

## Comparative mammalian choline metabolism with emphasis on the high-yielding dairy cow

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The present review examines the importance of choline in dairy cow nutrition. Choline is an essential nutrient for mammals when excess methionine and folate are not available in the diet. The requirement for choline can be met by dietary choline and by transmethylation reactions. Two types of functions for choline are known: functions of choline *per se*; functions as a methyl donor. The two principal methyl donors in animal metabolism are betaine, a metabolite of choline, and S-adenosyl-methionine, a metabolite of methionine. Choline and methionine are interchangeable with regard to their methyl group-furnishing functions. In adult ruminants, choline is extensively degraded in the rumen; for this reason dietary choline contributes insignificantly to the choline body pool and methyl group metabolism is generally conservative with a relatively low rate of methyl catabolism and an elevated rate of *de novo* synthesis of methyl groups via the tetrahydrofolate system. In dairy ruminants, the dietary availability of choline is still low, but the output of methylated compounds in milk is high, and precursors from the tetrahydrofolate pathway are limiting, especially at the onset of lactation. Therefore choline may be a limiting nutrient for milk production in high-yielding dairy cows.

**Choline: Methyl group: Methionine: Dairy cows**

### Introduction

Choline, the beta-hydroxyethyltrimethylammonium ion, is a strong base containing a trimethylated quaternary nitrogen. Choline occurs widely in biological materials as the compound itself, as acetylcholine and as various phospholipids (Kuksis & Mookerjee, 1978). Choline has been classified as one of the B-complex vitamins but it does not satisfy the standard definition of a vitamin (McDowell, 1989): it is synthesised endogenously and there is no evidence that it is an enzyme cofactor; furthermore, unlike other water-soluble vitamins, it is difficult to identify a

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**Abbreviations:** 2AMP, 2-amino-2-methyl-1-propanol; HCy, homocysteine; Met, methionine; NEFA, non-esterified fatty acids; PtdCho, phosphatidylcholine; RPC, rumen-protected choline; SAM, S-adenosyl-L-methionine; THF, tetrahydrofolate.

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deficiency syndrome for choline in healthy mammals because of its interrelation with methionine (Met), folic acid, and vitamin B<sub>12</sub> (Zeisel, 1988; Scott, 1999). However choline is a vital component of tissues and is required in the diet of non-ruminant species at much higher levels than the water-soluble vitamins (g v. mg) (Whitehead & Portsmouth, 1989). The presence of an endogenous synthetic pathway does not render choline dispensable and deficiency results in several dysfunctions when other nutrients are limiting (Zeisel *et al.* 1991).

*De novo* synthesis of choline occurs by the sequential methylation of phosphatidylethanolamine, the methyl groups being supplied by S-adenosyl-L-methionine (SAM) (Zeisel, 1988, 1992; Mato *et al.* 1994). Methyl groups may be derived from exogenous sources such as Met, betaine and also choline, but can arise *de novo* in the body from the tetrahydrofolate (THF) system (Zeisel, 1988, 1992); vitamin B<sub>12</sub> is involved in this process (Kennedy *et al.* 1992, 1995; Scott, 1999). Thus, dietary choline, Met and betaine, as well as folic acid and vitamin B<sub>12</sub>, all contribute to body requirements for choline. For this reason it has been suggested that choline may be an essential nutrient for mammals when excess Met and folic acid are not available in the diet (Zeisel *et al.* 1991; Zeisel, 2000).

The intention of the present paper is to review the nutrition and metabolism of choline, particularly its interactions with Met, and to appraise the importance of choline in dairy cow nutrition.

### Choline sources and bioavailability

Free choline, acetylcholine and choline-containing phospholipids are widely distributed in plant and animal tissues, and feedstuffs derived from them (Kuksis & Mookerjee, 1978; Zeisel, 1988, 1992). In feed ingredients and crude unprocessed fat sources (Table 1) most choline is present as phosphatidylcholine (PtdCho) (lecithin).

From the point of view of animal nutrition, relatively rich sources of choline are soyabean, soyabean meal, rapeseed meal, fishmeal and dried yeast (Whitehead & Portsmouth, 1989; Baker, 1995). However the dietary bioavailability of choline is considered 'moderate' (Whitehead & Portsmouth, 1989; Baker, 1995). Nutrient bioavailability to livestock depends mainly on two factors: (1) stability in premixes, diets and supplements; (2) utilisation efficiency (Baker, 1995). Choline is quite stable in mineral-vitamin premixes and in feeds generally, but

**Table 1.** Choline content of selected feedstuffs (mg/kg, DM)  
(Adapted from Whitehead & Portsmouth, 1989)

Feedstuff	Choline (mg/kg)
Barley	900
Cottonseed meal	2600
Fishmeal	3500
Herring-meal	3500
Lucerne	250
Maize	435
Maize gluten feed (20 % crude protein)	250
Meat and bone meal	1600
Millet	600
Oats	800
Peas	650
Rapeseed meal	6500
Soyabean meal	2500
Sunflower-seed meal	2600
Wheat	900

its bioavailability is variable. Bioavailability from soyabean meal, held to be an excellent choline source for non-ruminants (Baker, 1995), is, at 60–75 %, considerably higher than from most other feeds, and is as low as 24 % from rapeseed meal (Baker, 1995; Schutte, 1999). Choline chloride (87 % choline) is an alternative to feed sources but is hygroscopic and is therefore considered a stress agent for other nutrients present in vitamin–mineral premixes.

### Functions of choline

Two types of choline functions are known: as choline *per se*, for which the choline moiety is required; as a methyl donor.

#### *Choline per se*

Choline is an essential constituent of all cell membranes, where it is present mainly as the phospholipids PtdCho, lysophosphatidylcholine, and sphingomyelin. PtdCho is one of the most abundant phospholipids in higher plants and animals, and is the predominant phospholipid (>50 %) in most mammalian cell membranes (Kuksis & Mookerjee, 1978; Ruiz *et al.* 1983; Zeisel, 1988, 1992).

Choline is essential for the synthesis of the neurotransmitter acetylcholine (Kuksis & Mookerjee, 1978; Zeisel, 1988).

Choline also prevents the abnormal accumulation of fat in the liver (fatty liver) by promoting the removal of triacylglycerols from hepatocytes via their incorporation into lipoproteins, of which PtdCho is a component (Kuksis & Mookerjee, 1978; Zeisel, 1988, 1992). Choline is required to prevent haemorrhagic kidney lesions in rats (Kuksis & Mookerjee, 1978) and, together with other nutrients particularly Mn salts, is required to prevent perosis, a bone disease of poultry (Ruiz *et al.* 1983).

#### *Choline and lipid metabolism*

Choline plays a major role in metabolism, particularly in lipid transport. It is a lipotropic agent because of its ability to prevent or correct excess fat deposition in the liver generally arising as a result of its dietary deficiency (Kuksis & Mookerjee, 1978; Zeisel, 1988). Impaired triacylglycerol secretion to VLDL is considered a major cause of fatty liver in dietary choline deficiency (Zeisel, 1988).

The composition and metabolism of lipoproteins has been described in detail elsewhere (Eisenberg & Levy, 1975; Pullen *et al.* 1990; Fast & Vance, 1995). Two main types of lipoproteins are involved in plasma triacylglycerol transport, chylomicrons and VLDL (Eisenberg & Levy, 1975). In non-ruminants, VLDL are mainly synthesised and secreted by the liver, whereas chylomicrons and small quantities of VLDL originate in the intestine (Bell, 1981; Noble, 1981; Moore & Christie, 1984). PtdCho is an essential component of VLDL and cannot be substituted by other phospholipids (Moore & Christie, 1981; Fast & Vance, 1995). When dietary or synthetic choline availability is restricted, the rate of choline-containing phospholipid synthesis decreases. In the liver the result is that the rate of packaging of triacylglycerols into VLDL falls and their export from the liver declines (Yao & Vance, 1990; Fast & Vance, 1995; Van den Top *et al.* 1995; Gruffat *et al.* 1996). Fat therefore accumulates in hepatocytes.

Although in cattle the concentration of VLDL in plasma is extremely low, they appear to be essential as a primary source of fats for extrahepatic tissues, particularly during lactation (Bell, 1981; Gruffat *et al.* 1996). The long-chain fatty acids in milk are obtained from the blood triacylglycerols of VLDL, which arise either from absorbed fat or endogenously via mobilisation of adipose fat stores (Palmquist & Mattos, 1978; Grum *et al.* 1996). The requirement to increase the lipolysis of adipose fat stores is most critical during the first stage of lactation (McCarthy *et al.* 1968; Marcos *et al.* 1990; Breukink & Wensing, 1998). During this stage, fat mobilisation leads to increased blood levels of non-esterified fatty acids (NEFA), which are taken up by the liver and oxidised to ketone bodies or carbon dioxide or esterified to triacylglycerols. The liver normally packages the triacylglycerols in VLDL and secretes them, but sudden increases in plasma NEFA may not be adequately processed by the ruminant liver (Pullen *et al.* 1990; Gruffat *et al.* 1996).

Inability to rapidly increase VLDL production may be due to deficient synthesis of the essential components, cholesterol, phospholipids (particularly PtdCho) or apolipoproteins (Yao & Vance, 1990; Fast & Vance, 1995; Van den Top *et al.* 1995; Gruffat *et al.* 1996). Bovine VLDL phospholipids are mainly PtdCho, with smaller proportions of sphingomyelin and phosphatidylethanolamine (Moore & Christie, 1981). Thus, when massive mobilisation of fatty acids is associated with lipotropic factor deficiency (i.e. choline and Met) triacylglycerols accumulate in the liver and may lead to the development of fatty liver (Gruffat *et al.* 1996; Breukink & Wensing, 1998).

Choline-containing phospholipids (mainly PtdCho) of dietary and biliary origin also play an important role in the intestinal absorption of dietary fat and fat-soluble nutrients. Choline deprivation in rats leads to reduced PtdCho levels in bile, reduced total bile acid secretion, and reduced cholesterol secretion (Kuksis & Mookerjee, 1978). It is proposed that the PtdCho for bile salt micelles arises mainly from *de novo* synthesis, and since PtdCho synthesis is impaired in choline deficiency, the result is decreased secretion of bile salts and reduced lipid digestibility (Kuksis & Mookerjee, 1978). In this context, Moore & Christie (1981) have pointed out that in ruminants bile secretion accounts for a major fraction of choline use, consistent with the fact that PtdCho represents about 80 % of total lipid of ruminant bile, with lysophosphatidylcholine contributing a further 6.3 %. Phosphatidylethanolamine forms about 2.7 % of bile lipid (Moore & Christie, 1981; Noble, 1981). Moore & Christie (1981) reported that the rate of bile secretion is 1.45 ml/h per kg body weight in cattle.

However, choline-containing compounds are involved in another aspect of fat absorption: the stimulation of lipid processing within enterocytes (Koo & Noh, 2001). In fact, choline-containing phospholipids are necessary for the synthesis and release of chylomicrons and VLDL by intestinal villus cells (for references, see Kuksis & Mookerjee, 1978). In ruminants fatty acids absorbed by enterocytes are preferentially incorporated into VLDL triacylglycerols and as a result the phospholipid:triacylglycerol ratio of lymph lipids is usually higher in ruminants than non-ruminants (Moore & Christie, 1981, 1984; Noble, 1981). Unfortunately, the possible effects of choline deficiency and choline supplementation on lipid absorption in dairy cows remain largely unexplored.

#### *Choline in milk*

Choline is actively secreted into mammalian milk (Noble, 1981; Zeisel *et al.* 1986; Kaufmann & Hagemester, 1987; Rohlf *et al.* 1993). The major choline-containing compounds in human, bovine, and rat milk are non-esterified choline, PtdCho, and sphingomyelin (Rohlf *et al.* 1993).

Levels of choline secretion into milk are generally maintained even at the cost of depleting liver reserves of choline and its metabolites. Thus, compared with lactating rats fed a choline-adequate diet, lactating rats on a choline-deficient diet had 88 % lower hepatic phosphorylcholine. Furthermore the lactating choline-deficient rats had hepatic triacylglycerol levels seven times higher than non-mated females on a choline-sufficient diet, whereas levels were only four times higher in lactating rats on a choline-sufficient diet (Zeisel *et al.* 1995).

Kinsella (1973) reported that a bovine mammary gland yielding 25 litres milk secretes  $10 \pm 3$  g phospholipids/d, corresponding on average to 5 % of the phospholipids of the mammary tissue. The phospholipids of the membrane of the milk fat globule constitute the major choline-containing component of bovine milk (McPherson & Kitchen, 1983). However it is difficult to quantify precisely the total choline in bovine milk. If 60 % of milk phospholipids are assumed to contain choline, and phospholipids are 0.5–1 % of total milk lipids (Bitman & Wood, 1990; McPherson & Kitchen, 1983), then the concentration of choline-containing phospholipids is in the range 105–210 mg/l, in addition to the free choline (Kaufmann & Hagemester, 1987; Rohlf's *et al.* 1993). However this figure is likely to vary greatly with breed, fat content, fat globule size and lactation stage, and also with the methods and assumptions used in the estimation. In their study on milk phospholipids, Bitman & Wood (1990) showed that sphingomyelin plus PtdCho concentrations were 208 mg/l on the 7th day of lactation, decreasing to 138 mg/l at 42 d and 84 mg/l at 180 d.

Deuchler *et al.* (1998) measured choline in cows' milk in mid-lactation; they found that in animals receiving rumen-protected choline (RPC) or choline infusion, the choline content of milk was higher (91 and 116 mg/l, respectively) than in controls (72–86 mg/l) indicating that milk choline is sensitive to post-ruminal choline supply and bioavailability.

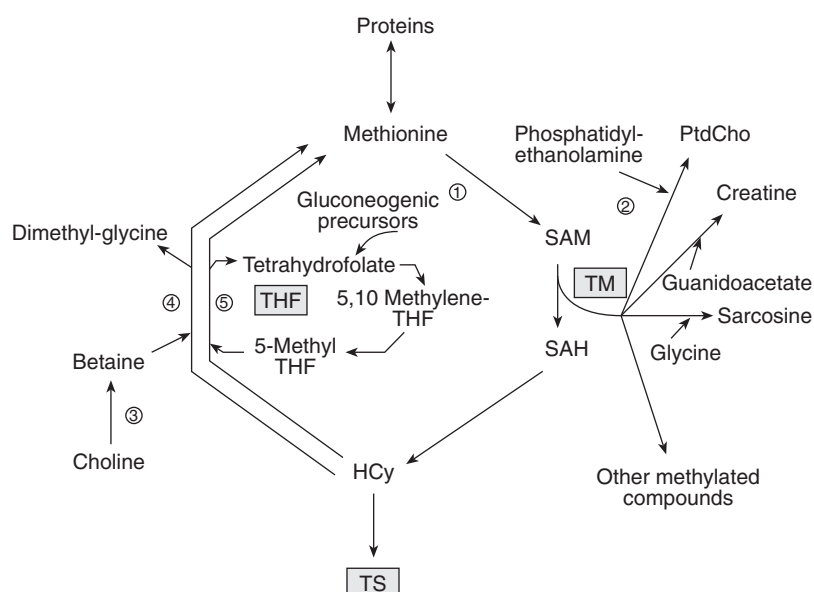
The above data suggest that choline is a limiting metabolite in lactating mammary tissue; it is used avidly when available, as the associated enzymes are highly active (Kinsella, 1973). The bovine mammary gland is therefore a target tissue for choline both for secretion in milk and for the maintenance of tissue integrity.

#### *Choline as methyl donor*

Choline is important as a source of labile methyl groups for the biosynthesis of other methylated compounds (Ruiz *et al.* 1983; Stipanuk, 1986; Xue & Snoswell, 1986*a,b*; Zeisel, 1992; Mato *et al.* 1994). The demand for choline as methyl donor is probably the main factor determining how rapidly choline deficiency induces a disease state (Zeisel *et al.* 1991).

The two principal methyl donors in animal metabolism are betaine, a choline metabolite, and SAM, a metabolite of Met. In these compounds the methyl groups are labile partly due to their attachment to hetero-atoms (nitrogen in betaine, sulfur in SAM), which can easily increase their covalent valency to become positively charged (Ruiz *et al.* 1983). Because they both contain labile methyl groups, choline and Met are closely interrelated metabolically (Stipanuk, 1986; Xue & Snoswell, 1986*a,b*; Zeisel, 1992; Mato *et al.* 1994), as shown in Fig.1.

SAM is a high-energy sulfonium compound, formed from ATP and Met in the first reaction of Met metabolism (Mato *et al.* 1994). SAM is both the methyl donor for transmethylation reactions and the precursor of decarboxylated SAM (S-adenosyl (5'-3-methylthiopropylamine), which is the aminopropyl donor for the synthesis of polyamines (Stipanuk, 1986; Mato *et al.* 1994). Under physiological conditions, the SAM methyl group is mainly used to form creatine (Stipanuk, 1986), but can also be channelled to PtdCho, sarcosine, carnitine, and other methylated compounds. In sheep not more than 55 % of the methyl groups of SAM are used for



**Fig. 1.** Metabolic pathways of methyl groups. SAM, S-adenosyl-L-methionine; SAH, Adenosylhomocysteine; HCys, Homocysteine; PtdCho, Phosphatidylcholine; 5-Methyl-THF, 5-Methyl-tetrahydrofolate; 5,10 Methylene-THF, 5,10-Methylene-tetrahydrofolate; TM, Transmethylation pathway; TS, Transsulfuration pathway; THF Tetrahydrofolate system; ① S-adenosyl-L-methionine synthetase, also called methionine-adenosyltransferase (EC 2.5.1.6); ② Phosphatidylethanolamine methyltransferase (EC 2.1.1.17); ③ Choline oxidase (EC 1.1.3.17); ④ Betaine-homocysteine methyltransferase (EC 2.1.1.5); ⑤ Methionine synthase (EC 2.1.1.13) (Adapted from Xue & Snoswell, 1986a; Mato *et al.* 1994; Armentano, 1994).

creatine synthesis, which is considerably less than in man (Xue & Snoswell, 1986b). By contrast, the proportion of SAM methyl groups used for choline synthesis is probably considerably greater in sheep than in man, as dietary choline is largely unavailable due to degradation by rumen micro-organisms (Henderson *et al.* 1983; Xue & Snoswell, 1986a,b). SAM methyl groups are transferred to phosphatidylethanolamine transforming it to PtdCho (Fig. 1). This methylation pathway, catalysed by the enzyme phosphatidylethanolamine-N-methyltransferase, makes new choline molecules by sequentially methylating phosphatidylethanolamine, using SAM as methyl donor (Zeisel, 1988).

When SAM transfers its methyl group to a methyl acceptor, the product is S-adenosyl-homocysteine, which is then hydrolysed to adenosine and homocysteine (HCy) (Stipanuk, 1986; Mato *et al.* 1994). *In vivo*, adenosine is rapidly transformed into inosine, while HCy is rapidly converted to cystathionine and then to cystathionine derivatives such as cysteine, glutathione, taurine, inorganic sulfate, etc., via the transsulfuration pathway (Stipanuk, 1986; Mato *et al.* 1994; Lobley *et al.* 1996). HCy can also be remethylated to Met, a reaction catalysed by Met synthase (EC 2.1.1.13) or by betaine-HCy methyltransferase (EC 2.1.1.5) (Stipanuk, 1986; Mato *et al.* 1994).

Met synthase is responsible for the *de novo* synthesis of Met methyl groups from one-carbon units furnished by THF (Fig. 1). The enzyme utilises methyl-THF as methyl donor and methylcobalamin as tightly bound coenzyme. *De novo* synthesis of methyl groups via this system appears to be minimal when labile methyl group intake is sufficient or excessive (Stipanuk, 1986). However in ruminants only small quantities of methyl group nutrients are available from the diet and Met synthase assumes a much more important role (Neill *et al.* 1978, 1979; Dawson *et al.* 1981; Kennedy *et al.* 1995).



In dairy ruminants producing large quantities of milk, Met is the first limiting amino acid. This means that the elevated requirement for Met for transmethylation reactions and milk protein synthesis may lead to altered methyl group metabolism (Lobley, 1991; LaCount *et al.* 1995, 1996; Lobley *et al.* 1996). The effects of lactation on methyl group metabolism have been examined in sheep (Xue & Snoswell, 1985); it was found that the activities of hepatic phospholipid methyltransferase (for PtdCho synthesis) and Met synthase were significantly higher (+33 and +34 %, respectively) in lactating than non-lactating ewes (Xue & Snoswell, 1985). Furthermore, the calculated Met intake was 2–4 times lower than the estimated minimum daily requirement for SAM-mediated transmethylation (18 mmol/d for non-lactating ewes), therefore the methyl groups used as donors for these syntheses must have been derived from THF system (Xue & Snoswell, 1986b). We conclude that the extra demand for methyl groups during lactation, for the secretion of choline, choline-containing compounds, creatine, carnitine, etc., into milk (LaCount *et al.* 1995, 1996), is met by an enhanced rate of *de novo* methyl group synthesis via the THF system (Xue & Snoswell, 1985; Armentano, 1994).

The general situation in ruminants, in fact, is one of conservative methyl group metabolism. This is an elevated rate of *de novo* methyl group synthesis from the one-carbon pool, flanked by a low rate of methyl catabolism (due to low activity of choline oxidase). This situation has been demonstrated in adult and in particular lactating sheep (Henderson *et al.* 1983; Robinson *et al.* 1984; Xue & Snoswell, 1986a,b; Snoswell & Xue, 1987) (Table 2). This state is probably an adaptation to the low availability of dietary choline in adult ruminants (Xue & Snoswell, 1986a).

Unlike Met synthase (EC 2.1.1.5; see Fig. 1), which is widely distributed in mammalian tissues, betaine-HCy methyltransferase (EC 2.1.1.13; see Fig. 1) is generally present in high concentrations in the liver only. The enzyme catalyses the remethylation of HCy to Met, when betaine is available as methyl donor (Stipanuk, 1986; Lobley *et al.* 1996). Significantly, hepatic betaine-HCy methyltransferase activity is increased in both Met-deficient rats, and also those fed high levels of Met, choline or betaine (for references, see Stipanuk, 1986).

In the pruruminant lamb the activities of liver choline-oxidase and betaine-HCy methyltransferase increase markedly after birth but subsequently decrease as the animals reach the

**Table 2.** Three physiological stages of methyl group metabolism in ruminants

Physiological stage	Availability	Enzyme activity
Pruruminant*	↑ Choline ↑ Betaine	↑ Choline oxidase ↑ Betaine-homocysteine-methyltransferase
Adult Ruminant	↓ Choline ↓ Betaine ↑ Methyl group synthesis via THF system	↑ Methionine synthetase
Lactating Ruminant	↓ Choline ↓ Betaine ↓ Methionine, folic acid, vitamin B <sub>12</sub> † ↓ Precursors of gluconeogenesis‡ ↑ Methyl group synthesis via THF system	↑ Methionine synthetase

↑, High; ↓, Low; THF, tetrahydrofolate.

\* The *de novo* synthesis of methyl groups via the THF system appears to be minimal in ruminants when labile methyl intake is sufficient or excessive, for example in the pruruminant lamb (Xue & Snoswell, 1986a; Snoswell & Xue, 1987).

† Methionine, folic acid and vitamin B<sub>12</sub>, which all contribute to meeting the requirement for choline, are often limiting nutrients in high-yielding dairy cows (McDowell, 1989; Girard, 1998; Girard & Matte, 1999; Petitclerc *et al.* 2000).

‡ It is also important to note that the precursors of gluconeogenesis, the primary sources of one-carbon units for methylneogenesis via the THF system, are often deficient in lactating ruminants (Armentano, 1994; Rukkwamsuk *et al.* 1999).

ruminant state (Xue & Snoswell, 1986a), a further indication of conservative methyl metabolism in the ruminant state; by contrast in rats the activities of these enzymes decrease slightly with age (Xue & Snoswell, 1986a). The increasing activities of liver choline oxidase and betaine-HCy methyltransferase in preruminant lambs are probably related to the abundance of choline-containing compounds in the milk (Xue & Snoswell, 1986a). Thus, the availability of choline and its metabolite betaine appear to influence the conversion or recycling of the HCy moiety of Met, through betaine-HCy methyltransferase activity (Stipanuk, 1986).

Emmanuel & Kennelly (1984) studied the interchangeability of choline and Met in lactating goats by infusing radiolabelled choline and Met. They estimated that 6 % of the choline pool (2.82  $\mu\text{mol/h}$  per kg body weight) was derived from Met. Since there is an endogenous pathway for the *de novo* biosynthesis of the choline moiety via the sequential methylation of phosphatidylethanolamine, they concluded that approximately 28 % of Met (8.46  $\mu\text{mol/h}$  per kg body weight) is used for choline synthesis. Of the total Met radioactivity in milk, 6 % was in deproteinised whey (of which lactose was the major labelled component) and 94 % was in protein, while the activity in the fat fraction was negligible. Following labelled choline infusion this was not recovered in the milk Met pool, but the contribution of choline to milk fat was ten times greater than that of Met. We can conclude therefore that, while Met may stimulate milk and milk fat production by enhancing lipoprotein synthesis, gluconeogenesis, and providing methyl groups for phospholipid synthesis in the liver, choline is required for the *de novo* synthesis of phospholipids in the mammary gland and to spare Met as a methyl donor (Kinsella, 1973; Emmanuel & Kennelly, 1984; Huber *et al.* 1984).

Lobley *et al.* (1996) used radiolabelled Met to investigate the importance of transmethylation reactions in sheep infused with choline and creatine. They found that the irreversible loss rate of Met decreased significantly in the presence of these methylated compounds and concluded that the irreversible loss of methyl groups as Met normally results in insufficient methyl groups to meet the demands of the sheep, and also that Met cycling is sensitive to the metabolic supply of methyl groups. Thus, it would seem that Met can replace choline and choline can 'save' Met from catabolism.

### Are choline and betaine interchangeable?

The first step in choline catabolism is oxidation to betaine (Ruiz *et al.* 1983; Zeisel, 1988). This is a two-step process in which choline is first oxidised to betaine aldehyde by choline-oxidase, which is further oxidised to betaine by betaine aldehyde dehydrogenase. It is betaine that is the methyl donor, not choline as such (Ruiz *et al.* 1983). For this reason it has been suggested that betaine might substitute for choline and *vice versa*. However, Lowry *et al.* (1987) showed in poultry that 75 % of the dietary choline requirement must be supplied as choline and only 25 % can be met by betaine. The authors suggested that the requirement for choline *per se* must be met as choline, and that betaine can substitute only the methyl donor function of choline, probably because betaine cannot be reduced to choline (Zeisel, 1988).

### The fate of choline in the rumen

As noted above, choline is rapidly and extensively degraded in the rumen of both cattle and sheep, both in its non-esterified form and as phospholipid (Neill *et al.* 1978, 1979; Dawson *et al.* 1981). Only a minimal quantity reaches the lower digestive tract and can be absorbed (Neill



*et al.* 1979). The choline methyl groups are metabolised to trimethylamine by rumen micro-organisms. Trimethylamine can accumulate in the rumen or be converted to methane, which is lost. The methanogenesis pathway is easily saturated by excess of substrates (trimethylamine, methylamine and Met) (Neill *et al.* 1978, 1979; Dawson *et al.* 1981). Quantitative studies in sheep showed that 76 % of [ $^{14}\text{C}$ ]choline injected into the rumen was expired as methane over 6 h, whereas approximately 15 % accumulated as trimethylamine. Under such conditions, less than 10 % of choline escapes degradation by incorporation, as PtdCho, into the structural membranes of ciliate protozoa (Neill *et al.* 1979). Nevertheless, the concentration of PtdCho in ruminal digesta was higher than in abomasal digesta, suggesting that protozoa are selectively retained in the rumen (Neill *et al.* 1979).

In sheep with a defaunated rumen, the concentration of PtdCho in the abomasal digesta was higher than in the rumen (Dawson *et al.* 1981), leading to the suggestion that some of this abomasal PtdCho was derived from non-dietary sources (regurgitation of bile from the lower digestive tract) (Robinson *et al.* 1984). In fact, intravenous injection of labelled choline in sheep indicated that the small amount of PtdCho present in abomasal digesta is largely (69 %) of non-dietary or ruminal origin (Dawson *et al.* 1981).

### Choline in dairy cow nutrition

The earliest investigations determined the effects of added dietary choline on milk fat synthesis. Erdman *et al.* (1984) reported that dietary supplementation with unprotected choline chloride had positive effects on the 4 % fat-corrected milk yield and milk fat percentage (Table 3). It was therefore suggested that choline might play a lipotropic role in preventing low fat syndrome (Erdman *et al.* 1984). Choline did not appear to have any effect on rumen pH or on the acetate:propionate molar ratio, so it was concluded that the increase in milk fat was not related to changes in rumen fermentation (Erdman *et al.* 1984; Atkins *et al.* 1988) and may have been due to improved lipid transport from adipose tissue via liver to mammary gland (Erdman *et al.* 1984). In later supplementation experiments using unprotected choline, neither milk production nor milk composition were affected (Table 3) due, it was suggested, to rapid choline degradation in the rumen (Atkins *et al.* 1988; Sharma & Erdman, 1988*b*). In fact the work of Atkins *et al.* (1988) indicated that choline chloride was more degradable than naturally occurring choline in feed, while increasing choline chloride intake from 23 to 326 g/d only raised duodenal choline flow from 1.2 to 2.5 g/d, so that recovery was low indeed (Sharma & Erdman, 1988*b*) (Table 3).

It was also reported that high amounts of choline (over 280 g/d) significantly reduced (from 18.4 to 16.7 kg/d) the DM intake (Sharma & Erdman, 1988*b*). Thus, work subsequent to the initial study clearly indicates that unprotected choline chloride supplementation is of little value in dairy cows.

However, others studies indicate that the administration of RPC or its post-rumen infusion, can influence both the yield and composition of milk (Tables 4 and 5) (Sharma & Erdman, 1988*a*; Erdman & Sharma, 1991; Erdman, 1994; DiCostanzo & Spain, 1995; Bonomi *et al.* 1996; Deuchler *et al.* 1998; Hartwell *et al.* 2000). For example, Sharma & Erdman (1988*a*) investigated abomasal infusion of choline or Met in dairy cows, either alone or with the choline synthesis inhibitor 2-amino-2-methyl-1-propanol (2AMP) (Table 4). They found that abomasal infusion of 30 g choline/d was more effective than abomasal infusion of 45.6 g Met/d (the molar equivalent of 30 g choline) in increasing milk yield and milk fat content. Additionally, milk production, milk fat percentage, milk fat yield, milk protein percentage, and milk protein

**Table 3.** Effects of unprotected choline supplementation on milk production, milk composition, and other parameters in dairy cows

Stage of lactation	Total choline supplemented (g/d)	Choline effects	Reference
Weeks 10–22	41.5	– Milk yield ↓ Plasma NEFA	Erdman <i>et al.</i> (1984)
Weeks 22–25	48.7	– Milk yield ↑ Milk fat content, milk fat yield ↑ 4 % fat-corrected milk	Erdman <i>et al.</i> (1984)
Weeks 22–25	73.2	– Milk yield ↑ Milk fat content, milk fat yield ↑ 4 % fat-corrected milk	Erdman <i>et al.</i> (1984)
Weeks 4–13	73.4	– Milk yield ↑ Rumen acetate	Atkins <i>et al.</i> (1988)
Mid-lactation	282	– Milk yield ↓ DM intake	Sharma & Erdman (1988b)
Late lactation	325	↑ Flow of choline in the duodenum ↓ Rumen pH ↓ Rumen propionate ↑ Rumen acetate:propionate ratio ↑ Rumen NH <sub>3</sub>	Sharma & Erdman (1988b)
Weeks 4–24	10	↑ Milk yield ↑ Milk fat content, milk fat yield ↑ Milk protein content, milk protein yield ↑ Plasma NEFA, methionine, and glucose ↓ Plasma glutamate-oxalacetate transaminase	Bonomi <i>et al.</i> (1996)

↑, Increase relative to control (no choline supplementation); ↓, decrease relative to control (no choline supplementation); –, no effect; NEFA, non-esterified fatty acids.

yield of cows infused with 2AMP plus Met were all lower than in cows infused with 2AMP plus choline (Sharma & Erdman, 1988a). These data suggest not only that supplemental choline is required to achieve maximal performance, but also that choline formed from Met (Emmanuel & Kennelly, 1984) may be partially responsible for these stimulatory effects on milk production (Sharma & Erdman, 1988a).

A subsequent study reported that RPC had positive dose-related effects on milk production that also depended on the protein level of the diet (Erdman & Sharma, 1991) (see Table 5). Increasing choline supplementation linearly increased milk yield, even though it did not consistently affect milk composition (Erdman & Sharma, 1991). When choline supplementation and dietary crude protein interactions were considered, a tendency for higher responses in milk production to choline supplementation with lower dietary crude protein was observed; indeed, the maximum responses were +2.1 and +3.1 kg/d in the 16.5 and 13.0 % crude protein diets, associated with 40 and 56 g of RPC intake respectively (Erdman & Sharma, 1991). In contrast DiCostanzo & Spain (1995) did not find any significant effects on milk production in the transition from late pregnancy to early lactation (Table 5). Bonomi *et al.* (1996), using RPC at three doses (2, 6 or 10 g choline chloride/d) and unprotected choline (10 g/d), found improvements in milk yield (6.75, 8.8, 10 and 5 %, respectively), milk fat (4, 4.3, 6 and 3.1 %, respectively) and milk protein content (4.7, 5.6, 7.5 and 3.5 %, respectively). These treatments also increased plasma choline, glucose, Met, threonine and isoleucine, while decreasing acetate, NEFA, phenylalanine, and glycine. Furthermore, both protected and unprotected choline reduced glutamate-oxalacetate transaminase and  $\gamma$ -glutamate transaminase activities (Bonomi *et al.* 1996).

**Table 4.** Effects of abomasal infusion of choline, methionine (Met), or both on milk production in dairy cows

Stage of lactation	Total infusion of choline	Choline effects	Methionine infusion (g)	References
Weeks 12–17	30 g/d	Choline v. Met* – Milk yield ↑ Milk fat content, milk fat yield ↑ Milk protein content	45·6 (control)	Sharma & Erdman (1988a)
Weeks 12–17	30 g/d + 2AMP	Choline v. Met* ↑ Milk yield ↑ 4 % fat-corrected milk ↑ Milk fat content, milk fat yield ↑ Milk protein content, milk protein yield	45·6	Sharma & Erdman (1988a)
Mid-lactation	60 g/d	– Milk yield† ↑ Choline secretion in milk	0	Deuchler <i>et al.</i> (1998)
Mid-lactation	25, 50 or 75 g/d	– Milk yield† ↑ Choline secretion in milk	0	Deuchler <i>et al.</i> (1998)

↑, Increase relative to control (no supplemented); ↓, decrease relative to control (no supplemented); –, no effect; 2AMP, 2-amino-2-methyl-1-propanol (inhibitor of choline synthesis).

\*Choline v. Met, choline effect compared with Met effect.

† The study was not designed to measure milk production effect or change (Deuchler *et al.* 1998).

**Table 5.** Effects of rumen-protected choline supplementation on milk production, milk composition, and other parameters in dairy cows

Stage of lactation	Total choline supplemented	Choline effects	Reference
Mid-lactation	From 18.5 to 56.9 g/d and 13.0 % crude protein	↑ Milk yield (linear response)	Erdman & Sharma (1991)
Mid-lactation	From 19.6 to 57.9 g/d and 16.5 % crude protein	↑ Milk yield ↑ Milk protein content	Erdman & Sharma (1991)
Early lactation	33 g/d	↑ Milk yield ↑ Milk fat content ↑ 4 % fat-corrected milk	Erdman (1994)
From 20 d prepartum to 100 DIM Weeks 4–24	From 5 to 45 g/d	No effects	DiCossanzo & Spain (1995)
		↑ Milk yield	
		↑ Milk fat content, milk fat yield	
	10 g/d	↑ Milk protein content, milk protein yield	Bonomi <i>et al.</i> (1996)
		↑ Plasma methionine and glucose	
		↓ Plasma NEFA	
		↓ Plasma GOT, $\gamma$ -GT activities	
Mid-lactation	50 g/d	– Milk yield* ↑ Choline secretion in milk ↑ Higher choline availability	Deuchler <i>et al.</i> (1998)
From 28 d prepartum to 120 DIM	12 g/d	↑ Milk yield	Hartwell <i>et al.</i> (2000)
From 21 d prepartum to 63 DIM	4.0 % RUP during prepartum 45, 60 or 75 g/d	↑ Body weight loss after calving ↑ Liver glycogen content (linear response) ↑ Liver fatty acid metabolism	Piepenbrink & Overton (2000)
From 14 d prepartum to 30 DIM	20 g/d	↑ Milk yield ↑ 3.5 % fat-corrected milk ↓ NEFA on parturition ↓ NEFA:cholesterol ratio on parturition ↑ $\alpha$ -Tocopherol in plasma	Pinotti <i>et al.</i> (2000) Pinotti <i>et al.</i> (2000) Pinotti <i>et al.</i> (2002)

↑, Increase relative to control (no supplemented); ↓, decrease relative to control (no supplemented); –, no effect; DIM, days in milk; NEFA, non-esterified fatty acids; GOT, glutamate-oxalacetate transaminase;  $\gamma$ -GT,  $\gamma$ -glutamate transaminase; RUP, percentage of rumen undegradable protein in diet.

\* The study was not designed to measure milk production effect or change (Deuchler *et al.* 1998).

These results seem to suggest that 10 g unprotected choline has the same effects as 2 g RPC, even though the higher response was observed with 10 g RPC/d (Bonomi *et al.* 1996). However, in this study (Bonomi *et al.* 1996) it appears that both the diet fed (containing 7.0–8.0 kg of lucerne hay) and the stage of lactation monitored (from 2nd to 6th month of lactation) could have influenced the response to choline supplementation.

More recently, Hartwell *et al.* (2000) found that 12 g RPC starting 28 d from calving improved milk yield (+2.6 kg) during the first 8 weeks of lactation when associated with a low level of rumen undegradable protein (4 % of the dietary DM). This response was considered in part due to a sparing effect of choline on Met catabolism in early lactation when it is extensively required for choline synthesis (Hartwell *et al.* 2000). In the study of Piepenbrink & Overton (2000) supplementation of RPC did not affect milk yield, but it was suggested that hepatic fatty acid metabolism is sensitive to the supply of the nutrient during the periparturient period since choline serves as a methyl donor in the synthesis of carnitine (Griffith, 1987) and carnitine is essential for fatty acid oxidation.

Pinotti *et al.* (2000, 2001, 2002) used 20 g RPC in cows around calving on a silage-based diet (Table 5) starting 14 d before the expected calving date and continuing to 30 d post-partum. They found an increase in milk production (+10 %), which was attributed to the functions of choline as a methyl donor, probably enhanced by the high proportion of silage in the diet (Chamberlain *et al.* 1989). These authors also reported lower NEFA concentration in plasma at parturition, suggesting improved lipid metabolism, as milk yield was increased at the same time (Pinotti *et al.* 2000, 2001). The NEFA:cholesterol ratio was also lower in the supplemented animal, a further indication of improved lipid metabolism since a high NEFA:cholesterol ratio is associated with increased risk of fatty liver (Holtenius, 1989). In the same study (Pinotti *et al.* 2000, 2001, 2002) the cows given RPC also had higher  $\alpha$ -tocopherol levels and higher  $\alpha$ -tocopherol:cholesterol ratios in plasma than controls. The increase of plasma  $\alpha$ -tocopherol is likely to be due, at least in part, to the improvement in fat absorption and transport induced by the choline supplementation, suggesting a novel choline–vitamin E interaction. Koo & Noh (2001) found that PtdCho hydrolysis is critical for improving intestinal absorption of  $\alpha$ -tocopherol in rats but, as noted previously, the absorption of fats and fat-soluble nutrients in ruminants differs from that in non-ruminant species.

We can conclude from these studies that the supply of choline is not always sufficient to maximise productivity of dairy cows. Although the requirement for choline in dairy ruminants can be satisfied in theory by other nutrients, mainly by excess Met, it is very unlikely that excess Met will be available, especially at the beginning of lactation and when maize silage-based diets are fed (Chamberlain *et al.* 1989; McDonald *et al.* 1991; DePeters & Cant, 1992) since Met is one of the limiting amino acids for milk production.

Nevertheless the data do not provide much of an indication as to the amount of choline needed in dairy cow nutrition. The supplementation of unprotected choline ranged from 10 to 326 g (Erdman *et al.* 1984; Atkins *et al.* 1988; Sharma & Erdman, 1988b; Bonomi *et al.* 1996), while levels of RPC ranged between 2 and 50 g (Erdman & Sharma, 1991; Erdman 1994; Bonomi *et al.* 1996; Deuchler *et al.* 1998; Hartwell *et al.* 2000; Pinotti *et al.* 2000, 2001, 2002).

### Implications

We have seen that choline and Met are metabolically interchangeable with regard to their ability to furnish labile methyl groups. In view of this and the fact that Met and other sources of

methyl groups are likely to be in short supply in high-yielding dairy ruminants, choline could be considered an essential and limiting nutrient. Supplementation of RPC would therefore seem to be essential for optimising high-quality milk production in high-yielding dairy cows, particularly in animals fed basal diets that limit post-ruminal Met supply. Moreover, a role of choline as methyl donor does not exclude its function *per se*, mainly in lipid metabolism. In spite of that, the optimal level of supplementation has not been established and further studies are required to determine the choline requirement during early lactation and under different feeding conditions (National Research Council, 2001). It is also evident that our knowledge of the interaction of choline with vitamins and other essential nutrients is incomplete in the dairy cow. For example there are indications that both folic acid and vitamin B<sub>12</sub> may spare Met, but Met re-synthesis via THF is blocked by lack of vitamin B<sub>12</sub> (McDowell, 1989; Kennedy *et al.* 1992, 1995; Girard & Matte, 1997, 1998, 1999; Girard, 1998; Petitclerc *et al.* 2000; National Research Council, 2001), which in turn are all connected with choline. These aspects not only reflect the inadequacy of a nutritional approach based on the supply and utilisation of individual nutrients, but also suggest that the requirements of B-complex vitamins in dairy cows should be reconsidered.

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