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"Improving Food Security : The Challenges for Enhancing Resilience to Climate Change"

Volume II

The University of Lampung

Indonesian SEARCA Fellow Association

Southeast Asian Regional Center for Graduate Study and Research in Agriculture

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## LIST OF CONTENTS

# DIFFERENCE OF CATION AND ANION DIETS ON LEUCOCYTES DIFFERENTIATION OF LAMB DURING ESTROUS CYCLE

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## ABSTRACT

Blood status of rumen is important to determine physiological conditions; especially those are in estrous cycle. It is also known that diets affect the blood status of rumens, therefore this study was determined to evaluate leucocytes differentiation of lamb during estrous cycle given different cation and anion ratio on their diets. Five different cation and anion ratio diets were given to the lambs which already experienced with pregnancies, they were -28, -18, 0, + 14 and +32 meq (of dietary cation and anion different/DCAD), each was replicated by 3. Group A (-28 meq) were given normal diet with 0.230 g S + 0.446 g Cl, group B (-18 meq) were given normal diet with 0.230 g S + 0.286 g Cl, group C (0 meq) were given normal diet with 14.259 g S, group D (+14 meq) were given normal diet only, group E (+32 meq) were given normal diet with 0.235 g Na + 0.523 g K. The blood variables were determined before and during estrus, they were the number of red blood cells (RBC/cells-$mm^{-3}$), Hb content (g %), hematocrit (%), and the leucocytes differentiation (leucocyte, monocyt, lymphocyt, eosinophil /cells $mm^{-3}$). ANOVA was applied to analyze the collecting data with α 5%, followed with LSD at α 5%. The result indicated that decreasing the cation on diet affected on lowering of RBC and Hb of the blood ($p \leq 0.05$) before estrus, while during estrus there was no significant different among blood variables.

**Key words:** Cation/anion, leucocytes, differentiation, lamb

## INTRODUCTION

Different cation and anion ratio on diet affect metabolism of animals, especially mammalian which undergo estrous cycle. This different cation and anion ratio on dietary is known improving the acid and base balance in which then effect the milk yield and feed intake (Hu and Murphy, 2004). Beside that cation and anion play important role in many

physiological activities, such as nerve function including hormone release, muscle contraction, etc.

Hematological parameters of ruminants particularly for goats and sheep are influenced by the biological condition of the animals themselves, such as age and sex (Egbre-Nwiyi *et al*, 2000). In which age and sex are correlated each other, particularly for the female that undergo estrous cycle. Therefore, this study was also try to evaluate any changes in blood/hematological parameters of the female lambs related to estrous and non estrous periods at given different cation and anion ratio on dietary.

## MATERIALS AND METHODS

Materials: The study was conducted in field work, Nutrition Lab of Animal Husbandry Faculty of Bogor Agriculture Institute (IPB) as well as in Lab of Rehabilitation Unit (URR) – Dept. Veterinary, Bogor Agriculture Institutes (IPB). Animal feed consisted of 65 days-Haway corn leaves, padi skin, cassava, corm meals, coconut and soy beans remnants, fish oil, minerals: Zn $SO_4$, $Na_2CO_3$, $K_2CO_3$, $CaCL_2$, and $CaSO_4$. Some medicinal substances provided by Nova Laboratories Sdn. Bhd. Sungai Pelek Sepang, Malaysia, *Vita vet* injectable solution, were also given, as well as Alben 10% as oral suspension for worm preventive. Hormone was also given, 0.3 g of progesterone EAZI-BREED™ (CIDR®). Others were cotton, alcohol, jelly (mix of 30.0 g carboxymetilcelulosenatrium, 100.0 g glicerol 85.0%, and 1000 ml renset vand metilparahydroxybenzoat 0.1%), glicerol, milique water, aquades. For animal sampels were used 15 ewes (*Ovis aries*) from Garut-West Java with average body weight of 22 – 36 kg.

Equipment used for this study food and drinking plates, buckets, O-Haus analytic balances with accuracy of 0.001 g, capacity of 2 kg, 10 kg, 100 kg. Tools for mixing the feed, syrink, venolject, vacuum tube lithium heparin 7 ml, micropipet ependorf (1.5 and 2.0 ml), plastic material, apron, speculum, CIDR setting aplicator, cortex, centrifuge (with 2500 rpm), Radiometer ABL 700 Series.

Methods: This conducting research used basic animal diet need with 15.0% crude protein based on Wodzicka-Tomaszewska *et al*. (1991). Proximate Analysis was applied to all animal diet and done in Laboratorium Pusat Antar Universitas (PAU) IPB. Mineral analysis of Na, K, Ca, Mg, P was conducted in Nutrition Lab of Animal Husbandry Faculty of Bogor Agriculture Institute (IPB) while Cl and S was done in Bogor – Center of Soil

Research (Puslitan – Bogor). *Dietary cation-anion different* (DCAD) was calculated based on Na, K, Cl, and S content of the total animal basic diet with formula generated by Tucker *et al*. 1992, as follow:

**DCAD (meq)= (Na+K) – (Cl+S) (meq/100 g BK diet)**

From this calculation the DCAD number was +14 meq/100 g of dry diet.

The treatment groups based on the cation-anion balance from basic diet then was modified with addition of cation or anion to form 28, -18, 0, dan +32 meq. The diet composition, amount of mineral added, and the nutrition content of treatment diets could be seen in Tabel 1.

**Tabel 1.** Diet composition, amount of additional mineral and nutrient content of treatment diets

| Criteria | DCAD treated diets (meq/100 g dry diet) | | | | |
|---|---|---|---|---|---|
| | -28 | -18 | 0 | +14 | +32 |
| Diet composition (% dry weight of basic diet) | | | | | |
| Corn leaves | 35.000 | 35.000 | 35.000 | 35.000 | 35.000 |
| Padi peels | 6.000 | 6.000 | 6.000 | 6.000 | 6.000 |
| Cassava | 9.500 | 9.500 | 9.500 | 9.500 | 9.500 |
| Corn meals | 18.500 | 18.500 | 18.500 | 18.500 | 18.500 |
| Coconut remnant | 7.000 | 7.000 | 7.000 | 7.000 | 7.000 |
| Soy beans remnant | 22.000 | 22.000 | 22.000 | 22.000 | 22.000 |
| Fish oil | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 |
| Minerals (g/kg dry weight of basic diet) | | | | | |
| $Zn\ SO_4$ | 0.124 | 0.124 | 0.124 | 0.124 | 0.124 |
| $CaSO_4$ | 9.881 | 9.881 | 9.700 | - | - |
| $CaCl_2$ | 6.974 | 4.965 | - | - | - |
| $Na_2CO_3$ | - | - | - | - | 4.015 |
| $K_2CO_3$ | - | - | - | - | 5.202 |
| Nutrient Content (% dry weight)* | | | | | |
| Dry weight* | 89.300 | 89.300 | 89.300 | 89.300 | 89.300 |
| Ash* | 8.118 | 8.118 | 8.118 | 8.118 | 8.118 |
| Crude Protein* | 15.003 | 15.003 | 15.003 | 15.003 | 15.003 |
| Crude Lipid/fat* | 5.118 | 5.118 | 5.118 | 5.118 | 5.118 |
| Crude Fiber* | 14.733 | 14.733 | 14.733 | 14.733 | 14.733 |
| Nitrogenless extraction * | 57.028 | 57.028 | 57.028 | 57.028 | 57.028 |

note:
* = Analyzed by Laboratorium Pusat Antar Universitas (PAU) IPB

Treatment diets of the research were:
1. A (-28 meq) = basic diet added with 14.375 meq (0.2300 g S) and 27.884 meq (0.4461 g Cl)
2. B (-18 meq) = basic diet added with 14.375 meq (0.2300 g S) dan 17.884 meq (0.286 g Cl)

3. C (0 meq) − basic diet added with 14.259 meq (0.2281 g S)
4. D (+14 meq)− basic diet only
5. E (+32 meq)− basic diet added with h 10.21 meq (0.235 g Na) dan 7.531 meq (0.5232 g K)

The animals were acclimated with mix diets for almost 2 months. Each animal was randomly put in metabolic cages and was fed and drink twice a day at morning (07.00 am) and afternoon (02.00 pm) *ad libitum*. A week later animals were given 10% suspension of Alben for worm preventive, 2 ml/animal.

Collecting data:

1. Data first collected when the animals were found nonpregnant for their body weights. They were fed-drink *ad libitum* and the amount of diets was determined and given for the next day.
2. At the 7th day, estrous cycle of animals were syncronized by using EAZI-BREED™ CIDR® (implantation in vagina was made for 13 day).
3. At the 13th day prior feeding period, blood was collected from jugularis vine, to determine blood parameters before estrous.
4. At the 13th day after implantation of CIDR, the CIDR was taken out from vagina of each animal.
5. At the 1st and 2nd day after CIDR was taken out, estrous condition of animals was observed and blood was collected from jugularis veins, for 2 – 3 ml and analyzed for the red blood cells (RBC), pack cell volume (PCV), Hemoglobin (Hb) and white blood cells (WBC), as well as their WBC differentiation. Blood collection followed methods presented by Jones and Allison (2007).

**Data Analysis**

All the collected data were analyzed by using ANOVA followed by LSD at 5% level in SPSS -17 program.

## RESULTS AND DISCUSSION

Effect of diets with different cation-anion ratio on blood parameters could be seen in the Table 2. Even though there was no statistical significant different among groups of different cation-anion ratio on diets for RBC, PCV, Hb, and WBC. There was decrease in RBC but increase in WBC during estrous. Meanwhile, the percentage of hemoglobin (Hb%) did not affected by different in cation-anion ratio of diets. All the blood parameters lay

within the normal number of blood cells (Kramer, 2000), in which the RBCs ranging from 8 – 18 $10^6$ cells/$mm^3$, PCV 22 – 38, and Hb 8 – 12 (g %).

**Table 2.** The effect of different cation-anion on diet in blood parameters

| Groups / Blood Parameters | | RBC ($10^6$)cells/$mm^3$ | PCV (%) | Hb (g %) | Leu/WBC ($10^3$) cells/$mm^3$ |
|---|---|---|---|---|---|
| Estrous | A | 11.56 ± 3.43 | 36.83 ± 5.77 | 11.33 ± 0.41 | 8.05 ± 1.33 |
| | B | 11.25 ± 1.57 | 34.92 ± 3.16 | 10.6 ± 1.22 | 9.22 ± 1.71 |
| | C | 10.77 ± 1.26 | 32.92 ± 0.88 | 9.8 ± 0.88 | 16.82 ± 10.33 |
| | D | 8.58 ± 1.16 | 33.0 ± 3.5 | 10.13 ± 0.42 | 19.68 ± 18.2 |
| | E | 9.57 ± 3.77 | 34.58 ± 4.84 | 10.47 ± 1.89 | 13.97 ± 4.58 |
| | $X_{ABCDE}$ | 10.34 + 0.62 | 34.45 + 0.77 | 10.39 + 0.26 | |
| Non-estrous | A | 12.29 ± 0.29 | 34.92 ± 2.13 | 11.27 ± 0.61 | 7.63 ± 3.36 |
| | B | 11.50 ± 0.76 | 34.75 ± 4.13 | 10.8 ± 1.51 | 6.46 ± 1.05 |
| | C | 10.28 ± 0.92 | 30.58 ± 1.23 | 9.60 ± 0.53 | 9.11 ± 1.48 |
| | D | 11.79 ± 1.21 | 33.67 ± 5.35 | 10.13 ± 1.03 | 8.13 ± 4.07 |
| | E | 11.69 ± 1.84 | 31.92 ± 2.27 | 10.13 ± 0.61 | 8.91 ± 5.68 |
| | $X_{ABCDE}$ | 11.51 + 0.30 | 33.17 + 0.86 | 10.47 + 0.28 | |

Increase in RBC of non-estrous groups could be accounted for preparation of estrous cycle, while fluctuation in WBC of each groups was not fully understood, but it likely to be affected by stress level of each animal, even though they were kept them in normal state and were acclimated in metabolic cages. In order to understand this phenomenon, the differentiation of the WBC was determined and can be seen in Table 3.

Given different cation-anion ratio on diets did not show any significantly different for differentiation of WBC in estrous groups, unlike those in non-estrous groups. However, both groups indicated that percentage of lymphocytes was the highest among other WBC differentiation. Different cation-anion ratio on diets affected the WBC differentiation of non-estrous groups. The percentage of neutrophils was far less in group given ration cation-anion +32 meq (E group) compared to others, but the number of lymphocytes was far more than others ($p<0.05$).

Tabel 3. Differentiation of leukocytes on different diets with different cation-anion ratio

| Groups Leucocytes Diff | | Neutophils (%) | Lymphocytes (%) | Monocytes (%) | Eosinophils (%) |
|---|---|---|---|---|---|
| Estrous | A | 39.00 ± 7.80 | 49.00 ± 3.00 | 3.33 ± 1.55 | 8.67 ± 5.50 |
| | B | 28.67 ± 8.02 | 51.00 ± 7.00 | 4.33 ± 1.53 | 16.00 ± 7.93 |
| | C | 30.00 ± 6.57 | 52.00 ± 7.00 | 3.00± 1.73 | 15.00 ± 3.00 |
| | D | 49.00 ± 34.22 | 34.67 ± 24.3 | 6.00 ± 6.00 | 10.33 ± 5.51 |
| | E | 37.33 ± 15.27 | 44.00 ± 16.64 | 3.67 ± 3.79 | 15.00 ± 5.29 |
| | $X_{ABCDE}$ | 36.80 ± 4.43 | 46.13 ± 3.49* | 4.07 ± 0.80 | 13.00 ± 1.46* |
| Non-estrous | A | 31.33 ± 15.04$^{ab}$ | 56.33 ± 10.12$^{b}$ | 7.00 ± 3.00 | 5.33 ± 3.05$^{b}$ |
| | B | 32.33 ± 17.39$^{ab}$ | 53.00 ± 9.64$^{b}$ | 6.00 ± 2.60 | 8.33 ± 5.51$^{b}$ |
| | C | 35.33 ± 8.38$^{ab}$ | 54.67 ± 8.08$^{b}$ | 5.00 ± 1.70 | 5.00 ± 2.00$^{b}$ |
| | D | 42.67 ± 0.58$^{a}$ | 50.33 ± 20.50$^{b}$ | 4.33 ± 1.53 | 15.67 ± 0.58$^{a}$ |
| | E | 14.33 ± 5.51$^{b}$ | 80.33 ± 3.06$^{a}$ | 4.37 ± 2.08 | 4.33 ± 1.16$^{b}$ |
| | $X_{ABCDE}$ | 31.20 ± 3.50 | 58.93 ± 3.88 | 5.33 ± 0.57 | 7.73 ± 1.30 |
| Normal [1] | | 30 – 48 | 50 – 70 | 0 – 4 | 1 – 8 |

$^{a,b}$ *Superscript at the means in the same column indicated significantly different LSD at 5%*
[1] Normal number based on Kramer, 2000
* Different at 5% between estrous and non estrous groups.

The number of neutrophils is likely affected by pathogen, like those in bovine in which the neutrophils of bovine mammary glands increased in cell numbers when *S. dysgalactiae* were injected to them (Blagitz *et al*, 2015). Since there was no infectious pathogen applied to these animals study, therefore, the normal number of neutrophils was gained for all of treatment groups, except the E group. Different cation-anion ratio on diet might be sufficient to control the neutrophils number. Meanwhile, the lymphocytes number was the highest among other WBC differentiation. It is known that lymphocytes are responsible for both humoral and cellular immunity, and in form of antibodies and in form of receptors of any antigen. The highest cation-anion ratio on diet seemed able to increase the production of lymphocytes in non estrous groups, but not during estrous. It seemed that production of lymphocytes was necessary prior estrous period. Seemingly, readiness of immunity of the female animals/lambs should be happened to ensure the breeding process. All treatment groups had the lymphocytes in nora, range based on Kramer (2000). Only those in group E,

the lymphocytes number was above the normal. No explanation could be generated for this effect. However, given different cation-anion ratio on diet (DCAD) with +32 mEq seemed affected in more alkaline, and might affect the dry matter intake of animals, just like those in Holsteins cows (Chan *et al*, 2005) and improved the amino acid availability for protein synthesis (Wildman, 2007), therefore the number of lymphocytes reached much greater than other treatment groups. Beside that, the production of WBC in lambs or goats is affected by certain minerals which absorbed in bone marrow and also by sex differences, in which male goats have higher lymphocyte number of cells as compared to females, while the females have a higher neutrophil number of cells as compared to the males (Tambuwal *et al*., 2002; Daramola *et al*., 2005).

When the sample animals were grouped from the estrous cycle, the number of eosinophils for both estrous and non estrous groups was significantly different. This could happen since the eosinophils work to fight bacterial or parasitic infectious as well as being produced to response with allergic. The estrous cycle then might affect this differences in cell numbers, even though both groups had number of cells still in normal range (Kramer, 2000) and no pathogenic particles

Based on the results we can conclude that the different cation-anion ratio on diet affected on the number of neutrophils and eosinophil and the increase in ratio cation-anion on diet of 32 mEq decreased in the number neutrophils but increased in the number of lymphocytes before estrous.

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