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NUTRIENTS INTAKE AND OVARIAN PROFILE AS AFFECTED BY CATIONIC ANIONIC DIETS IN *NILI-RAVI* BUFFALOES DURING WINTER

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ABSTRACT

The study was conducted to investigate the potential of cationic and anionic diets, on nutrients intake, involution period, ovarian activity, follicle growth and uterus tonicity in early lactating buffaloes during winter. Twelve early lactating buffaloes were randomly divided into four groups, three buffaloes in each. Four isocaloric and isonitrogenous diets were formulated to have -110, +110, +220 and +330 dietary cation anion difference (DCAD) mEq/kg dry matter and mar Ked as A, LC, MC and HC diet, respectively. Diet allocation to four groups was random. A linear increase in dry matter intake (DMI) was observed with increased DCAD level. Maximum and minimum DMI was observed in buffaloes fed HC and A diets, respectively. Similar trend was followed by crude protein, neutral detergent fiber and acid detergent fiber. Rectal palpation on 40th day post partum revealed that buffaloes fed HC diet had completed uterus involution period while buffaloes fed A and LC diets didn't complete involution period. Higher uterus tonicity was palpated in buffaloes fed HC diet compared to those receiving A, LC and MC diets. Lower ovarian activity was noticed in buffaloes fed A diet while its reverse was true in buffaloes on HC diet. Observed presence of corpus luteum in buffaloes fed HC diet was also higher in buffaloes fed HC diet. The findings of the present study indicated that high DCAD diet (HC diet) not only increased nutrients intake but also reduced involution period increased ovarian activity and uterus tonicity in early lactating buffaloes during winter.

Key words: DCAD, nutrients intake, ovarian activity, follicle growth, lactating buffalo.

INTRODUCTION

Parturition induces certain enzymatic, hormonal and physiological alterations (Bazer and First, 1983) to support the dairy animal's biological system, which undergoes tremendous changes, to ensure constant nutrient supply of macro and micro-nutrients for milk biosynthesis particularly in. However, during this critical period high yielding dairy animals experience negative energy balance (Bauman and Currie, 1980) due to insufficient availability of nutrients which not only adversely affect the milk production but also ovarian cyclicity and thus conception rate (Wathes *et al*., 2003; 2007). Reduced follicular growth is one of the major attributes of negative energy balance that leads to delayed onset of ovarian cyclicity with increased services per conception.

Under these circumstances, any nutritional tool that can enhance nutrients intake by animal would contribute significantly to escalate milk crop. This also ensures nutrients supply to initiate early ovarian activity. Many nutritional strategies have been documented to enhance nutrients intake with varying degree of success. Sufficient scientific literature indicates benefits of manipulation of primary nutrients to dissolve the impact of negative energy balance during early lactation in dairy animals. However, in last couple of decades, manipulation of micronutrients especially minerals got significant attention of dairy nutritionists to be used as a practical tool to enhance nutrients intake. In this context, difference between certain dietary cationic (Na^+ K^+) and anionic (Cl^- S^{--}) minerals usually referred as dietary cation anion difference (DCAD) revealed significant impact on nutrients uptake through manipulation of acid base status by diverting the biological reactions towards optimum animal productivity. Feeding high DCAD to exotic lactating dairy cows has been reported to improve energy status through enhanced dry matter up-take. Higher nutrients uptake has been reported to increase insulin like growth factor-I (IGF-I), a measure of body energy status, that acts as mediator between nutrition and reproductive performance (Jorritsma *et al*., 2003; Tibary, 2007). Improved energy status has been reported to increase ovarian cyclicity by improving growth and development of follicles (Beam and Butler, 1997).

The influence of DCAD on nutrients uptake, production and reproduction performance in exotic dairy cows has been extensively intensively studied. However, more research is needed before specific recommendations can be made regarding optimal DCAD for lactating dairy animals because an optimum DCAD value can be altered by numerous factors including breed, feed intake, season, potential of diet to produce acid and concentrations of other fixed ions etc. Furthermore, direct implementation of the scientific information obtained from the studies executed on exotic dairy cows to the lactating buffaloes, a distinct breed that differs in physiological aspects and reared under different feeding regime and environmental conditions would not be a scientific approach.

Keeping in view the desirable effects of DCAD, the present study was planned to investigate the potential of varying DCAD concentrations on nutrients intake, involution period and ovarian profile in early lactating buffaloes in winter.

MATERIALS AND METHODS

This experiment was conducted at Animal Nutrition Research Center, University of Agriculture, Faisalabad, Pakistan. Twelve early lactating *Nili-Ravi* buffaloes were used in the experiment. Four diets were formulated to have -11, +11, +22 and +33 mEq/100g DM DCAD and were represented by anionic (A), low cationic (LC), medium cationic (MC) and high cationic (HC) diets, respectively (Table 1). All diets were formulated to be iso-nitrogenous and iso-caloric using NRC (2001) values for energy and protein. Sodium bicarbonate and calcium chloride salts were used to attain the desired DCAD levels. Salts were first mixed in wheat bran and then with the rest of concentrate portion of the feed to mix it with 50% wheat straw of experimental ration. The DCAD was calculated by the following equation developed by Tucker *et al*. (1992)

$(Na^{+} + K^{+}) – (Cl^{-} + S^{--})$ -------- mEq/100g DM

These diets were allocated to buffaloes in a randomized complete block design (RCBD). The experiment was conducted during winter and lasted for five months (October to February). The diets were mixed daily and fed twice a day (07.00 a.m. and 07.00 p.m.) at *ad libitum*. Representative samples of feed were taken for analysis of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to their respective methods as described by AOAC (1990).

Post parturition rectal palpation was done on different random intervals to know the status of involution of uterus, uterus tonicity, ovarian cyclicity and ovarian follicles by following the procedure as described by Dawson (1975). Three conditions of uterus muscles were defined as flaccid, normal and tonic depending upon the presence of soft uterus tissues with no tone, soft uterus muscles with slight tone and slight hard with good tone in uterus muscles, respectively. Likewise, cyclic ovaries were defined by taking into account the presence of growing palpatable follicle, graffian follicle, and corpus luteum in its any form (i.e. regressed, mature etc.) while its reverse was true for non-cyclic ovaries. Palpation for corpus luteum was done according to the method used by Sharifuddin and Jainudeen (1983). Large follicles were defined as follicles that attained a diameter ≥10 mm and those with diameter < 10 mm were considered as small follicles. The data collected on nutrients intake, involution of uterus, uterus tonicity, ovarian cyclicity, ovarian follicles and corpus luteum were analyzed using general linear model procedure of SPSS (SPSS 10.0.1., 1999).

RESULTS AND DISCUSSION

Dry matter intake: There was a significant increase (P<0.05) in DMI as the DCAD level increased (Fig. A). Buffaloes fed on A, LC, MC and HC diets consumed 15.10, 16.22, 17.83 and 18.28 kg/d, respectively. The minimum (15.10 kg/d) and maximum (18.28 kg/d) DMI was recorded in buffaloes fed A and HC diets, respectively (Fig. A). Increased (P<0.05) crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were also observed as DCAD level increased in the diet (Fig.A). Similar findings have been reported by the Meta Analysis conducted by Hu and Murphy (2004). Increased nutrient intake with increased DCAD level by buffaloes was in concordance with Apper-Bossard *et al*. (2006). Reduced feed intake by buffaloes fed A diet might also be attributed to poor palatability of anionic salt (calcium chloride) used to attain required DCAD (-11 mEq/ 100 g DM) concentration. Increase DMI with increasing DCAD might be attributed to increased rumen pH at high DCAD level (Tucker *et al*., 1988). Furthermore, high DCAD diet tends to make the rumen pH alkaline which enhances the efficiency of ruminal cellulolytic microbes. Our findings were supported by Hu *et al*. (2007) who observed a linear relationship between DCAD and DMI. Other researchers also reported similar findings (Shahzad *et al*., 2008a,b; Sarwar *et al*., 2008; Sarwar *et al*., 2007 a,b,; Shahzad *et al*., 2007a,b; Oetzel and Barmore, 1993; Wang and Beede, 1992). However, results of our findings were not in agreement with Fredeen *et al*. (1988) who observed non-significant effect of DCAD on DMI. Roche *et al*. (2003) also reported reduced feed intake in early lactating cows fed high DCAD levels (21 to 127 mEq/100g DM). This contradiction of findings might be attributed to high very high range of DCAD used in their study.

Ovarian profile: Involution of uterus completed in 40 days in buffaloes fed HC diet while on 47 days post partum in buffaloes fed low A diet (Fig. 1 & 2). Higher uterus tonicity was noticed in buffaloes fed HC diet than those fed A and LC diets (Fig. 3 to 7). Ovarian cyclicity (Fig. 8 to 12) and follicles growth (Fig. 13 to 17) was also improved in buffaloes fed HC diet.

Early completion of uterus involution in buffaloes fed HC diet was due to increased energy status due to increased high DMI (Moore *et al*., 2000). A positive linear relationship between energy balance and ovarian activity is well documented (Butler, 2005). High-energy balance stimulates the ovarian activities (Pate, 1999). Increased DMI in buffaloes fed on high DCAD might have increased IGF-I (Moore *et al*., 2000) which has stimulatory effect on granulosa cells to produce more estradiol that in turn improves the ovarian activity (Spicer *et al*., 1990). The reduced ovarian efficiency at low DCAD diet might be associated with poor energy balance because of reduced DMI. Decreased feed consumption increases the occurrence of negative energy balance and postpartum anovulation (Canfield and Butler, 1991; Beam, 1996; Zurek, 1995). Furthermore, negative energy balance due to low nutrients intake causes less secretion of leutinizing hormone (Schillo, 1992) which results in reduced follicular growth and ovulation (Yoshimura *et al*., 1996).

Table 1. Ingredients and chemical composition of DCAD diets for early lactating buffaloes

Ingredient	A	Diets[1] LC	MC	HC
Wheat straw	50	50	50	50
Corn grain cracked	8.91	9.42	9.47	9.65
Canola meal	8.13	8.44	8.40	8.39
Sunflower meal	8.67	8.66	8.70	8.22
Wheat bran	8.70	8.75	8.97	8.50
Molasses	8.50	8.90	8.90	8.70
Vegetable oil	2.5	2.5	2.5	2.5
Urea	1.2	1.1	1.1	1.2
DCP	1.5	1.5	1.5	1.5
Salt	0.25	0.25	0.25	0.25
$NaHCO_3$	---	---	0.23	1.19
$CaCl_2$	1.74	0.48	---	---
		Chemical composition		
NE_L/kg	1.43	1.46	1.46	1.45
CP	14.1	14.1	14.1	14.1
NDF	47	47.2	47.3	46.9
ADF	30.6	30.7	30.8	30.6
Ca	1.28	0.86	0.69	0.69
P	0.65	0.66	0.66	0.65
Mg	0.27	0.27	0.27	0.27
Na	0.19	0.19	0.26	0.51
K	1.51	1.55	1.54	1.54
Cl	1.55	0.78	0.49	0.49
S	0.23	0.24	0.24	0.24
DCAD[2]	-11	+11	+22	+33
		mEq/100g, DM		

[1]A, LC, MC and HC stand for anionic, low cationic, medium cationic and high cationic [2] $(Na^+ + K^+) - (Cl^- + S^{--})$ mEq/100g DM

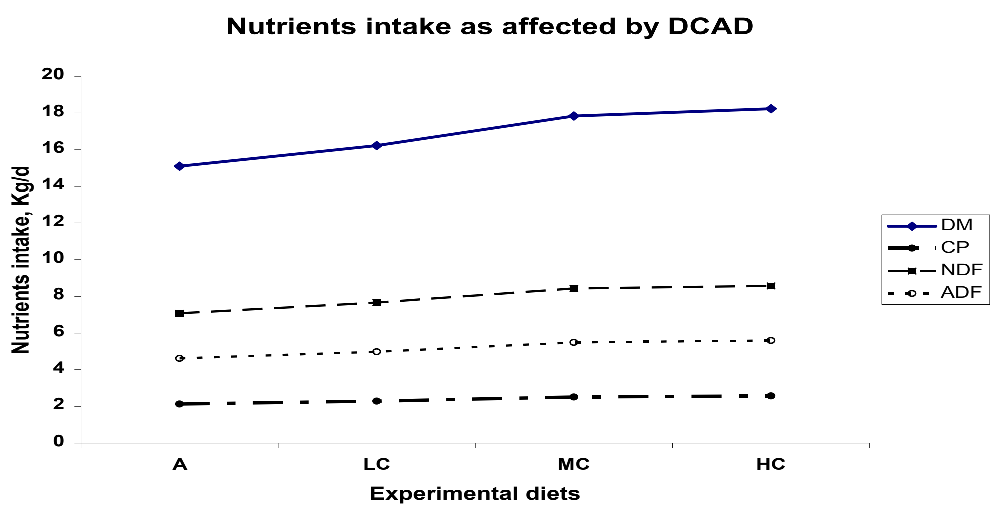


Fig. A. Involution of uterus

Fig 1: Involution of uterus 40 days postpartum

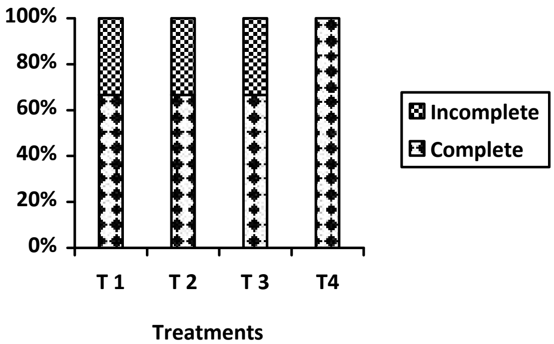
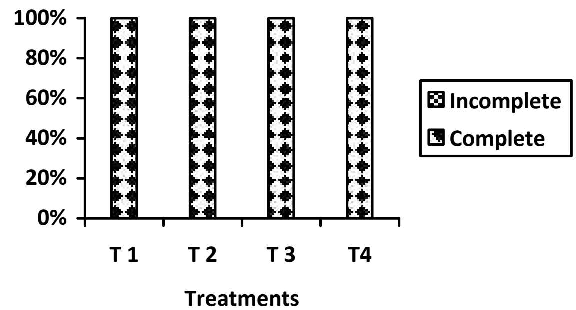


Fig 2: Involution of uterus 54 days postpartum



Uterus tonicity

Fig 3: Uterus tonicity 40 dayspostpartum

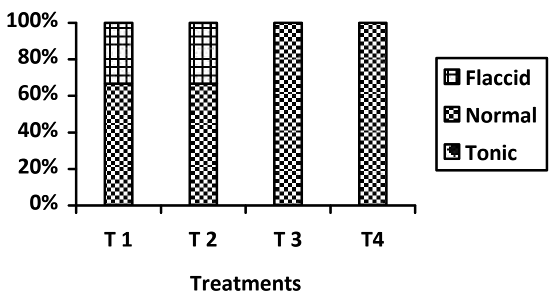


Fig 4: Uterus tonicity 54 dayspostpartum

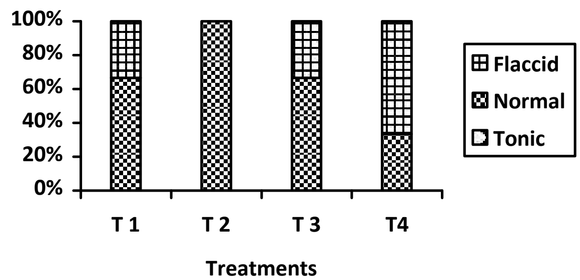


Fig 5: Uterus tonicity 68 days postpartum

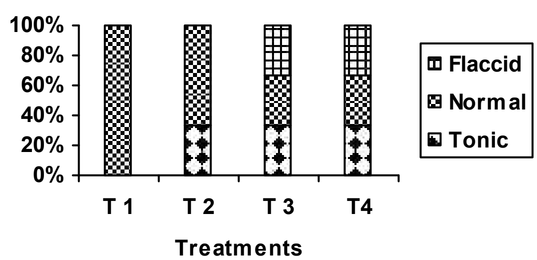


Fig 6: Uterus tonicity 97 days postpartum

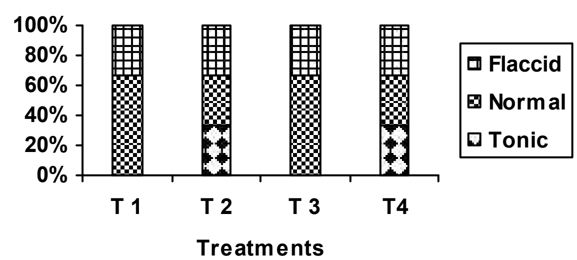


Fig 7: Uterus tonicity 127 dayspostpartum

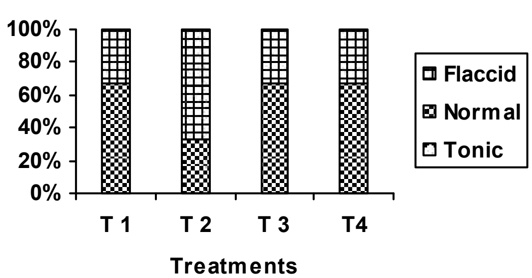
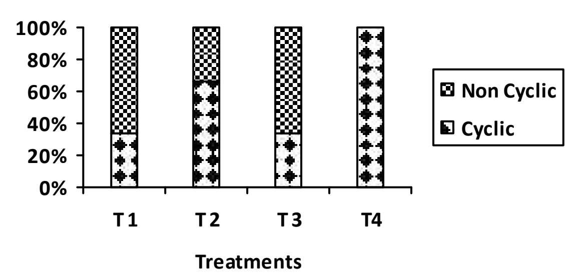


Fig 8: Ovarian cyclicity 40 days postpartum



Ovarian cyclicity

Fig 9: Ovarian cyclicity 54 days postpartum

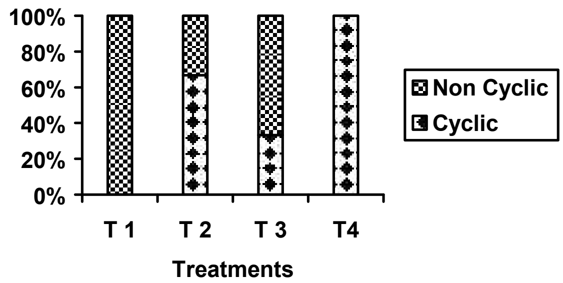


Fig 10: Ovarian cyclicity 68 days postpartum

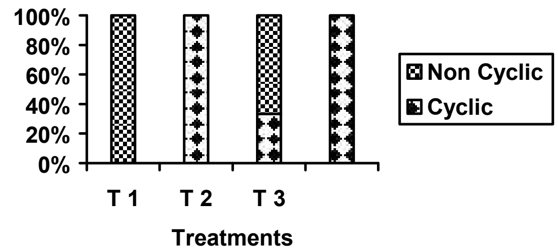


Fig 11: Ovarian cyclicity 97 days postpartum

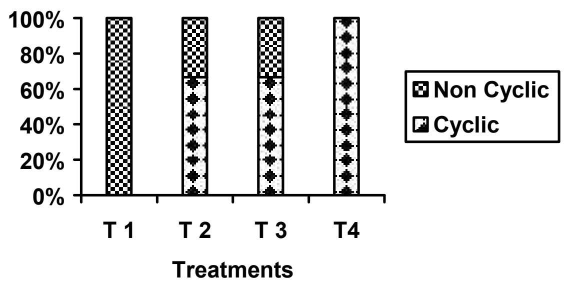
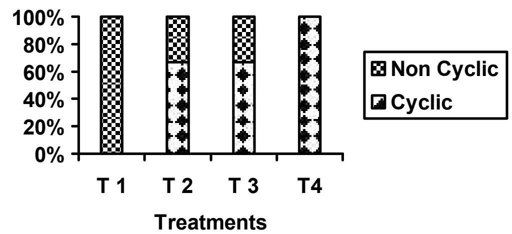


Fig 12: Ovarian cyclicity 127 days postpartum



Follicles

Fig 13: Follicles picture 40 dayspostpartum

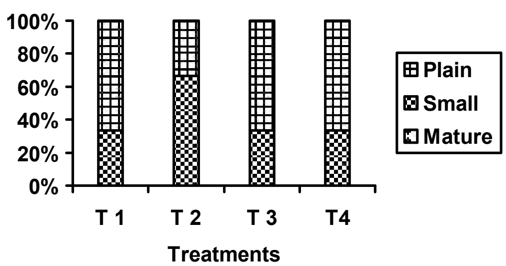


Fig 14: Follicles picture 54 dayspostpartum

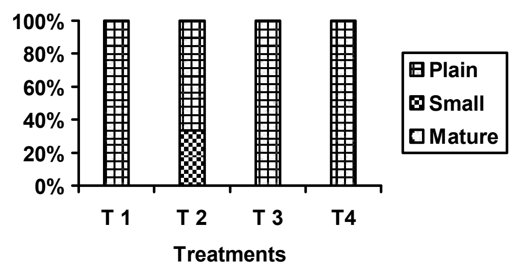


Fig 15: Follicles picture 68 day spostpartum

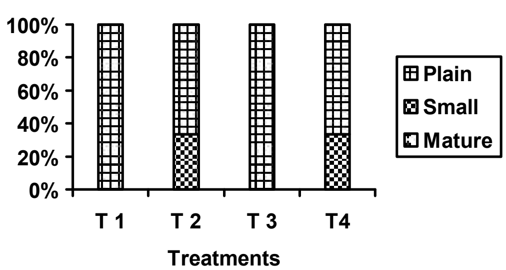


Fig 16: Follicles picture 97 days postpartum

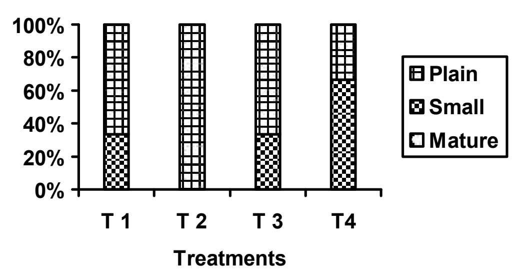
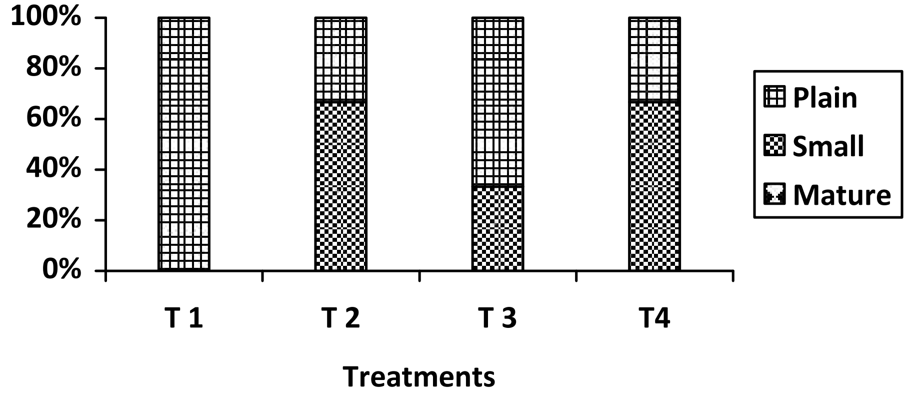


Fig 17: Follicles picture 127 days postpartum



In conclusion, increasing dietary anions can enhance the nutrients intake in early lactating buffaloes during winter. The findings confirmed that the ratio of fixed cation and anion in the diet can be used to improve ovarian activity and follicle growth of dairy buffaloes during winter season. However, more research involving large number of animals with wide DCAD range with different cationic salts is needed before making any recommendation to buffalo farming community

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