Research Article

Effect of Supplementing Bypass Fat with Rumen Protected Choline Chloride on Milk Yield, Milk Composition and Metabolic Profile in Crossbred Cows

M. R. Garg, B. M. Bhanderi* and P. L. Sherasia

Animal Nutrition Group, National Dairy Development Board, Anand - 388 001, Gujarat, India

Crossbred cows (n=24), yielding 8-14 kg milk/animal/day were divided into three groups of eight each, based on milk yield, fat per cent and stage of lactation. All animals were fed similar basal ration, comprising 14-15 kg oat green, 5-6 kg lucerne green and 4-5 kg wheat straw. Concentrate mixture was given according to the level of milk production, to meet the maintenance and milk production requirements. In addition to basal ration, cows in group II were fed 100 g bypass fat supplement, whereas, cows in group III were fed 100 g bypass fat and 10 g rumen protected choline (RPC) supplement per animal per day. Average increase in daily milk yield and fat in groups II and III over a 90 days experimental period were 1.48 kg (P<0.01) and 0.54% (P<0.05) and 1.77 kg (P<0.01) and 0.61% (P<0.05), as compared to group I. There was improvement (P<0.01) in poly-unsaturated fatty acids in milk of groups II and III. Total unsaturated fatty acids also increased by 15.29 and 15.71% in groups II and III, respectively. Non-esterified fatty acids (NEFA) in blood serum were reduced by 16.12 and 24.19% (P<0.01) in groups II and III, respectively. There was reduction (P<0.01) in cholesterol levels in blood serum in animals of groups II and III, as compared to group I. Blood glucose and urea nitrogen were not affected by the dietary treatments. On the basis of present study, it can be inferred that supplementing bypass fat helps improving milk and fat yield in crossbred cows, which can further be enhanced by fortification with rumen protected choline chloride.

***Keywords:** Bypass fat, rumen protected choline, milk production, fatty acids, crossbred cows*

INTRODUCTION

During early lactation, the amount of energy required for maintenance of body tissues and milk production often exceeds the amount of energy available from the diet (Goff and Horst, 1997), thus forcing mobilization of body fat reserves to satisfy energy requirement. Simultaneously, daily nutrient intake is insufficient to meet demands for milk production and energy balance is negative (Bell *et al.*, 1995). Due to depressed feed intake at the end of gestation, the period of negative energy balance often starts prior to calving (Van den Top *et al.*, 1995). The negative energy balance in early lactation affects peak milk yield and overall lactation yield apart from causing delayed post partum ovarian activity (Garnsworthy and Webb, 1999). The level of non-esterified fatty acids (NEFA) increases in plasma as a consequence of body fat mobilization (Reid and Collins, 1980) and leads to hepatic lipidosis. Calcium salts of long-chain fatty acids have been shown to be effective as ruminally inert fat supplements for lactating cows (Palmquist, 1991 and 1994; Grummer, 1995) and is a good source for increasing energy density of the diet to improve productive performances.

Choline, a component of phospholipid and methyl donor, plays an essential role in very low density lipoprotein synthesis and thereby contributes to fat export from the liver. Fat metabolism can be improved with the help of choline for better energy production. This also helps in improving milk production. Evidence suggests that the dietary supply of choline in early lactating dairy animals may be inadequate, even though choline can be synthesized by the animals (Pires and Grummer, 2008). As dietary choline gets degraded rapidly in the rumen, it must be supplemented in the protected form (Atkins *et*

* *Scientist-II (AN): Animal Nutrition Group, National Dairy Development Board, Anand - 388 001, Gujarat, India*
Tel: +91-2692-226270, E mail: bhanderi@nddb.coop
2012-051 Received:April 2012; Accepted:July 2012

al., 1988; Elek *et al.*, 2008). Therefore, rumen-protected form of choline has been developed to deliver choline to the small intestine for absorption. The aim of the present study was to determine the effect of bypass fat with or without rumen-protected choline (RPC) supplementation on milk production, milk composition and metabolic profile in crossbred cows.

MATERIALS AND METHODS

Trial Design and Treatments

This study was conducted during December to March months at Sarsa Farm, Anand district in Gujarat State, to evaluate the effect of supplementing bypass fat, with or without RPC on milk yield, composition, milk fatty acid profile and metabolic profile in early lactating HF crossbred cows. Crossbred cows (n=24), yielding 8-14 kg milk/animal/day were divided into three similar groups (I, II and III) of eight each, based on level of milk production (11 kg), fat (4.10%) and stage of lactation (2-3 weeks post partum). Cows in all the three groups were fed similar basal diet, comprising 14-15 kg oat green, 5-6 kg lucerne green and 4-5 kg wheat straw. Concentrate mixture was given according to the level of milk production, to meet the maintenance and milk production requirements (NRC, 2001). No supplement was offered to cows in group I. However, cows in group II were supplemented bypass fat @ 100 g per animal per day and in group III along with 100 g bypass fat, 10 g RPC was also fed to each of the animals. Feed and feed supplements to all the animals in three different groups were offered for three months and observations on daily feed intake, daily milk yield, fat per cent etc. were recorded.

Sample Collection and Analytical Methods

The chemical composition of feeds and fodder offered during the trial period was carried out as per AOAC (2005). Feeds and fodder were also analyzed for neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose as per Goering and Van Soest (1970). The degree of rumen protection in bypass fat and RPC supplements were measured as per procedures of Gulati *et al.* (1993) and Sharma and Erdman (1989). The milk yield of individual cow was recorded in the morning and evening. Milk samples from each cow, pooled from each milking were collected weekly for analysis of fat, protein, total solids and SNF contents by using Milko-scan. Blood samples were collected by jugular veni-puncture of each cow. The blood was allowed to clot and centrifuged at 1500 rpm for 10 minutes. The serum was then harvested for estimation of serum NEFA, triglycerides and cholesterol using commercially available test kits (Brishketu and Thakur, 2007).

Preparation of Fatty Acid Methyl Esters and Estimation by using Gas Chromatograph

Milk samples were also drawn for preparation of fatty acid methyl esters (FAME), during the trial period. Milk fat from 2 ml sample was saponified with ethanol and 5N sodium hydroxide, followed by acidification with 5N hydrochloric acid. Collected supernatant was then methylated using methanol. Methyl esters were extracted by adding 3 ml petroleum ether to the solution and removing a portion of the top phase into Gas Chromatograph (GC) vials. Individual fatty acids were determined by using a Perkin-Elmer Gas Chromatograph with flame ionization detector and fitted with BPX70 capillary column (50m x 0.32mm ID). Helium gas was used as a carrier and the detector temperature was set at 210°C (Ashes *et al.*, 1992).

RESULTS AND DISCUSSION

Analysis of Feed and Feed Supplements

Analysis of feeds and fodder offered to animals in various groups is given in Table 1. Total fat content in bypass fat supplement was 84.10% and the degree of rumen protection was 79.60%. The predominant fatty acid in bypass fat supplement was palmitic acid (44.20%), whereas, levels of oleic, linoleic and linolenic acids were 38.10, 9.80 and 0.22%, respectively. The degree of rumen protection of choline chloride supplement was 71.30%. Since animals in all the three groups were fed similar ration, there was no significant difference in daily dry matter intake (DMI) amongst the groups (14.05, 13.80, 13.70 kg). Other workers too have reported that daily dry matter intake of the ration had remained unaffected on supplementing rumen bypass fat (Tyagi *et al.*, 2009).

Milk Production and Composition

On supplementing bypass fat alone or with RPC in lactating crossbred cows, average increase in daily milk yield (kg) was 1.48 ($P<0.01$) and 1.77 ($P<0.01$) in groups II and III, respectively,

Table 1 : Chemical composition of feeds and fodder (% on DM basis)

Parameter	Concentrate mixture	Oats green	Lucerne green	Wheat straw
Crude protein	21.50	16.50	17.85	3.10
Ether extract	2.82	3.70	3.88	0.92
Acid detergent fibre	12.27	26.09	36.70	54.30
Neutral detergent fibre	19.98	39.80	48.51	68.80
Acid detergent lignin	1.45	0.80	7.53	6.10
Cellulose	9.10	25.90	26.85	45.90

as compared to control. Average fat per cent increased by 0.54 ($P<0.05$) and 0.61 units ($P<0.05$) in groups II and III, respectively (Table 2). As choline is used for phospholipid synthesis, its supplementation facilitates lipid absorption and transport, thereby favoring milk fat synthesis. However, milk protein content remained unaffected amongst the groups. Positive effects of supplementing bypass fat on milk production and daily fat yield in lactating animals have been reported earlier (Barley and Baghel, 2009; Sirohi *et al.*, 2010). Elek *et al.* (2008) and Lima *et al.* (2007) observed significant improvement in milk yield on supplementing RPC in dairy cows. It is also reported that RPC may improve the milk yield of dairy animals by elevating the export of triglycerides from the liver and by sparing methionine as a methyl donor. Collectively, the study indicated that further improvements in milk production in response to RPC supplementation may be attributed to a methyl donor sparing effect. Thus, enhanced intestinal supply of choline might have further improved milk production in crossbred cows.

Fatty Acid Profile of Milk

Calcium salts of fatty acids provide partial resistance from lipolysis and bio-hydrogenation in rumen by ruminal microbes and modify fatty acid profile of milk fat. Significant improvement was observed in poly unsaturated fatty acid (PUFA) content in groups II (@ 34.26%) and III (@ 37.45%). Total unsaturated fatty acids increased by about 15.29 and 15.71% in groups II and III, respectively. Long chain fatty acids (LCFA; $C_{16:0}$ to $C_{20:0}$) and mono-unsaturated fatty acids (MUFA; $C_{14:1}$, $C_{16:1}$ & $C_{18:1}$) contents were higher in experimental groups as compared to control (Table 3). There are several reports indicating that supplementation of bypass fat in the ration of cows and buffaloes increased the proportion of unsaturated and long chain fatty acids of milk fat (Garg *et al.*, 2008; Mahecha *et al.,* 2008; Sajith *et al.*, 2008).

NEFA - An Indicator of Energy Balance and Fat Mobilization

Presence of NEFA in blood is a direct indicator of energy balance and massive fat mobilization, suggesting more energy requirement than supplied in the diet. Changes in blood serum lipid profile in cows, subjected to the three feeding regimes are presented in Table 4. NEFA can be managed by optimizing the capacity of the liver to dispose of excess NEFA by exporting it back to the blood stream in the form of very low density lipoproteins (VLDL). In this process, the body uses VLDL for availing more usable energy for various body functions and health of the liver is maintained. For the formation of VLDL, the liver requires phospholipids, which are synthesized from choline. Choline supports

Table 2 : Effect of bypass fat with or without RPC on milk production and milk composition

Parameter	Group I	Group II	Group III
Dry matter intake (kg/day)	14.05±0.14	13.80±0.10	13.70±0.12
Milk yield (kg/day)	10.01^{d}± 0.42	11.49^{e} ± 0.36	11.78^{f} ± 0.46
Fat (%)	3.60^{a}±0.14	4.14^{b}±0.16	4.21^{c}±0.15
Protein (%)	3.55±0.10	3.58±0.13	3.57±0.14
SNF (%)	8.94±0.14	9.03±0.15	8.90±0.16
Total solids (%)	12.54^{a}±0.12	13.16^{b}±0.15	13.11^{ab}±0.21

a, b, c Means with different superscript in a row differ significantly ($P<0.05$)
d, e, f Means with different superscript in a row differ significantly ($P<0.01$)

Table 3 : Effect of bypass fat with or without RPC on milk fatty acid profile (% of total fatty acids)

Fatty acids	Group I	Group II	Group III
Caprylic acid ($C_{8:0}$)	$1.51^{de} \pm 0.02$	$1.45^{d} \pm 0.03$	$1.56^{e} \pm 0.02$
Capric acid ($C_{10:0}$)	$3.08^{d} \pm 0.03$	$2.86^{e} \pm 0.02$	$2.75^{f} \pm 0.02$
Lauric acid ($C_{12:0}$)	$3.60^{d} \pm 0.01$	$3.50^{e} \pm 0.02$	$3.53^{de} \pm 0.01$
Myristic acid ($C_{14:0}$)	$11.52^{d} \pm 1.10$	$10.48^{e} \pm 1.21$	$10.53^{e} \pm 1.08$
Myristoleic acid ($C_{14:1}$)	$1.21^{d} \pm 0.02$	$0.98^{e} \pm 0.01$	$0.99^{e} \pm 0.02$
Palmitic acid ($C_{16:0}$)	$32.10^{d} \pm 2.28$	$30.89^{e} \pm 3.04$	$30.58^{e} \pm 2.01$
Palmitoleic acid ($C_{16:1}$)	$1.71^{d} \pm 0.03$	$1.51^{e} \pm 0.05$	$1.40^{f} \pm 0.02$
Stearic acid ($C_{18:0}$)	$12.21^{d} \pm 0.82$	$10.80^{e} \pm 0.89$	$10.21^{f} \pm 0.81$
Oleic acid ($C_{18:1}$)	$25.52^{d} \pm 1.61$	$29.80^{e} \pm 1.19$	$29.95^{e} \pm 2.02$
Linoleic acid ($C_{18:2}$)	$1.71^{d} \pm 0.04$	$2.59^{e} \pm 0.05$	$2.70^{f} \pm 0.04$
Linolenic acid ($C_{18:3}$)	0.80 ± 0.05	0.78 ± 0.05	0.75 ± 0.03
Arachidic acid ($C_{20:0}$)	$0.60^{d} \pm 0.01$	$0.65^{e} \pm 0.00$	$0.68^{e} \pm 0.00$
Total saturated fatty acids	$64.60^{d} \pm 2.45$	$60.63^{e} \pm 2.32$	$59.84^{e} \pm 2.39$
Total unsaturated fatty acids	$30.93^{d} \pm 1.98$	$35.66^{e} \pm 2.10$	$35.79^{e} \pm 1.87$
LCFA ($C_{16:0}$ to $_{20:0}$)	$74.62^{d} \pm 2.56$	$77.02^{e} \pm 2.48$	$76.27^{e} \pm 3.14$
MUFA ($C_{14:1}$, $_{16:1}$, $_{18:1}$)	$28.42^{d} \pm 2.04$	$32.29^{e} \pm 2.15$	$32.34^{e} \pm 2.51$
PUFA ($C_{18:2}$, $_{18:3}$)	$2.51^{d} \pm 0.07$	$3.37^{e} \pm 0.06$	$3.45^{e} \pm 0.05$

$^{d, e, f}$ Means with different superscript in a row differ significantly (P<0.01)

Table 4 : Effect of feeding bypass fat with or without RPC on metabolic profile

Parameter	Group I	Group II	Group III
BUN (mg/dl)	21.08±1.05	22.83±1.20	21.98±0.85
Glucose (mg/dl)	56.83±3.76	57.42±1.05	57.06±1.36
Cholesterol (mg/dl)	143.99^{d}±1.81	146.39^{de}±1.28	149.58^{e}±2.80
Triglycerides (mg/dl)	39.18±1.34	42.21±2.44	45.51±1.56
NEFA (mmol/l)	0.62^{d}±0.03	0.52^{de}±0.04	0.47^{e}±0.02

$^{d, e}$ Means with different superscript in a row differ significantly (P<0.01)

phospholipids synthesis and thus helps in VLDL formation. Mean serum NEFA level (mmol/l) was reduced to 0.52 and 0.47 in groups II and III, respectively, as compared to group I (0.62). Significant reduction in serum NEFA level has been reported on feeding RPC (Zahra *et al.,* 2006). At the beginning of the lactation cycle, the blood NEFA originating from mobilization of adipose tissue is elevated, mainly due to a negative energy balance. Increased concentrations indicate lipolysis, which occurs in response to increased energy demand. Level of blood serum triglycerides (mg/dl) increased on feeding bypass fat with and without RPC and cholesterol level (mg/dl) reduced (P<0.01). Cholesterol is a component of the serum lipoproteins and its concentrations in serum gives an indication of overall lipoprotein concentrations. Zahra *et al.* (2006) observed reduction in the level of cholesterol on supplementation of RPC to dairy cows. The results obtained in this study are also in agreement with the previous findings (Janovick Guretzky *et al.*, 2006; Pinotti *et al.*, 2004).

CONCLUSIONS

It can be inferred from the present study that supplementing bypass fat helps in improving milk and fat yield in crossbred cows, which can further be enhanced by fortification with rumen protected choline chloride.

ACKNOWLEDGEMENTS

The authors are grateful to the management of National Dairy Development Board of India, for providing necessary facilities and financial support to carry out this study.

REFERENCES

A.O.A.C. 2005. *Official Methods of Analysis* (18th Edn.). Association of Official Analytical Chemists, Washington, DC.

Ashes, J.R., St-Vincent Welch, P., Gulati, S.K., Scott, T.W., Brown, G.H. and Blakely, S. 1992. Manipulation of the fatty acid composition of milk by feeding protected canola seeds. *J. Dairy Sci.*, **75**(4):1090.

Atkins, K.B., Erdman, R.A. and Vandersall, I.H. 1988.

Dietary choline effects on milk and duodenal choline flow in dairy cattle. *J. Dairy Sci.* **71**:109-112.

Barley, G.G. and Baghel, R.P.S. 2009. Effect of bypass fat supplementation on milk yield, fat content and serum triglyceride levels of Murrah buffaloes. *Buffalo Bull.,* **28**(4):173-175.

Bell, A.W., Sleptis, R. and Ehrdadt, R.A. 1995. Growth and accretion of energy and protein in the gravid uterus during late pregnancy in Holstein cows. *J. Dairy Sci.,* **78**:1954-1961.

Brishketu Kumar and Thakur, S. S. 2007. Effect of supplementing bypass fat on the performance of buffalo calves. *Indian J. Anim. Nutr.*, **24**(4):233-236.

Elek, P., Newbold, J.R., Gall, T., Wagner, L. and Husventh, F. 2008. Effects of rumen protected choline supplementation on milk production and choline supply of peri-parturient dairy cows. *Anim.*, **2**(11):1595-1601.

Garg, M.R., Sherasia, P.L., Bhanderi, B.M., Gulati, S.K. and Scott, T.W. 2008. Effect of feeding bypass fat supplement on milk production and characteristic of butter fat. *Indian J. Dairy Sci.*, **61**(1):13-23.

Garnsworthy, P.C. and Webb, R. 1999. The influence of nutrition on fertility in diary cows. Recent Advances in Animal Nutrition. pp. 39-57 (Eds.) Garnsworthy, P.C. and Wiseman J. Nottingham University Press.

Goering, H.K. and Van Soest, P.J. 1970. Forage fibre analysis (Apparatus, Reagents, Procedures and Some Applications), ARS U.S Dept. Agr. Handbook, No.379, Superintendent of Documents, U. S. Government Printing Office, Washington, D.C. 20402.

Goff, J.P. and Horst, R.L. 1997. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci.*, **80**:1260-1268.

Grummer, R.R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *J. Anim. Sci.,* **73**:2820-2833.

Gulati, S.K., Ashes, J.R. and Scott, T.W. 1993. Optimizing the nutritional value of oilseed proteins for ruminants. Proceedings of 90th American Oil Chemists Society Conference (A.O.C.S.). INFORM 10: 41.

Janovick Guretzky, N.A., Carlson, D.B., Garret, J.E. and Drackley, J.K. 2006. Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the peri-parturient period. *J. Dairy Sci.,* **89**:188-200.

Lima, F.S., Sa Filho, M.F., Greco, L.F., Susca, F., Magalhaes, V.J., Garrett, J. and Santos, J.E.P. 2007. Effects of feeding rumen protected choline (RPC) on lactation and metabolism. *J. Dairy Sci.*, **90**(Suppl. 1): 174.

Mahecha, L., Angulo, J., Salazar, B., Ceron, M., Gallo, J., Molina, C.H., Molina, E.J., Surarez, J.F., Lopera, J.J. and Olivera, M. 2008. Supplementation with bypass fat in silvo-pastoral systems diminishes the ratio of milk saturated/ unsaturated fatty acids. *Trop. Anim. Health Prod.*, **40**: 209-216.

NRC. 2001. Nutrient Requirements of Dairy Animals. 7th Edn., National Academy of Science - National Research Council, Washington DC.

Palmquist, D.L. 1991.Rumen synthesis, bio-hydrogenation and digestibility of fatty acids by dairy cows. *J. Dairy Sci.*, **74**:3025-3034.

Palmquist, D.L. 1994. The role of fats in efficiency of ruminants. *J. Nutr.*, **124**:1377-1382.

Pinotti, L., Campagnoli, A., Sangalli, L., Rebucci, R., Dell'Orto, V. and Baldi, A. 2004. Metabolism in periparturient dairy cows fed rumen protected choline. *J. Anim. Sci.*, **13**(l): 551-554.

Pires, J.A.A. and Grummer, R.R. 2008. Micronutrients and their impact on high performing dairy cows - A focus on niacin and choline. In Proc. Southwest Nutrition and Management Conference, Department of Animal Sciences, University of Arizona.

Reid, I.M. and Collins, R.A. 1980. The pathology of peri-parturient fatty liver in high yielding dairy cows. Invest. *Cell Pathol.*, **3**:237-249.

Sajith, P., Kumar, A. and Tiwari, D.P. 2008. Effect of feeding bypass fat (calcium salts of palm oil fatty acids) on performance of lactating crossbred cows. *Asian-Aust. J. Animal Sci.*, **21**:376-385.

Sharma, B.K. and Erdman, R.A. 1989. In vitro degradation of choline from selected feedstuffs and choline supplements. *J. Dairy Sci.*, **72**: 2772-2776.

Sirohi, S.K., Walli, T.K. and Mohanta, R.K. 2010. Supplementation effect of bypass fat on production performance of lactating crossbred cows. *Indian J. Anim. Sci.*, **80**(8): 733-736.

Tyagi, N., Thakur, S.S. and Shelke, S.K. 2009. Effect of feeding bypass fat supplement on milk yield, its composition and nutrient utilization in crossbred cows. *Indian J. Anim. Nutr.*, **26**(1): 1-8.

Van den Top, A.M., Geelen, M.J.H., Wentink, G.H., Van'tKlooster, A. and Beynen, A.C. 1995. Time trends of plasma lipids and hepatic triacyl glycerol synthesizing enzymes during post-partum fatty liver development in dairy cows with unlimited access during dry period. *J. Dairy Sci.*, **78**:2208-2220.

Zahra, L.C., Duffield, T.F., Lesilie, K.E., Overton, T.R., Putnam, D. and LeBlanc, S.J. 2006. Effects of rumen protected choline and monensin on milk production and metabolism of peri-parturient dairy cows. *J. Dairy Sci.,* **89**:4808-4818.