Effect of Anionic Transition Management on Health and Reproductive Status in Advanced Pregnant Mehsana buffaloes

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Abstract

The study was focused on evaluating effects of anionic-immunity booster supplementation during transition period in advanced pregnant Mehsana buffaloes. The buffaloes were grouped as treatment group (n=10) supplemented with Anions-Immunity booster preparation at 50 grams twice daily orally approximately ten days prior to expected parturition and control group (n=10) maintained on conventional feeding practice without any supplementation. The blood glucose, liver enzyme activity, serum biochemicals, minerals and electrolytes, oxidative stress and inflammatory cytokine were estimated in both groups. The serum albumin, SGOT, calcium, sodium, malondialdehyde and superoxide dismutase activity showed significant (P<0.05) differences between control and treatment groups. The total number of services per conception was higher in control compared to treatment group (5.75 *vs.* 2.57). The pregnancy rate was more than double in treatment group compared to control group (38.88 *vs.* 17.39 percent). The supplementation of anion-immunity booster for ten days prior to parturition improved reproductive performance in Mehsana buffaloes.

Keywords: Buffalo; reproductive performance; transition period

Introduction

The transition period is crucial in bovine reproductive life, as cow has to support advanced fetal growth and lactogenesis over her body growth and maintenance. This period is driven by hormonal changes, nutritional intake and metabolism (Batistel *et al*., 2018). Despite the remarkable extensive research on transition cow nutrition and physiology, metabolic disorders continue to occur (Burhans *et al*., 2003). They are related to diet fed during the pre-partum period (Curtis *et al*., 1985). The peri-parturient immunosuppression is multifactorial, mainly associated with parturition, lactation and metabolic factors (Overton and Waldron, 2004). The changes in blood constituents can reflect the physiological condition and nutritional and health status of cows; however, blood biochemical parameters vary during different physiological stages of animals (Ahmad *et al*., 2003). The reduced dry matter intake and higher energy need during the peri-parturient period results in negative energy balance and accumulation of liver triglycerides (Bertics *et al*., 1992). During early lactation, the observed negative energy balance becomes normalized about two months post-partum (Nogalski and Gorak, 2008). Regeneration of reproductive system after pregnancy and preparation for new fertilization, which are the most intensive at the early postpartum period, significantly impacts the intensity of metabolic processes (Meikle *et al*., 2004). Therefore, the present study was undertaken to check the effect of the anion-immune supplementation on reproductive performance of Mehsana buffaloes.

Materials and Methods

Twenty advanced pregnant Mehsana buffaloes were selected from milk shed area of Palanpur, Banaskantha district and grouped into two. Treatment Group (n=10) comprised of ten advanced pregnant Mehsana buffaloes maintained on conventional feeding and supplemented with Anions and Immunity booster preparation (Intabolite[a]) at 50 gram twice daily orally approximately ten days before expected parturition. Buffaloes of treatment group had pre-partum cervico-vaginal prolapse (02) and history of ketosis (02) and milk fever (01) in previous lactations, whereas, five buffaloes had no record of previous ailments. The control group (n=10) comprised of normal healthy buffaloes without history of metabolic and/or reproductive ailment maintained on conventional feeding practice and no supplements.

The blood samples were collected approximately ten days before expected parturition and ten days post-partum in both groups. The blood glucose was

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estimated using glucometer. The liver enzyme activity (SGOT), serum biochemicals (Creatinine, Albumin, Total Protein, Urea and Cholesterol), minerals and electrolytes (Calcium, Phosphorus, Sodium and Chloride) were estimated using commercial available Kits[b]. The inflammatory cytokine IL-6 was measured by ELISA kit[c]. Malondialdehyde (MDA), end product of lipid peroxidation was measured as per the method of Buege and Steven (1978) while superoxide dismutase activity (SOD) was estimated by Colorimetric assay kit[d].

The peri-parturient disorders like dystocia, milk fever, retention of placenta, prolapse, downers, ketosis, metritis and clinical endometritis, etc. were recorded. All buffaloes of both groups were monitored up to 180 days post-partum to record reproductive parameters such as service period, total number of services, service per conception and pregnancy. The service per conception was calculated by total number of services given to buffaloes divided by total number of pregnancies. The pregnancy rate (percent) was calculated by total number of pregnancies divided by total number of services. The pregnancy was confirmed by per rectal examination at two months of gestation.

All data were checked for normality using the Shapiro-Wilk test. The immuno-biochemical parameters were compared by independent t-test. The data were presented as Mean±S.E. and level of significance was 5 percent. Data were analyzed using statistical software SPSS (IBM® SPSS® statistics, version 20.0).

Results and Discussion

The results of immuno-biochemical parameters are shown in Table 1 and reproductive parameters are shown in Table 2. The observed detailed results of the study are discussed below.

Blood Glucose

The blood glucose concentration differed non significantly (P>0.05) between and within groups. The glucose concentration was lower during the lactation period than in pregnant period (Ashmawy, 2015). During early lactation, reduced blood glucose concentrations are attributed to blood glucose withdrawal by mammary gland for lactose synthesis (Nale, 2003). The blood glucose level indicates energy status (Hagawane *et al*., 2009). It is necessary for reproductive function because the metabolic inhibitor (2-Deoxy-D-Glucose) prevents the onset of estrus and corpora lutea formation (McClure *et al*., 1978). Although marginally higher concentration was noted in treatment group indicated better energy status, which has been reflected in reproductive performance over the control group during the study.

Serum Enzyme Activity

Serum Glutamic-Oxaloacetic Transaminase (SGOT)

The SGOT activity was increased during post-partum compared to pre-partum in both control and treatment groups. The increased (P<0.05) SGOT of treatment group's indicated better metabolism and physiological activities than control non-treated buffaloes. The higher serum AST levels in lactating than non-lactating or dry animals due to increased physiological stress during early lactation (Jambh *et al*., 2016). Contrarily, non-significant higher blood enzyme (AST, ALT and AlP) activities during pregnancy than lactation were reported by Ashmawy (2015) in buffaloes. This reduced activity of serum enzymes might be due to reduced physiological activities of reproductive organ as recorded in anestrus animals (Kalsotra *et al*., 2016).

Serum Biochemistry

Albumin

The albumin showed significant (P<0.05) increased level during post-partum compared to pre-partum in both groups. The liver synthesizes albumin to maintain the osmotic pressure within the circulatory system. The reduction in albumin levels are indicative of liver disease, kidney disease, inflammatory conditions and malnutrition (Lager and Jordan, 2012) which were not recorded in any groups of buffaloes during the study. However, buffaloes suffering from pre-partum prolapse were included in treatment group. Even though a significant (P<0.05) rise in albumin in treatment group indicated a beneficial effect of supplement in treated buffaloes.

Total Protein

The total protein showed non significantly higher trend in treatment group compared to control group during post-partum stage. The possibility for this may be the hemo-concentration and water losses due to parturition. The feed factor is assumed to have played a role in changes of total protein concentration (Heck *et al*., 2009). An increase in total protein levels is also associated with protein requirements for milk formation and immunoglobulin supply (Mohri Contrary to present study, the lower plasma urea concentrations during lactation period than pregnant

b - Brand of Agappe Diagnostic Ltd, Mumbai

c - Brand of IVD Technologies, California

d - Brand of Sigma-Aldrich, Germany

Table 1 : Immuno-biochemical parameters of buffaloes in control and treatment groups.

Sr No.	Parameters	Groups	Pre-partum (approx. 10th day before parturition)	Post-partum (10th day after parturition)
1	Blood glucose (mg/dL)	Control	56.70±1.94	55.50±3.05
		Treatment	59.90±1.82	61.00±1.41
2	Serum enzyme activity (SGOT; U/L)	Control	100.94±5.66	$108.82\pm5.09_{A}$
		Treatment	104.93 ± 7.51^{a}	$128.69\pm6.41^{b}{}_{B}$
3	**Serum Biochemical**			
	Albumin (g/dL)	Control	2.63 ± 0.50^{a}	3.78 ± 0.11^{b}
		Treatment	2.32 ± 0.52^{a}	3.82 ± 0.10^{b}
	Total protein (g/dL)	Control	7.92±0.28	7.97±0.18
		Treatment	7.32±0.41	8.22±0.25
	Urea (mg/dL)	Control	29.91±3.50	33.74±2.53
		Treatment	30.42±4.62	34.85±5.48
	Cholesterol (mg/dL)	Control	72.85±3.70	75.43±6.44
		Treatment	65.93±5.60	76.88±4.79
	Creatinine (mg/dL)	Control	1.48±0.07	1.49±0.07
		Treatment	1.42±0.10	1.41±0.08
4	**Minerals and Electrolytes**			
	Calcium (mg/dL)	Control	$7.86\pm0.23_{A}{}^{a}$	$7.12\pm0.15_{A}{}^{b}$
		Treatment	$6.93\pm0.35_{B}$	$7.70\pm0.14_{B}$
	Phosphorus (mg/dL)	Control	5.49±0.17	5.61±0.39
		Treatment	5.63±0.46	6.72±0.56
	Sodium (mEq/L)	Control	136.28±2.32	132.33±1.83
		Treatment	141.62 ± 3.60^{a}	129.09 ± 0.94^{b}
	Chloride (mEq/L)	Control	86.48±0.54	88.19±1.30
		Treatment	88.16±1.10	88.29±1.45
5	**Oxidative stress**			
	MDA (microM/L)	Control	0.75 ± 0.01^{a}	0.82 ± 0.03^{b}
		Treatment	0.71 ± 0.03^{a}	0.81 ± 0.01^{b}
	SOD activity (U/mL)	Control	9.86 ± 0.56^{a}	12.11 ± 0.58^{b}
		Treatment	10.67±0.70	11.07±0.48
6	Pro-Inflammatory Cytokine IL_6 (pg/mL)	Control	7.47±2.12	9.04±1.22
		Treatment	7.23±1.44	7.55±0.84

Subscript A,B differ significantly within column (P<0.05)

Superscript a,b differ significantly within row (P<0.05)

et al., 2007). However, the lower plasma total protein during lactation compared to gestation/dry period was recorded by Ashmawy (2015) in buffaloes.

Urea

The serum urea concentration showed a non-significant difference between and within groups.

Table 2 : Reproductive performance of post-partum buffaloes

Parameters	Control group (n=10)	Treatment group (n=10)
Service period	133±16.92 days (90-162)	103.14±12.29 days (68-165)
Total number of services	23	18
Number of pregnancies	4	7
Services per conception	5.75	2.57
Pregnancy rate (%)	17.39	38.88

period are reported by Ashmawy (2015). Increased deamination or protein intake might be the possible explanation for non-significant rise in urea level during the study, as suggested by Hagawane *et al.* (2009). Further, higher energy requirements for milk synthesis will increase metabolic activity during lactation, ultimately inducing protein catabolism (Piccione *et al.*, 2009). The catabolic effect was not evident in study while looking at total protein concentrations recorded.

Cholesterol

The serum cholesterol concentration showed non-significant rise at post-partum stage compared to pre-partum stage during the study. Contrary to present findings, lower cholesterol concentration during lactation period than pregnant period were reported by several workers (Ashmawy, 2015; Kristanto *et al.*, 2021). The higher rise in cholesterol concentration of treatment group than control group showed a positive effect of treatment ultimately resulted in superior reproductive performance. The cholesterol acts as a precursor of steroid hormones and indicates circulatory adequacy of hormones responsible for normal oestrus (Ramakrishna, 1997).

Creatinine

The serum creatinine level differ non significantly (P>0.05) between and within groups during the study. In agreement with this finding, Radostits *et al.* (2007) reported that mean serum creatinine in pregnant and lactating cows was similar to normal physiological level (1.0-2.0 mg/dL). The plasma creatinine concentrations were higher during lactation period than in pregnant period (Ashmawy, 2015). In group of pregnant cows, the creatinine level decreased (P>0.05) in lactation period (Kristanto *et al.*, 2021). However, increased creatinine during gestation, followed by decrease during lactation, was also reported in cows (Patel *et al.*, 2016).

Minerals and Electrolytes

Calcium

The serum calcium was significantly (P<0.05) differ between control and treatment group at both stages of study, moreover the significant (P<0.05) reduction in calcium was estimated in control group, contrarily treatment group showed non-significant rise. During the dry period, mechanisms for replenishing plasma calcium are relatively inactive (Ramberg *et al.*, 1984). During post-partum, calcium is directed towards galactogenesis; hence reduction is noticed compared to pre-partum stage (Hagawane *et al.*, 2009). Moreover, lower levels of post-partum calcium were noted than gestational period (Ashmawy, 2015). So, most cows experience some degree of hypocalcemia during the first days after calving and delays activation of calcium homeostasis. The reduction in calcium may be accompanied by impaired intestinal absorption, urinary losses and insufficient mobilization from bone (Sivakanesan, 1999). Buffaloes fed anionic diets had low incidence of parturient hypocalcemia (Shahzad *et al.*, 2008) and improved digestibility (Shahzad *et al.*, 2011) corroborated with present study. The recorded increment in treatment group revealed beneficial effect of supplement compared to control group.

Phosphorus

Similar to calcium, phosphorus is also directed to milk production, resulting in lower level during lactation as compared to gestation. The highest level of phosphorus at pre-partum and lowest at parturition was noted by Hussain *et al.* (2001). Hagawane *et al.* (2009) and Ashmawy (2015) reported lower phosphorous during lactation than in pregnant period in buffaloes. Contrarily, no such changes were noted and phosphorus was estimated to differ non-significantly in present study.

Sodium

The sodium level reduced from pre-partum to post-

partum stages in both groups under study. The reduction in treatment group revealed significant (P<0.05) difference during the study. Similarly, Ashmawy (2015) found increased sodium concentration during last week pre-partum with highest concentration on day of parturition, which significantly reduced at two weeks postpartum. Hussain *et al.* (2001) and Ashmawy (2015) reported higher sodium concentration during the pregnant period than in lactation period. Contrarily, Mordak and Nicpon (2006) have reported stability in sodium concentration during the last week before delivery and first week of lactation. Akhtar *et al.* (2010) reported a non-significant change in sodium level during pregnancy and early lactation in Nili-Ravi buffaloes. A decrease in sodium concentration during the first weeks of lactation may result from decreased plasma rennin activity in cows after calving (Ozgo *et al*., 2008) and a high concentration of prostaglandins (Asif *et al*., 1996) causes an increased sodium loss.

Chloride

The chloride concentration did not fluctuate in any group at any stage during the study. Contrary to present findings, Van Saun *et al.* (2004) showed higher chloride concentration during last month of gestation compared to early lactation. However, Akhtar *et al.* (2010) and Ashmawy (2015) reported that chloride concentrations varied non-significantly during pregnancy and lactation. The chlorides are abundant mainly in extracellular fluid associated with regulation of sodium concentration (Ozgo *et al*., 2008).

Oxidative Stress Status

Malondialdehyde (MDA)

The MDA showed significant (P<0.05) increased level during post-partum compared to pre-partum in both groups. Similarly, Khatti *et al.* (2017) and Colakoglu *et al.* (2017) observed increased MDA level post-partum compared to pregnancy in crossbred cows. The increase in post-partum MDA may be due to increased stress due to lactogenesis (Roche, 2006). With onset of lactation, energy and oxygen requirements reach much higher than pre-partum.

Superoxide Dismutase (SOD) Activity

The SOD activity was significantly (P<0.05) increased from pre-partum to post-partum stage in control group, indicating animals under severe stress. Whereas the SOD activity at a lower level in treatment group compared to control group indicates a beneficial treatment effect. Similarly, Khatti *et al.* (2017) observed increased SOD activity post-partum compared to pregnancy in crossbred cows. The SOD enzyme is the primary antioxidant defense component protecting the cells against increased ROS (Abd Ellah, 2016). The higher SOD activity in present study might be the result of homeostatic response to higher peroxide generation.

Pro-Inflammatory cytokine

Interleukin 6 (IL-6)

The immunity parameter IL-6, an inflammatory molecule, was non significantly differing between and within groups, however a non-significant rise in IL-6 on 10th day post-partum compared to pre-partum was notable in control group. The IL-6 level was higher in control group than treatment group during post-partum. A significant increase in IL-6 levels was observed at calving and in first two weeks post-calving (Gomma *et al*., 2021). Parturition is an inflammatory process, but failure to resolve these inflammatory reactions may adversely impact animal's productivity, health and fertility. The cows with metritis/ clinical endometritis had higher serum concentrations of IL-6 compared to normal cows suggesting prolonged uterine inflammation (Kasimanickam *et al*., 2013). IL6 reduces ovarian steroidogenesis, *in vitro*, through decreased LH receptor and STAR gene expression in bovine granulosa cells (Samir *et al*., 2017). Similarly, in present study, the higher IL6-pro-inflammatory cytokines in control group might have resulted in a longer service period than treated buffaloes.

Reproductive Performance

There was a difference in post-partum reproductive parameters between groups (Table 2). The service period was longer in the control group compared to treatment group. The total number of services per conception was higher in control compared to treatment group. The pregnancy rate was more than double in treatment group compared to control group. None of peri-parturient disorders were noted in both groups during the study period. Contrarily, the pre-partum prolapse in advanced pregnant Mehsana buffaloes (n=2) was resolved once the treatment was initiated. Moreover, buffaloes with history of recurrent ketosis (n=2) and milk fever (n=1) in all previous lactations were solved under the treatment group. The NEB predisposes to reduction in milk yield, infectious disease, metabolic disorders, and infertility (Esposito *et al*., 2014). The median interval between calving and onset of cyclicity was shorter and conception rates were higher for cows with better energy status at 7 and 14 days after calving (Whitaker

et al., 1993). In correlation to present study, dietary manipulation of transition period in cows improves fertility (Ulfina *et al*., 2015). Further, supplementation of Vitamin E and Selenium in late gestation improved reproductive performance in crossbred cows (Khatti *et al*., 2017) and buffaloes (Mavi *et al*., 2002).

Conclusion

The supplementation of anion immunity booster to advanced pregnant Mehsana buffaloes improved reproductive performance by maintaining calcium and sodium levels and reducing the oxidative stress. The higher pregnancy rates, shorter service period and lesser number of services per conception of treatment group indicate beneficial effect of pre-partum anion immunity booster supplementation for ten days before parturition in Mehsana buffaloes.

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