THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Effect of Ascorbic Acid Supplementation on Haemato-Biochemical and Oxidative Stress Parameters of Crossbred Malabari Does During Peripartum Period

Rejitha J.
MVSc Scholar, College of Veterinary and Animal Sciences, Mannuthy
Karthiayini K.
Associate Professor & Head, College of Veterinary and Animal Sciences, Pookode

Abstract:
This experiment was carried out to investigate the effects of ascorbic acid on haematological, biochemical and oxidative stress parameters of crossbred Malabari does during advanced pregnancy and early lactation period. Sixteen animals of 100-110 day of pregnancy were selected and eight does from them maintained on standard ration served as control group and eight does maintained on standard ration+ ascorbic acid 100mg/day/kg body weight orally served as experimental group of animals. Blood samples from all animals were collected and analyzed at 100-110 day of pregnancy and at fortnight interval up to kidding, within 12h of kidding and one week after kidding. The whole data were analyzed by one way ANOVA using computerized software program SPSS Ver. 17.0. The result showed that ascorbic acid supplementation had no effect on the haematological parameters. However, it acted as a good antioxidant and reduced oxidative stress especially on advanced pregnancy, on day of kidding and early lactation period. The effects of ascorbic acid on biochemical parameters were inconsistent. These showed that even though the ascorbic supplementation did not produce much effect on haemato-biochemical parameters, it can act as good antioxidantin does during advanced stages of pregnancy.

Keywords*: ascorbic acid, haematology, biochemical parameter, oxidative stress and Malabari goat*

1. Introduction

Pregnancy and early lactation are physiological states accompanied by high energy and oxygen demand that may cause increased level of oxidative stress (OS) and development of metabolic reproductive disorders in all living beings (Walsh, 1993). In late pregnancy and early lactation negative energy balance may be the reason for development of OS (Roche *et al.,* 2000); increased lipid peroxidation and reduced antioxidant activity are contributing to development of complications in pregnancy (Orhan *et al.*, 2003; Peuchant *et al.*,2004) such as endometriosis, spontaneous abortions and pre-eclampsia (Rizzo *et al.,* 2012). Increased OS in peripartum period of dairy cows and goats compromise animal performance and health, play an important role in development of metabolic diseases like ketosis, post parturient haemoglobinuria and post parturient paresis (Celi *et al.,* 2010). Antioxidants are the substances that can protect cells from damage caused by free radicals by acting at several different stages of oxidative sequence (Gutteridge, 1995). Antioxidant nutrients like ascorbic acid, vitamin E are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products (Anitra *et al.,* 2000). Ascorbic acid is one of the strong reductants and radical scavengers (Rice, 2000); it performs antioxidant function either by quenching various free radical species directly or by reducing membrane bound oxidized vitamin E at the membrane surface. There are so many studies conducted to study the effect of ascorbic acid as an antioxidant at various stress conditions in different species. But the reports were meager on the effect of ascorbic acid on haemato-biochemical and oxidative stress parameters of goats during peripartum period. Hence, the study was undertaken to assess the effect of ascorbic acid in goats during advanced pregnancy and early lactation period.

2. Materials and Methods

The study was done using animals retained in University Goat and Sheep Farm, Mannuthy. Sixteen apparently healthy crossbred Malabari pregnant does between one to six years of age and 100-110th day of pregnancy were selected at random from the flock maintained in the farm and divided into two groups (G1 and G2) with eight animals in each group. All animals were maintained on standard goat ration and allowed for 5h daily free range grazing and in addition to this G2 group of animals were supplemented with 100mg of ascorbic acid/day/kg body weight orally in feed.

Blood was collected from all animals at the start of the experiment, (100-110 day of pregnancy) at fortnight intervals, within 12h of kidding and one week after kidding. Whole blood and separated serum from each animal were used for estimation of haematological parameters, serum biochemical parameters and analysis of antioxidant status. Haematological Parameters such as Total erythrocyte count (TEC), haemoglobin (Hb) concentration and packed cell volume (PCV) were estimated and Erythrocytic indices were calculated as per the standard technique described by Schalm (1986). Biochemical Parameters such as glucose

concentration (using GLUCOSE LS kit, Euro Diagnostic Systems Pvt. Ltd, Chennai), serum total cholesterol concentration (using LyphoCHEK- Cholesterol kit, M/s. Agappe Diagnostics Limited, Kerala), concentration of creatinine (using ChemCHEK- Creatinine kit, M/s. Agappe Diagnostics Limited, Kerala), blood urea level (using Lyphochek- Urea-B kit, M/s. Agappe Diagnostics Limited, Kerala) were estimated using semiautomatic blood analyzer (Hospitex- Screen master T). Oxidative Stress Parameters such as level of lipid peroxides (Yagi, 1984) and ascorbic acid (Sonnenwirth and Jarett, 1980) level in serum, level of reduced glutathione in blood (Moron *et al.,* 1979) were estimated and oxidative stress factor (Bisla *et al*, 2003) was calculated. Serum mineral concentration of calcium (using ChemCHEK- CALCIUM kit, M/s. Agappe Diagnostics Limited, Kerala), magnesium (using ChemCHEK- INORGANIC PHOSPHOROUS kit, M/s. Agappe Diagnostics Limited, Kerala) and phosphorous were analysed in semiautomatic blood analyzer (using Hospitex- Screen master T). Data collected on various parameters were statistically analyzed using computerized software program SPSS Ver. 17.0. using one way analysis of variance .

3. Results and Discussion

There were no significant differences noticed in haematological parameters like Hb, PCV, MCV, MCH and MCHC (Table 1). Even though there was no significant difference in Hb concentration between the two groups of animals, a pattern of increment in Hb concentration was observed with advancement of pregnancy in all animals. El-Sherif and Assad (2001) also observed a similar pattern of increment of Hb concentration in Barki ewes with advancement of pregnancy. Ascorbic acid supplementation did not have any effect on haematological parameters.

Parameters	Groups	Days				
		100-110 day of pregnancy	114-124 day of pregnancy	128-138 day of pregnancy	Day of kidding	7 days after kidding
Hb concentration (g %)	G1	9.24^{a}±0.44	9.50^{a}±0.32	9.25^{a}±0.28	9.22^{a}±0.38	9.22^{a}±0.66
	G2	9.50^{a}±0.29	9.64^{a}±0.37	9.75^{a}±0.38	10.07^{a}±0.57	10.07^{a}±0.54
PCV(%)	G1	26.33^{a} ±1.69	25.00^{a} ±0.77	25.75^{a} ±0.80	24.56^{a}±1.25	23.67^{a}±1.51
	G2	26.50^{a} ±0.78	26.43^{a} ±1.02	26.83^{a} ±1.14	27.71^{a}±1.21	27.14^{a}±1.49
TEC (x 10^{6}/µL)	G1	12.09^{a} ±1.20	11.46^{a} ±0.58	11.89^{a} ±0.71	11.25^{a}±0.91	11.14^{a} ±1.01
	G2	12.70^{a} ±0.67	12.37^{a} ±0.87	12.87^{a} ±0.99	13.22^{a}±0.97	13.13^{a}±1.13
MCV (fL)	G1	22.13^{a} ±0.69	21.90^{a}±0.40	21.94^{a}±0.70	22.33^{a}±0.78	20.28^{a}±0.24
	G2	21.05^{a} ±0.54	21.70^{a}±0.84	21.22^{a}±0.99	21.37^{a}±0.98	21.23^{a}±1.17
MCH (pg)	G1	7.77^{a} ±0.63	8.35^{a} ±0.41	7.93^{a} ±0.41	8.59^{a} ±0.67	7.59^{a}±0.49
	G2	7.55^{a} ±0.25	7.92^{a} ±0.34	7.72^{a} ±0.38	7.70^{a} ±0.25	7.87^{a}±0.40
MCHC (g %)	G1	34.91^{a}±2.06	38.09^{a}±1.46	36.05^{a}±1.18	38.11 ±1.88	37.26^{a}±0.87
	G2	35.87^{a}±0.64	36.50^{a}±0.53	36.37^{a}±0.34	36.26^{a}±1.08	37.17^{a}±1.02

Table 1: Effect of vitamin C supplementation on haemogram of goats in peripartum period (Mean± SE, n=8).
G1- control group, G2- Vit. C supplemented group
a, b Means within a column with no common superscripts are significantly different at 5% level

Supplementation of ascorbic acid did not produce any significant change in blood glucose and serum cholesterol concentrations (Table 2) throughout the study. Blood glucose level could be taken as an indicator of metabolic state of animal. It is the major metabolite used by fetus (Firat and Ozpinar, 2002). In advanced pregnancy metabolic need of dam increased due to rapid growth of fetus and initiation of lactogenesis and associated hormonal influence. This increased need and reduced blood level of glucose might have resulted in mobilization of non esterified fatty acids from adipose tissue and increased total cholesterol, which was later on converted to glucose in liver. But in this study the supplementation might not modify the metabolic pathway and did not produce any significant change in blood glucose and serum cholesterol level. Even though ascorbic acid supplementation had significantly ($P \leq 0.05$) reduced serum creatinine concentration on 114-124 day of pregnancy it did not give a consistent result throughout the period. Blood urea nitrogen results were also inconsistent; no significant difference in BUN was observed in different groups studied except a significantly ($P \leq 0.05$) high BUN level (54.39±5.22 mg/dL) observed in non supplemented group of animals on 114-124 day of pregnancy. This result indicated that ascorbic acid might have some effect on the metabolic pathway which needs further detailed research.

Parameters	Groups	Days				
		100-110 day of pregnancy	114-124 day of pregnancy	128-138 day of pregnancy	Day of kidding	7 days after kidding
Glucose (mg/dL)	G1	37.83[a]±3.32	38.00[a]±4.46	37.88[a]±4.66	92.78[a]±21.82	45.78[a] ±4.68
	G2	35.00[a] ±2.03	38.43[a]±1.99	37.67[a]±0.92	79.29[a]±21.15	42.43[a] ±1.66
Cholesterol (mg/dL)	G1	55.04[a]±10.74	68.60[a]±7.70	50.22[a]±5.25	49.69[a]±4.62	60.99[a]±6.15
	G2	46.71[a]±7.94	58.24[a]±5.96	52.78[a]±8.21	50.39[a]±6.57	58.41[a]±5.14
Creatinine (mg/dL)	G1	1.89[a]±0.17	1.97[b]±0.09	1.80[a]±0.16	1.68[b]±0.06	1.63[a]±0.08
	G2	1.84[a]±0.09	1.52[a]±0.03	1.53[a]±0.06	1.40[a]±0.07	1.61[a]±0.10
BUN (mg/dL)	G1	46.31[a]±8.11	54.39[b]±5.22	45.92[a]±3.13	51.18[a]±3.49	44.83[a]±2.44
	G2	42.99[a]±2.43	43.22[a]±2.67	45.20[a]±3.43	43.44[a]±4.69	46.34[a]±1.95

Table 2: Effect of vitamin C supplementation on glucose, cholesterol, creatinine and blood urea nitrogen (BUN) concentrations of goats in peripartum period (Mean± SE, n=8).
G1- control group, G2- Vit. C supplemented group
a, b Means within a column with no common superscripts are significantly different at 5% level

Increased level of MDA is an indication of lipid peroxidation. From advanced stage of pregnancy (128-138 day) to one day after kidding the MDA level (Table 3) of control group of animals were more than supplemented group of animals. This result indicated that ascorbic acid was found effective to reduce the MDA level resulting from lipid peroxidation in advanced pregnancy and early lactation, thereby might be acted as a good antioxidant. Throughout the study it was seen that supplementation did not produce any significant change in GSH level (Table 3) except one week after kidding. Supplemented group of animals showed high ascorbic acid level (Table 3) than control group of animals during 128-138 day of pregnancy, on day of kidding and one week after kidding. The whole experiment was indicative of ascorbic acid supplementation increased serum ascorbic acid concentration from 128-138 day of pregnancy. Due to the antioxidant effect of ascorbic acid it reduced MDA level from 128-138 day of pregnancy and result in increased GSH concentration on seven day after kidding. Value of OSF was significantly reduced in supplemented group of animals from 128-138 day of pregnancy till the end of the study.

Parameters	Groups	Days				
		100-110 day of pregnancy	114-124 day of pregnancy	128-138 day of pregnancy	Day of kidding	7 days after kidding
MDA (n*M*/mL)	G1	5.08[a]±0.52	4.91[a]±0.87	4.29[b] ±0.48	12.63[b]±3.08	6.04[b]±1.37
	G2	7.14[a] ±1.47	2.92[a] ±0.68	2.73[a] ±0.31	4.27[a]±0.53	2.86[a] ±0.49
GSH (mg/dL)	G1	6.62[a] ±1.77	11.74[a]±1.66	9.53[a] ±1.21	8.01[a] ±1.10	7.02[a]±0.92
	G2	8.66[a] ±0.68	9.70[a] ±1.44	9.42[a] ±0.83	9.49[a] ±1.12	9.73[b]±0.96
OSF	G1	1.24[a]±0.34	2.13[a]±0.28	1.57[b]±0.24	3.79[b]±0.87	2.60[b]±0.73
	G2	3.14[a] ±0.98	2.85[a]±1.85	0.98[a]±0.18	1.45[a]±0.15	0.93[a]±0.13
Ascorbic acid (mg/dL)	G1	1.0[a]±0.12	1.10[a]±0.12	0.91[a]±0.08	0.89[a]±0.05	0.68[a]±0.07
	G2	0.97[a]±0.07	1.41[a]±0.05	1.66[b]±0.10	1.80[b]±0.21	1.54[b]±0.11

Table 3: Effect of vitamin C supplementation on malondialdehyde (MDA), reduced glutathione (GSH), ascorbic acid and oxidative stress factor (OSF) of goats in peripartum period (Mean± SE, n=8).
G1- control group, G2- Vit. C supplemented group
a, b Means within a column with no common superscripts are significantly different at 5% level

No significant difference in serum calcium concentration was noticed between the two groups of animals during the pregnancy period. But on day of kidding the control group of animals had a significantly ($P \leq 0.05$) high Ca concentration. During 128-138 day of pregnancy the phosphorus concentration of G2 group of animals were significantly ($P \leq 0.05$) high when compared to G1 group of animals. Throughout the period of study no significant difference in Mg concentration was observed between the control group of animals and supplemented group of animals in peripartum period. The inconsistent data of mineral concentration might be due to the changing metabolic need and individual variation in mineral status, which could not be interpreted and need further research to confirm the effect of ascorbic acid on mineral level.

Parameters	Groups	Days				
		100-110 day of pregnancy	114-124 day of pregnancy	128-138 day of pregnancy	Day of kidding	7 days after kidding
Calcium (mg/dL)	G1	10.34^{a}±0.92	10.04^{a} ±0.40	10.01^{a} ±0.45	12.15^{b}±0.64	9.47^{a}±1.02
	G2	9.95^{a} ±0.65	10.45^{a} ±0.72	10.27^{a} ±0.77	9.89^{a} ±0.42	8.86^{a} ±0.75
Phosphorus (mg/dL)	G1	9.82^{a}±0.99	9.26^{a} ±1.03	7.88^{a}±0.39	8.51^{a}±1.42	9.69^{a}±1.02
	G2	9.31^{a} ±0.71	10.41^{a} ±0.62	10.02^{b}±0.93	8.71^{a}±1.28	8.52^{a}±0.54
Magnesium (mg/dL)	G1	2.99^{a}±0.46	2.84^{a} ±0.38	2.35^{a} ±0.21	2.67^{a}±0.32	3.15^{a}±0.34
	G2	2.87^{a} ±0.32	2.29^{a} ±0.13	2.45^{a}±0.22	2.65^{a} ±0.21	3.55^{a} ±0.48

Table 4: Effect of ascorbic acid supplementation on serum levels of calcium, phosphorus and magnesium of goats in peripartum period (Mean± SE, n=8)

G1- control group, G2- Vit. C supplemented group .

a, b Means within a column with no common superscripts are significantly different at 5% level

4. Summary

The experiment showed that supplementation of ascorbic acid had no effect on haematological parametersof does in late pregnancy and peripartum period, but could reduce the oxidative stress parameters. The effect of ascorbic acid on biochemical parameter was inconsistent and need more research on it to reveal the actual metabolic pathway. Peripartum period demands high energy and during this period the modification of energy metabolism and increased oxygen consumption within the body resulted in an increased lipid peroxidation. Supplementation of antioxidants like ascorbic acid might be helpful to reduce oxidative stress during late pregnancy period in goats. Ascorbic acid supplementation @ 100mg/day/kg body weight in feed did not have effect on other blood haematological and biochemical parameters.

5. References

1. Walsh, S.W. (1993). Secretion of lipid peroxides by the human placenta. Am. J. Obstet. Gynecol. 169(6):1462-1466
2. Roche, J.F., Mackey, D. and Diskin, M.D. (2000). Reproductive management of postpartum cows. Anim. Reprod. Sci. 60: 703–712
3. Orhan, H., Onderoglu, L., Yucel, A. and Sahin, G. (2003). Circulating biomarkers of oxidative stress in complicated pregnancies. Arch. Gynecol. Obstet. 267:189–195
4. Peuchant,E., Brun, J.L., Rigalleau, V., Dubourg, L., Thomas, M.J., Daniel, J.Y., Leng, J.J. and Gin, H. (2004). Oxidative and antioxidative status in pregnant women with either gestational or type 1 diabetes. Clin. Biochem. 37: 293-298
5. Rizzo, A., Roscino, M.T., Binetti, F. and Sciorsci, R.L. (2012). Roles of Reactive Oxygen Species in Female Reproduction. Reprod. Dom. Anim. 47: 344–352
6. Celi, P., Trana, A.D. and Claps, S. (2010). Effects of plane of nutrition on oxidative stress in goats during the peripartum period. Vet. J. 184: 95–99
7. Gutteridge, J.M.C. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin. Chem. 41: 1819-1828
8. Anitra, C. C., Ben-Zhan, Z. and Frei, B. (2000). Potential anti-atherogenic mechanisms of ascorbate (vitamin C) and α-tocopherol (vitamin E). Circ. Res. 87:349-354
9. Rice, M.E. (2000). Ascorbate regulation and its neuroprotective role in the brain. Trends Neurosci. 23(5): 209-216
10. Schalm, O.W., Jain, N.C. and Carroll, E.J. (1986). Veterinary Haematology. Fourth edition. Lea and Febiger, Philadelphia. pp. 45-48
11. Yagi, K. (1984). Assay for blood plasma or serum. Methods Enzymol. 105: 328-331
12. Sonnenwirth, A.C. and Jarett, L. (1980). Gradwohl's Clinical Laboratory Methods and Diagnosis. Eighth edition. The C. V. Mosby Company, Torrento, 2339p.
13. Moron, M.A., De Pierre, J.W. and Mannervick, B. (1979). Levels of glutathione, glutathione reductase, glutathione- S-transferase activities in rat liver. Biochem. Biophys. Acta. 582: 67-68
14. Bisla, R.S., Singh, J., Singh, K. and Krishnamurthy, D. (2003). Assessment of vitamin E as an antioxidant in pre and post-operative treatment schedule of buffaloes subjected to trans-abdominal diaphragmatic herniorrhaphy. Indian J. Vet. Surg. 24: 11-15
15. El-Sherif, M.M.A. and Assad, F. (2001). Changes in some blood constituents of Barki ewes during pregnancy and lactation under semi arid conditions. Small Ruminant Res. 40: 269-277
16. Firat, A. and Ozpinar, A. (2002). Metabolic profile of pre-pregnancy, pregnancy and early lactation in multiple lambing Sakiz ewes. 1. Changes in plasma glucose, 3-hydroxybutyrate and cortisol levels. Ann. Nutr. Metab. 46: 57-61