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Effect of Nutrition on Production, Composition, Fatty acids and Nutraceutical Properties of Milk

Tripathi MK*

Division of Nutrition Feed Resources and Product Technology, Central Institute for Research on Goats, India

***Corresponding author:** Tripathi MK, Division of Nutrition Feed Resources and Product Technology, Central Institute for Research on Goats, Makhdoom, Farah 281 122, Mathura, India, Tel: 91 565 276 33 80; E-mail: mktripathi@gmail.com

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Abstract

The objective of this paper was to determine the opportunities of improving milk productivity and altering the milk composition through nutritional interventions. The increase in the nutraceutical value of milk is expected through dietary modifications of the animal. Feeding high concentrate diets combined with dietary fat can be used to modify the FA profile of milk, without negative effect on milk yield and milk fat or protein contents. Their effects may be complementary, at least for trans fatty acids. The profile of trans isomers relative to total trans-C18:1 seems dependent on the source of dietary fat. Feeding oilseeds and/ or novel fat supplements to ruminants can be used to modify the lipid metabolism in the mammary gland in modulating the secretion of fat and the profile of milk fatty acids. Feeding novel fatty acid sources the nutritive value of milk can be enhanced by decreased atherogenic saturated FA in milk and increased t_{11}-$C_{18:1}$ (trans vaccenic acid), and c_9,t_{11}-$C_{18:2}$ (geometric and positional isomer of conjugated linoleic acid (CLA) in milk, which are considered as positive for human health. Feeding bypass/ rumen protected sources of fatty acids and amino acids may help in meeting the need of higher milk production, and in modulating milk composition through increase availability of fatty acids and amino acids in circulation with synchronized extraction of desired nutrient by mammary gland for milk constituents synthesis. Milk may be used as delivery system of anticarcenogens (CLA and polyphenols) for human health. Therefore, nutritional manipulations have several opportunities in improving milk production, modifications of milk composition and fatty acid profile. The improved milk productivity is desired in line with increased human population with minimum environment degradation thereby nutritional manipulations are directed to enhance milk production without increasing dairy animal populations with reduced greenhouse gasses emissions from ruminant agriculture.

Keywords: Conjugated linoleic acid; Dairy animal; Diet; Milk fatty acid profile

Introduction

Milk and milk products are an important source of food and contributors of dietary energy requirements, high quality protein, minerals and vitamins, particularly in the vegetarian diets. Milk consumption is predicted to increase globally in the coming years in line with the increasing world population, a greater income potential exists for the availability of milk and milk products to meet human requirement. Present scenarios of animal production system imposing pressures on dairy animal production for enhancing milk production with available dairy animal numbers, or even on reduced population aiming for a minimal greenhouse gasses emission. The changes of dietary constituents of animals are therefore directed in enhancing milk productivity of an individual with increased availability and higher nutrient use efficiency of energy, protein and other essential nutrients. High energy supplements such as fat and oils are added to increase energy density of animal diets, while protein sources of better amino acid composition that are extracted at higher level for milk synthesis are being used in dairy animal diets. Nutritional modifications of dairy animal diets not only, aiming in enhancing milk production but also in reducing the content of high saturated fatty acids of milk. Recently more efforts are being associated with increased milk production through diet in combination of the manipulation of the milk composition. The major deriving forces for manipulating the composition of milk included the aims of improving the manufacturing and processing of milk and dairy products, altering the nutritional value of milk to conform the dietary specifications and using milk as a delivery system for nutraceuticals with known benefits to human health. The conjugated linoleic acid is known as the potential anticarcinogen in milk, which can be manipulated through dietary changes [1]. Recently ability of casein micelles has been demonstrated to deliver biologically active concentrations of polyphenols that display anticancer activities in colon cancer cells [2]. Therefore, dietary modifications of dairy animals may aim to improve bioefficacy of biologically active polyphenols in milk to deliver for human health. The dietary control of milk composition has been realized with great opportunities. The most sensitive component of milk to dietary manipulation was fat content, which could be changed over a range of 3 percentage units [3]. The principal fatty acids of the milk are uninfluenced by the composition of the diet, the digestive system and by the biosynthesis process of milk within the animal. It is shown that the lactose content could not be manipulated by dietary changes, except under extreme and unusual feeding situations. Milk protein is responsive to diet but less responsive than fat. During last several years, the greatest changes were made in milk fat and fatty acid composition. Although several factors including genetics and breed of animal, environment, stage of lactation, parity, and nutrition of animal are working together in determining the final composition of the milk [4]. The focus of present paper is on the nutrition of the dairy animal that aiming in influencing the production and composition of milk. The nutritional control for a change in milk composition is realized

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when one or more desired nutrients are incorporated into the diet, followed by absorption and transport of the nutrient to the mammary gland, and finishes with secretion of the nutrient in milk as either a desired component or as a regulator of milk synthesis. The ability of nutritional control of milk composition by dietary manipulation is known from the scientific contributions of the entire animal system, from practical studies on feeding systems to basic cellular work on mammary tissue metabolism.

Milk Production Performance

Nutrition is the single factor having the greatest influence on milk production performance of dairy animals. The diets providing essential nutrient in appropriate and adequate quality and quantity promoted milk production. Fat supplements are added in the diet of high producing dairy animals for increasing energy density without effecting fiber level. Fat sources such vegetable and animal fat [5], yellow grease [6,7] and plant and marine oils [8] have been reported to improve lactation performance without interfering in metabolism. Oilseeds and whole cottonseed are commonly used as primary dietary fat sources, that also providing protein and fiber. Proportion of unsaturated fatty acids in total fat has additional benefits; a high proportion increased the total and intestinal digestibility of fatty acids [8]. Glycerol is known to improve the energy balance in lactating dairy animals by increasing dry matter intake and reducing NEFA levels in blood. However, Kass et al. [9] did not find increased milk production in dairy cows even dry matter intake increased upon using glycerol at 0, 5, 10 and 15% in diet dry matter in replacement of barley meal on iso-energetic basis. The metabolic shift occurred upon glycerol supplementation that does not favor gluconeogenesis at the mammary gland from propionate. Rumen protected fat feeding above the basal diet at 0.7 and 1.5 percent improved milk production by 16.7 and 50.3 percent respectively in lactating buffaloes [3]. The nutrient density of diet and availability of desired in satisfying nutrient to support higher milk production are responsible in enhancing milk production of dairy animals.

Animal species	Production level (kg/d)	Milk constituent				Reference
		Fat (g/kg)	Protein (g/kg)	Lactose (g/kg)	Milk urea N (mg/dl)	
Cow						
	High (>28)	40.2	29.5-30.5	44.8-45.3	11.9-12.4	Van Zijderveld et al. [10]
		32.4-34.1	27.9-28.9	49.4-49.9	12.5-14.7	Pan et al. [11]
		36.7-39.9	29.0-30.7			Dean et al. [41]
		36.2-38.6	27.7-29.1	20.0-21.7	15.13-17.66	Kargar et al. [14]
	Medium (16-28)	19.3	39.3	32.6	48.1	Staerfl et al. [42]
		45.3-46.7	36.0-37.6	47.5-48.0	22.1-23.5	Kass et al. [9]
		40.2-42.7	30.9-32.5	44.6-45.3	12.0-13.2	Van Zijderveld et al. [10]
	Low (<16)	41.7	33.3	48.2	7.56	Staerfl et al. [42]
		37.3-37.8	33.2-34.6	51.6-53.2		Santra et al. [12]
Buffalo						
	High (>8)	8.2-9.0	4.5-5.1			Ranjan et al. [3]
	Low (<8)	81.6	43.0			Ranjan et al. [3]
Goat						
	High (>1.4)	43.2-44.6	36.5	49.4-49.9		Stella et al. [43]
		35.4	31.2	59.2		Miri et al. [1]
		39.4	35.9	48.6		Torres et al. [44]
		47.0	40.5	49.6		Mestawet et al. [59]
		32.8-36.5	33.6-38.7	48.7-49.0	21.7-23.1	Laudadio and Tufarelli [60]
	Medium (0.8-1.4)	33.6-36.3	26.3-29.1	55.0-60.9		Miri et al. [1]
		37.6-40.6	35.1-39.2	48.3-48.6		Torres et al. [44]
		36.5-56.5	40.8-48.0	49.2-49.7		Mestawet et al. [59]

		40.2-48.5	30.5-34.2	40.1-43.7		Renna et al. [61]
	Low (<0.8)	31.5-48.4	26.5-28.6	41.4-44.3		Durge [45]

Table 1: Milk composition of cow, buffaloes and goat.

Milk composition

Milk composition is a trait of animal species, which cannot be altered under normal production systems. Changes in composition are attributed through the altered genetic makeup and by the dietary modifications. Nutrition has direct and impact on milk composition. Level of milk production also has some influence on milk constituents (Table 1). Nutrition research of dairy animals broadened the opportunities altering milk composition through dietary manipulations. Dietary sources of nitrogen as urea or nitrate does not alter milk composition, yield of milk constituents and milk production of dairy cows [10]. Supplementation of protected fat above the basal diet at 0.7 or 1.4% increased fat and protein content [3]. Whereas, supplementation of *Radix Bupleuri* extract at 0.5 or 1.0 g/kg basal diet as a source of plant secondary metabolites, which are saponins and essential oil to combat heat stress in lactating dairy cows improved milk fat and protein yield, while milk protein and fat content of the milk did not change [11]. Similarly using *Piper betle* leaf feeding at 100 g per day in cows improved milk production and contents of fat, protein and lactose [12]. The *Piper betle* leaf is known for its ethano medicine properties because of high antioxidant, immunomodulation and anti-bacterial characteristics. The influence of diet on milk production and composition is presented in Table 2, the response of diet on milk production and composition is variable in relation to dietary changes in type of forage, level and sources of supplementations. The modification of the basal diet of animals, especially by lipid supplementation, is the most appropriate procedure for modifying the milk fatty acid profile. The increasing amount of fats and oils in the feed of ruminant animals reduce food consumption and alter the rumen microorganism, resulting in a decrease in the production of milk and, some occasions, its protein content. However, the inclusion of less than 4% oil in cattle and sheep feeds has no negative effects on the animal and increases the polyunsaturated fatty acid content of milk. The way in which the different lipid substrates are introduced in the animal diet may influence the levels of the different components in milk. The ruminants grazing on pasture having increased level if CLA in milk and meat.

	Fatty acid (g/100 g milk fatty acid)		
Fatty acids	**Cow**	**Goat**	**Ewe**
4:0	2.05-33.32	2.09-02.20	
6:0	1.92-2.78	2.13-2.15	1.72-1.86
8:0	1.5-2.37	2.39-2.43	1.68-1.72
10:0	3.23-4.47	10.05-10.28	5.25-5.55
12:0	3.50-4.50	5.24-5.32	3.44-3.75
14:0	11.22-3.12	10.63-11.28	7.76-8.20
16:0	26.47-31.38	30.51-31.09	34.96-36.84
16:1c	1.46-1.61	0.72-0.73	1.05-1.28
18:0	9.26-11.76	6.25-6.68	13.14-15.62
18:1c9	15.4-18.83	14.91-15.18	16.68-17.76
18:1t11 (vaccenic acid)		0.74-0.79	0.88-1.20
18:2c9,c12	2.72-3.30		2.71-3.04
18:3c9,c12,c15	0.35-0.42		
C18:3n-3/C18:2n-6	0.11-0.16		
Total CLA	0.71-1.06	0.39-0.48	0.64-0.81
18:2c9,t11	0.18-0.26	0.37-0.47	0.55-0.71
18:2t10,c12	0.05-0.08	0.012	0.086-0.096
18:2t10,t12	0.10-0.26		

18:2t8c10/c9,c11	0.08-0.14		
18:2c1,t13	0.11-0.16		
18:2t8c12/c11,c113	0.08-0.11		
18:2t11,t13	0.06-0.10		
22:6	0.09-0.11		
Short chain fatty acids	13.08-17.86	21.9-22.38	12.09-12.88
Medium chain fatty acids	43.17-48.33	41.86-43.10	43.77-45.96
Long chain fatty acids	34.23-42.52	34.52-36.24	41.16-44.14
Saturated fatty acids	65.4-70.28	70.24-69.51	72.72-73.24
Unsaturated fatty acids	29.63-34.86	19.80-20.99	24.18-25.46
Monounsaturated fatty acids	19.01-25.09	16.54-16.77	20.12-21.22
Polyunsaturated fatty acids	9.77-10.62	3.36-4.19	3.66-4.23
Desaturase index (%)			
12:0	7.2-9.9		
14:0	7.4-8.0	1.0-1.8	3.4-3.6
16:0	4.6-5.4	2.25-2.34	3.1-5.5
18:0	59.1-62.3	69.1-70.4	18.4-21.4
Anthrogenic index	2.22-2.99	2.51-3.05	2.81-3.10

Table 2: Milk fatty acid profile of cow [14], goat [1] and ewe [46]

CLA, conjugated linoleic acids; Short chain fatty acids (C4-C12); Medium chain fatty acids (C14-C16:1); Long chain fatty acids (C18:C22:6), {Desaturase index is [100×destuarted/(destaurated +saturated)]; Anthrogenic index is ([(C12+4)×(C14+C16)]/sum of unsaturated fatty acids), according to Chillard and Ferlay [26].

Effect of grain feeding and forage: concentrate ratio on milk composition

Cereal grains are used in dairy rations as a cost-effective source of digestible energy for maintaining high levels of milk production. High grain intake in dairy animals is also known to depress milk fat content and alter fatty acid composition. Grain feeding typically reduces the proportions of milk fatty acids having 6 to 16 carbons, and increases the proportion of 18-carbon unsaturated fatty acids. The milk fat depression with high grain diets is due to inadequate rumen production of acetate and butyrate to support milk fat synthesis and higher rumen propionate level stimulates circulating insulin concentration, which redirects metabolites away from mammary tissue. It is proposed [13] that milk fat depression on high grain diet was the result of altered fatty acid composition of milk and that the *trans* fatty acids were the causative agent of milk fat depression in dairy cattle. An inverse relationship between *trans* fatty acids in milk and milk fat content exists. However, several reports indicated a substantial increases in milk *trans* fatty acids without reductions in milk fat content, showing that not all *trans* fatty acid isomers were associated with milk fat depression. The milk fat depression was more closely associated with the production of *trans*-10 fatty acid isomers in the rumen than with *trans* isomers in general. Grain feeding enhanced the production of the *trans*-10 fatty acid isomers by ruminal microorganisms. Studies demonstrated severe milk fat depression in cows infused with *trans*-10, *cis*-12 Conjugated Linoleic Acid (CLA), but no depression following infusion of the *cis*-9, *trans*-11 CLA isomer. The conjugated dienes and trienes have failed to inhibit milk fat synthesis. Thus, the *trans*-10, *cis*-12 CLA is the most likely factor in milk fat depression. However, increasing the F:C ratio from 34:66 to 54:55 increased milk short chain fatty acids without effecting total milk fat CLA [14]. Therefore grain alone is responsible for milk fat depression, while mixed diets promoted the production of milk and milk protein.

Reducing the proportion of forage in the diet of a cow increases both protein content and yield. Milk protein content can be increased by 0.4 percentage units or more if forage proportion in the diet is reduced to 10% or less of the diet DM. A minimum concentration (40%) of forage is needed in dairy diets to avoid digestive and metabolic disturbances. Reducing the forage-to-concentrate ratio has not been a practical method of consistently enhancing milk protein content. In general forage is not the direct cause of milk protein depression but it is an indirect effect of decreasing energy intake. Rapidly fermentable dietary carbohydrate has been associated with milk protein content. The fermentation of starch to propionate production in the rumen explained how changes in the forage-to-concentrate ratio regulate milk protein production. When rapidly fermentable carbohydrate is fed, greater production of propionate and microbial protein is leading to signals in the cow's body to produce more milk and milk protein.

Milk fat and fatty acids

The milk fat being the greater sensitive to dietary manipulation than other milk constituents has received a great attention of nutritional control of milk fat content and fatty acid composition. Fatty acid composition of dairy animals is presented in Table 3, interspecies variations in fatty acid profile of animals occurred. However, diet and fermentation pattern are implicated as the source of variations in milk fatty acids within an animal species. Ruminal production of *trans* fatty acid isomers are linked with milk fat depression and that the rumen biohydrogenation of fatty acid providing insight on the origin of specific *trans* fatty acid isomers that are originating from ruminal biohydrogenation and uses of these modified isomers by the mammary enzymes for secretion in milk. Interest in manipulating the fat content of milk was emphasized reductions in total fat, saturated fat, and cholesterol. Animal products are the main source of saturated fat and cholesterol. While, in majority of dairy production systems the pricing system for milk offered to producers was the premium for high fat content during past, which continuing until now, whereas many consumers are focused on low-fat dairy products that conformed to healthy dietary guidelines. The interest of consumption of lower fat milks increasing in view of healthy dietary guidelines, moreover with the properties of nutraceutical of milk. Since, whole milk is having only 4%, but on a dry basis fat content is high as 27% with the majority (65%) of the fatty acids being saturated. About 50% of the calories in milk come from fat. The pressure to reduce total fat content of dairy products, as well as to reduce its saturation is continuing in meeting the dietary guidelines of health human food productions. The conflict between consumer and producer over fat content generated interests of manipulating properties of milk fat by the enhanced the concentration of fatty acids having beneficial health effects in humans, which is usually to increase one or more unsaturated fatty acids in milk. The manipulation of milk fatty acid composition efforts suggesting that an ideal milk would contain no more than 8% saturated fatty acids, less than 10% polyunsaturated fatty acids, and the remainder (82%) as monounsaturated fatty acids [4]. In addition, the health effects of unsaturated *trans* fatty acids, which are produced in the rumen, attracted interest in enhancing their concentration in milk. The ability of different diet formulations to reduce milk fat content or enhance the concentration of unsaturated fatty acids directed by several dietary factors including the amounts of grain and fat fed to dairy animal. Influence of fat supplements on the control of milk fat and fatty acid composition is complex because the transfer of dietary unsaturated fatty acids to milk can be reduced several factors including their destruction by ruminal microorganisms, poor rates of intestinal absorption, and their deposition in mammary fat and secretion in milk. The magnitude of changes in milk fatty acid profile are controlled by several including source and amount fat supplements, fatty acid biohydrogenation by ruminal microorganisms and the uptake and use of unsaturated fatty acids by the mammary gland.

Diet type	Diet specialty	Milk yield	Milk composition	Reference
Mixed forage concentrate diet	Water soluble carbohydrate level of roughage	Reduced milk yield with high WSC	No change	Staerfl et al. [42]
Mixed forage concentrate diet	Protected fat 0.7 and 1.4 %	Increased milk production	Increased fat and protein content	Ranjan et al. [3]
Mixed forage concentrate diet	Included *Piper betle* leaves, source of active secondary metabolite	Increased milk production	Increased fat, protein and lactose content	Santra et al. 12]
Mixed silage concentrate diet	Applied fibrolytic enzymes product comprised of xylanase and cellulose	No change	No change	Dean et al. [41]
Mixed silage, hay and concentrate	Yeast supplementation *Sccharomysec cerevisiae*	Increased milk production	Reduced fat content	Stella et al. [43]
Total mixed ration	Yeast supplementation *Sccharomysec cerevisiae*	No change	No change	Dean et al. [41]
Mixed forage and concentrate diet	Different roughage and concentrate	No change	Increasing forage reduced milk protein	Kargar et al. [14]
Mixed forage and concentrate diet	Supplemented fat	Increased milk production	Increased milk protein	Kargar et al. [14]
Mixed grass silage and concentrate	Crude glycerol added 52- 156 g/kg	No change	Increased milk protein content	Kass et al. [9]
Mixed legume hay and concentrate	Cumin seed extract supplemented1.3-2.5% in diet	Increased milk production	No change	Miri et al. [1]
Total mixed ration	Vegetable oil supplemented 106 g/kg	Increased milk production	No change	Castro et al. [46]
Total mixed ration	Pellet diet with High or low rumen degradable protein	No change	No change	Laudadio and Tufarelli [60]

Indoor to pasture feeding	Shifting of feeding from indoor to pasture diet	Improved milk production	Increased milk fat, protein, lactose content and yield. Promoted CLA and omega 3-fatty acids	Renna et al. [61]

Table 3: Effect of diet type on milk production and composition

Effect of fat supplements

Fat supplements are used in animal diets not only because it supplies essential fatty acids and fat-soluble vitamins, but also because it provides more energy, approximately twice that of carbohydrates for milk production that enhance productivity of milk. The actual choice of fat or oil and the form by which it is included in the feed is decided by a number of factors. These includes, the cost and availability of the raw material, both locally and globally, the impact of fat or oil form (oilseed or extracted oil) and its fatty acid composition on feed digestibility [15], the influence of consumers and retailers regarding the introduction of genetically modified material into the food chain and animal feed regulations regarding permitted supplements. Lipid may be supplied in an animal's diet via various forage sources, oil-rich cereals, oilseeds, or fish oil, and the tallow was offered as a significant energy source [16-19]. In recent years, novel oil sources such as marine algae, chia seed, lupin, hemp and camelina have been investigated as lipid sources in animal feeds. The vegetable oils either high low or in unsaturated fatty acids have only limited ability to alter milk fatty acid composition. This leads to the production of rumen bypass fats that minimized digestibility problems that often occurred when feeding unsaturated oils to dairy animals, calcium salts of fatty acids and products enriched in unsaturated fatty acids and that fat supplements could alter milk fatty acid composition. Supplementation of fat tended to increase milk fat content and yield. Milk fat content does not influence by the source of dietary fat. Supplementation of 2% hydrogenated palm oil or yellow grease in diet of lactating Holstein cows as source of fat improved milk fat content, and that the total fat yield increased with yellow grease compared to hydrogenated palm oil feeding [14]. The fatty acid profile of various fat/oil sources are presented in Tables 4, although there can be considerable variation in both the oil content and fatty acid profile due to botanical species and country of origin.

Biohydrogenation and milk fatty acids

Microbial biohydrogenation in rumen that transform dietary unsaturated fatty acids of dietary fat entering into rumen have only limited ability to alter milk fatty acid composition irrespective of the fatty acid composition of diet. Therefore, delivery of unsaturated fatty acids to mammary tissue is limited even when their dietary concentration is high. The ruminal microorganisms transform unsaturated fatty acids in a process called biohydrogenation, in which hydrogen addition via microbial enzymes removes double bonds in a fatty acyl chain converting it from unsaturated to saturate. The protected or rumen bypass fat technology was than evolved to seize dietary unsaturated fatty acids from biohydrogenation to enhance their absorption and delivery to the mammary gland. Rumen-protected fats were prepared by embedding unsaturated oils within a protein shell made resistant to microbial attack by cross-linking with formaldehyde. The formaldehyde-protected fats were had dramatic effects on enhancing unsaturated fatty acid in milk. Feeding of various forms of rumen-protected fats changes the oleic and linoleic acid concentrations in milk fat. Oleic acid concentration in milk fat varied from 18 to 24%, whereas linoleic acid concentration in milk normally ranges from 1.5 to 4% of total fatty acids when control rations containing no added fat were fed to cows. When rumen-protected fats were included in cow diets, oleic acid in milk varied from 18 to 48% and linoleic acid concentration to about 6.5%. Oilseeds are the sources of containing natural forms of rumen protected fat due to their hard outer seed coats, however oilseeds are commonly processed (ground, extruded, pelleted) to enhance their handling, intake, or digestibility, which can significantly reduce their resistance to biohydrogenation. Calcium salts of fatty acids also were examined for possible protection from biohydrogenation to avoid digestibility problems when feeding high amounts of fat to dairy animals, which to enhance the absorption of unsaturated fatty acids. Amides of unsaturated fatty acids enhanced unsaturated fatty acids in milk and that the protection of amides from biohydrogenation depending on the specific fatty acid and amide linkage. Oleamide fed to lactating cows enhanced oleic acid concentration in milk, but the same amide bond linkage with polyunsaturated fatty acids was less effective. The regulating pathway of fatty acid biohydrogenation by ruminal microorganisms plays an important role in achieving the successes in nutritional manipulation of milk composition. The intermediates of *trans* fatty acid are arising from the pathways of biohydrogenation which are than responsible for *trans* fatty acids and milk fat depression. Important biohydrogenation intermediates in milk fat is the CLA that had have beneficial effects on human health, importantly fighting properties against cancer. The *cis*-9, *trans*-11 CLA isomer having particular anticarcinogenic properties, were known to arise from the biohydrogenation of linoleic acid. The process in enhancing biohydrogenation intermediates in milk by determining the origin and possible enhancement of beneficial fatty acid isomers produced in the rumen was identified by a multitude of positional and geometric *trans* isomers produced in ruminal contents from lipid biohydrogenation. More than 10 positional isomers of *trans* monoene fatty acids and a dozen or more CLA isomers have been identified in intestinal contents of ruminants. A *trans*-10 double bond intermediate was formed from the biohydrogenation of linoleic and linolenic acids [13]. Rumen bacterium was capable of producing a *trans*-10, *cis*-12 CLA, and that the conversion of oleic acid to *trans* fatty acids occurs by mixed ruminal microorganisms, including the formation of a *trans*-10 isomer.

Diet and milk conjugated linoleic acid (CLA) contents

The discovery of Conjugated Linoleic Acid (CLA) as a potent anticarcinogenic agent attracted research interest on enhancing the CLA concentration in milk through nutritional manipulations. A number of a number of CLA and *trans* isomers in milk fat are indentified that are arising from range of dietary unsaturated fatty acid sources [20] and having a positive correlation between linoleic acid in the diet and of 18:1 *trans* isomers (vaccenic acid (t-11), t-13 + t-14, t-15 and t-16) in milk fat [21,22]. Seasonal variation in milk CLA content also occurred, which is ranging from 0.77 to 1.5 g/100 g total FA in winter with conserved forages and concentrates, and in summer when animals grazed fresh pasture [23]. The inclusion of vegetable oils in the dairy cow diet increased milk C18:1 trans fatty acids and the CLA isomer, c-9, t-11 more than offering extruded oilseeds, which in turn increased the levels of these fatty acids more than when raw oilseeds were offered [24]. Source of fat and fat supplementation increased and tended to increase total CLA concentrations in milk fat but changing the F:C ratio did not impact CLA levels of the milk. The main CLA isomers are not affected by dietary treatments that have fat supplementation and are relatively lower in content than reported [25], although they are within the reported range [26] of variations. Rumen pH has influence on rumen bio-hydrogenation and thereby effecting fatty acids availability for milk synthesis to the mammary gland. Changes in pH can alter ruminal fermentation and microbial population and thereby have key role in controlling the regulation of biohydrogenation and CLA synthesis. Reduced ruminal pH adversely affects microbial populations, especially cellulolytic bacteria, and reduces ruminal biohydrogenation [27]. Alfalfa hay as a sole source of forage in diets may alleviate effects of dietary treatments on rumen condition and rapid fermentation by inhibiting lactic acid producing bacteria and there by sustaining stable rumen environment. Oelker et al. [28] observed that any changes in rumen pH with alfalfa hay relative to corn silage based diets decreased the accumulation of biohydrogenation intermediates and thereby total CLA content in rumen of Holstein dairy cows. Rumen microbial enzymes are responsible for the isomerisation and hydrolysis of dietary lipid and the conversion of unsaturated fatty acids to various partially and fully saturated derivatives, including CLA (C18:2 *cis*-9, t-11) (c), *trans* vaccenic acid (C18:1 t-11) (VA) and stearic acid (C18:0). Although linoleic (C18:2 n-6) (LA) and linolenic (C18:3 n-3) (ALA) acids are the main unsaturated fatty acids in the diet of ruminants, the processes within the rumen ensure that the major fatty acid leaving the rumen is C18:0. The uptake of unsaturated fatty acids into the small intestine by ruminants differs to that of saturated fatty acids. The intestinal absorption coefficient of individual fatty acids is higher in ruminants ranging from 80% for saturated fatty acids to 92% for polyunsaturated fatty acids in conventional low fat diets. The higher absorption efficiency of saturated fatty acids by ruminants has been attributed to the greater capacity of the bile salt and lysophospholipid micellar system to solubilize fatty acids, as well as the acid conditions within the duodenum and jejunum (pH 3.0-6.0). The low pH is due to a low concentration of pancreatic hydrogen carbonate which reduces the conversion of saturated fatty acids into insoluble calcium salts (which cannot be absorbed by the enterocytes). However, triacylglycerol resynthesize in ruminants is by the glycerol-6-phosphate pathway due to the virtual absence of 2-monoacylglycerol. The resynthesized lipid is carried as lipoproteins, chylomicrons and very low density lipoproteins in the blood stream for uptake by the lipoprotein lipase enzyme. In ruminant animals, the long chain poly unsaturated fatty acids, C20 and C22 incorporated into the membrane phospholipids and there will be a very low transfer of the long chain poly unsaturated fatty acids into milk fat as the plasma phospholipids that carry these acids are poor substrates for the mammary gland lipoprotein lipase [29]. The two main CLA isomers of interest, *cis*-9, *trans*-11 C18:2 and *trans*-10, *cis*-12 C18:2, were not affected by fat source or forage, and concentrations of *cis*-9, *trans*-11 C18:2 tended towards the lower end of often reported concentrations [26]. Feeding fat in diets increased two *trans*, *trans* C18:2 isomers of CLA including *trans*-10, *trans*-12 C18:2 and *trans*-11, *trans*-13 C18:2, so that the former one increased in milk fat of cows fed yellow grease vs hydrogenated palm oil. Increasing the F:C ratio reduced *trans*-10, *trans*-12 C18:2 concentration, however concentration of *trans*-11, *trans*-13 C18:2 was not influenced by a greater amount of forage in the diet. Chilliard and Ferlay [26] reported that low fiber diets increase *cis*-11, *trans*-13, and *cis*-9, *cis*-11 CLA isomers, whereas linseed oil increases *cis*-9, *cis*-11 CLA, *trans*-11, *cis*-13 CLA, and trans-11, trans-13 CLA. A strong positive correlation also reported [30] between intakes of C18:1 c-9, linoleic acid and linolenic and a number of specific CLA isomers. Fish oils supplementation at a level were more effective in increasing milk CLA than vegetable oils. Combinations of fish oil and vegetable oils [31-34], which are rich in linoleic acid were investigated effective in increasing the CLA content of milk [35], when supplemented in the dairy cow diet at pasture with a low level of fish oil (105 g oil/day), sunflower oil (255 g oil/day), or a combination of sunflower oil/fish oil (255 g/52.5 g oil/day) provided in 3 kg concentrate mixture/day. Supplementation with sunflower oil or fish oil, alone or in combination, increased the content of C18:1 *trans* isomers (t-9 and t-11) and CLA c-9, t-11 above that in milk from pasture-fed cows. The highest value for C18:1 (t-9 and t-11) was 7.1 g/100 g total fatty acid with fish oil alone compared to 6.98 g/100 g total FA with sunflower oil/fish oil. The combination of oils resulted in the highest CLA c-9, t-11 levels, a value of 2.36 g/100 g total fatty acids, while fish oil inclusion resulted in a greater increase than sunflower oil alone. In a similar study, Abu Ghazaleh [36] reported slightly lower CLA and vaccenic acid contents when a basal grain diet (7.6 kg DM basis) supplemented with a combination of sunflower oil (300 g) and fish oil (100 g) was offered to grazing dairy cows. This author reported that supplementation with sunflower oil/fish oil significantly increased CLA c-9, t-11 from 0.83 to 1.55 g/100 g total fatty acids and vaccenic acid from 2.31 to 5.32 g/100 g total fatty acid in milk fat, compared to the control diet (grass, grain plus 400 g rumen inert animal fat). The increase in vaccenic acid and CLA in these studies is important because VA is converted to CLA in humans, thereby increasing the dietary contribution of CLA [37]. The concept of protected fats feeding was practiced in recent years that were designed to resist biohydrogenation and enhance the concentration of unsaturated fatty acids in milk. Yellow grease feeding at 2% in diet of cows increased milk fat CLA without effecting desatuarse indices [12], when compared with similar levels of hydrogenated palm oil. A higher iodine value, low proportion of saturated and higher proportion of unsaturated fatty acids of the yellow grease than these present in hydrogenated palm oil were responsible for such changes in fatty acids profile of milk.

Fatty acids uptake by the mammary gland and atherogenic index

The principles of nutrient uptake and use by the mammary gland made it possible to make changes in milk fat content through nutritional manipulations. The regulatory steps in fatty acid synthesis and, desaturation is the desaturase activity in the mammary secretory cell which converts stearic acid arising from ruminal biohydrogenation to oleic acid that is secreted in milk. Thus, enhanced activity of the Δ9-desaturase increased oleic acid at the expense of saturated fatty acids in milk. The Δ9-desaturase was the predominant source of the *cis*-9, *trans*-11 CLA isomer in milk, which has a number of benefits to human health (including anticarcinogenic properties). Trans-11 arising from biohydrogenation in the rumen is transferred to the mammary tissue and desaturated to *cis*-9, *trans*-11 CLA via the Δ9-desaturase. This has shifted attention to manipulating ruminal biohydrogenation to enhance the yield of the *trans*-11 isomer. Chilliard and Ferlay [26] reported that desaturase index as an indicator of mammary Δ9-desaturase enzyme activity was not influenced by dietary treatments. The atherogenic index of milk fat as an indicator of the propensity of the human diet to impact the accession of coronary heart disease was reduced by fat supplementation, especially by yellow grease relative to hydrogenated palm oil [14]. F:C ration did not have effect on atherogenic index of milk fat. This index is a precise indicator of cardiovascular disease, supplementation of dairy cow diets with yellow grease resulted in a healthier milk product for consumer [14]. Chilliard and Ferlay [26] reported that tallow favorably changes the nutritional quality of milk fatty acids by a sharp reduction in their antrogenic index.

Milk Protein Content

The nutritional factors that are having major influence on milk protein content are forage-to-concentrate ratio, the amount and source of dietary protein, and the amount and source of dietary fat. Fat supplements in lactating animal diets caused a decline in protein concentration and that attracted the research interest of the basic biology of milk constituent synthesis and regulation in controlling milk composition by dietary manipulation and its influences on the entire animal system from practical feeding to mammary tissue metabolism. The milk nitrogen fractions can be divided as casein, whey, and NPN. Casein comprises about 78% of the nitrogen in milk, whey N is 17% and NPN is approximately 5% [4]. Casein content of the milk is directly related for curd structure, curd firmness, and cheese yield. While manipulating the nitrogen composition of milk, it is important to distinguish between responses that affect protein content of milk and factor that affecting protein yield. It is reported that in general, the dietary changes having positive impacts on milk and protein yields cause negative effects on protein content. The object of dietary manipulations in most instances is to increase protein content while maintaining or increasing milk yields.

Influence of amount and source of protein and fat

The effects of amount and source of protein in the diet have only modest changes in the protein content of milk. Different protein sources have varying influence on milk protein content, yellow grease feeding at 2% in Holstein cows diet improved milk protein content and yield, while hydrogenated palm oil did not [14]. The milk protein content varies from 2.85 to 3.27% as protein content in the diet ranged from 15.0 to 19.5% that have had a wide variety of protein sources, including rumen-protected amino acids. A major factor is the low transfer efficiency (25 to 30%) of dietary protein to milk is accounting for the inability of diet to alter milk protein content. Involving feeding strategies for increasing milk protein content, the protein content of milk increased only about 0.02 percentage units for each 1 percentage unit increase in dietary protein. The low level of the amino acid capture by the mammary gland is a major factor for the low rates of transfer of amino acids; even adequate amino acids are delivered to mammary tissues through diet. Blood flow through the mammary gland is implicated as a key part of the overall process for the coordinated timing of nutrient delivery to the mammary gland. The mammary blood flow and amino acid use can adjust leading to enhanced milk protein production. This suggests that the mammary gland has the capacity to alter the uptake of substrates from the arterial supply in response to changes in arterial amino acid concentrations, mammary blood flow, and metabolic activity to improve milk protein production.

Fat supplements are used to increase energy density of diet and make enough energy available to dairy animal for enhancing milk production. However, feeding additional fat is often accompanied by a decline in milk protein content. Therefore, fat feeding in dairy animal diets had limited applicability, where milk pricing gave an incentive to protein content. On an average, protein content in milk declined 0.3 percentage units for each kg of supplemental fat intake, or about 0.1 to 0.3 percentage units for most typical levels of fat feeding. When fat supplementation reduced milk protein content, the casein fraction declined the most. Despite the decline in protein content of milk, total production of protein either increased or remained same upon a proper feeding of fat supplements that increased milk production. The mechanism of reduced milk protein content with a greater increase in milk yield was due to the elevated blood fatty acids supply from the fat supplement that decreased the release of somatotropin, which reduced mammary extraction of amino acids. Infusion of casein into the abomasum of lactating cows fed 4% yellow grease increased arterial amino acid concentrations but failed to prevent the milk protein depression [38]. A 7% drop in mammary blood flow was observed when cows were fed fat, thus preventing increased removal of critical amino acids as milk synthesis increased. It is proposed that fat supplements reduced milk protein concentration by reducing blood flow through the mammary gland causing reduced extraction of blood amino acids. Milk volume is known to increase by the higher fatty acids levels that inhibiting mammary de novo fat synthesis, causing a sparing of acetate for oxidation and more glucose available for lactose and milk synthesis. However, ssupplementation of diets containing added fat with rumen-protected methionine and lysine alleviated the total milk N [39] and casein-depressing effect of fat [40]. The response was observed only in the casein fraction [40]. Plasma lysine concentration did not increase, which suggested that lysine was limiting.

Feed resource	Fatty acid profile (g/100 g total fatty acids)					Reference
	C16:0	C18:0	C18:1	C18:2	C18:3	
Forages						
Barseem *Trifolium alexandrium*	18.65	10.64	21.58	14.62	45.17	Miri et al. [1]
Grass	20.80	3.29	5.74	14.0	49.2	French et al. [18]
Grass silage	24.0	2.90	6.32	15.4	46.2	French et al. [18]
Red clover (Autumn) *Trifolium pratense*	31.10	4.81	8.00	21.4	33.6	Loor et al. [19]
Red clover (Spring)	24.20	4.35	5.21	19.1	45.9	Loor et al. [19]

White clover *Trifolium repens*	3.99	0.50	0.34	3.41	4.21	Collins et al. [48]
Marine algae *Schizochytrium sp.*	18.0	0.33	0.11	0.31	0.25	Sardi et al. [49]
Marine algae *Nannochloropsis oculata*	17.9	0.70	5.60	7.40	6.70	Fredriksson et al. [50]
Oil seed and grains						
Lupin grain *Lupinus albus*	7.7	1.1	51.0	17.3	11.1	White et al. [51]
Oats grains *Avena sativa*	15.9	1.40	38.4	38.1	2.02	Givens et al. [52]
Linseed *Linum usitatissimum*	6.1	3.4	18.8	16.3	54.4	Glasser et al. [53]
Rapeseed *Brassica napus*	4.8	2.1	60.5	20.8	9.2	Glasser et al. [53]
Sunflower seed *Helianthus annuus*	5.1	4.3	21.6	66.8	0.2	Wood and Fearon [54]
Soyaseeds *Glycine max*	11.4	4.1	22.3	53.5	7.0	Wood and Fearon [54]
Camelina seed *Camelina sativa*	6.4	2.8	15.9	20.9	30.7	Budin et al. [55]
Hemp seed *Cannabis sativa L*	6.9	2.7	10.5	53.9	24.6	Gibb et al. [56]
Hia seed *Salvia hispanica*	6.5	2.9	7.2	20.3	62.0	Ayerza and Coates [57]
Essential Oil						
Cumin seed extract *Cuminum cyminum*	0.80	2.7	4.6	1.8	1.0	Miri et al. [1]
Fat supplements						
Hydrogenated palm oil	49.34	31.31	15.39	1.03	0.52	Kargar et al. [14]
	62.50	25.67	9.82	0.07		Castro et al. [46]
Yellow grease	11.55	4.05	25.68	51.59	6.11	Kargar et al. [14]
Sunflower oil	5.96	3.98	28.82	60.53	0.07	Castro et al. [46]
Fish oil			20.4	23.4	1.27	Baucells et al. [58]

Table 4: Fatty acid profile of different feed resources used in dairy animal feeding.

Conclusions and Recommendations

Nutritional manipulations of dairy animals enabled in enhancing milk production, alterations in milk fat and protein, and fatty acid profile (Table 4). Milk production can be improved upto 50% levels by altering the feed composition with addition of rumen protected supplements. Grain feeding is associated for milk fat depression, while mixed diets promoted milk production and milk protein content. Milk protein content can be increased by 0.4% unit by altering forage to concentrate ratio. Milk fat is more responsive to dietary alterations than protein and that the fat can be changed over 3% percent unit while protein content less than 1% unit. The each 1 percentage unit increase in dietary protein about 0.02 percent unit protein content of milk increases. Supplementation of protected fat above the basal diet at 0.7 or 1.4% increased fat and protein content of milk. Nutraceutical properties of milk were enhanced through increased content of t11-C18:1 (*trans* vaccenic acid), and c9, t11-C18:2, and their geometric and positional isomer of conjugated linoleic acid in milk by alterations in diet constituents and composition. Although, nutrition manipulations increased milk production, improved milk CLA content, however, efficient feed sources in respect of milk fat depression associated with dietary levels of CLA, rumen protected fatty acids and protein sources require intensive nutrition research. The biology of milk synthesis relative to circulating nutrient level and timing of delivery to mammary require great research interest.

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