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# INVESTIGATING THE IMPACT OF DIETARY CATION-ANION DIFFERENCE VARIATION ON PHYSIOLOGICAL RESPONSES AND RUMEN FERMENTATION IN HEAT-STRESSED MALE ZANDI SHEEP

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## Abstract

Recent attention has been drawn to the dietary cation-anion difference (DCAD), yet understanding remains limited regarding its influence on organic matter digestibility, blood parameters, and rumen fermentation in male Zandi sheep. This study aimed to elucidate the effects of varying DCAD levels on these parameters in male lambs experiencing heat stress. Forty male Zandi lambs, averaging 39 kg in body weight, were randomly assigned to five treatment groups, each with 8 replicates. Diets were formulated to contain different concentrations of DCAD (150, 300, 450, 600, and 750 mEq/kg dry matter). The experimental period spanned 100 days following a 21-day adaptation phase. Results indicated that the control group exhibited significantly higher values ($P<0.05$) for dry matter intake, crude protein digestibility, and dry matter digestibility. Additionally, ruminal pH was notably elevated in the control group ($P<0.05$). However, rumen fermentation parameters such as butyric acid, buffering capacity, acetic acid, propionic acid, the combined total of acetic acid and propionic acid, and the acetic acid to propionic acid ratio were unaffected by varying DCAD concentrations ($P>0.05$). Blood glucose parameters revealed significant differences ($P<0.05$), with the control group demonstrating the most significant impact on blood glucose levels. Conversely, no significant differences ($P>0.05$) were observed in other blood markers including potassium, magnesium, phosphate, cholesterol, and phosphate. Overall, our findings suggest that dietary manipulation of DCAD has no significant effect on rumen fermentation. However, it is noteworthy that lambs exposed to heat stress may sustain their apparent digestibility and output through increased DCAD intake.

**Keywords:** Zandi sheep, DCAD, Digestion, Rumen fermentation, Blood physiology, Heat stress

## Introduction

The term "dietary cation-anion difference" (DCAD) refers to the potential positive or negative alteration in non-metabolizable dietary ion combinations. Notably, Block (1984) observed that therapy with -172.3 mEq/kg DM DCAD helped prevent hypocalcemia compared to a control DCAD of +448.6 mEq/kg DM. This seminal finding marked the initial recognition of the benefits

associated with varying DCAD intake. Subsequent research has underscored the significant impact of DCAD levels on the health and economic outcomes of transition dairy animals. Recent investigations have primarily centered on understanding the interplay between DCAD and calcium (Ca) metabolism (Diehl et al., 2018; Collazos et al., 2018; Lopera et al., 2018; Rajeerad et al., 2020), along with its associations with vitamin D, 5-hydroxyl-tryptophan, and cholecalciferol/calcidiol (Collazos et al., 2018; Martinez et al., 2018b; Martinez et al., 2018a; Rodney et al., 2018). These studies have demonstrated the efficacy of modulating peripheral blood Ca homeostasis through DCAD reduction in conjunction with the aforementioned parameters. Heat stress exacerbates perspiration and urine output (linked to bicarbonate ions), respiratory CO2 loss (resulting in respiratory alkalosis), and Na and K depletion. Theoretically, altering DCAD levels can counteract shifts in blood acid-base balance and subsequent electrolyte imbalances. Optimal DCAD levels ranging from 200 to 370 mEq/kg DM have been recommended for nursing dairy calves to maximize milk production (Tucker et al., 1988; West et al., 1991; Caixeta et al., 2020), while diets containing 250 mEq/kg DM have been suggested for chickens and pigs to enhance growth rates (Mongin, 1981). Such dietary modifications may directly influence rumen pH in ruminants, consequently impacting the productivity of ruminal microbiota, dry matter intake, and digestion (Yang et al., 2021; Nguyen et al., 2020). Alterations in dietary salt concentrations affecting DCAD can promptly affect food digestibility and dry matter intake. However, the impact of DCAD adjustments on sheep performance remains relatively unexplored. Consequently, the specific effects of DCAD on blood metabolites, dry matter intake, organic matter digestibility, and fermentation in male lambs have not been adequately investigated.

Therefore, this study aimed to assess the impact of raising DCAD from 150 to 750 mEq/kg of DM on rumen fermentation and blood metabolites in male lambs. Furthermore, we sought to investigate whether positive DCAD levels in high-temperature environments could enhance dry matter intake, rumen fermentation, and lamb productivity.

**Materials and Methods Animal Management:**

Approval for animal breeding under the designation IUA-2020-P312 was granted by the Islamic Azad University's council on experimental animal ethics. Breeding occurred at the research farm between June 2020 and October 2021. Forty male Zandi lambs, indigenous to central areas of Iran, were divided into five treatment groups, each consisting of eight replicates using a completely random block design. Lambs were individually housed throughout the experiment and were provided diets containing varying levels of DCAD: +150 (control; group 1), +300 (group 2), +450 (group 3), +600 (group 4), and +750 (group 5).

A total mixed ration (TMR) with a 30:70 concentrate-to-roughage ratio was pelleted for feeding. Potassium carbonate (K2CO3) and sodium bicarbonate (NaHCO3) were added to manipulate DCAD levels. The experiment spanned 100 days, including a 12-week trial period preceded by a 3-week adaptation phase. Lambs were fed treatment diets twice daily at 9:00 and 18:00, with ad

libitum access to water. Nutritional ingredients and chemical components of the diets are detailed in Table 1.

**Monitoring of the Temperature-Humidity Index (THI)**

THI was monitored using wet and dry bulb thermometers placed at a height of 1.5 meters in the feeding barns. The THI was calculated daily using the formula: (Td + Tw) x 0.72 + 40.6, where Td and Tw represent the temperatures from the dry and wet bulb thermometers, respectively. Rectum temperature (RT) of each lamb was monitored daily using an electronic thermometer.

**Gathering Information and Determining Digestibility**

Daily dietary samples were collected from days 21 to 100 to determine chemical composition, including dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF), total fat (Ash), crude ash, and crude protein (CP), following AOAC (1990) guidelines. Sodium (Na), potassium (K), and chloride (Cl) levels were measured using atomic absorption spectrophotometry. DCAD was calculated using the formula: DCAD = Na (%) / 0.0023 + Cl (%) / 0.00355 + K (%) / 0.0039 + S (%) / 0.0016.

Individual lamb weights were recorded every 14 days throughout the 100-day period, and daily dry matter intake (DMI) was measured and calculated, accounting for refusals.

**Measurement of Metabolites in Plasma**

Monthly blood samples were collected via the jugular vein to assess biochemical parameters. Samples were placed in EDTA vacuum tubes, centrifuged to separate plasma, and stored for analysis. Glucose, potassium, sodium, cholesterol, phosphorus, and magnesium levels were measured using AOAC procedures.

**Collecting and Examining Rumen Fluid for Volatile Fatty Acids**

Rumen fluid samples were obtained from each lamb using a syringe and stomach tube, employing the numerous tubes technique to minimize saliva contamination. A mouth gag equipped with an outer rubber tube (inner diameter = 2.5 cm) and an inner collection tube (outer diameter = 1.2 cm, length = 110 cm) was inserted into the rumen for fluid collection.

Immediately following the morning feeding and after 2.5 hours, the pH of 25 mL of rumen fluid samples was measured using a pH meter. The ruminal fluid was then filtered through two layers of cheesecloth, and 1 mL of 6 N HCl was added for preservation before freezing the samples at -20°C for subsequent volatile fatty acid (VFA), osmolality, and NH3-N assays. Osmolality of the ruminal fluid was determined using an osmometer, while VFAs were analyzed according to Thammacharoen et al. guidelines. NH3-N levels were determined using salicylate-hypochlorite analysis.

**Analyzing Allantoin Excretion and Collecting Urine:**

Urine samples were collected concurrently with fecal samples (15 mL H2SO4 in 10% solution in 90 mL urine) each week. Urine was collected and measured in plastic containers with 10% sulfuric acid added to minimize nitrogen loss and maintain a pH below three. At the conclusion of the experiment, 30 mL of urine from each day was pooled, frozen at -20°C, and analyzed for nitrogen and allantoin excretion using colorimetric techniques, following procedures outlined by Chen and Gomes. Nitrogen retention was calculated by determining the difference between nitrogen output and nitrogen uptake (from feed intake).

**Statistical Analysis**

Experimental data were analyzed using MIXED models in SAS 9.4 (SAS Institute Inc.) employing a randomized complete block design. The statistical model used was:

$$Yij = \mu + Ti + Bj + Eij$$

Where Yij represents the value of each variable, Ti denotes the treatment effect, Bj represents the block effect, and μ represents the mean of the associated trait, with Eij representing the error rate. Post-hoc comparisons of means were conducted using the LSD test method, with significance set at $P<0.05$.

**Results**

**Lambs' Rectal Temperature and THI**

Throughout the study period, the dry bulb thermometer recorded a mean temperature of 33.94°C, ranging from 29.03°C to 39.25°C. Wet-bulb temperatures ranged from 20.16°C to 30.01°C, with a mean of 25.61°C. The calculated THI varied between 75.13 and 86.55, with a mean of 81.23. Notably, THI consistently remained above 75 throughout the experiment. Rectal temperatures did not exhibit significant differences among the various DCAD groups compared to the control group. No appreciable differences in rectal temperature were observed across the different DCAD treatment groups.

**Influence of DCAD on Plasma Metabolites**

Analysis of variance for blood biochemical parameters in lambs revealed significant differences in salt, cholesterol, and glucose parameters across treatments ($P<0.05$, see Table 2). The results indicate that the control group had the most pronounced impact on blood glucose levels ($P<0.05$). However, there were no significant differences in blood glucose levels among groups 3, 4, and 5. Group 5 exhibited a substantial difference in blood cholesterol compared to other groups, with the highest cholesterol levels observed. Blood levels of potassium, magnesium, and phosphorus showed no significant changes ($P>0.05$). Nevertheless, group 5 demonstrated the highest levels of blood phosphorus, magnesium, and potassium, while the control group exhibited the lowest levels (see Table 2).

**Parameters of Rumen Fermentation and the DCAD Effect**

A significant difference in ruminal pH between treatments was observed at $P<0.01$. The control group exhibited the lowest ruminal pH, while group 5 demonstrated the highest ruminal pH ($P<0.05$) (refer to Table 3). However, total volatile fatty acids, including butyrate, propionate, acetate, isovaleric acid, valeric acid, acetate/propionate ratio, ammonia, and urea nitrogen remained unaffected by variations in DCAD levels. Despite this, numerically, the control group displayed the highest levels of ammonia, total volatile fatty acids, urea nitrogen, isovaleric acid, valeric acid, butyrate, and acetate/propionate ratio. Conversely, group 5 exhibited the lowest levels of ammonia, urea nitrogen, butyrate, valeric acid, isovaleric acid, acetate/propionate ratio, and total volatile fatty acids (refer to Table 3).

**Impact of DCAD on Dry Matter Intake and Digestibility**

Significant variations were observed across treatments regarding crude protein digestibility and dry matter intake ($P<0.05$, refer to Table 4). Dry matter intake and crude protein digestibility were notably lowest ($P<0.05$) in the control group. Conversely, group 4 exhibited the highest apparent digestibility of organic matter, crude protein, and dry matter intake ($P<0.05$). Table 4 highlights that Group 1 had the lowest crude protein digestibility ($P<0.05$).

**Discussion**

The dietary cation-anion difference (DCAD), expressed as mEq/kg DMI, is a critical parameter defined as the ratio of primary cations (Na+ and K+) to principal anions (Cl- and S2-) per kilogram of dry matter (DM) (Riond, 2001). In recent years, research by Shahzad et al. (2008), Edwards et al. (2010), Nrgaard et al. (2014), Heer et al. (2017), Cardoso et al. (2020), and Hassanien et al. (2022) has underscored the growing significance of DCAD in formulating animal diets. Furthermore, recent meta-analyses conducted by Santos et al. (2019) and Lean et al. (2019) have delved into the diverse impacts of DCAD on cattle health, productivity, and performance. Their findings suggest that prenatal DCAD adversely affects total blood calcium levels during labor and may contribute to decreased overall health outcomes among cattle.

The investigation revealed notable differences in dry matter intake (DMI), crude protein, and organic matter digestibility among the treatment groups. Notably, the lambs in the control group exhibited the lowest DMI, suggesting a preference for drier feed components. Similarly, Group 5 displayed the lowest intake of dry matter. Moreover, Group 5 exhibited the lowest crude protein digestibility and the highest organic matter content compared to the other groups. These findings underscore the impact of supplementation on the nutritional digestibility of male lambs. The observed increase in digestibility may be attributed to the absence of rumen microbial population requirements in the basic diet, as suggested by Mallaki et al. (2015).

Various studies (Bougouin et al., 2019; Glosson et al., 2020; Samarin et al., 2022) have identified several factors influencing nutrient digestibility, including supplement concentration, supplement

source (organic or mineral), diet balance (forage to concentrate ratio), and base diet supplement concentration. In our study, we observed substantial variation in ruminal pH among treatments. However, total volatile fatty acids, including butyrate, propionate, acetate, acetate/propionate ratio, isovaleric, valeric, ammonia, and urea nitrogen, did not show significant alterations across treatments, based on the data analysis. Interestingly, the ruminal pH was highest in the control group, while the lowest ruminal pH was observed in Group 5. These findings suggest that while supplementation influenced ruminal pH levels, it did not significantly impact volatile fatty acid concentrations.

The results of our study highlight the direct impact of the cation source fed to lambs on the anion-cation balance of their diet, influencing ruminal fermentation characteristics. A crucial indicator for assessing rumen fermentation conditions is the level of ammonia nitrogen generated in the rumen. A balanced diet aims to minimize waste associated with excess ammonia while providing adequate non-degradable protein and nitrogen for optimal microbial protein synthesis. Ammonia serves as one of the key nitrogenous substances utilized by rumen microorganisms for protein synthesis. Studies by Afsahi et al. (2020) and Lee et al. (2020) elucidate that urea in saliva, nitrogenous components in the feed, and urea penetrating the rumen wall all contribute to the production of rumen ammonia. These findings underscore the intricate dynamics of rumen fermentation and the importance of maintaining optimal nitrogen balance for microbial protein synthesis and overall rumen health.

The observed decrease in ammonia concentration as the DCAD level rises suggests a corresponding increase in ruminal acidity. This increase in acidity creates a favorable environment for further synthesis within the rumen. The breakdown of crude protein significantly influences ammonia production by microorganisms in the diet and contributes to the decomposition of the microbial population, especially under adverse conditions where nitrogen recycling occurs. Studies by Dudareva et al. (2004) have demonstrated that reducing rumen ammonia production can enhance microbial protein synthesis or decrease rumen protein degradation. Similarly, findings by Doepel and Hayirli (2011) align with our study, indicating that incorporating sodium bicarbonate into the diet has no significant effect on ruminal ammonia levels. These insights emphasize the intricate interplay between dietary factors, ruminal acidity, and microbial protein production within the rumen ecosystem.

The disruption of the energy cycle between bacteria and protozoa occurs when protozoa are reduced or removed from the rumen, leading to a decrease in the breakdown of bacterial proteins. Consequently, there is a decrease in the concentration of ammonia due to an increase in the flow of microbial nitrogen from the rumen, as highlighted by studies conducted by Kavanagh et al. (2019) and Thanh et al. (2020). In line with the findings of our study, Harrison et al. (2012) demonstrated that potassium carbonate can serve as an effective buffer, enhancing dry matter intake, promoting the synthesis of volatile fatty acids, maintaining ruminal pH, and increasing the

acetate-to-propionate ratio. While DCAD influences the acetate to propionate ratio, it does not have a discernible effect on the molar proportion of propionate, as reported by Iwaniuk and Erdman (2015). These findings underscore the potential role of potassium carbonate in modulating rumen fermentation dynamics and improving nutrient utilization within the rumen environment.

According to Shahzad et al. (2008), elevating the dietary level of anion-cation difference preserves the fermentation pattern, leading to balanced amounts of butyrate and acetate. This balanced fermentation pattern, in turn, enhances the synthesis of volatile fatty acids, including butyrate and acetate, which contribute significantly to milk fat production, accounting for up to 25% of milk fat content. Consequently, raising the DCAD level has been found to mitigate environmental pollution caused by methane generation, a significant consideration in contemporary practical nutrition strategies aimed at sustainability and environmental responsibility. Earlier research, such as that conducted by Apper-Bossard et al. (2010), suggests that adding potassium carbonate to raise DCAD levels results in an increase in dry matter intake. These findings highlight the potential benefits of manipulating DCAD levels in animal diets to optimize rumen fermentation patterns, enhance milk production efficiency, and mitigate environmental impact. It can be inferred that the addition of potassium carbonate to the diet of dairy cows enhances rumen fermentation processes and supplies essential nutrients such as potassium and calcium to the microbial population. This supplementation ultimately leads to an increase in dry matter digestibility. Studies, such as those conducted by West et al. (1987) and Zhang et al. (2022), have demonstrated that the treatment containing potassium carbonate and magnesium carbonate with a DCAD level of 905+ mEq exhibits the highest apparent dry matter digestibility and an enhanced area below the digestibility curve, indicating fluctuations in dry matter digestibility at different times. Additionally, research by Iwaniuk and Erdman (2015) corroborates these findings by indicating that as the DCAD level increases, so does dry matter digestibility. These collective results underscore the beneficial effects of supplementing dairy cow diets with potassium carbonate in improving rumen fermentation dynamics and enhancing nutrient utilization, ultimately leading to improved dry matter digestibility and overall animal health and performance.

Funk et al. (1986) investigated the effects of adding lasalocid and potassium on the digestibility of insoluble fiber in neutral detergent and found that simultaneous supplementation with both substances improved the digestibility of insoluble fiber in neutral detergent. Our study demonstrates that increasing DCAD levels enhances ruminal acidity, consequently improving the digestion of insoluble fiber in neutral detergent. This improvement in dry matter digestibility aligns with earlier research findings, as reported by Martinez et al. (2018). Additionally, assessing the metabolic health of animals can be achieved through blood measurements. Our study observed significant variations in the levels of sodium, cholesterol, and glucose among the different dietary regimens, indicating potential metabolic effects associated with DCAD levels and dietary supplementation. These findings underscore the multifaceted impacts of dietary interventions on rumen fermentation dynamics, nutrient digestibility, and metabolic health in ruminant animals.

The control group exhibited the most pronounced effect on blood glucose levels compared to the means of the treatments, while groups 3, 4, and 5 did not demonstrate substantial differences. This observation is consistent with previous research indicating that the concentration of propionate in ruminal fluid significantly influences blood glucose levels in ruminants (McDonald et al., 2010). Moreover, blood cholesterol levels did not exhibit significant differences across groups 1, 2, 3, and 4, with group 5 showing the greatest impact. While the concentrations of potassium, magnesium, and phosphorus in blood plasma did not significantly differ among the groups, group 5 demonstrated the highest concentrations of potassium, magnesium, and phosphorus numerically. Conversely, the control group had the most notable effect on blood salt levels. These findings underscore the varied metabolic responses associated with different dietary treatments and highlight the intricate relationship between rumen fermentation dynamics and blood metabolite concentrations in ruminant animals.

Alterations in blood parameters can result from various factors influencing ruminal propionate synthesis, such as diet composition, supplement source, and dosage (Spears et al., 2004). Minor discrepancies in plasma sodium and potassium levels could be associated with dietary alterations in these minerals, as excess sodium and potassium are excreted by the kidneys. Similarly, findings by West et al. (1991) align with our study, demonstrating no noticeable changes in plasma potassium and sodium levels with the elevation of DCAD from -116 to +312 mE/kg DM. These observations highlight the nuanced interplay between dietary components, rumen metabolism, and blood chemistry, underscoring the complexity of nutritional interventions in ruminant physiology.

## Conclusion

In conclusion, this study elucidated the intricate relationship between dietary cation-anion difference (DCAD) and various physiological parameters in male Zandi sheep under heat stress conditions. Our findings underscored the significant impact of DCAD on rumen fermentation characteristics, blood metabolite levels, and nutrient digestibility. Notably, supplementation with different DCAD concentrations resulted in distinct responses in dry matter intake, crude protein digestibility, and ruminal pH among treatment groups. The observed variations in blood metabolites, including glucose, cholesterol, and electrolyte levels, further highlight the systemic effects of DCAD manipulation on metabolic homeostasis in sheep. Moreover, our results suggest that alterations in DCAD levels can modulate rumen fermentation dynamics, influencing the synthesis of volatile fatty acids and ammonia production. The enhanced dry matter digestibility observed with specific DCAD treatments underscores the potential of dietary interventions to optimize nutrient utilization and metabolic efficiency in ruminant livestock. Furthermore, the differential responses in blood parameters emphasize the importance of considering DCAD as a crucial determinant of metabolic health and performance in sheep. It is important to note that additional research is warranted to elucidate the underlying mechanisms governing the observed physiological responses to DCAD manipulation. Longitudinal studies evaluating the long-term effects of DCAD supplementation on growth performance, reproductive outcomes, and overall

health parameters would provide valuable insights into its practical implications for sheep production systems. Furthermore, exploring the synergistic effects of DCAD with other dietary interventions, such as fiber sources and microbial additives, could offer novel strategies for enhancing nutrient utilization and mitigating heat stress in sheep populations.

**Author Contributions**

In this work, M Khani, A Fattah, S Joezy-Shekalgorabi, and SR Ebrahimi-Mahmoudabad planned, carried out, and contributed to the data collection and interpretation of the experimental outcomes all research procedures and animal studies. GJ Cho helped with the manuscript's composition. After reading the published version of the manuscript, all writers have given their approval.

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**Conflict of interest**

The authors declare no conflict of interest. Acknowledgments

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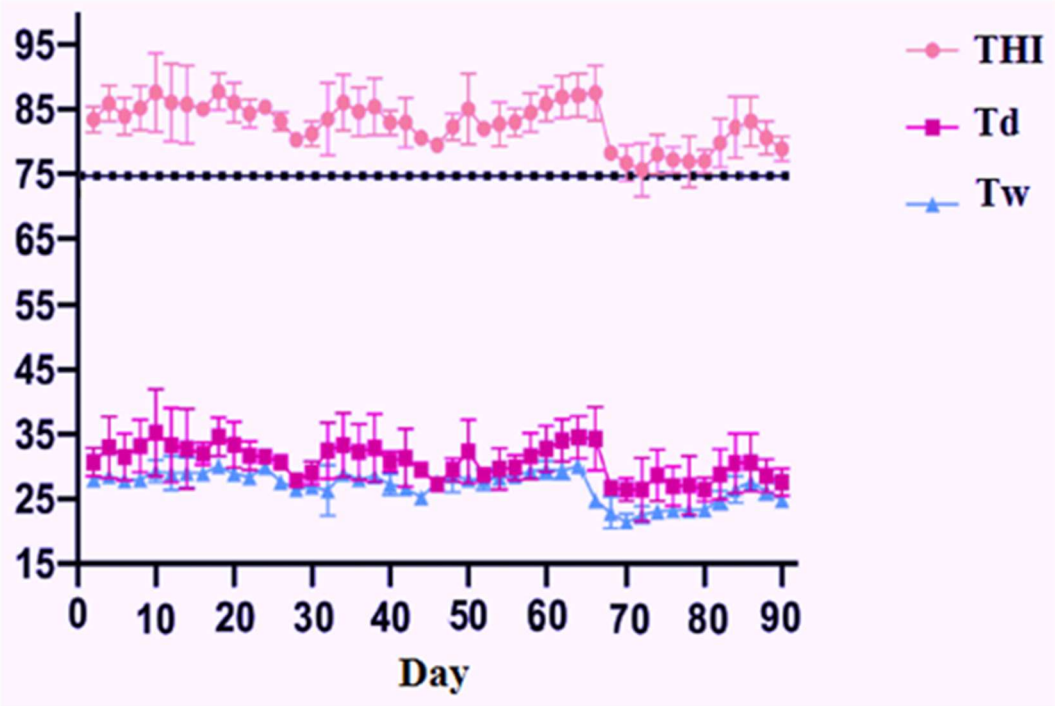


**Fig. 1: The daily average temperature-humidity index (THI), dry bulb temperatures (Td) and wet bulb temperatures (Tw) during the experimental period. The horizontal line at 75 indicates the threshold for heat stress.**

**Table 1: Ingredients and chemical components of diet for lambs (diets were formulated according to the recommendations of NRC 2001 and gradually provided to lambs. This experiment included five diets with the same energy and protein levels).**

| Items | Group 1 (+150 mEq/kg DM) | Group 2 (+300 mEq/kg DM) | Group 3 (+450 mEq/kg DM) | Group 4 (+600 mEq/kg DM) | Group 5 (+750 mEq/kg DM) |
|---|---|---|---|---|---|
| **Ingredients (gr/d)** | | | | | |
| Alfalfa hay | 400 | 400 | 400 | 400 | 400 |
| Wheat straw | 100 | 100 | 100 | 100 | 100 |
| Corn meal | 300 | 300 | 300 | 300 | 300 |
| barley | 300 | 300 | 300 | 300 | 300 |
| Corn silage | 350 | 350 | 350 | 350 | 350 |
| wheat bran | 120 | 120 | 120 | 120 | 120 |
| Soybean meal | 100 | 100 | 100 | 100 | 100 |
| Salt | 10 | 10 | 10 | 10 | 10 |
| $Na_2CO_3$ | - | 7 | 15 | 23 | 31 |
| NaHCO3 | - | 7 | 15 | 23 | 31 |
| Chemical components | | | | | |
| Dry matter | 93.91 | 93.01 | 93.23 | 92.98 | 92.89 |
| Crude protein | 13.41 | 13.48 | 13.51 | 13.54 | 13.4 |
| Neutral detergent fiber | 39.94 | 39.75 | 39.52 | 39.44 | 39.32 |
| Acid detergent fiber | 30.75 | 30.64 | 30.52 | 30.44 | 30.41 |
| Crude ash | 12.28 | 12.12 | 12.17 | 11.98 | 11.85 |
| DCAD[1] (mmol/kg DM) | +150 | +300 | +450 | +600 | +750 |

1DCAD, dietary cation-anion difference

**Table 2: Effect of varying dietary cation-anion difference on the plasma metabolites and nitrogen balance of male lambs.**

| Items | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Group 1 (+150 mEq/kg DM) | Group 2 (+300 mEq/kg DM) | Group 3 (+450 mEq/kg DM) | Group 4 (+600 mEq/kg DM) | Group 5 (+750 mEq/kg DM) | p-Value |
| Ca (mmol/L) | 2.82[a] | 2.71[ab] | 2.64[ab] | 2.61[ab] | 2.42[b] | <0.05 |
| Glc (mmol/L) | 5.15[a] | 4.21[b] | 3.36[c] | 3.55[c] | 3.40[c] | <0.05 |
| CHOL (mmol/L) | 16.46[b] | 16.55[b] | 15.22[b] | 15.13[b] | 18.12[a] | <0.05 |
| P (mmol/L) | 1.45 | 1.56 | 1.57 | 2.05 | 2.17 | 0.75 |
| Mg (mmol/L) | 1.13 | 1.00 | 1.76 | 1.30 | 1.76 | 0.82 |
| K (mmol/L) | 3.39 | 3.30 | 3.44 | 3.50 | 3.52 | 0.84 |
| Na(mmol/L) | 124[a] | 108[abc] | 111[ab] | 94[bc] | 84[c] | <0.05 |
| Cl (mmol/L) | 112.50 | 112.00 | 111.50 | 111.00 | 110.23 | 0.32 |
| Nitrogen balance (g/d) | 9.65 | 9.40 | 11.80 | 12.39 | 12.06 | 0.83 |

DCAD, dietary cation-anion difference; CHOL, cholesterol; Na, sodium, K, potassium; Mg, magnesium; P, phosphorus. K, potassium; Mg, magnesium; P, phosphorus.

**Table 3: Effects of DCAD and concentrate level (conc) on the rumen fermentation parametersof male lambs.**

| Items | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Group 1 (+150 mEq/kg DM) | Group 2 (+300 mEq/kg DM) | Group 3 (+450 mEq/kg DM) | Group 4 (+600 mEq/kg DM) | Group 5 (+750 mEq/kg DM) | p-Value |
| pH | 6.54$^{b}$ | 6.68$^{ab}$ | 6.64$^{ab}$ | 6.71$^{ab}$ | 6.82$^{a}$ | <0.05 |
| Total VFA (mmol/L) | 75.15 | 72.21 | 71.36 | 72.65 | 73.40 | 0.33 |
| Acetate (%) | 61.46 | 56.55 | 58.22 | 55.13 | 54.12 | 0.44 |
| Propionate (%) | 19.44 | 19.10 | 18.53 | 18.01 | 17.83 | 0.24 |
| Butyrate (%) | 13.60 | 13.31 | 13.10 | 13.04 | 13.04 | 0.75 |
| Valeric (%) | 0.34 | 0.33 | 0.33 | 0.31 | 0.30 | 0.84 |
| Isovaleric (%) | 1.14$^{a}$ | 0.96$^{b}$ | 0.98$^{b}$ | 0.84$^{b}$ | 1.01$^{ab}$ | <0.05 |
| A/P[1] | 3.74 | 3.20 | 3.10 | 3.08 | 3.01 | 0.86 |
| Ammonia (mg/L) | 112.50 | 112.00 | 111.50 | 111.00 | 110.23 | 0.32 |

1A/P: Acetic acid/propionic acid.

**Table 4. Effects of dietary cation and anion difference on dry matter intake, organic matter digestibility and crude protein digestibility in lambs.**

| Items | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Group 1 (+150 mEq/kg DM) | Group 2 (+300 mEq/kg DM) | Group 3 (+450 mEq/kg DM) | Group 4 (+600 mEq/kg DM) | Group 5 (+750 mEq/kg DM) | p-Value |
| DMI (g/kg BW) | 31.60[b] | 36.75[a] | 38.11[a] | 38.21[a] | 37.11[a] | <0.05 |
| Nutrient intake (g/kg BW/d) | | | | | | |
| Organic matter | 30.46[b] | 34.67[a] | 35.35[a] | 35.13[a] | 34.12[a] | <0.05 |
| Crude protein | 4.44[b] | 5.46[a] | 6.53[a] | 6.42[a] | 6.83[a] | <0.05 |
| Neutral detergent fiber | 16.60 | 16.90 | 19.10 | 19.07 | 19.04 | 0.09 |
| Acid detergent fiber | 8.34 | 8.56 | 9.33 | 9.59 | 9.30 | 0.84 |
| Apparent digestibility (%) | | | | | | |
| Dry matter | 73.74[b] | 74.40[a] | 77.10[a] | 78.75[a] | 80.01[a] | <0.05 |
| Organic matter | 61.50[b] | 68.05[a] | 73.50[a] | 74.86[a] | 76.23[a] | <0.05 |
| Crude protein | 65.87[b] | 73.03[a] | 77.05[a] | 78.08[a] | 78.22[a] | <0.05 |