Ration with Different Dietary Cation Anion to Mineral Status of Blood and Urine Garut Ewes

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ABSTRACT

The objectives of the present experiment were to evaluate the effect of dietary cation-anion difference (DCAD) on mineral status in blood and urine. Rations with DCAD value of -28, -18, 0, +14, and +32 mEq were offered to 15 ewes in a randomized complete block design. On day 21, blood samples were taken anaerobically using heparinized syringes from the coccygeal jugular venipuncture. Each syringe was capped and placed on ice immediately following collection to determine on plasma Na, K, Cl, Ca, and P concentrations. The DCAD values had no effect on plasma Na, K, Cl, Ca, and P concentrations indicating that there was homeostasis to maintain the physiological status of the body. The DCAD value of -18, 0, +14 and +32 mEq resulted in the normal blood with Na:K ratio closed to 20:1. Plasma Cl concentration was associated with plasma Na, but the concentration of Cl was lower than that of Na. The DCAD values significantly influenced P urine, but had no effect on urinary Na, K, Cl, S, and Ca. The DCAD value of -28 and -18 mEq resulted in the low acidity of urine at level of 5.73 ± 0.20 and 5.84 ± 0.27, respectively. The DCAD value of 0, +14, dan +32 mEq resulted in normal urinary pH. Rations with DCAD values of -18, 0, +14, and +32 mEq in garut ewes had normal ratio of plasma's Na^+:K^+ and were able to perform regulation of minerals control inside their blood to be homeostatis, and some excessive minerals would be secreted through urine. Rations with DCAD values of -28 and -18 mEq in garut ewes had the highest ratio of plasma's Ca^{2+}:P^{2-} which was 2.2:1.0, so it could be used as an action to prevent milk fever.

Key words: dietary cation-anion difference, blood, urin, ewe

INTRODUCTION

Consumed ration will affect physiological condition of the livestock. According to Stewart (1983), addition of anions (Cl and S) into the ration would lower the pH of body fluid. Blood condition is the result of acid base balance in body fluid and regulation of nutrition metabolism inside it. Blood is consisted of cells and plasma. Plasma contains water as much as 90% and anorganic minerals in form of soluted ion as electrolytes, proteins, metabolic waste products, respiration gases and hormones. Concentration of this combined ion is important for maintenance of blood osmotic balance. Acid base balance is highly affected by the function of lungs and kidneys.

Kidneys have vital role as controller of volume and composition of blood chemicals by secreting solution and water selectively. Vital functions of kidneys are done by the filtration of plasma through *glomerulus* followed with reabsorption some amount of solution and water with correct volume along the kidneys tubulus. The excess of solution and water will be secreted out as urine through collector system. Epitelic cells help maintaining constant pH of body fluid by controlling secretion of hydrogen ion. Secretion of acid in urine as the result of potential acid and H^+ formation rate from blood buffer. Acidic urine is also secreting Ca^{2+}.

In this research, the rations experimented with DCAD values of -28, -18, 0, +14, and +32 mEq. The objectives of this research was, to identify the effect of different DCAD to mineral in blood and urine of Garut ewes (*Ovis aries*).

MATERIALS AND METHODS

This experiment was conducted at the Pen Field Laboratorium A of Animal Husbandry Faculty and Integrated Laboratorium of Veterinary Faculty Bogor Agricultural University on January 11th - July 14th 2007.

Experimental Design and Animal Care

Fifteen Garut ewes were 2.50 ± 0.25 years old were assigned randomly to randomized complete block design. The ewes were blocked into groups of 3 according to (I) ewes previously had twin female offsprings; (II) ewes previously had twin male offsprings; and (III) ewes previously had twin male and female offsprings. Ewes were housed and fed in individual cage. The composition of basal ration contained 89.30% dry matter, 8.12% ash, 15.00% crude protein, 5.12% ether extract, 14.73% cude fiber, and 57.03% nitrogen free extract (Tabel 1).

Determination of crude protein ration contents of 15.00 % based on Wodzicka-Tomaszewska *et al*. (1991), Na mineral of 0.09 - 0.18 %, K of 0.50 - 0.80 % (maximum 3.00 %), Cl had no clause (based on NRC, 1985).

The value of basal rations DCAD was +14 mEq/100 g of DM and treatment rations in this research with five dietary cation anion difference values (DCAD).

1. -28 mEq = basal ration added with 14.375 mEq S and 27.884 mEq Cl
2. -18 mEq = basal ration added with 14.375 mEq S and 17.884 mEq Cl
3. 0 mEq = basal ration added with 14.259 mEq S
4. +14 mEq = basal ration
5. +32 mEq = basal ration added with 10.21 mEq Na and 7.531 mEq K

Method of operating decreasing of DCAD value to 0 mEq/100 g of DM with basal ration was added $CaSO_4$ (Brataco Chemika, Cikarang, Jakarta). Decreasing DCAD to -28 and -18 mEq/100 g of DM with basal ration were added $CaCL_2$, dan $CaSO_4$ (Brataco Chemika, Cikarang, Jakarta). The value of DCAD increased to +32 by addition of Na_2CO_3 and K_2CO_3 in basal ration (Brataco Chemika, Cikarang, Jakarta). Treatment rations had been offered for three weeks before the samples were collected.

Sample Collection and Analysis

On 21st day after treatment rations were offered, blood samples were taken anaerobically using heparinized syringes (Franklin Lakes NJ USA) from the coccygeal jugular venipuncture. Then, the blood were centrifuged for 15 minutes at 3000 rpm. Afterward, the plasma were taken to be analyzed for its Na, K, and Ca contents by using Automatic Absorbance Spectrofotometer (AAS), while Cl, P, and S by titration.

Sample of urine were collected by using plastic apron in the morning around 07.00 - 08.00 o'clock. The urine were tested for its pH by using pH-meter pocket HANNA, then the urine were analyzed for its Na, K, Ca, Cl, P, and S mineral contents by using the same method with the blood sample.

Statistical Analysis

Data were analyzed with GLM procedure in SAS System for Windows 6.12. Treatments effects were compared using the multiple comparation approach of Duncan Multiple Range Test. Regression analyses were conducted with the Proc REG procedure, whereas correlation corficients were obtained from the Proc CORR procedure of SAS (Mattjik and Sumertajaya, 2006).

Tabel 1. Composition and nutrient content of basal ration base on dry matter

Feed type	Percent	Proximate analysis (%)					Minerals (%)			
		Ash	CP	EE	CF	NFE	Na	K	Cl	S
Corn forage	35.0	2.81	3.24	0.43	10.18	18.34	0.020	0.305	0.011	0.070
Rice bran	6.0	0.63	0.59	0.84	0.65	3.30	0.000	0.079	0.014	0.008
Onggok	9.5	1.83	0.26	0.13	0.83	6.45	0.007	0.017	0.002	0.003
Corn meal	18.5	0.26	1.48	0.55	0.42	15.79	0.001	0.068	0.002	0.013
Coconut meal	7.0	0.43	1.17	1.01	1.08	3.31	0.007	0.144	0.053	0.017
Soybean meal	22.0	1.98	8.24	0.37	1.58	9.84	0.001	0.458	0.111	0.041
Fish oil	2.0	0.18	0.03	1.79			0.003	0.006	0.000	0.000
Total	100	8.12	15.00	5.12	14.73	57.03	0.039	1.077	0.192	0.152

Note: CP = crude protein Na = natrium EE = ether extract K = kalium CF = crude fiber Cl= chlor
NF = nitrogen-free extract S = sulfur

RESULTS AND DISCUSSION

Blood Acidity and Blood Plasma Mineral Status

Average data of Na^+, K^+, Cl^-, S^{2-}, Ca^{2+}, and P^{2-} of garut ewes' blood plasma offered with rations with various DCAD values were presented on Table 2. The DCAD values had no effect (P>0.05) on plasma's Na^+. The differences of DCAD values were consumed by garut ewes had no effect on plasma's Na^+ (P>0.05). It means that, the ewes succeeded in performing homeostatis. Average values of Na^+ of experimented ewes's plasma were ranging 17308 ± 3281 to 18397 ± 1915 ppm (Table 2). The amount of plasma's Na^+ was not related to DCAD value (r = 0.01). Hu and Murphy (2004) stated that there was no effect of DCAD on Na^+ of blood plasma. However, Roche *et al*. (2005) reported that the increase of DCAD value, would increase Na^+ of blood plasma. Ewes offered with rations with DCAD value of -28 mEq produced acidic blood (Fathul *et al.,* 2008) so the Na^+ of blood plasma in ewes offered with rations with DCAD value of -28 mEq were relatively lower than those offered with rations with DCAD values of 0, +14, dan +32 mEq.

The DCAD values had no effect (P>0.05) on plasma's K^+. The differences of DCAD values were consumed by garut ewes had no effect on plasma's K^+ (P>0.05). It means that, the ewes also succeeded in performing homeostatis. Average values of K^+ of experimented ewes' plasma were ranging 811 ± 268 to 983 ± 183 ppm (Table 2). The amount of plasma's K^+ was not related to DCAD value (r = -0.16).

At normal condition, extracellulic fluid performed balance between Na^+ and K^+ at constanta of 20:1 (Georgievskii, 1982). The ewes on this research performed homeostatis, the contents of Na^+ and K^+ of blood plasma were not affected by DCAD. But, the body regulated K^+ value inside the plasma to always lower than Na^+ value. The relatively highest average value of plasma's K^+ (983 ± 183 ppm) was found in ewes offered with rations with DCAD value of -28 mEq. This was because in those ewes there were occured metabolic acidosis indicated by the decrease of blood's HCO_3 concentration (-2.53±2.42 mmol/L) (Fathul *et al.,* 2008). Therefore, the decrease of blood's HCO_3 concentration would be followed by alteration of plasma's K^+ so plasma's K^+ became relatively highest.

On Table 2, ewes offered with rations with DCAD values of -28, -18, 0, +14, and +32 mEq had each ratio plasma's Na^+:K^+ of 18:1, 20:1, 20:1, 20:1, and 19:1, respectively. The values ratio plasma's Na^+:K^+ in ewes offered with rations with DCAD value of -18, 0, +14, and +32 mEq (approximately 19 : 1 − 20:1) were close to normal because the normal ratio plasma's Na^+:K^+ was 20:1. Ewes offered with rations with DCAD values of -28 had ratio plasma's Na^+:K^+ of 18:1, it meant that its plasma's Na^+ was lower and its plasma's K^+ was the highest compared with ewes offered with other DCAD values. Odongo *et al.* (2006) stated that metabolic acidosis was acid-base upset caused by the decrease of blood's [HCO_3^-] and generally, followed by alteration of [K^+] to become hyperkalemia (Weiderseiner *et al*., 2004).

Ewes offered with rations with DCAD value of -28 mEq had the lowest blood's HCO_3^- and included in metabolic acidosis (Fathul *et al*., 2008), so it had relatively the most K^+ content. High content of K^+ in blood was called hyperkalemia.

Table 2. Average of Na^+ , K^+, Cl^-, S^{2-}, Ca^{2+}, and P^{2-} of garut ewes' blood plasma offered with different DCAD

Variables	Dietary cation-anion difference (mEq)				
	-28	-18	0	+14	+32
Na^+ (ppm)	17308 ± 3281	18547± 1661	18397 ± 4940	16520 ± 516	18397 ± 1915
K^+ (ppm)	983 ± 183	926 ± 174	918 ± 104	811 ± 268	945 ± 55
Cl^- (ppm)	4580 ± 646	4449 ± 82	4509 ± 268	4307 ± 102	4698 ± 182
S^{2-} (ppm)	63 ± 18^a	35 ± 2^b	33 ± 2^b	32 ± 5^b	29 ± 3^b
Ca^{2+} (ppm)	473 ± 27	471 ± 22	449 ± 20	421 ± 44	426 ± 43
P^{2-} (ppm)	211 ± 114	217 ± 51	320 ± 50	331 ± 51	339 ± 25
Na^+:K^+	18 : 1	20 : 1	20 : 1	20 : 1	19 : 1
Ca^{2+}:P^{2-}	2.2 : 1.0	2.2 : 1.0	1.4 : 1.0	1.3 : 1.0	1.3 : 1.0

Note: value with different letter on same row mean different (P<0.05).

This, maybe because the ewes offered with rations with DCAD value of -28 mEq had very acidic blood pH and there was occured metabolic acidosis, eventually ratio Na^+:K^+ was not at normal condition.Ratio Na^+:K^+ had to be performed by the livestocks in order homeostatis. Determination of Na^+:K^+ homeostasis mechanism inside the body was done by kidneys. Regulation of Na^+:K^+ homeostasis was involving corticoid-aldosterone and deoxycorticosterone mineral which acted on K^+ secretion in consequence of reabsorption of Na^+ ion inside the kidneys' ducts. Corticoid mineral was also likely affecting the regulation of membrane permeability and Na^+:K^+ pair mechanism (Pratas, 1992).

Block (1994) explained that the unbalance of one ion with another, will caused poisoning that produced alkalosis or acidosis. This was likely because the unbalance of HCO_3^- and H^+ variables. If the presence of Na^+ was not enough to initiate absorption of NaCl (neutral), the excess of HCO_3^- in blood vessels could drive to acidosis condition. Further explained by Horst *et al*. (1997) that Cl^- was absorped more than SO_4^{2-} so Cl^- was a stronger aciditive to acidified the blood. Acid-base balance was related to exchange of H^+ ion internal media component which was able to donate or recieve ion. Substances that was able to donate ion was acid, while the one that was able to bind hydrogen was base.

The DCAD values had no effect (P>0.05) on plasma's Cl^-. The differences of DCAD values consumed by garut ewes had no effect on plasma's Cl^- (P>0.05). It means that, the ewes succeeded in performing homeostatis. Average values of Cl^- of experimented ewes' plasma were ranging 4307 ± 102 to 4698 ± 182 ppm (Table 2). The amount of plasma's Cl^- was not related to DCAD value (r = 0.07). The content of plasma's Cl^- was following presence trend of plasma's Na, but the amount of Cl^- was lower than Na and the presence of Na^+ to form NaCl (neutral). Ewes offered with rations with DCAD value of -28 mEq had the lowest plasma's Na so to perform neutralization with Cl^- was relatively fewer than ewes offered with rations with other DCAD values.

The DCAD values had very high effect (P<0.01) on plasma's S^{2-}. Ewes offered with rations with DCAD value of -28 mEq had the highest plasma's S^{2-} (P<0.05) as much as 63.38 ± 17.94 ppm. Ewes offered with rations with DCAD value of -18, 0, +14, dan +32 mEq had no differences on plasma's S^{2-} (P<0.05). The amount of plasma's S^{2-} was highly related to DCAD value (r = -0.67).

The DCAD values had no effect (P>0.05) on plasma's Ca^{2+}. The differences of DCAD values consumed by garut ewes had no effect on plasma's Ca^{2+} (P>0.05). It meant that, the ewes also succeeded in performing homeostatis. Average values of Ca^{2+} of experimented ewes' plasma were ranging 421 ± 44 to 473 ± 27 ppm. The relatively highest average value of plasma's Ca^{2+} (473 ± 27 ppm) was found in ewes offered with rations with DCAD value of -28 mEq. This was because the ewes offered with rations with DCAD values of -28 and -18 mEq had very acidic blood. That acidic condition would increase cells of intenstines tissue's sensitivity to paratyroid hormone (PTH) so the absorption of Ca^{2+} on intestines increased. In addition, acidic condition increased synthesis of 1.25 dihydroxyvitamin D3 from 25 hydroxyvitamin D3 by 1α-hydroxylase enzyme in the kidneys so increasing reabsorption of Ca^{2+} from glomerular filtrate. Therefore, ewes offered with rations with DCAD value of -28 and -18 mEq had more plasma's Ca^{2+} than those offered with other DCAD values. Block (1994) stated that the increase of ration's anion, would increase reabsorption of osteoclastic bones and increase synthesis of 1.25 dihydroxyvitamin D3 regulated by PTH. Paratyroid hormone also regulated reabsorption of Ca^{2+} and HPO_4^{2-}.

Ewes offered with rations with different DCAD values performed ratio Ca^{2+}:P^{2-} inside the blood therefore obtained different number of ratio, depended on cells of intenstines tissue's sensitivity to PTH, 1.25 dihydroxyvitamin D3, and PTH utilization depended on its blood acidity. Experimented ewes offered with rations with various DCAD values had ratio of plasma's Ca^{2+}:P^{2-} ranging from 1.3:1.0 to 2.2:1.0 (Table 2). Ewes offered with rations with DCAD values of -28 and -18 mEq had the highest ratio of plasma's Ca^{2+}:P^{2-} (2.2:1.0) than ewes offered with rations with other DCAD values. Ratio of normal plasma's Ca^{2+}:P^{2-} balance was 2:1 (Georgievskii et al. 1982). If the ratio of plasma's Ca^{2+} : P^{2-} < 2:1, it was likely that thelivestock would have milk fever especially diary cattles with high diary production. Therefore, rations supplies at late months pregnancy (dry condition) could act as prevention to milk fever. The amount of plasma's Ca^{2+} was highly related to DCAD value (r = -0.59) and less related to plasma's K (r = 0.47).

Block (1994) explained that low DCAD value could reduce hypokalsemia peripartum by the increase of Ca^{2+} ion in blood and urine. Addition of Ca^{2+} in blood was caused by the decreasing DCAD value (low) which causing Ca^{2+} had homeostatis by increasing absorption on intestines so that also increasing the secretion (Schonewille *et al.*, 1994; Roche *et al.*, 2003b). Findings of this research were appropriate with ideas of Moore et al. (2000), Roche *et al*. (2003), and Charbonneau *et al.* (2006) who stated that the decrease of DCAD value would cause increase of blood Ca^{2+}. Relation between plasma's Ca^{2+} and plasma's K was explained by Yingst *et al*. (2001) that K^+ ion increased the pump of Na^+ in order to increase the concentration of Ca^{2+} so that free Ca^{2+} in blood was increased by the pump of Na^+ in some cells. In the other part, polarization affected the decrease of K^+ concentration in order to perform K^+ balance (Quinn et al., 1987).

The DCAD values had no effect (P>0.05) on plasma's P^{2-}. The differences of DCAD values consumed by garut ewes had no effect on plasma's P^{2-} (P>0.05). It meant that, the ewes were trying to perform homeostatis. Average values of P^{2-} of experimented ewes' plasma were ranging 211 ± 44 to 473 ± 27 ppm. The amount of plasma's P^{2-} was quite highly related to DCAD value (r = 0.67) but not related to blood pH (r = 0.17).

In this research, the order of plasma's mineral from the most to the less were Na^+ which then followed by Cl^-, K^+, Ca^{2+}, P^{2-}, and S^{2-}. Isnaeni (2006) stated that in extracellulic fluid or blood plasma the order of mineral from the most to the less were Na^+, Cl^-, K^+, Ca^{2+}, P^{2-}, and Mg^{2+}.

Urinary Mineral Status

The DCAD values had high effect (P<0.01) on urinary pH. Ewes offered with rations with DCAD values of -28 and -18 mEq had the lowest urinary pH (P<0.05) each of 5.73 ± 0.20 and 5.84 ± 0.27, because in rations with DCAD values of -28 and -18 mEq there were addition of $CaCl_2$ and $CaSO_4$ anionic minerals. It was known that body of livestock perform homeostatis so the excess of Cl^- and S^- were secreted outside the body. Secretion of S^- dan Cl^- excess was through urine so the urine would become more acidic because Cl^- dan S^- were acidic. Value of urinary pH was the picture of cation-anion of consumed rations. If the livestock consumed excessive anion, its urine will be acid. Otherwise, if the Na^+ was consumed excessively, urinary pH will became base. Rations with DCAD values of -28 and -18 mEq there were many addition of acidic anionic salts, while in rations with DCAD value of +32 mEq there were many addition of base cationic salts. So ewes offered with rations with DCAD values of -28 and -18 mEq had the lowest urinary pH than those offered with other DCAD values, but ewes offered with rations with DCAD values of +32 mEq had the highest urinary pH. This had been explained by Chan *et al.* (2006) that the decrease of urinary pH was reflection of the effect from anion contained in the rations. Urinary pHvalues caused by consuming ratios with DCAD value of -28 and -18mEq were 5.7373 ± 0.20 dan 5.84 ± 0.27, respectively; those urinary pH were acid because the urinary pH were <6.0. Ewes offered with rations with DCAD values of 0, +14, and +32 mEq had urinary pH of 7.60 ± 0.51, 7.51 ± 0.78, and 8.28 ± 0.33, respectively; those urinary pH were normal because the urinary pH were between 7.50 - 8.50. Pratas (1992) stated that acidic urinary pH was <7.50; normal urinary pH was between 7.50 - 8.50; base urinary pH was >8.50. Moore *et al.* (2000) reported that if urinary pH was lower than 6.0, than the rations offered contained excessive anionic salts. Based on those facts above, the addition of anionic salts into the rations wih DCAD values of -28 dan -18 were excessive. Low urinary pH showed blood pH was also low (Vagnoni and Oetzel, 1998). This matched with this research, ewes offered with rations with DCAD value of -28 mEq had very acidic blood as well as the urinary pH. The urinary pH was highly related to DCAD value (r = 0.89). Roche *et al.* (2002) reported that the increase of K^+ consentration in rations would increase urinary pH. The increase of K^+ consentration in rations meant that there was an increase of DCAD value. This was similar to the findings by West *et al*. (1992), Moore *et al*. (2000), Riond (2001), Dersjant-Li *et al.* (2002), Roche et al. 2003, Borucki Castro *et al*. (2004), Hu and Murphy (2004), Roche *et al*. (2005), Apper-Bossard *et al*. (2006), Charbonneau (2006), and Kienzle *et al*. (2006); they stated that the increase of DCAD value would also increase urinary pH.

Body would always balancing its body fluids, in this case, the one that took role was kidneys. The excess of anion or cation carried by rations would be regulated by kidneys to be secreted through urine. Renal tubular cells responded directly to the changes in blood pH and intracellular pH. Kalium ion was moving

from cells into the blood by releasing H^+. The body cells pumped the excess of H^+ ion into the urine. Ion of H^+ caused the decrease of pH. In this research, changes in urinary pH were matched with its rations' cation-anion balance. Escobasa *et al.* (1984) reported that the increase of Cl^- consumption on cattle would decrease its urinary pH.

The DCAD values had no effect (P>0.05) on urinary Na^+. The average values of experimented ewes' urinary Na^+ were ranging from 68 ± 10 to 907 ± 734 ppm (Table 3). Although the urinary Na^+ was not affected by DCAD value, but it close related (r = 0.66) with DCAD value. In Rations with DCAD value of +32 mEq there was addition of Na_2CO_3 salt, but there were no differences in plasma's Na^+ and urinary Na^+ (P>0.05) and normal urinary pH, so it meant that the addition of that Na_2CO_3 was not excessive.

The DCAD values had no effect (P>0.05) on urinary K^+. The average values of experimented ewes' urinary K^+ were ranging from 21258 ± 8874 to 39895 ± 12109 ppm (Table 3). Urinary K^+ content was not too related to DCAD (r = 0.35). In Rations with DCAD valeu of +32 mEq there was addition of K_2CO_3 salt, but there were no differences in plasma's K^+ and urinary K^+ (P>0.05) and normal urinary pH, so it meant that the addition of that K_2CO_3 was not excessive.

The DCAD values had no effect (P>0.05) on urinary Cl^-. The average values of experimented ewes' urinary Cl^- were ranging from 1400 ± 1329 to 3072 ± 990 ppm (Table 3). Urinary Cl^- content was not related to DCAD (r = 0.06), Cl^- consumption (r = 0.04), and blood Cl^- (r = 0.11).

The DCAD values had no effect (P>0.05) on urinary S^{2-}. The average values of experimented ewes' urinary S^{2-} were ranging from 66 ± 23 to 977 ± 456 ppm (Table 3). Urinary Cl^- content was highly related to DCAD (r = -0.72).

The DCAD values had no effect (P>0.05) on urinary Ca^{2+}. The average values of experimented ewes' urinary Ca^{2+} were ranging from 17 ± 27 to 2295 ± 1733 ppm (Table 3). Urinary Ca^{2+} content was highly related to DCAD (r= -0.65). Roche (1999) stated that there was an increase in absorption of Ca^{2+} and secretion of Ca^{2+} in diary cattle, if anionic salts were added in its DCAD rations.

The DCAD values had no effect (P>0.03) on urinary P^{2-}. The lowest urinary P^{2-} contents (P<0.05) were found in ewes offered with rations with DCAD values of -28, -18, 0, and +14 mEq each of 120 ± 67, 95 ± 30, 96 ± 36, and 203 ± 93 ppm, respectively, while the highest value (P<0.05) was found in ewes offered with rations with DCAD values of +32 mEq, which was 407 ± 191 ppm. This was because urinary P^{2-} was highly related (r = 0.82) to DCAD value. Secretion through urine was the main homeostatis in the regulation of Na^+, K^+, and Cl^- (Maltz and Silanikove, 1996) in order to maintain constant Na^+:K^+ ratio in extracellular fluids. On Table 2 appeared that ratio plasma's Na^+ : K^+ was close to normal balance (as much as 20:1) in rations with DCAD values of -18, 0, +14, and +32 mEq. It meant that the ewes consuming rations with DCAD values of -18, 0, +14, and +32 mEq were able to perform regulation of minerals control inside thier blood to be homeostatis, and some excessive minerals would be secreted through urine. Bannink *et al.* (1999) and Nennich *et al.* (2006) stated that that urine secretion was directly related with consumption of Na^+, K^+, and N. Maltz and Silanikove (1996) explained that urine secretion was the main method of homeostatis regulation. Furthermore, according to Price and Wilson (1995), minerals filtrated by kidneys were Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-.

Table 3. Average values of pH , Na^+, K^+, Cl^-, S^{2-}, Ca^{2+}, and P^{2-} in urine of garut ewes offered with different DCAD

Variables	Dietary cation-anion difference (mEq)				
	-28	-18	0	+14	+32
pH	5.73 ± 0.20[b]	5.84 ± 0.27[b]	7.60 ± 0.51[a]	7.51 ± 0.78[a]	8.28 ± 0.33[a]
Na^+ (ppm)	68 ± 10	94 ± 43	121 ± 82	460 ± 661	907 ± 734
K^+ (ppm)	27397± 8162	33039±6704	21258 ± 8874	36697 ± 16258	39895±12109
Cl^- (ppm)	2374 ± 2528	2870 ± 3014	1400 ± 1329	2192 ± 778	3072± 990
S^{2-} (ppm)	977 ± 456	1274 ± 676	630 ± 574	7 ± 117	66 ± 23
Ca^{2+} (ppm)	2295 ± 1733	2119 ± 1951	224 ± 323	272 ± 118	17 ± 27
P^{2-} (ppm)	120 ± 67[b]	95 ± 30[b]	96 ± 36 [b]	203 ± 93[b]	407 ± 191[a]

Note: values with different letters on same row mean different (P<0.05).

CONCLUSIONS

Rations with DCAD values of -18, 0, +14, and +32 mEq in garut ewes resulting normal ratio of plasma's Na^+:K^+ and were able to perform regulation of minerals control inside their blood to be homeostatis, and some excessive minerals would be secreted through urine. Rations with DCAD values of -28 and -18 mEq in garut ewes resulting highest ratio of plasma's Ca^{2+} : P^{2-} which was 2.2:1.0, so it could be used as an action to prevent milk fever.

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