Open Access

Check for updates

Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.3.542-546

Effects of rumen-protected methionine and lysine on antioxidant status and lactationperformance in crossbred dairy cattle during transition

B. Ragupathi[1], T.R. Sreekumar[1], Zarina Aziz[1], V. Ramnath[1] and K.M. Syammohan[2]

[1]Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, [2]University Livestock Farm and Fodder Research Station, Mannuthy, Thrissur, Kerala Veterinary and Animal Sciences University, Kerala, India

Citation: Ragupathi, B., Sreekumar, T.R., Zarina, A., Ramnath, V. and Syammohan, K.M.2024. Effects of rumen-protected methionine and lysine on antioxidant status and lactation performance in crossbred dairy cattle during transition. *J. Vet. Anim. Sci.* **55** (3):542-546

Received: 24.02.2024 Accepted: 24.05.2024 Published: 30.09.2024

Abstract

Dairy cows undergoing the transition phase often face nutritional challenges, impacting their antioxidant status and lactational performance. To address these challenges, the incorporation of rumen-protected methionine and lysine in the diet has emerged as a strategic intervention.Twelve crossbred cows inthe transition period were randomly divided into two groups of six animals. The control group and treatment group were fed with a standard diet (ICAR, 2013) while the treatment group was supplemented with rumen-protected methionine and lysine. Throughout the trial period, all the animals were subjected to consistent management and feeding conditions.Glutathione peroxide(GPx)levels exhibited a statistically significant difference between groups, with the treatment consistently showing higher GPx levels, indicating an improvement in antioxidant capacity, where it helps regulate systemic oxidative stress.The recorded milk yield data was utilized to compute the daily milk production for each individual for the experimental period. Milk yield remained unaffected, but the treatment group consistently exhibited higher milk yield, suggesting positive influences on postpartum lactation performance.

Keywords: *Rumen-protectedmethionine, rumen-protectedlysine,transition period, antioxidant status*

The transition period in dairy cattle, spanning the three weeks before and after calving, represents a critical juncture characterized by substantial physiological changes and increased metabolic demands (Drackley, 1999). This period is characterised by dynamic changes in nutrient requirements, metabolic processes, and immune function, all of which influence the health, productivity, and profitability of dairy herds (Bernabucci *et al*., 2005).Transition cows are particularly vulnerable to metabolic and infectious diseases due to the complex interplay of physiological and nutritional challenges they face during this critical period(Lykkesfeldt and Svendsen.,2007). Recognizing the multifaceted nature of these nutritional intricacies, a targeted approach involves the integration of rumen-protected methionine and lysine into the dietary regimen.

Methionine and lysine are identified as the first limiting amino acids during the transition period in cattle. Methionine is crucial for cellular integrity and shielding tissues from oxidative stress, especially relevant in high-producing dairy cows facing such stress during lactation. Lysine iscritical for milk production due to its role in L-carnitine synthesis. L-carnitine

#Part of M.V.Sc. thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala.
**Corresponding author: sreekumartr@kvasu.ac.in, Ph. 9496441351*

facilitates fatty acid transport within liver cells' mitochondria, crucial for fat metabolism and energy generation in milk production.The inclusion of rumen-protected methionine and lysine in the diet serves as an essential antioxidant source, contributing to improved health and productivity (Khan *et al.,* 2023).Supplementation of these limiting amino acids in rumen-protected form canconfer health benefits to cattle in transition by alleviating oxidation stress and decreasing inflammation. This study intends to investigate the effects of such supplementation on the antioxidant status and lactational performance of crossbred dairy cattle, contributing valuable insights to the optimisation of nutritional strategies for dairy herds during the transition period.

Materials and methods

Experimental animals

Twelve crossbred cows from the University Livestock Farm and Fodder Research Station, Mannuthy were selected as experimental subjects. The cows were identified three weeks before their expected calving date. The animals were randomly assigned into two groups, T_1 (control) and T_2 (treatment), each with six animals. During the trial, all animals were maintained under uniform management and feeding conditions.

Experimental feed

The experimental feed was formulatedas perstandards outlined by the Indian Council of Agricultural Research (ICAR, 2013).TheT_1group animals were fed withpellet feed containing 20 per cent crude protein (CP) 68 per cent total digestible nutrients (TDN) and a crude fibre content of 12 per cent. In the T_2 group, animals were fed a compound feed mixture (CFM) in a mashed form containing 20per cent CP, 68per cent TDN, and 12per cent crude fibre. Additionally, the feed was supplemented with 15g of rumen-protected methionine (RPM) and 15g of rumen-protected lysine (RPL). Hybrid Napier grass served as the roughage component of the diet. Additionally, all the animals had continuous access to clean, fresh drinking water. The feeding plan comprised of a base maintenance ration of 1.5kg, with an extra 400g per kg of milk produced. Thefeed composition and proximate analysis of the experimental diet are given in Tables1and 2.

Table 1. Composition of ingredients of concentrate ration

Sl. No.	Items	Amount (%)
1	Maize	12
2	Corn gluten fibre	15
3	Tapioca starch waste	4
4	Coconut oil cake	15
5	Alfalfa	11
6	Rice polish	9
7	Black gram husk	14
8	De-oiled rice bran	17
9	Calcite	1.5
10	Salt	1
11	*Mineral mixture	0.5
	TOTAL	100

*Each kg contained calcium 250 g, phosphorous 120 g, magnesium 7 g, iron 5 g, zinc 4500 mg, manganese 1500 mg, copper 1200 mg, iodine 275 mg, cobalt 150 mg and potassium 100 mg.

Collection of blood samples

Blood samples were systematically collected at specific prepartum and postpartum intervals: 21st day before calving, day zero,14th dayand 21st dayafter calving by jugular venipuncture from both T_1 and T_2 groups.These samples were collected in vacutainerscoated with lithium heparin to enable immediate evaluation of antioxidant status.

Estimation of glutathione peroxidase activity (GPx)

The glutathione peroxidase activity was determined photometrically in a semiautomatic biochemical analyser (Hospitex- Screen Master T) according to the methodby Paglia and Valentine (1967) using the RANSEL kit supplied by RANDOX Laboratories Ltd, U.K.The GPx activity was calculated based on the change in absorbance over time, using the calibration curve generated from the standards. The results were expressed U/L of haemolysate.

Milk production

The animals were milked twice a day, in the morning and the afternoon. The recorded milk yield data from these sessions was utilised to compute the daily milk production for each individual for the experimental period.

Table 2. Proximate composition of concentrate feed

Sl. No.	Parameters	Composition (%)
1	Dry matter	92.82
2	Crude protein	20.99
3	Total ash	9.99
4	Acid insoluble ash	2.47
5	Crude fibre	9.97
6	Ether extract	4.95
7	Nitrogen free extract	54.10

The feeding trial extended for a total of 62 days, which included a 10-day adaptation period, 30 days before calving (prepartum) and a 22-day period after calving (postpartum).

Table 3. TheGPxactivity of control and treatment(Mean±SE) (n=12)

Sample	Day -21 (U/L)	Day Zero (U/L)	Day 14 (U/L)	Day 21 (U/L)	F value (p-value)
Control (T_1)	1091.98^{a}±44.38	921.950^{bcB}±46.20	945.067^{cB}±39.42	1035.467^{abB}±67.74	8.774* (0.007)
Treatment (T_2)	1205.45^{a}±44.38	1081.683^{bA}±46.20	1169.617^{aA}±39.42	1182.517^{aA}±67.74	3.152 (0.086)^{ns}
F value (p-value)	3.268 (0.101)^{ns}	5.975* (0.035)	16.217** (0.002)	5.346* (0.0463)	

* ns. non – significant. ** significant at 0.01 level. * significant at 0.05 level. Means bearing same superscript within a row (a-c) and column (A-B) do not differ significantly (p<0.05).

Table 4. Lactation yield of control and treatment (Mean±SE) (n=12)

Sample	7th day (Litres/day)	14th day (Litres/day)	21st day (Litres/day)	28thday (Litres/day)	35th day (Litres/day)	F value (p-value)
Control	12.050^{b}±1.41	13.417^{b}±1.52	13.600^{b}±1.72	12.350^{b}±1.09	14.933^{a}±1.25	2.361 (0.470)^{ns}
Treatment	10.750^{c}±1.41	13.767^{b}±1.52	14.900^{b}±1.72	14.050^{b}±1.09	16.200^{a}±1.25	7.515 (0.277)^{ns}
F value (p- value)	0.421 (0.531)^{ns}	0.027 (0.874)^{ns}	0.287 (0.604)^{ns}	1.198 (0.299)^{ns}	0.511 (0.491)^{ns}	

* ns – Non – significant. Means bearing same superscript within a row (a-b) do not differ significantly (p<0.05).

Statistical analysis

Results were expressed as means±SE. The statistical significance of the difference or relation between the two groups were analysed by repeated measures of two-wayANOVA

Results and discussion

Assessment ofglutathione peroxidase (GPx) activity

The mean values of glutathione peroxidase (GPx) activity in the blood of the control and treatment group of crossbred transition cattle were investigated to assess the impact of supplementing rumen-protected methionine and lysine in their diet. The results, as presented in Table 3 and Fig.1 revealed a significant (p< 0.005) difference between the control and the treatment groups on the zeroth day, 14th day, and 21st day after calving.

The T_2 group consistently exhibited higher GPx levels compared to the T_1 group throughout all days of data collection. Intensive hepatic non-essential fatty acids oxidation following body fat mobilization during the transition period produces higher amounts of reactive oxygen species (ROS), which leads to the development of oxidative stress (Turk *et al*.,2013).

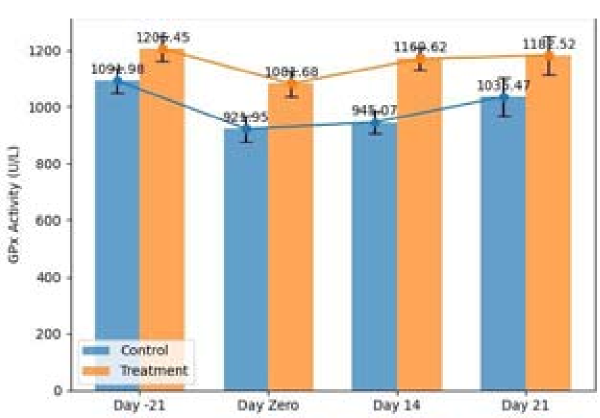

Fig.1.The GPx activity of the control and treatment groups over time

Glutathione, a key antioxidant, is predominantly synthesized in the liver (Trevisan *et al*., 2001). It is a tripeptide composed of three amino acids: glutamine, cysteine, and glycine. Methionine supplementation in transition dairy cows has been observed to elevate liver glutathione levels.This increase in glutathione goes beyond its function as a hepatic antioxidant; it can also be released into the bloodstream, where it helps regulate systemic oxidative stress. The transition period, particularly post-calving, often sees a depletion of glutathione in the liver, a phenomenon supported by studies conducted by Osorio *et al.* (2014), Zhou *et al.* (2016) and Batistel*et al.* (2018). In bovine mammary epithelial cells, methionine has been shown to enhance GSH-Px production. Furthermore, it has been demonstrated that increased RPM modifies the hepatic gene expressions in the transition cow methionine cycle, increasing the generation of antioxidants such as taurine and glutathione (Sun *et al*., 2016).

The observed decline in liver glutathione postpartum indicates that the metabolism of transition dairy cows heavily relies on this amino acid reservoir for crucial functions, particularly in combating oxidative stress. To elaborate, the reduction in key proteins, such as glutathione and albumin, synthesised in the liver suggests a compromised state of liver protein synthesis during the transition period. The supplementation of limiting amino acids, like methionine, has the potential to reverse these conditions, facilitating a smoother transition from pregnancy to lactation in dairy cows.

Milk yield

Table4 and Fig.2display the average milk yield levels in crossbred transition cattle, comparing those with and without supplementation of rumen-protected methionine and lysine in their diet. The results suggest that there is no statistically significant (p>0.05) difference in milk yield levels between the T_2 and T_1 groups. Additionally, no noteworthy variations in milk yield levels were observed across different days within both the control and treatment groups. The T_2 group consistently exhibited higher milk yield compared to the T_1 group throughout all days of data collection.

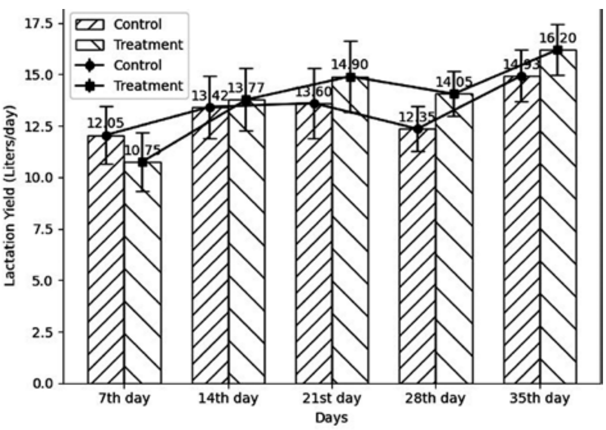

Fig.2 Lactation yield of control and treatment group over time

The findings strongly indicated a positive outcome associated with the addition of rumen-protected methionine and lysine to the diet, resulting in enhanced milk production.Methionine is considered the first limiting amino acid for milk production. Therefore, the enhanced milk yield observed with methionine supplementation can be attributed to its increased availability, supporting milk production processes. Lysine is also identified as the second limiting amino acid,contributing to improved lipid export during liver metabolism.The synthesis of L-carnitine, facilitated by lysine, is crucial for fatty acid transport within liver cells' mitochondria, ultimately supporting efficient fat metabolism and energy generation essential for milk production. Therefore, incorporating rumen-protected forms of methionine and lysine into dairy cow diets holds significant potential for optimising milk yield and overall herd performance.These findings align with the results reported byNoftsger and St-Pierre (2003), Davidson *et al.* (2008), Broderick and Muck (2009) and Wang *et al.* (2010) all of whom observed enhanced milk yield in multiparous cows with the supplementation of rumen-protected methionine and lysine. In contrast, several researchers, including Benefield *et al.* (2009), did not observe any significant impact on milk production with the supplementation of rumen-protected methionine. Additionally, Robinson*et al.* (2011) reported no discernible effect on milk production when supplementing with rumen-protected lysine alone.

Conclusion

Feeding RPM and RPL to crossbred dairy cattle during the transition period is a targeted nutritional strategy that aims to enhance antioxidant status, support milk protein synthesis, and improve overall lactational performance. However, it is essential to base nutritional decisions on scientific research and consult with nutritionists or veterinarians to optimize the diet according to specific herd requirements.

References

Batistel, F., Arroyo, J.M., Garces, C.I.M., Trevisi, E., Parys, C., Ballou, M.A., Cardoso, F.C. and Loor, J.J. 2018. Ethyl-cellulose rumen-protected methionine alleviates inflammation and oxidative stress and improves neutrophil function during the periparturient period and early lactation in Holstein dairy cows. *J. Dairy Sci.* **101**: 480-490.

Benefield, B.C., Patton, R.A., Stevenson, M.J. and Overton, T.R. 2009. Evaluation of rumen-protected methionine sources and period length on performance of lactating dairy cows within Latin squares. *J. Dairy Sci.* **92**: 4448-4455.

Bernabucci, U., Ronchi, B., Lacetera, N. and Nardone, A.2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *J. Dairy Sci.* **88**: 2017-2026.

Broderick, G.A. and Muck, R.E. 2009.Effect of alfalfa silage storage structure and rumen-protected methionine on production in lactating dairy cows. *J. Dairy Sci.***92**:1281-1289.

Drackley, J.K. 1999. Biology of dairy cows during the transition period. *J. Dairy Sci.* **82***:* 2259-2273.

Davidson, S., Hopkins, B.A., Odle, J., Brownie, C., Fellner, V. and Whitlow, L.W.2008. Supplementing limited methionine diets with rumen-protected methionine, betaine, and choline in early lactation Holstein cows. *J. Dairy Sci.* **91**:1552-1559.

Khan, M.Z., Liu, S., Ma, Y., Ma, M., Ullah, Q., Khan, I.M., Wang, J., Xiao, J., Chen, T., Khan, A. and Cao, Z. 2023. Overview of the effect of rumen-protected limiting amino acids (methionine and lysine) and choline on the immunity, antioxidative, and inflammatory status of periparturient ruminants. *Front. Immunol*. **13**:1042895.

Lykkesfeldt, J. and Svendsen, O., 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. *Vet. J.* **173**: 502-511.

Noftsger, S.and N. R. St-Pierre. 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. *J. Dairy Sci*. **86**: 958-969.

Osorio, J.S., Trevisi, E.R.M.I.N.I.O., Ji, P., Drackley, J.K., Luchini, D., Bertoni, G. and Loor, J.J. 2014. Biomarkers of inflammation, metabolism, and oxidative stress in blood, liver, and milk reveal a better immune metabolic status in peripartal cows supplemented with Smartamine M or MetaSmart. *J. Dairy Sci*. **97**:7437-7450.

Paglia, D.E. and Valentine W.N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*. **70**: 158–169.

Robinson, P.H., Swanepoel, N., Shinzato, I. and Juchem, S.O. 2011. Productive responses of lactating dairy cattle to supplementing high levels of ruminally protected lysine using a rumen protection technology. *Anim. Feed Sci.Technol.* **168**:30-41.

Sun, F., Cao, Y., Cai, C., Li, S., Yu, C. and Yao, J. 2016. Regulation of nutritional metabolism in transition dairy cows: energy homeostasis and health in response to post-ruminal choline and methionine. *PloS one*. **11**: 0160659.

Trevisan, M., Browne, R., Ram, M., Muti, P., Freudenheim, J., Carosella, A.M. and Armstrong, D. 2001. Correlates of markers of oxidative status in the general population. *Am.J. Epidemiol.* **154**:348-356.

Turk, R., Podpecan, O., Mrkun, J., Kosec, M., Flegar-Mestric, Z., Perkov, S.andZrimsek, P. 2013. Lipid mobilisation and oxidative stress as metabolic adaptation processes in dairy heifers during transition period. *Anim.Reprod.Sci.* **141**:109-115.

Wang, C., Liu, H.Y., Wang, Y.M., Yang, Z.Q., Liu, J.X., Wu, Y.M., Yan, T. and Ye, H.W. 2010. Effects of dietary supplementation of methionine and lysine on milk production and nitrogen utilization in dairy cows. *J. Dairy Sci*. **93**: 3661-3670.

Zhou, Z., O. Bulgari, M. Vailati-Riboni, E. Trevisi, M. A. Ballou, F. C. Cardoso, D. N. Luchini. and J. J. Loor. 2016. Rumen-protected methionine compared with rumen-protected choline improves immune metabolic status in dairy cows during the peripartal period. *J Dairy Sci.* **99**:8956-8969.

■