anions \text{HPO}_4^{2−} and \text{H}_2\text{PO}_4^{−}. More specifically, the basic (cationic) amino acids (lysine, arginine and histidine) yield neutral end-products plus a proton while sulphur-containing (methionine and cysteine) amino acids are also acidogenic because they generate sulphuric acid upon cellular oxidation; the dicarboxylic (anionic) amino acids (aspartate and glutamate, but not asparagine and glutamine) consume acid when oxidized and thus reduce the acid load of the diet$^{52}$. When Greppi et al.$^{53}$ fed horses varying amounts of dietary protein for 27 days there was no effect of treatment on [PP], an expected result since ingested protein is broken down into amino acids. In contrast, Graham-Thiers et al.$^{54}$ found that when horses were fed a diet with low protein content (7.5%) they had a higher plasma pH and [SID] and lower PCO$_2$ and [lactate] than when fed a high-protein (14.5%) diet. While DCAD (calculated as DCAD = (\text{Ca} + \text{Mg} + \text{Na} + \text{K}) − (\text{Cl} + \text{S} + \text{P})) was 182 and 260 meq kg$^{-1}$ DM for the high- and low-protein diets, respectively, it is not likely that this modest difference in DCAD can fully account for the observed differences. As none of the horses in the latter study showed signs of diminished protein status, restriction of dietary protein, with addition of essential amino acids, may be a useful means for reducing the acidogenic properties of the feed as opposed to increasing DCAD by addition of K$^+$ or Na$^{2+}$.$^{54}$

**Fat**

An increased amount of dietary fat has no effect on acid–base state at rest, but leads to increased plasma [lactate] and reduced acidosis during high-intensity exercise$^{55–57}$. The mechanisms responsible for these responses appear to be complex and have yet to be determined.

**Areas for further research**

The acute blood acid–base responses to abrupt alterations in diet composition remain largely unknown. Of particular relevance to acid–base balance in horses is the impact of abrupt increases or decreases in DCAD on:

- the immediate time course of acid–base responses; and
- the time course of adaptation to the new diet. It may be hypothesized that, when a horse accustomed to a normal DCAD of \sim 200 meq kg$^{-1}$ DM is changed abruptly on to a diet with DCAD of 0, the post-feeding acidosis will be more pronounced than that seen after the horse has been on the low DCAD diet for 2–3 weeks.

**Chronic responses to feeding**

Several studies have examined the changes that occur with respect to whole-body electrolyte balance and acid–base status in response to 2–3 weeks on diets with varying DCAD (Table 3). While many of these studies report acute responses to feeding, it must be kept in mind that these responses are after the 2–3 weeks of conditioning on a particular diet. This design facilitates comparisons among different diets in ‘diet-adapted’ animals, but does not provide information on the acute responses to an altered diet and only limited information regarding the time course of adaptations and regulatory responses that occurred during the conditioning period. In general, lowering the DCAD of a horse’s normal diet (DCAD
of $\sim 200$ meq kg$^{-1}$ DM) has a chronic acidifying effect, while elevations in DCAD above $200$ meq kg$^{-1}$ DM do not appear to have as pronounced an effect on blood acid–base parameters. Baker et al.\textsuperscript{27} and McKenzie et al.\textsuperscript{48} investigated effects of varying DCAD on mineral balance in sedentary horses, horses with recurrent exertional rhabdomyolysis (REX)\textsuperscript{48} and horses receiving anaerobic exercise training 6 days per week for 3 weeks\textsuperscript{27}. Sodium balance was significantly greater in sedentary horses consuming high-DCAD diets than in those consuming low-DCAD diets. Also, varying DCAD had a significant impact on plasma acid–base variables. Plasma [SID] and pH were significantly lower in all horses consuming the low-DCAD diet, compared to the medium- and high-DCAD diets. Plasma [Cl$^-$] was significantly increased in horses fed the low diet. Plasma [HCO$_3^-$] and [TCO$_2$] were also significantly greater in horses consuming the high diet, and significantly less for horses fed the low-DCAD diet. There was no effect of RER on the plasma parameters and mineral balance\textsuperscript{49}. The exercised horses had greater sodium balances when consuming the highest-DCAD diet ($352$ meq kg$^{-1}$ DM) while chloride balance was elevated in exercised horses consuming the lowest-DCAD diet ($24$ meq kg$^{-1}$ DM), with no effect on sulphur balance\textsuperscript{27}.

The studies listed in Table 3 demonstrate that varying the DCAD will change plasma acid–base status in horses. As alterations in plasma [Na$^+$], [K$^+$] and [Cl$^-$] induced by diet affect plasma [SID], alterations in the balance of these minerals affect the overall acid–base status of an animal. A low DCAD produces a persistent metabolic acidosis characterized by lower plasma pH, [Na$^+$], PCO$_2$, [HCO$_3^-$] and TCO$_2$ with elevated [Cl$^-$], while high-DCAD diets tend to induce a mild alkalosis characterized by increased plasma pH, [Na$^+$], PCO$_2$, [HCO$_3^-$] and TCO$_2$ with decreased [Cl$^-$]. It can be concluded that manipulation of DCAD has direct and predictable effects on the acid–base status of horses.

By way of example, the results in this paragraph – from two independent laboratories – describe the acute responses to feeding a diet to which the horses have been conditioned. Baker et al.\textsuperscript{26} and Stutz et al.\textsuperscript{30} fed mature horses diets with four different DCADs (see Table 3) for a 21-day adjustment period. On the 22nd day, jugular venous blood samples were drawn every hour up to 17 h post-feeding. Plasma pH, PCO$_2$ and [HCO$_3^-$] were decreased with low-DCAB diets, while high-DCAB diets did not differ with respect to each other. The rate and magnitude of change in these parameters were inversely proportional to DCAD. High-DCAD diets resulted in a transient increase in [HCO$_3^-$] after feeding, followed by a decrease approximately 3 h post-feeding, and then an increase to a new steady state. The low-DCAD diets showed a lower PCO$_2$ and [HCO$_3^-$] post-feeding, and these gradually returned to their pre-feeding values (see also Fig. 4). The reductions in PCO$_2$ and [HCO$_3^-$] were suggested to be due to an increased rate of alveolar ventilation associated with decreased plasma pH\textsuperscript{26}; however, ventilation was not measured. Similar changes in these parameters have been demonstrated by Mueller et al.\textsuperscript{58}, who also controlled for starch source (rolled corn vs oats) and intake, comparing diets with $\sim 4$ g starch kg$^{-1}$ body mass with $\sim 0.3$ g kg$^{-1}$. Aside from the DCAD effects, they found no effect of starch source or quantity on the plasma acid–base responses.

**Mixed ration with control for DCAD – exercising horses**

Some studies have examined the effects of chronic dietary manipulation of DCAD on blood acid–base responses to exercise. In general, responses to altered diets during periods of exercise are difficult to ascertain because exercise itself produces a substantial and complex acid–base disturbance. Stutz et al.\textsuperscript{30} fed four different diets with DCAD (Na + K – Cl) of 5 (L), 107 (ML), 201 (MH; control treatment) and 327 (H) meq kg$^{-1}$ DM for 21 days. Two hours before the morning feeding, horses performed anaerobic exercise consisting of a 1.6 km sprint on a track to elicit a peak heart rate of $\sim 200$ beats min$^{-1}$. In contrast to the effects of diet on resting horses, the different DCAD treatments did not significantly affect measures of acid–base status in horses performing strenuous, anaerobic exercise; however, the data showed a trend towards lower plasma pH (7.15 vs 7.19) and [HCO$_3^-$] (14 vs 16 mmol l$^{-1}$) with diet L compared with MH. Cooper et al.\textsuperscript{52} also found that DCAD had a minimal effect on plasma acid–base parameters directly following or during recovery from anaerobic exercise, nor was there an effect on the performance of the horses. An interesting observation of Stutz et al.\textsuperscript{50} occurred in a pilot study where horses exercised 4 h after eating a high-DCAD meal. In contrast to performing exercise 2 h before feeding, plasma pH was higher during and immediately following exercise. Poplewell et al.\textsuperscript{35} exercised horses anaerobically 2 h after the morning feedings of diets with DCAD (Na + K – Cl – SO$_4$) of 10 (L), 95 (ML), 165 (MH) and 295 (H) meq kg$^{-1}$ DM. On the last day of the 15-day experimental period, the horses performed a standard exercise test 2 h after the morning feed. Arterial and venous blood samples were taken pre-exercise, immediately after exercise, and at 1–60 min of recovery. Diets with low DCAD resulted in a significantly lower plasma pH and [HCO$_3^-$], both at rest and at 60 min of recovery, but not at the end of exercise. While a direct effect on
plasma acid–base balance was not evident during exercise, the horses that consumed diet H showed improved exercise performance (faster running speed) and faster recovery of heart rate. Kronfeld’s group\textsuperscript{19,56,57} has been interested in determining if adaptation (4 months) to increased dietary fat (10% corn oil by weight) leads to improvements in anaerobic exercise performance and, if such an improvement occurs, its relationship to acid–base status. Adaptation to a high (10%) fat diet was associated with increased rate of decrease of jugular venous plasma PCO\(_2\), attenuated decrease in plasma pH and increased lactate threshold, compared with controls. These results indicate primarily long-term metabolic adaptations at the level of skeletal muscle, as differences were not evident until after 6 weeks of adaptation. The nature of these adaptations, and the mechanisms by which they affect acid–base balance, have yet to be determined.

**Daily variation**

As mentioned above, there are effects of circadian variation on blood acid–base parameters that are independent of feeding and exercise influences. Circadian rhythm refers to a pattern of cyclical, biological variation in response to light–dark cycles over a 24-h period, while daily variation refers to biological responses to daylight (diurnal) and night (nocturnal)\textsuperscript{51,55}. Many animals and plants are influenced by diurnal and circadian variations and, in animals, many blood parameters appear to fluctuate with \(\sim\) 24-h periodicity. Because there is known daily variation in plasma electrolyte and protein concentrations, it may be expected that blood acid–base parameters will also exhibit daily variation and should thus be taken into consideration when assessing acid–base balance.

Horses exhibit daily variations in heart and respiratory rates, blood pressure and hormones, plasma protein, PCV, electrolytes and serum enzymes\textsuperscript{49,51,53,59,60}. Increases in these parameters during the daylight period are associated with increased activity and alertness, with increased heart and respiratory rates to meet daytime increases in tissue oxygen demand and hence supply compared with sedentary, restful periods at night\textsuperscript{56,49,53,61,62}. The daytime increase in plasma protein content contributes oncotically to an increased plasma volume, aiding in the circulatory responses to periods of increased activity\textsuperscript{61–63}. For example, Greppi et al.\textsuperscript{53} reported a gradual decrease of [PP] after the evening meal and then a progressive increase 4 h after the morning meal through to the next feeding. It may be that some of the change is due to feeding however, as feeding causes a transient net movement of water into the GI tract, followed by re-uptake of water with nutrients. These changes in plasma volume will affect measured [PP] and PCV.

In mammals, many plasma strong ion blood concentrations also exhibit daily and circadian variation, including \([\text{Ga}^2\text{+]}, [\text{K}^+], [\text{Na}^+]\), \([\text{Cl}^-]\) and \([\text{HPO}_4^{2-}\])\textsuperscript{19,51,61–63}. Reports of daily variation in plasma \([\text{Na}^+]\) and \([\text{Ca}^{2+}\]) are inconsistent, with some studies showing minimal change\textsuperscript{52,65}, or increases\textsuperscript{53} or decreases\textsuperscript{61} at night. Plasma \([\text{K}^+]\), however, appears to show more distinct increases at night\textsuperscript{56,51,62}. This observation is consistent with a decreased activity of cellular Na,K-ATPase activity during night-time periods of inactivity and rest. A major confounder of all of these studies is that all animals were fed at some point during the 24-h period, with plasma strong ion concentrations therefore affected by the diet as well as possible circadian fluctuations.

Studies designed to examine diurnal or circadian variation must control for variables such as food, activity and environment, otherwise a true diurnal or circadian variation may not be found or a false one reported. It is also important that studies be conducted over a minimum period of 25 h, with blood samples taken with sufficient frequency (30–120 min intervals depending on time of day and feeding). Inadequate sampling frequency will result in missed parameter fluctuations and lead to misinterpretations. Despite these important limitations, the present literature indicates that blood parameters that determine acid–base status in the horse exhibit diurnal or circadian variation, and these need to be considered when assessing blood acid–base status. Another consideration that may impact on diurnal variation, particularly when diurnal variation is studied at different times of the year, are the effects of season and/or lunar cycle on physiological responses to light and daytime activities.

**Practical applications**

The practical applications of dietary manipulation for optimizing growth, health and production in domestic food animals are obviated by the economic costs of producing and maintaining thriving animals. Amongst horses, in addition to optimizing growth and health, the practical benefits that may accrue from a detailed consideration of dietary composition are also extended to exercise performance. Different types of activity provide different metabolic stresses on horses, and, accordingly one may expect that nutritional requirements for achieving optimal performance in specific disciplines will vary. An increased reliance on dietary and stored fat may be a benefit for the endurance horse, but a detriment for the track horse. Optimizing recovery from training work and from competition may also require unique, discipline-related nutritional requirements. It is understood that
the composition of the diet, with respect to macronutrients and DCAD, and the amount of time that a horse is kept on a dietary regimen, will have an impact on acid–base balance, performance and overall health. In domestic food animals, and seemingly too in horses, diets with DCAD greater than 200 meq kg\(^{-1}\) DM are beneficial for growth and health compared with low-DCAD diets.

A further consideration for Standardbred and Thoroughbred racehorses is that high-DCAD diets cause an elevation in plasma \([\text{HCO}_3^-]\) and TCO\(_2\). There is also a generations-old practice amongst trainers of racehorses of supplementing feed with a ‘small handful’ of sodium bicarbonate – anecdotally trainers perceive health benefits. The concern with elevated plasma \([\text{HCO}_3^-]\) and TCO\(_2\) is that horses are closer to the testing threshold for determining if a horse was administered an alkalinizing agent for the purpose of enhancing exercise performance\(^4\). TCO\(_2\) in racehorses not supplemented with alkalinizing agents shows considerable hour-to-hour variation by as much a 10 mmol l\(^{-1}\) (MI Lindinger, unpublished results; as may be expected from feeding and other influences) and there is considerable variability amongst horses – the normal distribution reported in the literature is from 23 to 38 mmol l\(^{-1}\). The DCAD of the diet, with respect to both the acute and chronic effects of feeding, clearly requires consideration when horsemen require that their horses do not test high for bicarbonate or TCO\(_2\).

**Summary and conclusions**

Acid–base status in horses is affected by the composition of the diet, timing of feeding and diurnal/circadian variation. The chronic effects of varying the DCAD of a horse’s diet are clear. When horses are fed a low-DCAD diet there is a more pronounced post-feeding acidosis and, with long-term feeding, there is a persistent chronic systemic, strong ion acidosis compared with horses on high-DCAD diets. On the other hand, high-DCAD diets produce a less pronounced acidosis post-feeding and, in the long term, confer a mild systemic strong ion alkalosis that may be characterized by elevated plasma \([\text{Na}^+]\), \([\text{HCO}_3^-]\), TCO\(_2\) and pH and decreased plasma \([\text{Cl}^-]\). The timing of feeding also has an effect on acid–base status, as horses undergo acute changes in blood parameters following the feeding of a meal. Plasma pH, \([\text{HCO}_3^-]\) and PCO\(_2\) show significant fluctuations post-feeding, due primarily to changes in plasma \([\text{Na}^+]\), \([\text{Cl}^-]\) and [protein]. Many blood parameters that influence acid–base state also show daily variation that may be affected by circadian variation. A comprehensive study, with proper controls for the confounding influences of feeding and activity, of the effects of daily variation on acid–base variables is needed.

The acute and chronic acid–base responses to diet, feeding and circadian variation have important implications with respect to diagnosis of acid–base disturbances and testing for administration of alkalinizing substances by racing jurisdictions. Single-statistic reports, in the absence of additional information, may yield erroneous or incomplete interpretations of a horse’s acid–base status. Consideration should be given to the time since the last feeding, the type of food eaten, the activity pattern of the horse and the time of day.

Although acid–base status is obviously affected by feeding, many questions still remain and more research on the subject is required to answer them. The studies by Kerr and Snow\(^3\) and Clarke et al.\(^3\) are really the only experiments conducted that specifically examined acute changes in blood parameters post-feeding; while many others have measured and reported such responses, horses were preconditioned to the diets for at least 2 weeks. There do not appear to be any studies that have examined the acute responses to an alteration of DCAD, and the time course of adaptation to the altered diet also remains to be determined. The impact of abrupt changes in DCAD on exercise performance and acid–base status during and following exercise require investigation. Research specifically examining acute changes in all acid–base variables in the blood post-feeding is needed. This research should focus on the factors that cause changes in pH, \([\text{HCO}_3^-]\) and [TCO\(_2\)], and should include blood samples taken frequently post-feeding. Finally, with respect to exercise conditioning, recovery from exercise and race performance, it remains to be determined whether diets with a high DCAD are beneficial compared with those with low DCAD. Some of these studies must be of long duration by design and will require a concerted effort by industry and researchers.

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**References**

Feeding and daily variation on acid–base balance


Appendix – Determination of DCAD from elemental percentages within feed

This appendix demonstrates the steps involved in converting percentages of sodium (Na⁺), potassium (K⁺), chloride (Cl⁻) and sulphate (SO₄²⁻) into ionic equivalents for the purpose of calculating the dietary cation–anion difference (DCAD). The quantity of the electrolytes used should be expressed in the feed analysis table on a dry matter basis. For example, a typical pelleted ration for horses contains 0.33% sodium, 0.87% potassium, 1.03% chloride and 0.2% sulphur (assumes sulphur is in the sulphate form). A step-by-step example is provided next for Na⁺ and then solutions are given for the other electrolytes and DCAD.

- **Step 1.** Convert % Na⁺ to g Na⁺ kg⁻¹ feed:
  
  \( 0.33\% = \frac{0.33\;\text{g}}{100\;\text{g}} = 3.3 \;\text{g kg}^{-1} \).

- **Step 2.** Convert g of Na⁺ to mol of Na⁺. Dividing the amount of Na⁺ \( 1\;\text{kg}^{-1} \) by the molecular weight for Na⁺ (23 g mol⁻¹) gives:
  
  \( \frac{3.3 \;\text{g kg}^{-1}}{23 \;\text{g mol}^{-1}} = 0.143 \;\text{mol kg}^{-1} \).

- **Step 3.** Convert mol kg⁻¹ to meq kg⁻¹. An ‘equivalent’ represents the magnitude of the positive or negative charge on the electrolyte. Na⁺, K⁺ and Cl⁻ are monovalent ions (single charge electrolytes).
while \( SO_2^- \) is divalent. Multiplying mol kg\(^{-1}\) by the ionic charge (eq mol\(^{-1}\)) gives, for Na\(^+\):

\[
(0.143 \text{ mol kg}^{-1}) \times (1 \text{ eq mol}^{-1}) = 0.143 \text{ eq kg}^{-1}.
\]

Then, dividing by 1000 to convert eq to meq yields:

\[
(143 \text{ eq kg}^{-1})/(1000 \text{ meq eq}^{-1}) = 143 \text{ meq kg}^{-1}.
\]

Using the molecular weights of 35 g mol\(^{-1}\) for Cl\(^-\), 43 g mol\(^{-1}\) for K\(^+\) and 32 g mol\(^{-1}\) for S\(^2-\), the following values are obtained: Cl\(^-\), 294 meq kg\(^{-1}\); K\(^+\), 202 meq kg\(^{-1}\); and S\(^2-\), 125 eq kg\(^{-1}\).

Thus, for DCAD = Na + K – Cl, one obtains:

\[
\text{DCAD} = 143 + 202 - 294 = 51 \text{ meq kg}^{-1}.
\]

Note that, because Cl\(^-\) carries a negative charge, it is subtracted from the sum of the electrolytes with positive charge.

If one calculates DCAD = Na + K – Cl – S, then:

\[
\text{DCAD} = 143 + 202 - 294 - 125 = -74 \text{ meq kg}^{-1}.
\]

As noted in the text, however, it is inappropriate to use sulphur, or for that matter calcium (Ca\(^{2+}\)), directly because their absorption by the GI tract is much less than that for the three monovalent ions. The dietary forms of sulphur and calcium determine the rates and magnitude of GI absorption. In many pelleted feeds, the total amounts of sulphur and calcium are similar, so their effects nearly balance out. A reasonable approximation of DCAD is therefore obtained as Na + K – Cl.