



Effect of Ration Balancing on Milk Production, Microbial Protein Synthesis and Methane Emission in Crossbred Cows under Field Conditions in Chittoor District of Andhra Pradesh

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ABSTRACT

Effect of balancing the ration on milk production, microbial protein synthesis and methane emission was studied in 30 crossbred cows under field conditions in Chittoor district of Andhra Pradesh. Nutritional status of animals was analysed by ration balancing software and methane emission by sulphur hexafluoride technique. Microbial protein synthesis was calculated by estimation of purine derivatives excreted in urine. Initially, baseline data for all the animals were estimated and thereafter their ration was balanced as per their nutrient requirements by using ration balancing software. After 30 days of feeding again all the parameters were estimated. Analysis of the feeding practices revealed that dietary intake of TDN was adequate but CP intake was lower (10.44%) than their requirements. The calcium and phosphorus were also deficient by 47.81 and 46.52%, respectively. Balancing the ration did not affect body weight, dry matter intake, TDN intake and concentrate roughage ratio. CP intake was improved significantly ($P < 0.05$) after balancing the ration. Though milk fat was not affected, balancing of ration significantly ($P < 0.05$) improved the milk yield and reduced methane production. The average methane emission reduction was 11.17 and 19.62%, in terms of g/d and g/kg DMI, respectively, besides reducing ($P < 0.05$) the gross energy lost as methane. The calculated microbial nitrogen supply (g/d) was also significantly higher ($P < 0.05$) after balancing the ration. It was concluded that ration balancing has the potential to improve milk production, microbial protein synthesis and reduce methane emission from lactating crossbred cows.

Key words: Ration Balancing, Methane Emission, Lactating Cows, Field Trial.

INTRODUCTION

Global dairy sector contributes 4.0 per cent to the total anthropogenic greenhouse gas emissions (FAO 2010). Methane is the most important greenhouse gas produced from livestock sector and enteric fermentation from ruminants is responsible for 30% of the global anthropogenic methane emissions (USEPA 2006). Indian livestock sector emitted 10.37 million MT of

methane, contributing 50 per cent of total methane emissions from India in the year 2007 (INCCA 2010). Reducing methane emission is a key element of sustainable milk production. Improving efficiency of microbial protein production (EMP) and high proportional propionic acid production in comparison with acetic acid production proportionally reduces methane loss (Blummel et al. 2010). Though increasing concentrate mixture in the diet improves propionic acid

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production and reduces methane emission, the strategy is not practical in crop residue based small holder dairy production system like India because of shrinkage in resources like land and water (Blummel et al. 2010). Though supplementation of bromochloromethane (Lalu et al. 2009) and plant extracts (Patra et al. 2006) are known to reduce methane emission, their usefulness as a strategy to reduce methane emission in small holder dairy production system is limited. Recent studies suggest that feeding balanced ration as per the nutrient requirement of animals reduces methane emissions due to improved feed utilization and enhanced overall production efficiency (Capper et al. 2009; GHGMP 2005). Field surveys conducted by different workers reported the imbalance of energy and protein (Singh et al. 2002; Mudgal et al. 2003, Gupta et al. 2006) and minerals deficiency (Garg et al. 2000; Bakshi et al. 2009) in the traditional feeding practices followed by farmers which results in poor productive and reproductive performance of the animals (Garg et al. 2000). Hence, in crop residues based feeding system, ration balancing could be an important strategy to make the best use of available resources with nutritional and environmental benefits.

Keeping this in view, the present study was carried out to know the effect of ration balancing on milk production, microbial protein production and methane emission in lactating crossbred cows under field conditions in Chittoor district of Andhra Pradesh.

MATERIALS AND METHODS

Thirty crossbred cows, in their second to fourth lactation with a production of 6.0 to 13.0 kg milk per day with 3.5 to 4.5% milk fat, belonging to twenty eight farmers were chosen for the study from six villages of Chittoor district in Andhra Pradesh state. The feed was offered by the farmers to animals twice a day i.e. morning & evening. The feed intake of each animal was measured and representative sample was taken for proximate and

detergent fiber analysis. Thereafter, the ration of all the animals was balanced for total digestible nutrients (TDN), crude protein (CP), calcium and phosphorus using the ration balancing software developed by National Dairy Development Board (NDDB), which is based on NRC (1989) for cows. The balanced diet was fed to all the animals for 30 days. Blood samples were collected before and after balancing the ration. Dry matter intake, milk yield and milk fat were recorded daily during methane gas sampling period. On the spot urine (100 ml) samples were collected from all experimental animals before and after balancing the ration and preserved with sufficient quantity of 10% H₂SO₄ to maintain pH below 3. The urine samples were diluted in such a way that the concentration in the final sample would fall within the range of standards used in the assays for estimation of purine derivatives (IAEA 1997). The body weights of the animals were calculated using Shaeffer's formula (Sastry et al. 1982).

$$\text{Body weight (kg)} = \left(\frac{[(\text{heart girth in inches})^2 \times \text{length of the body in inches}] / 300}{1000} \right) \times 0.4536$$

Methane measurement was done using sulfur hexafluoride tracer technique (Johnson et al. 1994). All the selected animals were covered under the insurance for a period of one year, in view of farmer's reluctance to allow insertion of permeation tube in the lactating animals. A small permeation tube containing sulfur hexafluoride (SF₆) was taken and kept at 39°C. The release rate of SF₆ from the permeation tube was determined and when it became constant, the tube was ready for the insertion into the rumen of cow. A halter fitted with a capillary tube was placed on the animal's head and connected to an evacuated sampling canister. One week before start of sample collection, permeation tubes with known SF₆ release rate were inserted in the rumen of all the animals selected for the experiment and all the animals were acclimatized to wearing the halter and canister. The breath samples of all the experimental

animals were collected daily for four consecutive days in canisters and brought to the laboratory for methane and SF₆ analysis at the start of the study. After one month of experimental feeding, methane emission was measured again using similar method. Methane and SF₆ concentrations were then determined by gas chromatography. Methane emission rate was calculated as the product of the permeation tube emission rate and the ratio of CH₄ to SF₆ concentration in the sample. Samples were analyzed in duplicate. The gas chromatograph was fitted with a molecular sieve 5A column for SF₆ and a Porapak N for methane. The column temperature was maintained at 50°C and nitrogen was used as a carrier gas, with a flow rate of 30ml min⁻¹. Prepared standards were used to standardize the gas chromatograph for SF₆ (39ppt and 79ppt, Scott-Marrin Inc., Riverside, CA, USA) and methane (10ppm and 100ppm, Scott-Marrin Inc., Riverside, CA, USA).

Methane emission rate was calculated as under:

$$Q \text{ CH}_4 = Q \text{ SF}_6 \times (\text{CH}_4) / (\text{SF}_6)$$

Where

QCH₄ - Methane emission rate (g/min)

QSF₆ - Known release rate of SF₆ from permeation tube (g/min)

CH₄ - Methane concentration of collected sample in canister (µg/m³)

SF₆ - SF₆ concentration of collected sample in canister (µg/m³)

Proximate composition (AOAC 1995), acid detergent fiber (ADF) and neutral detergent fiber (NDF) of feeds and fodders were determined (Van Soest et al. 1991). The milk samples were analysed for milk fat (IS: 1224 1977). The stored spot urine samples were assayed for purine derivatives and creatinine (IAEA 1997). Purines absorbed and microbial nitrogen supply were calculated from the daily urinary purine derivatives excreted (IAEA 1997). Gross energy of feed and fodder samples were calculated from the prediction equation of Guenther (1979). Energy content of methane was taken as 13.34 kcal per g (Brouwer 1965). The data were statistically analyzed using paired student's t-test (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

Farmers used cattle feed, groundnut cake, rice bran and paddy straw as feeds for the animals. Body weight and feed intake of the experimental cows before and after ration balancing are presented in Table 1. Analysis of the feeding practices revealed that though the dietary intake of TDN was adequate, CP intake was lower

Table 1. Body weight, plane of nutrition and milk yield of the experimental cows

Parameter	Cows	
	Baseline	After ration balancing
Body weight (kg)	319.40 ± 5.67	321.97 ± 5.37
DM intake (kg/day)	10.47 ± 0.74	11.57 ± 0.30
Concentrate : Roughage ratio	28.30 : 71.70	32.07 : 67.73
DMI (kg /100 kg B. Wt)	3.28 ± 0.12	3.59 ± 0.08
DMI (g/ kg W ^{0.75})	138.58 ± 4.86	152.22 ± 3.24
CP intake (g/day)*	972.63 ^a ± 17.13	1082.13 ^b ± 16.23
TDN intake (g/day)	5588.18 ± 92.17	5714.32 ± 77.14
Blood urea N (mg/100ml)	10.78 ^a ± 1.02	12.5 ^b ± 0.92
Milk yield (kg/day) *	8.43 ^a ± 0.43	8.85 ^b ± 0.42
Milk fat (%)	4.10 ± 0.05	4.14 ± 0.05

^{a,b} Values with different superscript in a row differ significantly (P<0.05).

(10.44%) than their requirements. The calcium and phosphorus were also deficient by 47.81 and 46.52%, respectively. Deficiency of CP and minerals in the diet of lactating cows in traditional feeding practices in different parts of India were reported by different workers (Singh et al. 2002; Mudgal et al. 2003; Dutta et al. 2010). Though TDN intake was similar, the CP intake was improved ($P < 0.05$) significantly after balancing the ration of selected cows. Concentrate and roughage intake was also similar before and after balancing the ration as evident from similar concentrate: roughage ratio. Ration balancing did not affect body weight of cows.

Effect of Ration Balancing on Milk Yield and Milk Fat Initially, average daily milk production was 8.43 kg/d and the milk fat content was 4.10% in cows. Implementation of ration balancing programme improved the milk yield by 0.42 kg milk/ animal/ day which was significantly ($P < 0.05$) higher than before implementation of the programme (Table 1). The improvement in milk yield may be due to balancing of nutrients, which might have alleviated the deficiency of protein and minerals as good quality mineral mixture was used for balancing the ration. Many on farm studies also reported improvement in milk yield following the strategic supplementation of deficient nutrients in the diet. On farm feeding trials of feeding balanced ration improved milk yield in cattle and buffaloes in Gujarat

(Garg et al. 2009) and strategic supplementation of limiting nutrients increased milk yield in buffaloes in Uttar Pradesh (Dutta et al. 2010) and Maharashtra (Khochare et al. 2010).

Methane Emission

Baseline methane production from cows was 187.28 g/day (Table 2). These values are similar to those reported earlier (Holter and Young, 1992; Madhu Mohini and Singh, 2010). Holter and Young (1992) found methane emission from 358 lactating Holstein cows varying from 175 to 299 g/day/cow. Madhu Mohini and Singh (2010) reported methane emission varying from 160.90 to 223.45 g/day for lactating cows yielding 4.36 to 7.18 kg milk/ day. Average methane emission (g/kg DMI) was 17.89 ± 0.28 which was similar to the values reported by Madhu Mohini and Singh (2010). Due to ration balancing, average reduction of 11.17% and 19.62% methane emission in terms of g/day and g/kg DMI, were observed which were significantly lower than the baseline emissions ($P < 0.05$). The dietary gross energy loss as methane was also reduced significantly ($P < 0.05$) from 5.96 to 4.76 %.

Purine derivatives excretion and microbial nitrogen supply

The mean concentration of allantoin, uric acid, total purine derivatives and creatinine (mM/l) are

Table 2. Effect of ration balancing on methane emission in lactating crossbred cows

Parameter	Cows	
	Base line	After ration balancing
Methane emission (g/animal/day) *	187.28 ^b ± 4.56	166.36 ^a ± 3.93
Dry matter intake (kg/day)	10.47 ± 0.74	11.57 ± 0.30
Methane emission (g/kg DMI) *	17.89 ^b ± 0.28	14.38 ^a ± 0.25
Organic matter intake (kg/day)	9.59 ± 0.71	10.39 ± 0.29
Methane emission (g/kg OMI) *	19.52 ^b ± 0.27	16.01 ^a ± 0.24
Gross energy intake (Mcal/day)	41.98 ± 1.34	46.63 ± 1.20
Energy loss as methane (Mcal/day) *	2.50 ^a ± 0.06	2.22 ^b ± 0.05
Energy loss as methane (% of gross energy) *	5.96 ^a ± 0.09	4.76 ^b ± 0.08

^{a,b} Values with different superscript in a row differ significantly ($P < 0.05$).

presented in Table 3. Total purine derivatives excreted (mM/d) were significantly lower ($P < 0.05$) before balancing the ration. Balancing the ration significantly increased purine derivatives excretion and the microbial nitrogen (g/d) supply to the animals, which may be attributed balancing of nutrients in the ration of cows, as it alleviated the dietary deficiency of protein and minerals.

The reduction in the methane emission observed in the present study may be attributed to the balancing of nutrients, which might have changed rumen fermentation towards production of more microbial biomass which is the major protein source for lactating animals fed on crop residue based ration. There was deficiency of CP in the diet of cows, which was confirmed by their low blood urea nitrogen level (Table 1). The increased nitrogen supply after ration balancing might have provided the required fermentable nitrogen for efficient microbial protein synthesis as evident from the more purine derivatives excreted and more microbial nitrogen supply after balancing the ration (Table 3). The minerals supplied in the diet also could have enhanced the microbial cell growth since ash content of microbial matter is 13% (Czerkawski 1986). The reduction in methane emission observed in the study following the ration balancing was consistent with the earlier reports (Leng, 1991). Madhu Mohini and Singh

(2010) also observed supplementation of nutrients to cows fed on low plane of nutrition through concentrate mixture or urea molasses mineral block, improved digestibility of nutrients as well as decreased methane emission.

In crop residue based diets, as practiced in India, the limitation for growth of microorganisms on diets is probably due to inadequate concentration of ruminal ammonia and deficiency of trace and macro minerals besides low feed intake. Depending on the efficiency of utilization of ATP for microbial cell synthesis, the amount of carbohydrate converted to microbial cells can be highly variable, which controls the production of methane and volatile fatty acids (Leng 1991; Blummel et al. 2010). Therefore, feeding as per the nutrient requirement of animals provides an effective measure for reducing methane emission as recorded in dairy cattle in USA (Capper et al. 2009) and in beef cattle in Canada (GHGMP 2005) due to improved feed utilization and enhanced overall production efficiency of the herd.

These findings suggested that the ration balancing has the potential to improve milk production, microbial protein supply as well as to reduce methane production in crossbred cows under field conditions. More experiments are needed under different conditions of feeding and management to generate more information.

Table 3. Effect of ration balancing on rumen microbial protein synthesis in lactating crossbred cows.

Parameter	Cows	
	Baseline	After ration balancing
Average metabolic b. wt. (kg $w^{0.75}$)	75.55 ± 1.01	76.01 ± 0.95
Uric acid (mM/l)	2.52 ± 0.07	2.79 ± 0.06
Allantoin (mM/l)	12.12 ± 0.28	14.48 ± 0.30
Purine derivatives concentration (mM/l)	14.64 ± 0.28	17.27 ± 0.30
Creatinine concentration (mM/l)	7.91 ± 0.17	7.48 ± 0.19
PDC index	139.83 ± 1.01	175.49 ± 0.95
Total PD excreted* (mM/d)	137.04 ^a ± 0.99	171.98 ^b ± 0.94
Absorbed purine* (mM/d)	148.16 ^a ± 0.99	189.18 ^b ± 0.94
Intestinal flow of microbial nitrogen* (g/d)	107.71 ^a ± 0.72	137.54 ^b ± 0.67

a,b Values with different superscript in a row differ significantly ($P < 0.05$).

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REFERENCES

- AOAC, 1995. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, D.C.
- Bakshi, M.P.S., M. Wadhwa, K. Kaur and J. Kaur. 2009. Nutritional status of animals of western plain zone of Punjab. *Indian J. Anim. Nutr.* 26:199-203.
- Blummel, M., L.A. Wright and N.G. Hegde. 2010. Climate change impacts on livestock production and adaptation strategies: A global scenario. In: National Symposium on Climate Change and Rainfed Agriculture. P 136-145. Central Institute for Dryland Agriculture, Hyderabad, India.
- Brouwer, E. 1965. Report of subcommittee on constants and factors. Proc. III EAAP Symp. In: Energy Metabolism, EAAP Publ.No.11, Academic Press, London. pp. 441-443.
- Capper, J.L., R.A. Cady and D.E. Bauman. 2009. The environmental impact of dairy production: 1944 compared with 2007. *J. Anim. Sci.* 87: 2160-2167.
- Czerkawski, J.W. 1986. An Introduction to Ruminant Studies. Pergamon Press. Oxford. England.
- Dutta, N., K. Sharma, A.K. Pattanaik, Anand Singh and Amit Narang. 2010. Effect of strategic supplementation of limiting macro nutrients on the lactation performance of buffaloes. P27. Proc. VII Animal Nutrition Association Conference, Bubhaneshwar, India.
- FAO. 2010. Green House Gas Emissions from Dairy Sector-A Life cycle Analysis. Food and Agricultural Organization of United Nations, Rome, Italy.
- Garg, M.R., S.A. Biradar and A. Kannan. 2009. Assessment of economic impact of implementing ration balancing programme in lactating cows and buffaloes under field conditions. *Indian J. Anim. Nutr.* 26:146-150.
- Garg, M.R., S.P. Arora, B.M. Bhandari, P.L. Sherasia and D.K. Singh. 2000. Mineral status of feeds and fodders in Khaira district of Gujarat. *Indian J. Dairy Sci.* 53: 291-297.
- GHGMP. 2005. Greenhouse Gas Mitigation Program for Canadian Agriculture. Report to the Cattle Industry. Canadian Cattlemen's Association, NE Calgary, Canada.
- Guenther, K.D. 1979. Nettoenergie Lactation (NEL) die neue energetische Futterbewertung für milchkihe. *Kraftfutter.* 62: 310-311.
- Gupta, R.S., S.G. Vahora, D.C. Patel, B.R. Devalia, C.R. Bhatt, Y.J. Patil, M.B. Pande and S. Parnerkar. 2006. Nutritional status of dairy cows in Vadodara district of middle Gujarat. Proc. XII Animal Nutrition Conference on Technological Interventions in Animal Nutrition for Rural Prosperity, Anand, Gujarat, India.
- Holter, J.B. and A.J. Young. 1992. Methane production in dry and lactating Holstein cows. *J. Dairy Sci.* 75: 2165-2172.
- IAEA. 1997. Estimation of Rumen Microbial Protein Production from Purine Derivatives in Urine. IAEA-TECDOC-945, International Atomic energy Agency, Vienna, Austria.
- INCCA. 2010. India: Greenhouse Gas Emissions - 2007. Indian Network for Climate Change Assessment, Ministry of Environment and Forests, Government of India.
- IS: 1224, 1977. Indian Standards Methods of Test for Dairy Industry Part-I, Chemical Analysis of Milk, Indian Standards Institute, New Delhi, India.
- Johnson, K.A., M.T. Huylar, H.H. Westberg, B.K. Lamb, and P. Zimmerman. 1994. Measurement of methane emissions from ruminant livestock using a SF₆ tracer technique. *Environ. Sci. & Technol.* 28: 359.
- Khochare, A.B., V.D. Kank, G.M. Gadegaonkar and S.C. Salunke. 2010. Strategic supplementation of

- limiting nutrients to medium yielding dairy animals at field level. Proc. VII Animal Nutrition Association Conference, Bhubaneswar, India. P 30
- Lalu, K., R. Bhar, A. Das and A.B. Mandal. 2010. Effect of bromochloromethane supplementation and dietary energy restriction on methane production and efficiency of energy utilization in rams. *Indian J. Anim. Nutr.* 26: 97-102.
- Leng, R.A. 1991. Improving Ruminant Production and Reducing Methane Emissions from Ruminants by Strategic Supplementation. US EPA. Washington D.C.
- Madhu Mohini and G.P. Singh. 2010. Effect of supplementation of urea molasses mineral block on the milk yield and methane production in lactating cattle on different plane of nutrition. *Indian J. Anim. Nutr.* 27: 96-102.
- Mudgal, V., M.K. Mehta, A.S. Rane, and S. Nanavati. 2003. A survey on feeding practices and nutritional status of dairy animals in Madhya Pradesh. *Indian J. Anim. Nutr.* 20: 217-220.
- NRC. 1989. Nutrient Requirement of Dairy Cattle. 6th edn. National Research Council, National Academy Press, Washington, D.C., U.S.A.
- Patra, A.K., D.N. Kamra and Neeta Agarwal. 2006. Effect of plant extracts on *in vitro* methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. *Anim. Feed Sci. Technol.* 128: 276-291.
- Sastry, N.S.R., C.K. Thomas and R.A. Singh. 1982. Farm Animal Management and Poultry Production, 2nd edn. Vikas Publishing House Pvt. Ltd., New Delhi.
- Singh, D., A.S. Yadav and K.R. Yadav. 2002. Feeding practices of lactating buffaloes in Mohindergarh district of Haryana. *Indian J. Anim. Nutr.* 19: 153-155.
- Snedecor, G.W. and W.G. Cochran. 1989. Statistical Methods, 9th edn. The Iowa State University Press, Ames, IA.
- USEPA. 2006. Global Anthropogenic Emissions of Non-CO₂ Greenhouse Gases 1990-2020 EPA Report 430-R-06-003. US Environmental Protection Agency, Washington, USA.
- Van Soest, P.J., J.B. Robertson and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3583-3597.

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