Credit No.: 5074-IN

For office use only

## **Evaluating the Impact of Ration Balancing on Methane Emissions in Dairy Animals**

# **FINAL REPORT**



## Anand Agricultural University, Anand-388 110, Gujarat



National Dairy Plan-I (National Dairy Support Project) National Dairy Development Board, Anand-388 001



August, 2016

Project Team Leader	Dr Subhash Parnerkar Research Scientist & Head
	Dr P R Pandya Research Scientist
	Dr M A Shekh Assistant Research Scientist
Team Members	Dr B R Devalia Assistant Research Scientist
	Dr R S Gupta Research Scientist
	Dr D C Patel Research Scientist

Animal Nutrition Research Station Anand Agricultural University, Anand - 388 110, Gujarat, India Contact details: (O) 02692 263440 E mail: parnerkar@aau.in

## INDEX

Sr. No.	Contents	Page No.
1	Acknowledgement	1
2	Executive summary	2
3	Introduction	5
4	Background of the study	7
5	Methodology	7
6	Results and discussion	13
7	Limitations of the study and future possibilities of research in the area	18
8	Conclusion	18
9	References	19
10	Annexure I	22

#### ACKNOWLEDGEMENT

The team of scientists duly acknowledges the overall support and guidance from honourable Vice Chancellor, Dr. N. C. Patel; Dr. K. B. Kathiria, the Director of Research and Dr. A. M. Thaker, the Dean, Veterinary Faculty, Anand Agricultural University, Anand for conducting the present study. The financial support from National Dairy Development Board (NDDB) is also duly acknowledged. We express a special gratitude to Dr M R Garg, General Manager (AN), Sh Aditya Jha, General Manager (CMC), Dr Pankaj L Sherasia, Scientist-II (AN) and Sh Arvind Kumar, Manager (CMC) from NDDB for their continuous help during the entire study period. Thanks are also due to the office and technical staff of the department for their continuous efforts for smooth running of the study. Last but not the least, the continuous support of farmers of Jahangirpura and Bhumel villages during the work deserves special thanks.

Date:30/08/2016 Place: Anand

2000 (Subhash Parnerkar)

Project Team Leader and Research Scientist & Head Animal Nutrition Research Station College of Veterinary Science & A.H. Anand Agricultural University, Anand

(K. B. Kathiria) Director of Research

& Dean, PG Studies

Anand Agricultural University, Anand

#### **EXECUTIVE SUMMARY**

India is the largest milk producing country in the world, having estimated production of 155.5 million tonnes of milk in 2015-16. Emerging trends indicate that milk demand is growing rapidly and is likely to reach between 200 and 210 million tonnes by the year 2021-22. For domestic supply to meet the projected demand, incremental annual milk production of about 6 million tons per annum is needed over the next 15 years. If milk production fails to increase at the required pace, the demand-supply gap would continue to widen, which could lead to dependence on imports.

The ruminant livestock contribute up to 50% of the total methane (CH<sub>4</sub>) emission in India (INCCA, 2010). Over a wide range of diets, enteric methane accounts for 2 to 12% of dietary gross energy intake (Johnson and Johnson, 1995) which represents a significant loss of energy that could otherwise potentially be repartitioned toward tissues or the mammary gland. Due to the concerns of increase in greenhouse gases emissions into the environment and its potential effects on global warming, there is a need to develop strategies to lower methane emission from ruminants to secure and develop more sustainable ruminant food production systems. Manipulating diet composition to induce changes in rumen fermentation characteristics remains the most feasible approach to achieve reduction in methane emission (Bayat and Shingfield, 2012).

Imbalanced feeding is widely prevalent in the smallholder dairy systems of tropical countries, like India. Imbalanced feeding not only produces less milk at a higher cost, but also produces more methane per litre of milk production. Livestock fed imbalanced rations produce more methane, as most of the dietary organic matter (OM) is fermented to produce acetate and butyrate, resulting into more CH<sub>4</sub> production (Blummel, 2000). On the contrary, Leng (1991) has reported that if the ration is balanced for all essential nutrients, OM is fermented to produce more microbial biomass and less of CH<sub>4</sub>. Changing plane of nutrition through balanced feeding improves rumen fermentation pattern and thus reduces methanogenesis in ruminants.

The present study was planned to evaluate the impact of ration balancing on methane emissions in dairy animals in western region of India. In this region, Anand district of Gujarat state is considered to have a significant importance for dairying, hence its two villages Jahangirpura and Bhumel were selected for the study. Thirty seven early lactating buffaloes were shortlisted. A structured schedule/ Questionnaire was prepared and the data on feeding practices of the animals followed by the dairy farmers were recorded through Personal Interview. Sulfur hexafluoride (SF<sub>6</sub>) tracer technique was used for measurement of methane emission from milch animals under field conditions. Permeation tubes were filled with SF<sub>6</sub> gas and its release rate was recorded. Permeation tubes with known release rate of SF<sub>6</sub>gas were inserted in the rumen of experimental animals.

To measure methane emission before feeding a balanced ration, dummy canisters and halters were fitted to individual animal for 3 days for adaptation. After adaptation period, breath samples (24 hours basis) were collected from individual animal for four consecutive days in evacuated canisters. The canisters were filled with nitrogen gas for maintaining neutral pressure and were brought to AAU, Anand for estimation of methane emissions. The samples of feed, fodder and milk of individual animal were also collected before feeding a balanced ration. All the canisters were analyzed by gas chromatography in the laboratory at Animal Nutrition Research Station, AAU, Anand for methane and  $SF_6$  concentration in the breath samples.

After completing the baseline methane emission measurement (before ration balancing), the ration of individual animal were balanced for energy, protein, calcium and phosphorus using software developed by NDDB. The farmers were advised to feed balanced ration to their buffaloes for a period of 30 days. After this period was over, again the breath samples were collected from individual animal for 4 consecutive days in evacuated canisters and analyzed for methane and SF<sub>6</sub> as described above. Milk yield and milk fat content of individual animal on daily basis during the methane collection period was determined before and after balancing the ration on individual animal.

Methane emission was calculated as per the product of the permeation tube release rate and the ratio of  $CH_4$  to  $SF_6$  concentration in the breath samples. All samples were analyzed in duplicates. Methane emission (g/kg milk yield) was calculated before and after feeding a balanced ration. Statistical analysis of data was done using SAS software version 9.3.

In the present study, the daily average milk yield before ration balancing was 8.68 kg, which increased significantly (P<0.05) to 9.11kg after feeding a balanced ration. The Milk fat content also increased from 6.79 to 7.02% (P<0.05) after feeding a balanced ration. The average methane emission from buffaloes was 214.59 and 192.73 g/ day before and after feeding a balanced ration, respectively. The average methane emission in terms of g/kg milk yield was reduced significantly from 25.51to 21.63 in buffaloes after feeding a balanced ration. The balanced feeding reduced average methane emission (g/kg milk yield) by about 15.21% in experimental buffaloes. Thus, ration balancing helped in improving productivity of lactating buffaloes with concomitant reduction in enteric methane emission in western region of India.

#### INTRODUCTION

Feeding is the foundation of livestock systems and accounts for more than 70% of the total cost of milk production. It affects the entire livestock sector, including animal productivity, health and welfare, and the environment. Devendra and Leng (2011) have stated that the locally available feed resources act as the key driving force for improving the productivity of animals in developing countries. Feeding as per the nutrient requirement of animals, using locally available feed resources is imperative for improving the genetic potential of low yielding dairy animals in India. Therefore, to maximize profitability from the dairy animals, one needs to ensure that the dairy animals receive required quantity of protein, energy, minerals and vitamins, preferably from locally available feed resources. This would result in improved production and nutrient use efficiencies. The balanced nutrition approach is also one of the most promising ways to reduce methane emissions in ruminants.

It is documented that the most relevant methane mitigation strategy for smallholder mixed crop-livestock systems in tropical countries is to increase individual animal productivity as a consequence of providing nutritionally balanced feeds (Bayat and Shingfield, 2012; Hristov *et al.* 2013).

In India, most of the farmers follow traditional feeding practices, which often lead to either excess or deficient intake of protein, energy and minerals as compared to requirement of the animals. Imbalance of protein, energy and minerals exists widely in dairy animals and severity of the excess or deficiency depends upon the type of diet, age, physiological status of animals and the agro-climatic conditions of the region (Underwood and Suttle, 1999). Various studies conducted in India show that either there is deficiency of energy (Mudgal *et al.*, 2003) or in excess (Singh *et al.*, 2002) in the ration of dairy animals. Similarly, protein is either deficient (Mudgal *et al.*, 2003; Singh *et al.*, 2002) or in excess (Gupta *et al.*, 2006) in the diet of animals.

Mineral deficiencies are frequently encountered in the ration of dairy animals in most of the developing countries (Underwood and Suttle, 1999; McDowell *et al.*, 1993). Excess or deficiency of minerals in soil are directly reflected in animals because livestock in tropics are maintained only on forages without additional

mineral supplementation (McDowell, 1985) and most occurring mineral deficiencies in dairy animals are area specific (Ramana *et al.*, 2001; Gowda *et al.*, 2002; Garg *et al.*, 2005). Farmers in India often do not feed adequate quantities of mineral mixtures to their animals due to non-availability and lack of knowledge about the benefits of feeding mineral mixtures or higher cost.

The concept of ration balancing is already in place in most of the developed countries, where the feed resources are available in abundance with good sources of protein, energy and minerals. The herd sizes are much bigger and the livestock owners are better versed with the scientific practices of feeding and management. In most of the tropical countries, herd sizes are smaller and dairy farmers follow traditional feeding practices, causing imbalance of nutrients in terms of protein, energy, minerals and vitamins. In view of this, the concept of ration balancing for smallholder dairy farmers in most of the tropical countries has been a challenge owing to their lack of knowledge and skills to prepare a balanced ration. Also the smallholder farmers are not in a position to hire specialists for preparing balanced rations.

The National Dairy Plan Phase-I (NDP-I) is a central sector scheme of Government of India, assisted by the World Bank and implemented by National Dairy Development Board (NDDB), Anand with the help of numerous End Implementing Agencies (EIAs). NDP-I is a scientifically planned multi state initiative to increase milk production by increasing milch animal productivity in existing herds through a focussed approach to feeding and breeding. The Ration balancing programme (RBP) is being implemented by the NDDB in different states of the country. To quantify the effect of ration balancing on enteric CH<sub>4</sub> emissions under field conditions, NDDB had undertaken various CH<sub>4</sub> emission measurement studies in different agro-climatic regions of the country, using SF<sub>6</sub>tracer technique (Johnson et al., 1994). The results of study conducted by Garg et al., (2014) indicate that balanced feeding has reduced methane emissions (g/kg milk yield) by 17.30% (P<0.05) and 19.50% (P<0.01) in lactating cows and buffaloes, respectively. Under NDP I, Anand Agricultural University (AAU), Anand as an external agency to the project conducted the present study for evaluating the impact of ration balancing on methane emissions in dairy animals in western India.

#### **BACKGROUND OF THE STUDY**

Emerging trends indicate that milk demand is growing rapidly and has been reachedabout 155.5 million tonnes by 2016-17 (the end year of 12<sup>th</sup> Five Year Plan). It is further projected that milk demand could reach between 200 and 210 million tonnes by the year 2021-22. For domestic supply to meet the projected demand, incremental annual milk production of about 6 million tones per annum is needed over the next 15 years (compared to actual achievement of about 3 million tonnes annually over the last 15 years). If milk production fails to increase at the required pace, the demand-supply gap would continue to widen, which could lead to dependence on imports.

Over a wide range of diets, enteric methane accounts for 2 to 12% of dietary gross energy intake (Johnson and Johnson, 1995) which represents a significant loss of energy that could otherwise potentially be repartitioned toward tissues or the mammary gland. Due to the concerns of increase in greenhouse gases emissions into the environment and its potential effects on global warming, it is obligatory to develop strategies to lower methane emission from ruminants and develop more sustainable ruminant food production systems. Manipulating diet composition to induce changes in rumen fermentation characteristics remains the most feasible approach to achieve reduction in methane emission (Bayat and Shingfield, 2012). The milk production targets could be achieved provided the available feed resources are utilized efficiently and also the genetic potential of animals for milk production is realised to the maximum possible extent.

#### METHODOLOGY

In order to evaluate the impact of ration balancing on methane emissions in dairy animals, 37 early lactating buffaloes (up to 100 days post calving) were shortlisted in Jahangirpura village of Anand district and Bhumel village of Kheda district. Insurance of these animals were taken from United India/Oriental Insurance Company Limited, Anand for one year period. The permeation tubes with known release rate of  $SF_6$  gas were inserted into the rumen of these experimental buffaloes through mouth. Sulfur hexafluoride ( $SF_6$ ) tracer technique for measurement of

methane emission from ruminants under field conditions is being followed (Johnson *et al.*, 1994).

The study was conducted in three phases taking 17, 10 and 10buffaloes in phase-I, II and III, respectively. Study in Phase-I (n=17) was conducted in Jahangirpura village of Anand district, whereas, Phase-II (n=10) and Phase-III (n=10) were conducted in Bhumel village of Kheda district. A structured questionnaire was prepared and the data on feeding practices forwarded by the dairy farmers in the two selected villages were recorded through Personal Interview (Photo 1). During before ration balancing period, animals were fed the ration as per the farm feeding practices followed. The Milk production and composition were recorded during this period. After one month of observation, the measurement of methane emission was done by collecting breath samples from the animals. After collecting the breath samples for methane emission measurements, the ration was balanced for individual animals as per the RBP software developed by NDDB. The farmers were advised to feed the balanced ration for 30 days. Regular monitoring and execution was followed during this period. After one month of feeding a balanced ration, the milk yield was recorded and the samples of milk and breath samples were collected again from the same animals.



Photo 1: Collecting information in a structured questionnaires

#### Estimation of baseline methane emission

#### Standardizing SF<sub>6</sub> Release Rate

About 100 permeation tubes were filled with pure (99.9%) sulfur hexafluoride (SF<sub>6</sub>) gas under liquid nitrogen. Permeation tubes containing SF<sub>6</sub> gas were kept at 39°C in water bath for 5 weeks period. The release rate of SF<sub>6</sub> from each permeation tubes was monitored weekly. After standardizing the release rate, 37 permeation tubes containing known release rate of SF<sub>6</sub> (2.79  $\pm$  0.05 mg/day) were inserted in the rumen of each experimental buffalo through mouth.

#### Preparation of canisters and halters

An evacuated PVC canisters having 2-2.5" ID and 200 psi pressure, PVC end caps (10 kg/cm3 pressure) and a 90° elbow were used for breath sample collection from each buffalo. A short (4") piece of  $\frac{1}{4}$ " teflon tubing was attached to the valve with female  $\frac{1}{4}$ " quick connect on the upstream end to allow attachment to the halter. The prepared canisters are shown in **Photo 2**.

#### **Collection of breath samples**

Methane emission measurement from all 37 buffaloes fed under traditional feeding practices was undertaken. Dummy canisters and halters were tied to individual buffaloes for 3 days. After this period, breath samples (24 hour basis) were collected from individual buffaloes for four consecutive days by tying canisters and halters with necessary accessories (Photo 3). After collection of breath samples for baseline measurement of methane emissions (control period), these canisters were analyzed in the laboratory for methane and SF<sub>6</sub> concentration in the breath samples.



Photo 2: Preparation of canisters for collecting breath samples



Photo 3: Collection of breath samples before ration balancing for methane analysis

#### Feeds, fodder and milk

During control period, feeding of milch animals was in accordance with the prevailing feeding practices that the farmers followed. Feeds and fodder samples of these animals were collected and analyzed for proximate constituents in our laboratory (AOAC, 2005). The daily milk yield and milk fat content were recorded for four consecutive days.

#### Estimation of methane emission

The breath samples of all buffaloes were collected daily for 4 consecutive days in canisters and analyzed for  $CH_4$  and  $SF_6$  gases, using Gas Chromatograph instrument (Photo 4), fitted with a Porapack N column for  $CH_4$  and molecular sieve 5A for  $SF_6$  analysis (Johnson *et al.*, 1994). The column temperature was maintained at 50°C and nitrogen was used as a carrier gas, with flow rate of 30 ml/min. The  $CH_4$ emission rate was calculated as the product of the permeation tube emission rate and the ratio of  $CH_4$  to  $SF_6$  concentration in the sample.



Photo 4: Analysis of breath samples in GC

#### Estimation of methane emission after feeding a balanced ration

After completing the measurement of methane emission before feeding balanced rations, the ration of individual animal was balanced for energy, protein, calcium and phosphorus as per the RBP software developed by NDDB. Based on the details of animal like body weight, milk yield, milk fat content, pregnancy status, lactation number etc. balanced rations of these 37 buffaloes were formulated. Intake and requirement of nutrients as well as recommended nutrients for all buffaloes are given in Annexure I. The details about feeding of balanced rations were provided to animal owners in local language. The owners were advised to feed these balanced rations at least for 30 days. The members of research team and skilled persons regularly visited the farms and monitored the feeding of experimental buffaloes. After feeding balanced ration for 30 days, again methane emission was measured from these animals for four consecutive days (Photo 5). Daily milk yield and milk fat content were measured during 4 days period after feeding balanced ration.



Photo 5: Collection of breath samples after feeding a balanced ration

#### Calculation

Methane emission was calculated as the product of permeation tube emission rate and the ratio of methane to  $SF_6$  concentration in the breath samples. All samples were analyzed in triplicate, using Gas Chromatograph instrument located at Animal Nutrition Research Station, Veterinary College, AAU, Anand. The Methane emission rate was calculated as under:

$$Q CH_4 = Q SF_6 \times (CH_4)/(SF_6)$$

Where,

 $QCH_4$  = Methane emission rate (g/min)

 $QSF_6$  = Known release rate of  $SF_6$  from permeation tube (g/min)

 $CH_4$  = Methane concentration of collected sample in canister ( $\mu g/m^3$ )

 $SF_6 = SF_6$  concentration of collected sample in canister ( $\mu g/m^3$ )

The amount of methane emission (g/ kg milk yield) was also calculated before and after feeding a balanced ration.

#### **Statistical analysis**

Completely Randomized Design was followed for Statistical analysis of data as given in Snedecor and Cochran (1994). SAS software version 9.3., one way ANOVA and Paired t test was used for test of significance for observing the statistical difference between the baseline methane emission and emission after balanced feeding of dairy animals.

#### **RESULTS AND DISCUSSION**

#### Chemical composition of feeds and fodders

The data for proximate composition and calcium and phosphorus content of feeds and fodders samples were found to be within normal range.

#### Milk production

There is a significant potential for increasing milk production to achieve the genetic potential of dairy animals in India. Milk production potential from ruminants is linked to genetic merit, balanced nutrition and good management practices. If a dairy

animal with high genetic merit for milk production is fed rations that are unable to meet her nutritional requirements, she will not produce milk as per her potentials. Feeding nutritionally balanced rations play a vital role in realization of the genetic potential of dairy animal for milk production.

The average daily milk yield and milk fat (%) of all 37 buffaloes, before and after feeding a balanced ration is presented in Table 1 and depicted as Figure 1. In the present study, the average daily milk yield before ration balancing was 8.68 kg, which increased significantly (P<0.05) to 9.11 kg after feeding a balanced ration in buffaloes. Similarly, milk fat content increased significantly from 6.79 to 7.02% after feeding a balanced ration.

Similar to our findings, improvement in milk yield due to supplementation of limiting nutrients in dairy animals has been reported by many authors in developing countries (Dutta *et al.*, 2010; Khochare *et al.*, 2010). A study conducted by Garg *et al.* (2013a) in 12,518 lactating animals showed that the implementation of balanced feeding approach under field conditions improved (P<0.05) daily milk yield by 2 - 14% and its fat content by 0.2 - 15% in cows and buffaloes, and at the same time decreasing ration cost by 5 - 11%. The average increase in net daily income of farmers has been reported to increase by 6 - 60% per animal on account of the increase in milk yield and milk fat content, as well as decrease in cost of feeding.

#### Methane emissions

Enteric methane emissions are closely related to the feeding regime, particularly feed quantity and quality, and ultimately the productivity of dairy animal. The fraction of feed converted to CH<sub>4</sub> emissions generally decreases as both the amount of feed intake and the feed quality increases (US EPA, 2006). Improper feeding not only leads to productivity losses but also increases emission of pollutants in the form of methane, nitrogen and phosphorus release in soil, water and environment (IAEA, 2008). Mekonnen and Hoekstra (2012) reported that the achieving higher milk production from the same amount of feeds would also decrease carbon footprint of milk.

In the present study, average daily methane emission was 214.59 and 192.73 g in buffaloes before and after feeding a balanced ration, respectively (Table 1 and Figure 2). Average methane emission in terms of g/ kg milk yield was reported as 25.51 in before ration balancing, whereas, it was reduced to 21.63 after feeding a balanced ration in buffaloes. The results indicated that the balanced feeding significantly reduced average methane emission (g/kg milk yield) by about 15.21% in lactating buffaloes.

Similar to present findings, Mohini and Singh (2010) also reported lower methane emissions (197.5 vs 223.4 g/day and 29.9 vs 40.0 g/kg milk yield) after balancing the ration of cows. The NDDB has undertaken various methane measurement studies in different agro-climatic regions of the country, using sulfur hexafluoride tracer technique. Methane emission measurements were carried out in early lactating cows (n=80) and buffaloes (n=82), before and after feeding a balanced ration. The methane emissions reduction on feeding a balanced ration was measured per kg of milk production. The study (Garg *et al.*, 2014) indicated that balanced feeding has reduced methane emissions (g/kg milk yield) by 17.3% (P<0.05) and 19.5% (P<0.01) in lactating cows and buffaloes, respectively.

A methane emission measurement study conducted by Kannan *et al.* (2011) in Chittoor district of Andhra Pradesh state revealed that by feeding a balanced ration, methane emission in terms of g/kg milk yield reduced significantly by 15.35% (P<0.05) in lactating crossbred cows (n=30). A study in Banaskantha district in Gujarat State revealed that methane emission (g/kg milk yield) reduced significantly by 13.45% (P<0.05) in crossbred cows (Garg *et al.*, 2013b).Enteric methane emission was reduced by 19.5% in lactating buffaloes (n=61) after feeding a balanced ration in different parts of the country (Sherasia *et al.*, 2014). Sherasia *et al.* (2016) also reported that ration balancing helps in reducing methane emission by 18.1% (g/kg milk yield) in lactating cows.

In the present study, balancing of protein, energy and minerals might have shifted the rumen fermentation pattern towards higher microbial cell production, resulting in lower acetate and butyrate production, on account of higher propionate production, thereby reducing methane emissions. Changing the plane of nutrition

through a balanced nutrient approach might have improved nutrient digestibility and thus reduced methane production. A greater efficiency of microbial protein synthesis and a higher proportion of propionate relative to acetate reduced digestive carbon losses through methane.

The Present findings indicate that there is significant effect of ration balancing on improving milk yield and reducing methane emission in buffaloes, suggesting the positive impact of ration balancing under field conditions. Thus, ration balancing helped in improving productivity of dairy animals while reducing enteric methane emission in western region of India.

## Table 1. Effect of ration balancing on milk production and methane emissionsin buffaloes (n=37)

Parameters	Before RBP	After RBP	% change
Milk yield (kg/day)	8.68 <sup>a</sup> ± 0.28	9.11 <sup>b</sup> ± 0.27	+4.95
Milk fat (%)	$6.79^{a} \pm 0.08$	$7.02^{b} \pm 0.07$	+3.39
Methane emission (g/day)	214.59 <sup>b</sup> ± 8.61	192.73 <sup>a</sup> ± 6.03	-10.19
Methane emission (g/kg MY)	25.51 <sup>b</sup> ± 1.21	21.63 <sup>a</sup> ± 0.81	-15.21

a,b (P<0.05)

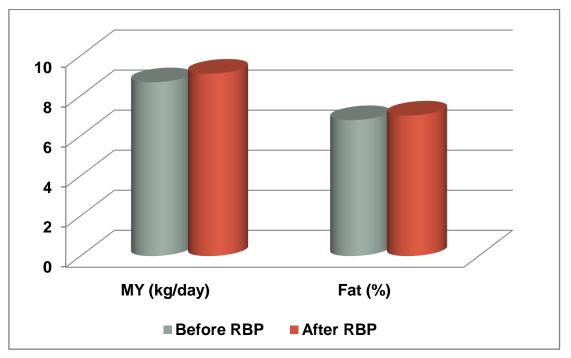


Figure 1. Effect of ration balancing on milk production

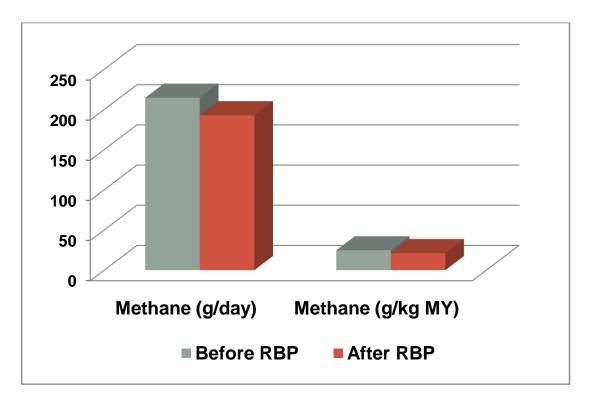


Figure 2. Effect of ration balancing on methane emissions

#### LIMITATIONS OF THE STUDY

- High variation in SF<sub>6</sub> release rate from the permeation tubes may affect the results of methane emission. There is a wide variation in methane estimation values within the animals selected for the study, at any given time. A small error might result in variation in calculation of methane release from the animals.
- The participating farmers have apprehensions about the adverse effects on the health and well being of their animals in future on account of insertion of permeation tube in the rumen. This needs a lot of persuasion of farmers. There is always a possibility of some of them backing out of the study. Therefore, it is prudent to take more number of farmers in the beginning in order to have sufficient observations.

#### FUTURE POSSIBILITIES OF RESEARCH IN THE AREA

The studies on methane emissions from other species of animal viz. indigenous and crossbred cows, sheep and goats be taken up.

#### CONCLUSION

The present study demonstrated that the ration balancing improved milk production with concomitant decrease in the enteric methane emission in lactating buffaloes under field conditions.

#### REFERENCES

- AOAC (2005) Official methods of analysis. Association of Official Analytical Chemists. Washington DC;18th ed.
- Bayat, A. and Shingfield, K. J. (2012) Overview of nutritional strategies to lower enteric methane emissions in ruminants. *MaataloustieteenPäivät,* 1-7.
- Blummel, M. (2000) Predicting the partitioning of fermentation products by combined in vitro gas volume and true substrate degradability measurements: opportunities and limitations. In: Proc. Brit. Soc. of Anim. Sci. on gas production: Fermentation kinetics for feed evaluation and to assess microbial activity. pp 48-58.
- Devendra, C. And Leng, R.A. (2011) Feed resources for animals in Asia: issues, strategies for use, intensification and integration for increased productivity. *Asian-Aust J Anim Sci.*, 24(3):303–321.
- Dutta, N., Sharma, K., Pattanaik, A.K., Anand, S., Amit, N. (2010) Effect of strategic supplementation of limiting macro nutrients on the lactation performance of buffaloes. Proceedings of the 7th Biennial Animal Nutrition Association Conference; 2010 December 17-19, Bhubaneshwar, India.
- Garg, M.R., Bhanderi, B.M. and Sherasia, P.L. (2005) Assessment of adequacy of macro and micro mineral content of feedstuffs for dairy animals in semi-arid zone of Rajasthan. *Animal Nutrition and Feed Technology*, 5:9-20.
- Garg, M.R., Sherasia, P.L., Bhanderi, B.M., Phondba, B.T., Shelke, S.K. andMakkar, H.P.S. (2013a) Effects of feeding nutritionally balanced rations on animal productivity, feed conversion efficiency, feed nitrogen use efficiency, rumen microbial protein supply, parasitic load, immunity and enteric methane emissions of milking animals under field conditions. *Animal Feed Science and Technology*, 179:24-35.
- Garg, M.R., Sherasia, P.L., Phondba, B.T., Shelke, S.K. and Patel, C.T. (2013b) Effect of feeding balanced ration on milk production, enteric methane emission and metabolic profile in crossbred cows under field conditions. *Indian J. of Dairy Science*, 66(2): 113-119.
- Garg, M.R., Sherasia, P.L., Phondba, B.T. and Hossain, S.A. (2014) Effect of feeding a balanced ration on milk production, microbial nitrogen supply and methane emissions in field animals. *Animal Production Science*, 54:1657-1661.

- Gowda, N.K.S., Prasad, C.S., Ramana, J.V. and Shivaramaiah, M.T. (2002) Assessment of mineral status in hilly and central zones of Karnataka and ways to supplement them. *Indian Journal of Animal Science*, 72:165-170.
- Gupta, R.S., Vahora, S.G., Patel, D.C., Devalia, B.R. Bhatt, C.R. andPatil, Y. J. (2006) Nutritional status of dairy cows in Vadodara district of middle Gujarat. Proceeding of the 12th Animal Nutrition Conference on Technological Interventions in Animal Nutrition for Rural Prosperity; 2006 January 7-9; Anand, Gujarat, India.p.92.
- Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P.S., Adesogan, A.T., Yang, W., Lee, C., Gerber, P.J., Henderson, B. and Tricarico, J.M. (2013) Special Topics - Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *J. Anim Sci*, 91:5045-5069.
- IAEA (2008) Guidelines for efficient manure management in Asia, International Atomic Energy Agency-TECDOC-1582, Vienna, Austria, pp.137.
- INCCA (2010) India: Greenhouse Gas Emissions 2007. Indian Network for Climate Change Assessment, Ministry of Environment and Forests, Government of India.
- Johnson, K.A. and Johnson, D.E. (1995) Methane emissions from cattle. J. Anim. Sci., 73: 2483-2492.
- Johnson, K.A., Huyler, M.T., Westberg, H.H., Lamb, B.K. and Zimmerman, P. (1994) Measurement of methane emissions from ruminants livestock using a SF<sub>6</sub> tracer technique. *Environmental Science and Technology*, 28: 359-362.
- Kannan, A., Garg, M.R. and Mahesh Kumar, B.V. (2011) 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. *Indian J. Anim. Nutr.*, 28(2): 111-123.
- Khochare, A.B., Kank, V.D., Gadegaonkar, G.M. and Salunke, S.C., (2010) Strategic supplementation of limiting nutrients to medium yielding dairy animals at field level, pp. 30. In: Proc. VII Animal Nutrition Association Conf., Bhubaneswar, India.
- Leng, R. A. (1991) Improving ruminant production and reducing methane emissions from ruminants by strategic supplementation. USEPA. Washington D.C.

- McDowell, L.R. (1985) *Nutrition of grazing ruminants in warm climates*. Academic Press, New York.
- McDowell, L.R., Conrad, J.H. and Glen Hembry, F. (1993) *Minerals for grazing ruminants in Tropical regions*. Animal Science Department, Centre for tropical agriculture, University of Florida. The US Agency for International Development and Caribbean Basin Advisory Group (CBAG), pp.7-15.
- Mekonnen, M.M. and Hoekstra, A.Y. (2012) A global assessment of the water footprint of farm animal products. *Ecosystems*, 15:401-15.
- Mohini, M. and Singh, G. P. (2010) Effect of supplementation of urea molasses mineral block (UMMB) on the milk yield and methane production in lactating cattle on different plane of nutrition. *Indian J. Anim. Nutr.*, 27, 96-102.
- Mudgal, V., Mehta, M.K., Rane, A.S. andNanavati, S. (2003) A survey on feeding practices and nutritional status of dairy animals in Madhya Pradesh. *Indian Journal of Animal Nutrition*, 20(2):217-220.
- Ramana, J., Prasad, C.S., Gowda, N.K.S. and Ramachandra, K.S. (2001) Levels of micro-nutrients in soil, feed, fodder and animals of North East transition and dry zones of Karnataka. *Indian Journal of Animal Nutrition*, 18:235-242.
- Sherasia, P.L., Garg, M.R. and Bhanderi. B.M. (2014) Effect of feeding balanced rations on production efficiency and enteric methane emission in lactating buffaloes under tropical conditions (2014). *Buffalo Bulletin* (December 2014), Vol. 33 (4): 323-331.
- Sherasia, P.L., Phondba, B.T., Hossain, S.A., Patel, B.P. and Garg, M.R. (2016) Impact of feeding balanced rations on milk production, methane emission, metabolites and feed conversion efficiency in lactating cows. *Indian J of Animal Research*, DOI:10.18805/ijar.8595.
- Singh, D., Yadav, A.S. and Yadav, K.R. (2002).Feeding practices of lactating buffaloes in Mohindergarh district of Haryana. *Indian Journal of Animal Nutrition*, 19:153-155.
- Snedecor, G.W. and Cochran, W.G. (1994) *Statistical Methods*, 8th edn.lowa State University Press, Ames, Iowa.
- Underwood, E.J. and Suttle, N.F. (1999) *The mineral nutrition of livestock*. 3rd ed. CAB International Publishing Company.
- US EPA.(2006) *Global mitigation of non-CO*<sub>2</sub> *greenhouse gases*. United States Environmental Protection Agency. EPA 430-R-06-005. Washington DC, USA.

## Annexure I

## Requirement, availability and recommended nutrients (g) using ration balancing software for experimental buffaloes (n=37)

Sr. No.	Ear tag No.	Nutrients	Nutrient requirement	Nutrient available from feed	Surplus/ Deficiency	Recommended nutrients
		TDN	9850	9020	-830.00	10356.01
	240055645242	СР	1993.50	1588	-405.50	1993.54
1	340055645342	Ca	75.40	46.06	-29.34	75.40
		Р	46.45	28.69	-17.76	51.83
		TDN	6870	5905	-965	6869.94
	240004640464	СР	1359	1034.50	-324.50	1358.98
2	340004619164	Са	51.28	30.77	-20.51	55.61
		Р	31.25	15.10	-16.15	31.21
		TDN	7130	6582.50	-547.50	7129.82
	340004619563	СР	1372	1142.50	-229.50	1371.93
3		Са	54.20	32.06	-22.14	56.76
		Р	32.55	16.86	-15.69	32.52
		TDN	6244	3890	-2354	6244.08
1	240055000116	СР	1256	1297.50	41.50	1276.12
4	340055090116	Ca	44.90	10.96	-33.94	53.43
		Р	28.52	7.69	-20.83	28.52
		TDN	8040	8732	692	8301.26
5	240055000428	СР	1577	1562	-15	1577.05
5	340055090138	Ca	61.40	39	-22.40	61.32
		Р	37.40	31.39	-6.01	41.93
		TDN	6410	6132	-278	6409.77
6	240055645426	СР	1235	1484	249	1281.62
6	340055645136	Ca	46.93	22.97	-23.96	46.90
		Р	28.75	35.42	6.67	33.45

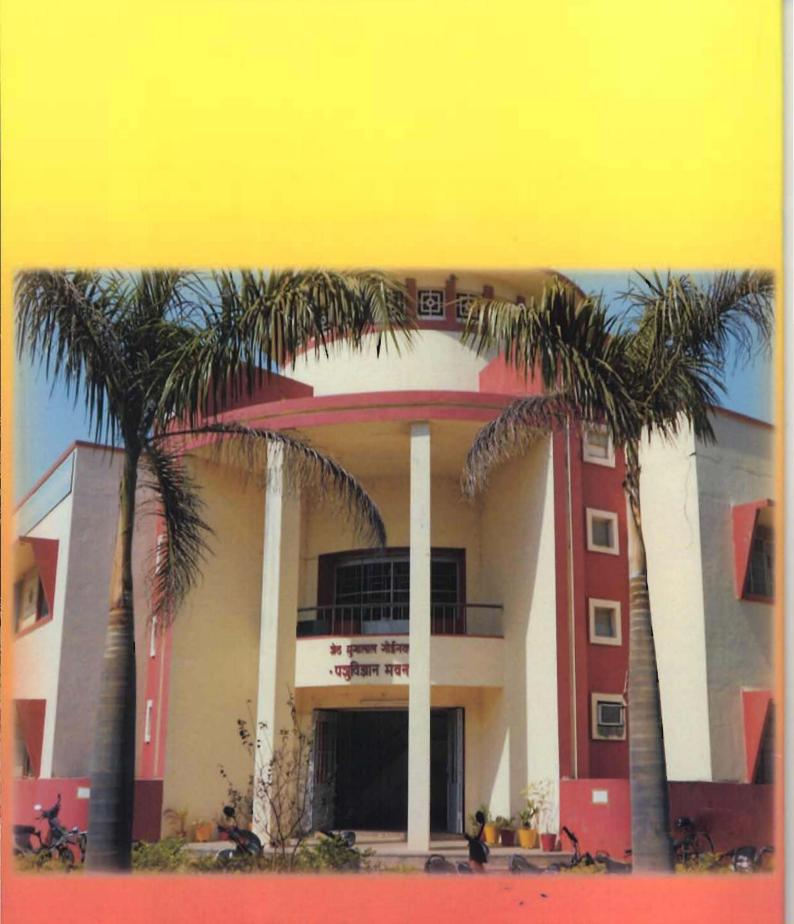
Sr. No.	Ear tag No.	Nutrients	Nutrient requirement	Nutrient available from feed	Surplus/ Deficiency	Recommended nutrients
		TDN	6940	8116.50	1176.50	6939.96
7	340004619404	CP	1380	1528.50	148.50	1379.98
/	340004019404	Ca	52.20	29.96	-22.25	53.58
		Р	32.20	17.43	-14.77	32.16
		TDN	8140	8315	175	8140.04
8	340004619483	CP	1690	1396	-294	1690.04
0	340004019403	Ca	63.70	37.64	-26.06	63.69
		Р	38.70	21.70	-17	39.53
		TDN	7080	7880	800	7427.39
9	340004619506	CP	1394	1296	-98	1394.02
9		Ca	53.60	35.04	-18.56	53.69
		Р	32.90	21.20	-11.70	34.82
		TDN	7380	10210	2830	7380
10	340004619756	CP	1435	2213	778	1434.96
10	340004619756	Ca	56.60	39.15	-17.45	61.02
		Р	33.90	32.80	-1.10	33.91
		TDN	7100	8173	1073	7100.17
11	240004610779	СР	1504	1615	111	1504.02
	340004619778	Ca	52.80	22.09	-30.71	52.90
		Р	33.20	19.71	-13.49	35.93
		TDN	9680	8885	-795	9679.80
10	240004610825	СР	1983	2556	573	2015.44
12	340004619825	Ca	77.80	23.85	-53.95	77.77
		Р	45.70	27.29	-18.41	53.90
		TDN	6770	7463	693	8158.03
10	240004640046	СР	1351	801	-550	1351.01
13	340004619916	Са	51.60	14.89	-36.71	51.69
		Р	31.90	9.33	-22.57	34.08

Sr. No.	Ear tag No.	Nutrients	Nutrient requirement	Nutrient available from feed	Surplus/ Deficiency	Recommended nutrients
		TDN	8080	6835	-1245	8080.12
1.1	340019241978	СР	1646	1389	-257	1881.37
14	340019241976	Ca	63.20	26.95	-36.25	63.16
		Р	38.30	17.01	-21.29	44.92
		TDN	6800	7177.50	377.50	6881.06
15	340055645216	СР	1380	1776.50	396.50	1379.97
15	340055645216	Ca	50.45	34.06	-16.39	51
		Р	31.50	35.69	4.19	31.46
		TDN	7080	7880	800	7427.39
16	340004619472	СР	1394	1296	-98	1394.02
10		Ca	53.60	35.04	-18.56	53.69
		Р	32.90	21.20	-11.70	34.82
	340004619494	TDN	7080	7880	800	7427.39
17		СР	1394	1296	-98	1394.02
		Ca	53.60	35.04	-18.56	53.69
		Р	32.90	21.20	-11.70	34.82
		TDN	11790	12218	428	11790.19
10	70106088	СР	2432	2455	23	2432.13
18	79196988	Ca	79.8	52.01	-27.79	107.55
		Р	54.4	30.48	-23.92	54.35
		TDN	9300	10715	1415	9299.98
10	70400000	СР	1920	1896	-24.0	1919.94
19	79198622	Са	72.8	54.6	-18.2	87.73
		Р	44.8	24.43	-20.37	44.81
		TDN	9300	10715	1415	9299.98
	70400400	СР	1920	1896	-24.0	1919.94
20	79198132	Са	72.8	54.6	-18.2	87.73
		Р	44.8	24.43	-20.37	44.81

Sr. No.	Ear tag No.	Nutrients	Nutrient requirement	Nutrient available from feed	Surplus/ Deficiency	Recommended nutrients
		TDN	8260	9905	1645	8260.34
21	79197047	СР	1664	1832	168	1664.12
21	79197047	Ca	63.32	50.93	-12.27	77.06
		Р	39.2	23.35	-15.85	39.19
		TDN	7220	7249	29	7220
22	79198883	СР	1408	1745	337.5	1408
22	79190003	Ca	53.6	32.86	-20.74	61.63
		Р	33.6	18.4	-15.2	33.64
		TDN	6580	5947.5	-630.25	6580.39
23	79197014	СР	1268	1129.25	-138.75	1268.12
23		Ca	48.8	24.34	-24.46	48.7
		Р	30.2	15.26	-14.94	31.91
		TDN	6580	5974.5	-630.25	6580.39
24	79197344	СР	1268	1129.25	-138.75	1268.12
24	79197344	Ca	48.8	24.34	-24.46	48.7
		Р	30.2	15.26	-14.94	31.91
		TDN	5580	5031	-549	5580.45
25	79198360	СР	1016	1101	85.5	1016.17
25	79198300	Ca	39.2	19.37	-19.83	47.16
		Р	24.8	7.02	-17.78	24.78
		TDN	7494	5178	-402	5579.9
26	79197492	СР	1016	1071.5	55.5	1016.01
20	13131432	Са	39.2	24.19	-15.01	46.73
		Р	24.8	10.91	-13.89	24.82
		TDN	6940	7686	746	7265.43
27	79198267	СР	1380	1078.5	-301.5	1380.05
~/	19190201	Са	52.2	36.71	-15.49	52.3
		Р	32.2	19.58	-12.62	32.26

Sr. No.	Ear tag No.	Nutrients	Nutrient requirement	Nutrient available from feed	Surplus/ Deficiency	Recommended nutrients
		TDN	9260	9635	375	9260.11
28	79197138	СР	1867	2426	559	1867.06
20	79197130	Ca	73	52.67	-20.33	90.57
		Р	43.40	34.08	-9.32	43.40
		TDN	9900	10040	140	9899.73
29	79197311	СР	2005	2462	457	2004.85
29	79197311	Са	77.80	55.37	-22.43	96.32
		Р	46.80	34.51	-12.29	46.75
		TDN	7880	7430	-450	7880.14
30	79198735	СР	1561	1244	-317	1561.04
30	79198735	Са	61.4	34.54	-26.86	61.31
		Р	36.6	28.04	-8.56	45.91
	79197652	TDN	10768	10850	82	10768
31		СР	2303	2534	231	2714.29
51		Ca	89.5	60.77	-28.73	89.49
		Р	52.24	35.38	-16.86	61.71
		TDN	6856	5623	-1233	6856.14
32	70108028	СР	1309	1412	103	1433.12
52	79198928	Ca	51.80	32.22	-19.58	51.71
		Р	31.08	17.36	-13.72	31.02
		TDN	5960	6765	805	5960.04
33	79197765	СР	1065	1478.5	413.5	1064.98
55	79191705	Ca	42.20	43.49	1.29	58.25
		Р	26.20	18.14	-8.06	26.24
		TDN	8040	8092	52	8040.16
		СР	1577	1885	308	1861.02
34	79198724	Са	61.40	35.74	-25.66	61.77
		Р	37.40	20.88	-16.52	37.39

Sr. No.	Ear tag No.	Nutrients	Nutrient requirement	Nutrient available from feed	Surplus/ Deficiency	Recommended nutrients
		TDN	9160	8720	-440	9189.92
35	79197617	СР	1917	1768	-149	1916.95
- 55	79197017	Ca	73.60	41.47	-32.13	76.61
		Р	43.60	21.74	-21.86	43.62
	79197776	TDN	7380	7910	530	7380.22
36		СР	1435	1704	269	1435.02
30		Ca	56.6	37.8	-18.80	59.88
		Р	33.9	20.66	-13.24	33.95
	79198974	TDN	6952	6733	-219	6952.06
37		СР	1315	1497	182	1315.02
		Ca	51.80	43.96	-7.84	67.23
		Р	31.56	18.12	-13.44	31.53



Animal Nutrition Research Station Anand Agricultural University, Anand - 388 110, Gujarat, India