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Influence of diet cation-anion difference (DCAD) on plasma acid-base status in pregnant sheep

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Summary

The last trimester of pregnancy in sheep is frequently associated with pregnancy toxemia, usually connected with ketonemia and metabolic acidosis. Because a changed diet cation-anion difference (DCAD) is able to change the values of plasma acid-base balance (ABB) the authors sought to determine what kind of shift in ABB values is induced by diets with different DCAD. The authors also compare the influence of two diets with a different DCAD on some reproduction parameters in sheep. Although both diets used in our experiment had a positive DCAD, one of them was more alcalemic (high-DCAD) and the other more acidemic (low-DCAD). In the group of sheep fed with a +214.5 mEq/kg DM DCAD diet, blood pH was higher than under +46.2 mEq/kg DM condition. Significant changes, especially in the 15th and 16th week of pregnancy were observed. Under a high-DCAD condition, anion gap (AG) was elevated to 19.6 ± 2.62 mEq/l. No significant changes of AG in low-DCAD fed animals were observed. The values of AG in this group ranged between 18.02 ± 3.68 mEq/l – 19.15 ± 1.79 mEq/l. The feeding of sheep with +214.5 mEq/kg DM essentially resulted in negative values of a strong ion gap (SIG). In the second group, fed with lower DCAD, positive SIG (3.43 ± 0.55 mEq/l) occurred already in the 16th week of pregnancy. In this group of sheep, the litter size was higher and reached 1.29 ± 0.7. By employing forage with a known value of DCAD it is possible to influence not only on the health of sheep during the course of pregnancy but also to improve their reproductive parameters.

Keywords: diet cation-anion difference, metabolic acidosis, pregnancy, sheep

The plasma acid-base status of the extracellular fluids is directly affected by the concentration of strong basic cations and anions in the diet (9, 16, 18, 19). In recent years the measuring of the cations (Na^+, K^+) and anions ($Cl^–$, $S^{2–}$) have been introduced for the evaluation of diet cation-anion difference (DCAD) (1, 24). When animals are fed a more anionic (acidogenic) or more cationic (alkalogenic) diet, different acid-base balance responses may be evoked (7, 14). For example an anionic diet characterized by low DCAD led to an increase of blood H^+ and decrease of blood $HCO_3^–$, pH and urine pH (19, 20). Furthermore, many obtained results indicated that feeding a more anionic diet e.g. in late pregnancy, maintaining the sheep in mild metabolic acidosis, consequently leads to an increase of plasma ionized calcium and osteocalcin levels (6, 23). In this way a dam is protected against periparturien hypocalcaemia, which might exerts an additional depressive effect on endogenous glucose production in the presence of hypercetonemia (12, 15, 17, 18). The limiting of glucose availability is a crucial factor for the development of pregnancy toxemia occurring in the last trimester of sheep pregnancy.

It should be taken into account that there are only a few reports devoted to the influence of anionic and cationic diets given for longer pre-partum periods on the plasma acid-base status and mineral metabolism in sheep (4, 5, 10). Thus, the aim of our study was to compare the influence of two diets with different DCAD on the acid-base balance and some reproduction parameters in late-pregnant sheep.

Material and methods

Study design and diets. Sixteen clinically healthy pregnant Polish Lowland Sheep were used in our experiment. The animals were divided into two groups and fed with a different kind of diet. The mean body weight in each of groups reached 58.4 ± 6.2 kg (group I) and 79.5 ± 5.3 kg (group II). The diet of the first group contained hay (51%), sugar-beet (34%) and oat (15%). In the second group the diet was composed with sugar-beet pulp (46.3%), corn (46.35) and hay (7.4%).

Analytical procedures. To obtain a constant dry weight of diet samples, each of them was dried at 105°C. For the analysis of diet sulfur content to 0.5 g each of samples, 18 ml of digested mixture (70% perchloric acid: 60% nitric acid [5 : 1 v/v]) was added. After a double phase mineralization process (phase I 180°C/20 min, phase II 220°C/90 min), samples were transferred to a calibrated tube and the volume was brought to 25 ml with deionized water. The final concentration of sulfur (mg/kg) was performed by inductively coupled plasma spectrometry using Spectrometer ICP PS 950 (Leeman, USA) (8).

For sodium and potassium determination, 0.5 g each of dried samples was digested by concentrated nitric acid and hydrochloric acid (3 : 1 v/v) using a microwave oven (Multiwave 3000, Anton Paar). Afterwards the samples were quantitatively transferred into plastic flasks and taken for atomic absorption spectrometry measurements (Avanta PM, GBC). Absorbance for Na at 589 nm and for K at 766.5 nm was read.

Determination of diet chloride concentration (preparation, procedure and results calculation) was performed by the Volhard method according to the binding research protocol PB-Nr 03/CH/07.

Blood was collected three time in the 15^{th}, 16^{th} and 17^{th} week of pregnancy. Blood samples were drawn from the jugular vein into heparinized tubes. Gasometry and serum minerals concentration (Na, K, C and Cl) was performed by blood gas analyzer ABL80 Flex (Radiometer, Copenhagen).

The serum inorganic phosphate level was determined calorimetrically by the Fiske-Subbarow method, based on the reaction between inorganic phosphate and acidified ammonium molybdate. Absorbance against blank samples at 660 nm (Spectrometer UV-160A, Shimadzu) was measured (25).

Serum albumin was analyzed by the colorimetric bromocresol green method, with absorbance at 628 nm. Final albumin concentration was calculated as follows:

$$\Delta A_{sample}/\Delta A_{standard} \times C_{standard} = C_{sample}$$

A = absorbance,

C = concentration.

Anion gap (AG) was calculated from $(Na^+ + K^+) - (Cl^- + HCO_3^-)$ and strong ion gap (SIG) from AG – {[albumin g/dl] (1.2 × pH-6.15) + [phosphate mg/dl] (0.097 × pH-0.13)}(3).

To obtain the value of the difference between strong ions the simple formula: SID = $[HCO_3^-] + [Pr^{x-}] + [Pr^{y-}]$ was used, where Pr^{x-} is the value of the albumin electric charge, Pr^{y-} is value of inorganic phosphate electric charge (21).

Results and discussion

Both diets used in our experiment had a positive cation-anion difference (tab. 1). However, DCAD in the second group of animals was significantly ($p \leq 0.05$) lower than in the first group. Concomitantly with the lower DCAD a decrease in daily dry matter intake was observed. +46.2 mEq/kg DM of DCAD results in 0.01 ± 0.002 kg/day/sheep of DMI. There were marked differences in mineral concentrations between high- and low-DCAD diets. Those in the second group, with sugar-beet pulp, corn and hay, were characterized by a greater concentration of Na (990 mEq/kg DM), Cl (1868 mEq/kg DM) and S (229.8 mEq/kg DM). In the group of sheep fed with a +214.5 mEq/kg DM DCAD diet, the blood pH was higher than under +46.2 mEq/kg DM condition (fig. 1). Significant changes especially in 15^{th} week of pregnancy (I^{st} gr. – 7.43 ± 0.05; II^{nd} gr. – 7.39 ± 0.06) and 16^{th} (I^{st} gr. – 7.45 ± 0.04; II^{nd} gr. – 7.37 ± 0.06) were observed.

Tab. 1. Ingredients and composition of diets for pregnant sheep

	Experimental diets	
	I^{st} group	II^{nd} group
DCAD[a] (mEq/kg of DM)	214.5	46.2*
% composition of diets	Hay 51 Sugar-beet 34 Oat 15	Sugar-beet pulp 46.3 Corn 46.3 Hay 7.4
DM[b]	1.994	1.272*
mean DMI (kg/day/sheep)	0.03 ± 0.002	0.01 ± 0.002
ME[c] (Mcal/kg DM)	8.32	7.47
CP[d] (Mcal/kg DM)	317	302
NDF[e] (g/kg DM)	1126	1400
NFC[f] (g/kg DM)	1966	1661*
Na (mEq/kg DM)	705	990*
K (mEq/kg DM)	1414	1432
Cl (mEq/kg DM)	1698	1868*
S (mEq/kg DM)	196.8	229.8*

Explanations: * – $p \leq 0.05$; a – dietary cation anion difference; b – dry matter; c – metabolizable energy; d – crude protein; e – neutral detergent fiber; f – non-fermentable carbohydrates

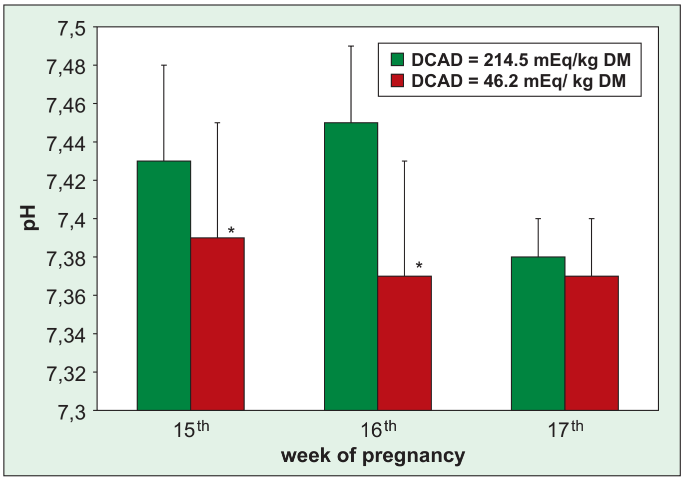

Fig. 1. Influence of different DCAD diet on blood pH values in pregnant sheep (n = 8; $\bar{x}$ ± SD)
Explanations: *significantly differences at $p \leq 0.05$ (vs. pH values obtained in I^{st} group of sheep)

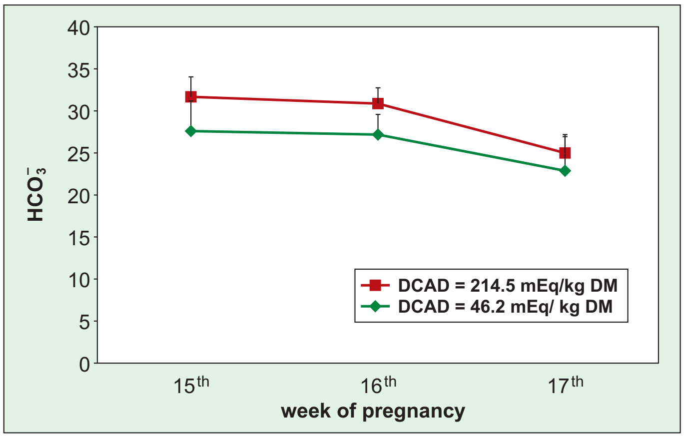

Fig. 2. Effect of high- and low-DCAD diet on plasma HCO_3^- concentration (n = 8; $\bar{x} \pm SD$)

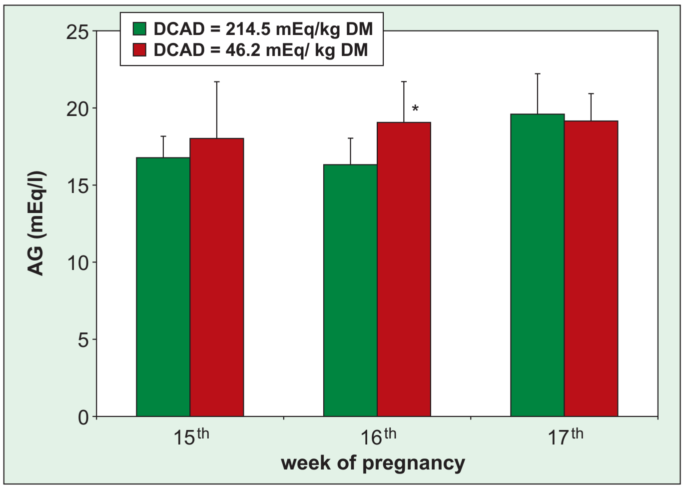

Fig. 3. The values of anion gap obtained under high- and low--DCAD condition (n = 8; $\bar{x} \pm SD$)
Explanations: *significantly differences at $p \leq 0.05$ (vs. AG values obtained in I[st] group of sheep)

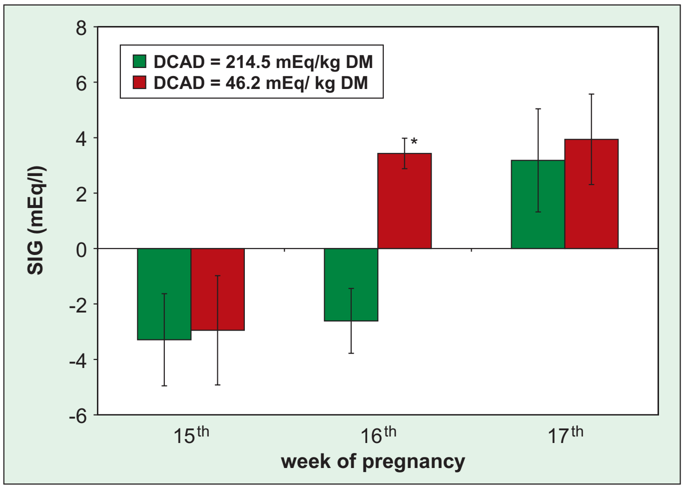

Fig. 4. Influence of different DCAD diet on strong ion gap values (n = 8; $\bar{x} \pm SD$).
Explanations: *significantly differences at $p \leq 0.05$ (vs. SIG values obtained in I[st] group of sheep)

In first and second groups of animals, serum HCO_3^- concentration subsequently dropped from 27.6 ± 3.5 mEq/l and 31.67 ± 2.36 mEq/l in the 15th week of

Tab. 2. Comparison of mean body weight and litter size in group of sheep fed with different DCAD diet

	DCAD = 214.5 mEq/kg DM	DCAD = 46.2 mEq/kg DM
Mean body weight of ewes	58.4 ± 6.2 kg	79.5 ± 5.3 kg
Mean litter size	1.18 ± 0.5	1.29 ± 0.7

pregnancy to 22.87 ± 4.31 mEq/l and 25 ± 1.94 mEq/l in the 17th week of pregnancy (fig. 2). During the duration of the experiment, sheep fed +214.5 mEq/kg had a lower bicarbonate level than those fed +46.2 mEq/kg DM.

Under a high-DCAD condition, only in the last instance of blood collection, the anion gap elevated to 19.6 ± 2.62 mEq/l (fig. 3). No significant changes of AG in low-DCAD fed animals were observed. Values of AG in this group ranged between 18.02 ± 3.68 mEq/l – 19.15 ± 1.79 mEq/l.

The feeding of sheep with +214.5 mEq/kg DM resulted in negative values of strong ion gap, maintained until the 16th week of pregnancy (fig. 4). A positive 3.18 ± 2.86 mEq/l value of SIG was observed only in the 17th week of pregnancy. In the second group, fed with a lower DCAD, a positive SIG (3.43 ± 0.55 mEq/l) occurred earlier, already in the 16th week of pregnancy. In this group of sheep, a higher (attaining 1.29 ± 0.7) litter size was observed (tab. 2).

The AG has been used in the evaluation of acid--base disorders in many animal species including sheep (13). The reference range for sheep has been reported to be 10-16 mEq/l (9). AG has been maintained at upper limits in those of our experimental sheep which consumed the diet with low DCAD (46.2 mEq/l). More stable, closer to the normal AG, were shown in sheep which consumed a diet with higher DCAD (214.5 mEq/kg DM). AG provides an estimate for the concentration of unmeasured strong anions like lactate, β-hydroksy butyrate (β-OHB), acetoacetate, nonesterified fatty acids, sulphate and anions associated with uremia, but under some limitation: e.g. if the level of cation remains comparable. So it would only be speculating on the authors' part that our pregnant sheep of group II had more unmeasured anions in their blood, including ketone bodies. But because in this group of sheep the level of cations was higher than in group I such a situation was highly unlikely. Moreover, as has been seen in tab 2. the group of sheep fed with a low DCAD diet delivered a higher litter size.

To obtain more accurate insight into the changes in unmeasured ions the authors performed another analysis, namely SIG. Anion gap reflects the difference between all unmeasured anions and cations concentrations, whereas SIG reflects the difference between strong cations (SC) and strong anion (SA) concentrations (2). Thus, for clinical purposes unmeasured strong anions are better expressed by the estimation of SIG than AG. In the experimental study reported here, the normal SIG for sheep was established to be approximately 3 mEq/l. The positive value of SIG takes place if plasma contains more cations such as calcium and magnesium but at a negative value of SIG plasma anions (e.g. βOHB, NEFA) exceed. Because changes in SIG can only be caused by changes in unmeasured SC and SA, the SIG value over 4 obtained in group II (which consumed a diet with lower DCAD = 46.2 mEq/kg DM) suggests that strong cations such as calcium have increased, whereas a SIG < –3 mEq/l (seen in the group I – DCAD 214.5 mEq/l) suggests that such anions as βHOB or NEFA have increased (2). The above mentioned relation finds its reflection in the higher litter size in group II than in group I. A diet with lower DCAD consumed by sheep consequently induced higher calcium mobilization whereas higher values of DCAD (214.5 mEg/kg DM given in group I) induced more undesirable unmeasured anions. According to Espino et al. (4, 5), feeding sheep with a low DCAD diet induces mild hypercortisolism, which may lead to bone absorption and osteo-porosis during late pregnancy. Such a response has two desirable purposes. Firstly, it secures the optimal calcium supply to the rapidly growing fetus in late pregnancy and, secondly, prevents hypocalcaemia around the periparturient period.

During the sampling period of the present study, the blood pH was also significantly lower in the group of pregnant sheep consuming a diet with low DCAD. The increase in plasma [H^+] following feeding with a more anionic diet (46.2 mEq/kg DM) corresponds with Stutz et al. (22) who found that consuming diets with either a medium or low DCAD experienced an increase in [H^+]. It may be assumed that [H^+] was higher with the consumption of a low DCAD diet. It should be stressed that in our first group of sheep which consumed an acidogenic diet, a higher litter size was observed. It is known that the level of Ca is among the factors influencing ovulation rate and fertilization (11). In both groups of pregnant sheep HCO_3^- values had a tendency to drop during the course of the experiment. Such behavior of blood bicarbonates may reflect the progression of pregnancy and growing metabolic acidosis.

Conclusion

Relaying on the positive value of SIG obtained at low DCAD the authors can suggest that in this group of pregnant sheep mild metabolic acidosis has been evoked. From one perspective such a state reflects the elevation of the unmeasured strong anions level e.g. βOHB, from another it is known to mobilize more essential cations like calcium and magnesium during pregnancy. By employing forage with a known value of DCAD it is possible to influence not only on the health of sheep during the course of pregnancy but also to improve their reproductive parameters.

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