Effect of Feeding Bypass Fat Supplement on Milk Production and Characteristics of Butter Fat

M. R. Garg*, P. L. Sherasia*, B. M. Bhanderi*, S. K. Gulati[1] and T. W. Scott[2]

Modified feeding of dairy cattle has been described frequently as an appropriate tool to improve rheological properties of fat based dairy products, especially butter. Controlled feeding experiments with subsequent small-scale butter-making trial was performed to determine potential effects of bypass fat/ protein supplement feeding on physical and chemical properties of butter. Six multiparous cows, yielding 12-15 kg milk/animal/day (HF x Jersey) were fed a basal diet, comprising 15-17 kg green jowar fodder and 5-6 kg paddy straw. Concentrate mixture was given according to their level of milk production. In addition to basal ration, animals were fed 1.0 kg bypass fat supplement (Fat 32%, CP 27%, NDIN 0.52%, ADIN 0.31%). Degree of fat protection was 74% of the total fat, whereas, protein protection was 76% of total CP. Average increase in milk yield, fat and protein content was 1.34 kg, 0.43 and 0.26 percent, respectively, compared to base levels, recorded at the time of starting experimental feeding. Increase in milk yield and fat per cent was significantly ($p<0.05$) higher. However, no significant effect was observed on level of protein percent in milk. Butter manufactured by conventional method was evaluated for its fatty acid composition and rheological properties. Saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) reduced @ 12.0% and unsaturated fatty acids ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$) increased @ 20.0% in butter made from milk, on feeding bypass fat supplement. Significant reduction was observed in $C_{16:0}$ (@ 19.0%), whereas, $C_{18:1}$ increased (@ 14.0%) significantly ($p<0.05$). On feeding bypass fat/protein supplement, level of $C_{18:2}$ increased significantly in experimental group (3.77, $p<0.01$), as compared to control group. Average fat and moisture contents in butter of control period were 85.35 and 11.78 per cent, whereas, it was 85.81 and 12.53 per cent in experimental period. On feeding bypass fat supplement, penetration (mm) and iodine values (g/100 g of fat) in butter increased from 4.72 ± 0.22 to 5.64 ± 0.26 ($P<0.05$) and 30.81 ± 0.67 to 34.38 ± 0.73, respectively. This study demonstrates the feasibility of producing high quality products from milk supplemented with bypass fat / protein supplement.

Keywords: Bypass fat/protein, fatty acids, butter, iodine value, penetration test, cow

INTRODUCTION

Animal studies and epidemiological investigations indicate potential nutritional benefits of polyunsaturated fatty acids (PUFA) in human diets (Simopoulos, 1991; Gibson *et al.* 1996; McGuire *et al.* 1999; Pariza *et al.* 2000). Feeding special diets to dairy cows can modify milk fat composition. In ruminants, if increased amount of polyunsaturated fats are fed, they are utilized by microorganisms in the rumen or metabolized by these organisms to form saturated and mono-unsaturated fatty acids; as a result the milk fat do not shown any increase in polyunsaturated fatty acids. A group of scientists in Australia, coated polyunsaturated oils with a protein and then protected these particles from microbial attack in the rumen by treatment with formaldehyde (Scott *et al.* 1970). The coated oils passed through the rumen (pH 6-7) and into the abomasums and omasum (pH 2-3), where the more acid conditions hydrolyzed the protein-formaldehyde coat, releasing the intact dietary polyunsaturated oil, which could then be absorbed and incorporated into milk fat. In view of this, a study was undertaken to see whether, it was possible to alter the fatty acid profile of milk fat by feeding protected fat / protein supplement to dairy cows.

MATERIALS AND METHODS

Experimental Design and Diets

A feeding trial was conducted on 6 lactating crossbred (HF X Jersey) cows, yielding 12-15 kg milk per animal per day. Feeding trial was conducted for 4 weeks at Jersey farm, Anand

* *Productivity Systems - Animal Nutrition Group, National Dairy Development Board, Anand 388 001, Gujarat, India.*
1 *Faculty of Veterinary Science (B19), University of Sydney, NSW 206, Australia.*
2 *Rumentek Industries, Parkside, 5001, South Australia.*
2008-009 Date received:May 2006; Accepted:October 2007

Agricultural University (AAU), Anand. Animals were selected based on milk yield, fat per cent and stage of lactation (80-90 days post calving). Each animal was fed on a basal diet, comprising 15-17 kg green jowar fodder and 5-6 kg paddy straw per day. Concentrate mixture, having 21.52% CP; 2.82% EE: 9.85% CF and 2.90% AIA, was given according to level of milk production, to meet the maintenance and milk production requirements (NRC, 2001). Feeding was done twice daily in the morning and evening and animals were offered ad-lib clean drinking water thrice a day. After recording baseline information (1 week), animals were supplemented with one kg bypass fat/protein (Fat 32%; CP 27%) supplement for 4 weeks, per animal per day. Degree of fat protection was 74% of the total fat, whereas, protein protection was 76% of the total CP (Table 2).

Analytical Method

The chemical composition of feeds and fodder (Table 1) was carried out as per AOAC (1995). Feeds and fodder were also analyzed for NDF, NDIN, ADF, ADIN, cellulose, hemi-cellulose and acid detergent lignin as per Goering and Van Soest (1970). The data were analyzed statistically (Snedecor and Cochran, 1968). The milk samples were drawn weekly and were analyzed for fat (IS: 1224, 1977) and protein (IS: 1479, 1961) contents. Fatty acid analysis (Ashes *et al.* 1992) was carried out by Gas Chromatography (Perkin-Elmer, auto sampler). Following conditions were used to separate the fatty acids in methyl esters. BPX70 capillary column (50m x 0.32 mm ID x 0.25 µm, SGE). Split-splitless injection. Column temperature programmed: 150°C for 40 min. Carrier gas- helium.

Small Scale Butter Making

Cream was separated from milk, using cream separator and its fat content was standardized to 35-40% w/w. The cream was stored overnight at 4° C and then churned in at 8°C room using a one litre capacity laboratory churn. Agitation was stopped 10 seconds after emulsion inversion. The butter was transferred to a plastic container with drainage holes in the walls and worked. Penetration value of butter was measured by using FPN_3 cone penetrometer (Associated Instrument Manufacturers Pvt. Ltd., India). Butter was tested for penetration value by adjusting cone of the penetrometer exactly above the surface of the sample and allowing the cone to freely penetrate exactly for 5 seconds. The depth of penetration was measured in 0.1 mm units on the dial of the instrument. These penetrations were made on each sample at different points and the closest three readings were averaged. Butter was analyzed for fat and moisture (IS: 3507-1966) and iodine value (IS: 3508-1966).

RESULTS AND DISCUSSION

Effect on Milk Parameters

Daily milk yield, weekly fat and protein per cent in control and experimental period are shown in Table 4. On feeding 1.0 kg bypass fat / protein supplement to dairy cows, average milk yield (kg) increased from 14.01 ± 1.45 to 15.35 ± 1.33 ($p<0.05$). The fat and protein per cent increased from 3.90 ± 0.65 to 4.33 ± 0.58 ($p<0.05$) and 3.00 ± 0.43 to 3.26 ± 0.41, respectively. Significant effect of supplementing bypass fat on milk production and daily fat yield in Holstein Friesian

Table 1: Chemical Composition (% on DM basis) of Feeds and Fodder Fed During Trial Period

Particular	Jowar green	Paddy straw	Cattle feed
Crude protein	4.28	2.42	21.52
Ether extract	1.21	0.51	2.82
Acid detergent fibre	43.64	51.28	21.37
Acid detergent insoluble nitrogen	0.34	1.63	2.16
Neutral detergent fibre	69.36	67.37	27.42
Neutral detergent insoluble nitrogen	0.53	1.91	2.76
Acid detergent lignin	4.55	4.32	5.62
Cellulose	34.87	41.62	12.85
Hemi-cellulose	25.72	16.09	6.05
Total ash	10.26	17.28	14.33
Silica	4.22	5.34	2.90

Table 2: Nutrient Profile of Bypass Fat Supplement

Particular	(%)
Fat	
Total fat	32.00
Rumen undegradable fat	23.68
Rumen degradable fat	8.32
Palmitic acid (C16:0)	5.31
Stearic acid (C18:0)	2.43
Oleic acid (C18:1)	51.68
Linoleic acid (C18:2)	27.34
Linolenic acid (C18:3)	8.56
Protein	
Total protein	27.00
Rumen undegradable protein	20.52
Rumen degradable protein	6.48

cows has been reported earlier (Wost and Hill, 1990; Knapp and Grummer, 1991; Ashes *et al.* 1995; Gulati *et al.* 1997; Garg and Mehta, 1998; Garg *et al.* 2002a; Garg *et al.* 2002b). The significant improvement in milk production performance could be due to the increased supply of amino acids at the tissue level. There are reports by several workers that, formaldehyde treatment caused an increased supply of amino acids at the lower tract (Antoniewicz *et al.* 1992; Xu *et al.* 1998). Chalupa and Sniffen (1996) also reported that the increased supply of essential amino acids in protected form causes an increase in milk production. Methionine in particular, plays a significant role as a methyl donor during milk fat synthesis, and is also the precursor for phospholipids component i.e. choline synthesis. The improved supply of amino acids in the presence of sufficient metabolizable energy, might have also improved the protein-energy balance and created a better balance of precursors for milk synthesis, resulting in increased milk production.

Fatty Acid Analysis and Modified Fat

Levels of essential fatty acids in milk fat in both groups are given in Table 3. On feeding bypass fat/ protein supplement, levels of saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) reduced @ 12.0% and unsaturated fatty acids ($C_{18:1}$, $C_{18:2}$, $C_{18:3}$) increased @ 20.0% in butter made from milk (Gulati *et al.* 2000). Significant reduction was observed in $C_{16:0}$ (@ 19.0%), whereas, $C_{18:1}$ increased (@ 14.0%) significantly ($p<0.05$). On feeding bypass fat/ protein supplement, level of $C_{18:2}$ increased significantly in experimental group (3.77, $p<0.01$) as compared to control group. The fatty acids of milk fat originate from three main sources: Preformed fatty acids from food fat transferred to the mammary gland via blood and lymph in the form of triglycerides and free fatty acids. For the most part, these are acids of 16 or more carbons. These form the major portion of milk fat. Fatty acid synthesized by the gland from acetate and β-hydroxybutyrate produced by rumen bacterial (C_4-C_{14} and part of C_{16}). Manipulation of composition of milk fat is possible through feeding practices for dairy cows (Grummer, 1991; Palmquist *et al.* 1993). Feeding fat that is rich in 18 carbon fatty acid increases $C_{18:0}$ and $C_{18:1}$ content of milk fat and reduces

Table 3: Average Fatty Acid Composition (%) of Milk Fat

Fatty acid	Control	Experimental
Caprylic acid ($C_{8:0}$)	1.20 ± 0.05	1.17 ± 0.05
Capric acid ($C_{10:0}$)	2.59 ± 0.08	2.35 ± 0.08
Lauric acid ($C_{12:0}$)	2.83 ± 0.16	2.75 ± 0.21
Myristic acid ($C_{14:0}$)	10.71 ± 0.22	10.35 ± 0.18
Myristoleic acid ($C_{14:1}$)	1.15 ± 0.14	1.08 ± 0.14
Palmitic acid ($C_{16:0}$)	32.07 ± 0.29	25.86* ± 0.66
Palmitoleic acid ($C_{16:1}$)	1.69 ± 0.05	1.57 ± 0.13
Stearic acid ($C_{18:0}$)	12.03 ± 0.31	11.58 ± 0.45
Oleic acid (C18:1)	25.10 ± 0.24	28.64* ± 0.39
Linoleic acid ($C_{18:2}$)	1.59 ± 0.09	3.77** ± 0.21
Linolenic acid ($C_{18:3}$)	0.42 ± 0.06	0.89 ± 0.07
Arachidic acid ($C_{20:0}$)	0.54 ± 0.12	0.52 ± 0.03

*P<0.05; **P<0.01

the short chain fatty acids (SCFA) content of milk via reduction of *de novo* fatty acid synthesis. Oleic acid ($C_{18:1}$) content of milk can be increased substantially if the cow is fed high levels of substrate ($C_{18:0}$) for stearoyl-CoA desaturase (Bickerstaffe *et al.* 1972). The most significant changes in milk fat quality relate to rheological properties, which influence numerous aspects of character and quality of manufactured dairy products (Mortensen, 1983). The type of fatty acids present in milk fat can influence the physical and chemical properties of dairy products (Stegeman *et al.* 1992; Sundstol, F. 1974; Warner, R.G. 1960).

fat and moisture percent were 85.45 to 85.94 and 12.44 to 12.87 respectively, in experimental period (4 weeks). Penetration values of butter were measured using FPN_3 cone penetrometer (Haighton, 1959; Mortensen and Danmark, 1981). Penetration values (mm) increased from 4.72 ± 0.22 to 5.64 ± 0.26 ($p<0.05$). Consistency is the most important characteristic among the many physical properties of butter. The most commonly used test to measure consistency is the cone penetration test (Fig.1). The instrument used for determining the consistency and penetration properties of butter is called a penetrometer.

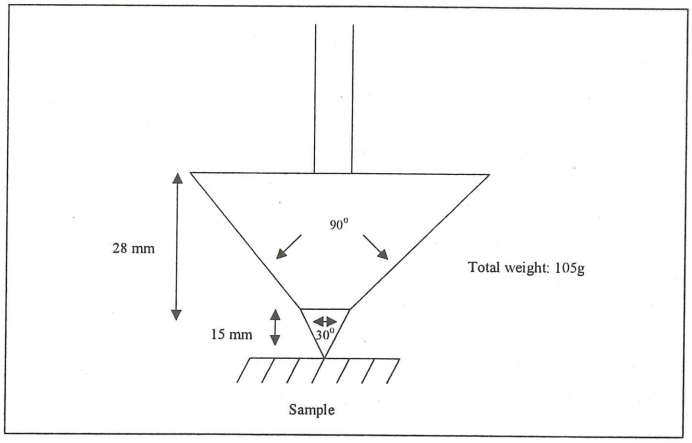
Table 4: Effect of Treatment on Quantity and Quality of Milk and Butter

Particular	Control		Experimental	
	Range	Mean ± SE	Range	Mean ± SE
Milk				
Milk yield (kg)	12.45 – 15.68	14.01 ± 1.45	12.53 – 16.85	15.35* ± 1.33
Fat (%)	3.53 – 4.56	3.90 ± 0.65	3.50 – 4.83	4.33* ± 0.58
Protein (%)	2.86 – 3.34	3.00 ± 0.43	3.11 – 3.57	3.26 ± 0.41
Butter				
Moisture (%)	10.86 – 11.95	11.78 ± 1.63	12.44 – 12.87	12.53 ± 1.14
Fat (%)	85.12 – 85.86	85.35 ± 1.87	85.45 – 85.94	85.81 ± 1.66
Iodine value				
(g/100g of fat)	30.12 – 31.63	30.81 ± 0.67	32.18 – 35.54	34.38 ± 0.73
Penetration value (mm)	4.04 – 6.22	4.72 ± 0.22	4.93 – 7.88	5.64* ± 0.26
*P<0.05				

Penetration Testing

Butter contained fat and moisture percent in the range of 85.12 to 85.86 and 10.86 to 11.95, respectively in control period (1 week), whereas,

Figure 1: Diagram Showing Cone Details for Penetration Testing



The penetration measurement is the depths in tenths of millimeters to which a standard penetrant such as cone sinks into butter under defined conditions of sample size, penetrant weight, geometry and time. The softer the sample is the deeper the penetrant will sink into the sample and thus the higher the penetration number will be. The penetration cone is placed at the surface of the sample and the test stated with the press of a button. Once the penetrant is released, it falls into the sample under the influence of gravity.

It is important to remember that the penetration measurement is the distance traveled by the penetrant inside the sample under the influence of gravity for a fixed period of time.

Iodine Value Estimation

Iodine value was also affected by supplementing protected fat. Iodine value was estimated by the

Wijs method (IDF, 1959). Iodine value of milk fat (g/100g of fat) of control group ranged from 30.12 to 31.63, with a mean value 30.81 ± 0.67. In experimental group, iodine value (g/100g of fat) increased and reached a mean value of approx. 34.38 ± 0.73 (Table 4). The iodine value indicates the number of double bonds, or degree of unsaturation. It can be used as an estimate of oxidative stability of a lipid. As butter has a low iodine value, it is less likely to undergo oxidation (Bruun, H. 1971; Palmquist and Jenkins, 1980). Studies have demonstrated a significant and prompt change in the iodine number of milk fat when the diet fat was changed from a high to a low degree of unsaturation. The modest increase in unsaturation was probably due to an increase in the oleic acid ($C_{18:1}$) with little increase in linoleic acid ($C_{18:2}$). The rumen micro-flora is apparently able to hydrogenating only one double bond. The most significant changes in milk fat quality relate to rheological properties, which influence numerous aspects of character and quality of manufactured dairy products (Mortensen, 1983). The type of fatty acids present in milk fat can influence the physical and chemical properties of butter (Stegeman *et al.* 1992).

From the present study, it is evident that it was possible to significantly increase milk volume, as well as modify composition of butter fat in cows on feeding rumen bypass fat/protein supplement and the same can be exploited commercially.

ACKNOWLEDGEMENT

Financial assistance and necessary facilities provided by the management of National Dairy Development Board, Anand for undertaking this study, are gratefully acknowledged.

REFERENCES

Antoniewicz, A. M.; Van Vuuren, A. M.; Vender Keeled, C. J. and Kosmala, I. 1992. Intestinal Digestibility of Rumen undegraded Protein of Formaldehyde Treated Feedstuffs Measured by Mobile bag and *in vitro* Technique. *Animal Feed Sci. and Tech.* **39**:111-124.

AOAC 1995. Official Methods of Analysis (14th edn.) Association of Official Analytical Chemists, Washington, D.C.

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**:1090.

Ashes, J. R.; Gulati, S. K. and Scott, T. W. 1995. The Role of Rumen Protected Proteins and Energy Sources in the Diet of Ruminants. In: Animal Science Research and Development. (Ed. Ivan, M., Centre for Food and Animal Research Agriculture and Agri-Foods Canada). pp. 177.

Bickerstaffe, R.; Noakes, D. E. and Annison, E. F. 1972. Quantitative Aspects of Fatty Acid Biohydrogenation, Absorption and Transfer into Milk Fat in the Lactating Goat, with Special Reference to the Cis and Trans-Isomers of Octadecenoate and Linoleate. *Biochem. J.* **130**:607-617.

Bruun, H. 1971. Iodine Value and Improvement in Butter Quality. Cited from *Dairy Sci. Abstr.* **33**:4322.

Chalupa, W. and Sniffen, C. J. 1996. Protein and Amino Acid Nutrition in Lactating Dairy Cattle – Today and Tomorrow. *Anim. Feed Sci. & Tech.* **58**:65-75.

Garg, M. R. and Mehta, A. K. 1998. Effect of Feeding Bypass Fat on Feed Intake, Milk Production and Body Condition of Holstein Friesian Cows. *Indian J. Anim. Nutr.* **15**:242-245.

Garg, M. R.; Sherasia, P. L.; Bhanderi, B. M.; Gulati, S. K. and Scott, T. W. 2002a. Effect of Feeding Rumen Protected Nutrients on Milk Production in Crossbred Cows, *Indian J. Anim. Nutr.* **19**:191-198.

Garg, M. R.; Sherasia, P. L.; Bhanderi, B. M.; Gulati, S. K. and Scott, T. W. 2002b. Effect of Feeding Rumen Protected Nutritents on Milk Production in Cows and Buffaloes. *Indian J. Dairy Sci.* **55**:281-285.

Gibson, R. A., Neumann, M. A. and Makrides, M. 1996. Effect of Dietary Docosahexaenoic Acid on Brain Composition and Neural Function in Terms Infants. *Lipids.* **31**:S1777-S181.

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, Superintendented of Documents, U. S. Government Printing Office, Washington, D.C. 20402.

Grummer, R. R. and Carroll, D. J. 1991. Effects of Dietary Fat on Metabolic Disorders and Reproductive Performance of Dairy Cattle. *J. Anim. Sci.* **69**: 3838.

Gulati S. K.; Byers, E. E.; Byers, Y. G.; Ashes, J. R. and Scott, T. W. 1997. Effect of Feeding Fat Supplements on the Fatty Acid Composition of Goat Milk. *Anim. Feed. Sci. & Tech.* **66**:159.

Gulati, S. K.; Kitessa, S. M.; Ashes, J. R.; Fleck, E.; Byers, E. B.; Byers, Y. G.; Scott, T. W. 2000. Protection of Conjugated Linoleic Acids from Ruminal Hydrogenation and Their Incorporation into Milk Fat. *Anim. Feed Sci. and Tech.* **86**:139-148.

Haighton, A. J. 1959. The Measurement of the Hardness of Margarine and Fats with Cone Penetrometer. *J. Am. Oil Chemists' Society,* **36**:345.

IDF 1959. International Standard 8. Determination of the Iodine Value of Butter Fat by The Wijs Method. Brussels: International Dairy Federation.

ISI:1224. 1977. Indian Standards Methods of Test for Dairy Industry Part – I, Chemical Analysis of Milk,

Part – I, Indian Standards Institute, New Delhi, India.

IS: 1479. 1961. Indian Standards Methods of Test for Dairy Industry Part – I, Chemical Analysis of Milk, Part-II, Indian Standards Institute, New Delhi, India.

IS: 3507. 1966. Methods of Sampling and Test for Butter. Indian Standards Institute, New Delhi, India.

IS: 3508. 1966. Methods of Sampling and Test for Ghee (Butterfat). Indian Standards Institute, New Delhi, India.

Knapp, D. M. and Gummer, R. R. 1991. Responses of Lactating Dairy Cows to Fat Supplementation During Heat Stress. *J. Dairy Sci.* **74**:2573.

McGuire, M. K.; McGuire, M. A.; Ritzenthaler, K. and Shultz, T. D. 1999. Dietary Sources and Intakes of Conjugated Linoleic Acid in Humans. Page 369-377 in Advances in Conjugated Linoleic Acid Research Volume I.P. Yuraweez, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza and G. J. Nelson eds. AOCS Press, Champaign, Illinois.

Mortensen, B. K. 1983. Physical Properties and Modification of Milk Fat. In P.F. Fox, (Ed.) Developments in Dairy Chemistry-2. Lipids. *Applied Science,* London, pp. 159-194.

Mortensen, B. K. and Danmark, H. 1981. Firmness of Butter Measured with a Cone Penetrometer. *Milchwissenschaft* **36**: 393.

NRC, 2001. "Nutrient Requirements of Dairy Cattle, National Academy of Science- National Research Council, Washington, D.C.

Palmquist, D. L. and Jenkins, T. C. 1980. Fat in Lactation Rations: Review, *J. Dairy Sci.* **63**: 1.

Palmquist, D. L.; Beaulieu, A. D. and Bari, D. M. 1993. ADSA Foundation Symposium: Milk Synthesis and Modification: Feed and Other Factors Influencing Milk Fat Composition. *Dairy Sci.* **76**:1753-1771.

Pariza, M. W.; Park, Y. and Cook, M. E. 2000. Mechanisms of Action of Conjugated Linoleic Acid: Evidence and Speculation. *Proc. Soc. Exp. Biol. Med.* **223**:8-13.

Scott, T. W.; Cook, L. J.; Ferguson, K. A.; McDonald, I. W.; Buchanan, R. A. and Loftus. Hills, G. 1970. Production of Polyunsaturated Milk Fat in Domestic Ruminants. *Aust. J. Sci.* **32**: 291.

Simopoulos, A. P. 1991. Omega-3 Fatty Acids in Health and Disease and in Growth and Development, *Am. J. Clin. Nutr.* **54**: 438-463.

Snedecor, G. W. and Cochran, W. G. 1968. Statistical Methods, 6th ed., Oxford and IBH Publishing Company, Calcutta.

Stegeman, G. A.; Bear, R. J.; Schingoethe, D. J. and Casper, D. P. 1992. Composition and Flavour of Milk and Butter from Cows Fed Unsaturated Dietary Fat and Receiving Bovine Somatotropins. *J. Dairy Sci.* **75**: 962-970.

Sundstol, F. 1974. Hydrogenated Marine Fat as Feed Supplement. IV. Hydrogenated Marine Fat in Concentrate Mixtures for Dairy Cows. *Sci. Rep.* Agri. Univ. Norway 53 (Nr. 25).

Warner, R. G.1960. The Place of Added Fat in Ruminant Rations. Page 88 in Proc. Cornell Nutr. Conf. Feed Manuf.

Wost, J. N. and Hill, G. M. 1990. Effect of a Protected Fat Product on Productivity of Lactating Holstein and Jersey Cows. *J. Dairy Sci.* **73**:3200.

Xu, S., Harrison, J. M.; Chalupa, W.; Sniffen, C.; Julien, W.; Sato, H.; Fuvieda, A.; Watanabe, K.; Veda, T.; and Suzuki, H. 1998. The Effect of Ruminal Bypass Lysine and Methionine on Milk Yield and Composition of Lactating Cows. *J. Dairy Sci.* **81**: 1062-1077.