Quantitative analysis of acid-base balance in *Bos indicus* steers subjected to transportation of long duration


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**ABSTRACT:** There is a lack of information pertaining to the effects of transport stress on the acid-base physiology of ruminants. The effect of transportation and/or feed and water deprivation on acid-base balance was studied using 19 2-yr-old *Bos indicus* steers. The steers were allocated to one of three groups: 1) control, offered ad libitum access to feed and water (n = 8); 2) water and feed deprived, offered no feed or water for 60 h (n = 6); and 3) transported, offered no feed or water for 12 h, and then transported for 48 h (n = 5). Blood gases, electrolytes, lactate, total protein, albumin, anion gap, strong ion difference, and total weak acids were determined at the conclusion of transportation. Arterial blood pH did not differ among the experimental groups. Partial pressure of carbon dioxide (pCO2) was lower for the water and feed deprived (P = 0.023) group than for the control group. Plasma total protein, albumin and total weak acid concentrations were higher for the transported (P = 0.001, P = 0.03, P = 0.01) and water- and feed-deprived (P = 0.000, P = 0.003, P = 0.001) groups, respectively, compared with the control group. Transported animals had a lower plasma concentration of potassium (P = 0.026) compared with the control animals. This study demonstrates that although blood pH remains within normal values in transported and fasted steers, the primary challenge to a transported or feed- and water-deprived animal is a mild metabolic acidosis induced by elevated plasma proteins, which may be the result of a loss of body water. The loss of electrolytes had little effect on the acid-base balance of the animals.

Key Words: Acid Base Equilibrium, *Bos indicus*, Cattle, Electrolytes, Transport

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**Introduction**

The standard management practices of transport, assembly, mixing, handling, and the associated deprivation of feed and water are significant contributors to transport stress syndrome, characterized by loss of appetite and body mass (Hutcheson and Cole, 1986) and compromised immune function (Murata, 1989; Atkinson, 1992). Transport stress has led to live weight loss (Phillips et al., 1991) en route, and greater carcass shrink (Schaefer et al., 1992), whereas it is also accepted that animals dehydrate with increasing transit time (Sinclair et al., 1992; Tarrant et al., 1992; Knowles et al., 1999). Management strategies for dealing with the problems caused by transport stress have included preconditioning regimens (Pritchard and Mendez, 1990), rest periods during and after transport (Wythes et al., 1988), the use of supplemental potassium (Hutcheson et al., 1984), and the use of electrolyte solutions (Gortel et al., 1992; Phillips, 1997; Schaefer et al., 1997).

The use of electrolyte solutions for minimizing the effects of stressors on animals in the marketing process has been advocated in the sheep and beef industries without a full understanding of the effects of transport stress on the acid-base physiology of ruminants (Schaefer, 1997). Studies have shown transportation stress to have no effect on the pH of the bovine animal’s blood (Schaefer et al., 1988; 1992); however, there have been small but significant changes in some electrolytes that make up the strong ion group. Since the strong ion group plays an important role in regulating plasma pH, the changes seen in the plasma electrolyte status of transported animals must be minimal or are compensated by another system to maintain pH within normal values. This study was undertaken to assess the compensatory mechanisms involved in the maintenance of acid-base balance in *Bos indicus* steers subjected to transportation of long duration.
Materials and Methods

Animals and Management

Nineteen 2-yr-old Bos indicus steers (276 ± 14.65 kg of mean BW) were sorted in ascending order of live weight and allocated to one of three treatment groups: 1) control, offered ad libitum feed and water (n = 8); 2) water and feed deprived, offered no feed or water for 60 h (n = 6); and 3) transported, offered no feed or water for 12 h and then transported for 48 h (n = 5). Animals in the control group were offered a commercial dietary cube: 8.5 MJ of ME, 12% CP, and 31.1% CF per kilogram of DM (Cane Fibre Products, Brandon, QLD, Australia). Animals in the feed- and water-deprived and transported groups had their water and feed withdrawn 12 h prior to departure of the transported group. The transported group was trucked for 48 h (3,600 km) before being unloaded and sampled. The animals did not have access to feed and water in the yards while waiting to be sampled and were immediately processed upon exiting the unloading ramp. The transported animals were conveyed in a rigid truck with an 8-t tare. The truck was equipped with an adjustable gate separating the holding compartment into two areas. The transported animals were loaded into the forward compartment at a density of 0.86 m²/animal. At sampling, all animals were forced into a race where the animals were captured in a cattle head bail and restrained. A halter was placed on the individual animals, and their heads were then restrained to the side with an attendant holding the head while samples were taken. When cattle are captured and restrained, abnormal physiological reactions to the restraint may be expressed as an increase or decrease in respiration rates. Subsequently, the blood gas parameters of the animal become affected. Although the possibility exists for an abnormal measurement in blood gas parameters to have taken place in an animal from this study, it is unlikely when the current results are compared to those of other authors (Fisher et al., 1980; Mirakhur et al., 1985). Furthermore, the animals used in the present study were accustomed to being handled, and behaved in a quiet and amicable manner when sampled.

The mean daily temperature-humidity indices during the experimental period for d 0, 1, and 2 were 74, 74, and 73, respectively. The control and water- and feed-deprived groups were housed in outdoor pens with minimal shade.

All experimental procedures were reviewed and approved by the animal ethics committee at James Cook University (approval No. A730-02).

Sample Collection

After 48 h of transportation, 22.5 mL of blood was manually collected by jugular venipuncture from all groups; 20 mL was collected into 2 × 10-mL tubes containing lithium heparin (Disposable Products Pty Ltd., Adelaide, SA, Australia) and a 1 × 2.5-mL tube containing fluoride oxalate (Sarstedt Australia, Technology Park, SA, Australia). The samples containing fluoride oxalate were used for the analysis of plasma lactate, and the tubes containing lithium heparin were used for all other analyses. Blood samples were immediately placed into an ice water slurry, centrifuged at 200 × g for 15 min, and plasma was poured off within 2 h and frozen (−20°C) for analysis at a later date.

A 22-gauge (0.9 × 25 mm) intraarterial catheter (Optiva, Johnson & Johnson Int., Belgium) was used with a 2-mL blood gas syringe containing lithium heparin (Sarstedt Australia, Technology Park, SA, Australia) to sample arterial blood gases. Arterial blood samples for blood gas analysis were obtained from the caudal auricular artery (Riley and Thompson, 1978). Blood gas syringes were capped and placed into an ice-water slurry for immediate analysis of blood gases. All blood gas assays were performed within 0.5 h of collection.

Measurement

Arterial blood pH, partial pressure of carbon dioxide (pCO₂) and bicarbonate (HCO₃⁻) were measured using a blood gas analyzer (Ciba Corning model 278, Bayer Diagnostics, Bisbane, Australia). Plasma concentrations of Na and K were measured using ion-selective electrodes (Lablyte System 830, Beckman Instruments Inc, Brea, CA). Sodium and K samples were completed on singular samples and quality control samples (Liquichek controls; 16171 and 16172, Bio-Rad Laboratories, Regents Park, NSW, Australia) were performed every 10 samples. Lactate, P, albumin, total protein, Ca, and Cl concentrations in plasma were measured using a Mira Autoanalyzer (Roche Diagnostics, Brisbane, Australia) with standard enzymatic and spectrophotometric kits (lactate, Roche Diagnostics, Australia; P, TR30025; albumin, TR36025; total protein, TR34025; Ca, TR29248; and Cl, TR38025, Trace Scientific Ltd., Noble Park, Australia).

Anion gap (AG) (Polancic, 2000) was obtained from the equation:

\[ AG (\text{mEq/L}) = \left[ (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- - \text{HCO}_3^-) \right] \]

whereas strong ion difference (SID) (Stewart, 1983) was obtained from the following equation:

\[ SID (\text{mEq/L}) = \left[ (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- - \text{lactate}) \right] \]

Total weak acids (A_total) were calculated from the equation by Figge et al., (1992):

\[ A_{\text{total}} (\text{mEq/L}) = \left[ \text{albumin} \times (1.23 \times \text{pH} - 6.31) + \left( P (0.309 \times \text{pH} - 0.469) \right) \times 10/30.97 \right] \]

Statistical Analysis

Least squares means and standard errors are presented. Data were analyzed by one-way ANOVA with
Table 1. Least squares means ± SEM for blood pH, blood gases, plasma lactate, electrolytes, albumin, total protein, anion gap (AG), strong ion difference (SID) and total weak acids (A_{total}) in *Bos indicus* steers offered ad libitum feed and water (Control), subjected to fasting alone (Water and feed deprived), or subjected to 48 h of transportation and fasting (Transported).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n = 8)</th>
<th>Water and feed deprived (n = 6)</th>
<th>Transported (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.44 ± 0.01</td>
<td>7.46 ± 0.01</td>
<td>7.46 ± 0.02</td>
</tr>
<tr>
<td>pCO2, mm Hg</td>
<td>42.63 ± 1.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.08 ± 1.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.82 ± 1.82&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCO3, mmol/L</td>
<td>28.87 ± 1.16</td>
<td>25.47 ± 1.34</td>
<td>27.08 ± 1.47</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>0.59 ± 0.11</td>
<td>0.75 ± 0.12</td>
<td>0.62 ± 0.13</td>
</tr>
<tr>
<td>Na&lt;sup&gt;+&lt;/sup&gt;, mmol/L</td>
<td>139.48 ± 1.83</td>
<td>141.13 ± 2.11</td>
<td>140.72 ± 2.32</td>
</tr>
<tr>
<td>K&lt;sup&gt;+&lt;/sup&gt;, mmol/L</td>
<td>4.41 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.10 ± 0.14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.86 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;++&lt;/sup&gt;, mmol/L</td>
<td>2.30 ± 0.06</td>
<td>2.48 ± 0.07</td>
<td>2.34 ± 0.07</td>
</tr>
<tr>
<td>Cl&lt;sup&gt;-&lt;/sup&gt;, mmol/L</td>
<td>97.75 ± 1.82</td>
<td>102.67 ± 2.10</td>
<td>98.80 ± 2.30</td>
</tr>
<tr>
<td>PO&lt;sub&gt;4&lt;/sub&gt;, mmol/L</td>
<td>2.17 ± 0.18</td>
<td>2.80 ± 0.20</td>
<td>2.69 ± 0.22</td>
</tr>
<tr>
<td>AG, mEq/L</td>
<td>17.26 ± 3.33</td>
<td>17.10 ± 3.85</td>
<td>18.70 ± 4.21</td>
</tr>
<tr>
<td>SID, mEq/L</td>
<td>49.02 ± 3.00</td>
<td>45.80 ± 3.47</td>
<td>48.73 ± 3.80</td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>64.13 ± 1.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>81.33 ± 2.43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>78.60 ± 2.22&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>35.12 ± 1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.17 ± 1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.60 ± 1.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>A_{total}, mEq/L</td>
<td>11.07 ± 0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.46 ± 0.40&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.95 ± 0.44&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Within a row, means that do not have a common superscript letter differ, *P* < 0.05.
<sup>c,d</sup>Within a row, means that do not have a common superscript letter differ, *P* < 0.01.

Results and Discussion

Stewart (1983) proposed a comprehensive quantitative method of acid-base analysis that required the distinction between independent and dependant variables involved in acid-base balance. That author demonstrated that acid-base homeostasis in plasma is regulated by changes in three independent variables, pCO2, SID, and A_{total}, that can be changed independently of each other (Stewart, 1983). Whereas pCO2 is regulated by the respiratory system, SID is mainly regulated by transmembrane ionic exchanges, and A_{total}, although it has a significant influence on acid-base status, is not primarily regulated to maintain acid base homeostasis (Aguilera-Tejero et al., 2000).

This approach to acid-base analysis has offered an excellent qualitative framework for clinical interpretation of acid-base disorders in a number of species (Weinstein et al., 1991; Pieschl et al., 1992; Frischmeyer and Moon, 1994). It offers researchers and clinicians the ability to identify the mechanisms involved in changing acid-base status, thereby focusing an appropriate treatment on the inciting cause (Constable, 2002).

Blood gases, plasma electrolytes, and metabolites are presented in Table 1. There was no difference in the pH of arterial blood in the treatment groups, confirming other data (albeit on venous blood) that transportation stress causes no difference in the acid-base status of transported vs. nontransported ruminants (Schaefer et al., 1988; 1992). Arterial pH values recorded in all treatment groups in the present study were similar to those reported by Mirakhur et al. (1985) in normal *Bos indicus* cattle (7.47 ± 0.04) and also by Fisher et al. (1980) for *Bos taurus* cattle (7.43 ± 0.03).

The water- and feed-deprived animals had a lower pCO2 compared with the control animals (*P* = 0.023). However, the pCO2 values for the transported animals did not differ from those of the control group (*P* = 0.126). A lowering of the pCO2 concentration in the blood of the water- and feed-deprived animals and a trend toward the same in the transported group demonstrates a compensatory mechanism used to buffer against a mild metabolic acidosis caused by dehydration. The primary pathophysiology for metabolic acidosis, a low HCO3 concentration, results in a low pH that stimulates respiration. This produces a low pCO2, which reverts the pH towards normal. However, this process reaches a compensatory limit at approximately 12 h (Walmsley et al., 1988). If the inciting cause of the acidosis persists, then it is the renal system, which reverts the pH back to normal via the reabsorption and production of HCO3<sup>-</sup> and secretion of H<sup>+</sup> ions (Guyton and Hall, 2000). It is difficult to state which compensatory system contributed to the differences seen in pCO2 concentration in the water- and feed-deprived and transported groups. However, we would speculate that due to the duration of feed and water deprivation and transit, that at 48 h, it is a metabolic system that is influencing pCO2 concentration.

There were no differences in HCO3<sup>-</sup> concentrations in the treatment groups, and all groups remained within...
normal limits for cattle (20 to 30mmol/L) (Blood and Radostits, 1989). Schaefer et al. (1988; 1990) demonstrated a decrease in pCO₂ and subsequently in HCO₃⁻ concentrations in the venous blood of cattle exposed to the marketing process and feed and water deprivation, respectively. Total protein or albumin concentrations were not reported in these studies; however, animals were withheld from water and feed for up to 72 h, and Schaefer et al. (1990) recorded increases in hematocrit, hemoglobin, and red blood cells, indicating a state of dehydration. Thus, the changes seen post-treatment by Schaefer et al. (1988; 1990) are more likely the result of a respiratory and/or metabolic compensation for a mild metabolic acidosis secondary to water loss.

As a consequence of dehydration, the biosynthesis of the L-isomer of lactic acid from anaerobic glycolytic metabolism by skeletal muscle may be increased (Nappert and Johnson, 2001). There was no difference between treatment groups for plasma lactate concentrations. Transportation or water and feed deprivation in the present study failed to elevate lactate concentrations to those demonstrated by Mitchell et al. (1988) or Schaefer et al. (1988). Mitchell et al. (1988), working with Bos indicus × Bos taurus steers and heifers, demonstrated a difference for lactate values between handling (3.1 ± 1.8 mmol/L), transport for 2 h (4.0 ± 2.2 mmol/L), and animals that had not been handled or transported (0.3 ± 0.2 mmol/L). Schaefer et al. (1988), who transported Bos taurus steers and heifers for 6 h, obtained plasma lactate levels of 5.53 mmol/L prior to the stress of transportation, and 6.50 mmol/L at slaughter.

The collection of blood for lactate analysis, during the studies of Schaefer et al. (1988) was not recorded as using an antiglycolytic agent in the collection tubes. As such, the lactate data reported by Schaefer et al. (1988), especially the pretransplant values, may be artefactually elevated due to this preanalytical error. Anaerobic glycolysis may have occurred within blood samples if tubes containing Li heparin were used by Schaefer et al. (1988) (Polancic, 2000).

The difference seen between studies for lactate concentrations may be due to time in transit. Tarrant (1990) indicated that 24 h of transportation fatigued steers enough to induce resting behaviors in transit. Although we cannot confirm that all transported animals rested in transit in our study, resting may have been sufficient to decrease lactate concentration in the transported steers compared with that expressed in the other groups housed in pens. Tarrant (1990) further stated that during short transit times, cattle tend not to lie down in trucks while they are moving. The constant standing and bracing during short haul transit may elevate lactate concentrations in cattle during these journeys. This is consistent with other reports that indicate that the major effects of transport stress take place during the early portions of transport (Cole et al., 1988).

Mitchell et al. (1988) noted that the animals used in their study were unaccustomed to being handled. By contrast, the animals used in the present study had been extensively handled by experienced stockmen. The lactate concentrations reported here may also reflect the beneficial effects of a sound management program in minimizing lactate accumulation in cattle subjected to transportation of long duration.

In agreement with the work of Galyean et al. (1981), plasma Na was not influenced by treatment and remained within normal values for cattle (Blood and Radostits, 1989). Transported animals had lower concentrations of plasma K compared with the control animals (P = 0.026). It is well recognized that stressor-induced activation of the hypothalmo-pituitary-adrenal axis stimulates the secretion of cortisol, resulting in the excretion of K (Parker et al., 2003). The hypokalemia associated with the transported group may also be the result of a lack of feed intake; however, this was not replicated by the water- and feed-deprived group.

Plasma concentrations of Ca, Cl, and P did not differ between groups. However, there was a trend (P = 0.07) for plasma concentrations of P to be higher in the water- and feed-deprived group than in the control group. Galyean et al. (1981) reported plasma P concentrations to be higher in fasted vs. transported animals at 32 h; however, the changes, as in the present study, were small and within physiological limits.

The AG is a diagnostic concept that demonstrates the difference between unmeasured anions and unmeasured cations (Guyton and Hall, 2001). Usually, the unmeasured anions exceed the unmeasured cations, with the AG for cattle ranging between 14 to 26 mEq/L (Blood and Radostits, 1989). Strong ions move between body fluids through membranes, and the resulting changes in SID values provide the major mechanism for acid-base interactions between fluids (Stewart, 1983). Despite the small but significant changes in the K concentration of plasma in the transported animals, there were no differences between groups for the AG or SID calculations. This would suggest that electrolyte solutions fed to these steers post-transport would provide little benefit in correcting their acid-base balance compared to water alone. In support of this suggestion, AG did not differ significantly between low- and moderate-stress groups of cattle offered water when compared with moderately stressed cattle offered only an electrolyte solution (Schaefer et al., 1994).

The application of electrolyte solutions to minimize transport stress in cattle has been extensively investigated (Schaefer et al., 1988; Gortel et al., 1992; Phillips, 1997). There is a trend in the literature for increases in the extracellular fluid, carcass weight, and body weight of cattle when electrolyte solutions are fed vs. when no fluids offered post-transport. The effects of the electrolyte solutions fed in these studies were to replenish lost total body water in the animals involved (Schaefer et al., 1992; 1997; Gortel et al., 1992). Similarly, the same effects can be seen when cattle are offered water post-transport (Wythes et al., 1980; 1983).
electrolyte solution and water as treatments, demonstrated no difference ($P < 0.05$) in carcass yield as a proportion of farm weight, rumen weights, extracellular fluid volume, plasma volume, hematocrit, serum Na, K, glucose or β-hydroxybutyric acid between the water- and electrolyte-fed groups. Lower values for plasma osmolality, serum Cl, and serum lactate were found between the water and electrolyte groups, respectively. This was a reflection of the amount of fluid consumed post-transport between the treatments. There was a difference recorded for hot carcass weight as a proportion of the preslaughter weight between the electrolyte- and water-treated groups; however, this difference could be attributed to the higher intake of fluid by the water group, causing the animals to be heavier at slaughter than the electrolyte group. Subsequently, the carcass yield as a proportion of the preslaughter weight would be lower for the group offered water if carcass weights were similar. Unfortunately, carcass weights were not reported in that study.

Plasma total protein, albumin, and $A_{\text{total}}$ concentrations were higher for the transported ($P = 0.001$, $P = 0.03$, $P = 0.01$) and water- and feed-deprived ($P = 0.001$, $P = 0.003$, $P = 0.001$) groups, respectively, than for the control group. Transport stress has been observed to cause dehydration and may manifest itself as a hypoproteinemia (Atkinson, 1992; Schaefer et al., 1997). Serum proteins, especially albumin, act as weak acids in plasma. The role of proteins in acid-base balance has practical importance: hypoproteinemia and hyperpro- plasma. The role of proteins in acid-base balance has practical importance: hypoproteinemia and hyperproteinemia by themselves cause metabolic alkalosis and acidosis, respectively (Figge et al., 1991; 1992). Hemo-concentration secondary to dehydration elevates total protein and is a contributing factor toward metabolic acidosis (Walmsley et al., 1988; Figge et al., 1991; 1992; Nappert and Johnson, 2001).

Transportation and water and feed deprivation resulted in an increase in $A_{\text{total}}$ compared with control animals due to an elevation in albumin concentration. The changes seen in plasma albumin, and hence total protein concentrations, in the water- and feed-deprived and transported groups are likely to be due to hemo-concentration secondary to water loss. The increase in albumin, and hence total protein and $A_{\text{total}}$, would contribute to a mild metabolic acidosis. The resulting hypovolemia and low tissue perfusion may also cause a limited supply of oxygen to tissues and a decrease in H excretion by the kidneys (Nappert and Johnson, 2001).

It would appear in the present study that the water- and feed-deprived group incurred a greater deviation from the control group in some of the parameters measured compared with the transported group, in spite of the winter temperature-humidity index for the region being mild for tropically adapted cattle. This may imply that the tractable genotype used in this study found feed and water deprivation to be a greater challenge to acid-base homeostasis than transportation itself. Further to this, the increased airflow created by transportation may have had a cooling effect on the animals, thereby decreasing the amount of water loss and, in turn, the degree to which the animals compensatory acid-base mechanisms were employed.

**Implications**

We conclude from the results of this study that Bos indicus steers transported for 48 h are able to maintain their acid-base balance within normal values. The primary challenge to these animals seems to be the elevation of total weak acids via an increase in plasma albumin concentration as a result of dehydration. This was compensated for by the respiratory and renal systems decreasing the pCO$_2$ concentration in arterial blood. Plasma electrolytes were selectively altered; however, the strong ion difference and anion gap did not differ between the control, water- and feed-deprived, and transported groups. Offering electrolyte solutions to dehydrated, transported, nutrient-deprived, and stressed Bos indicus cattle is unlikely to resolve the physiological stressors more efficiently than water alone.

**Literature Cited**


