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Review Article

Folic acid in ruminant nutrition: a review

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Folic acid plays an essential role in DNA and methionine metabolism. Micro-organisms in the rumen can synthesise folates, but it has not been verified that these amounts are sufficient to achieve the best efficiency of dairy cows. However, the amount of folates synthesised in the rumen could possibly, to some extent, be affected by the forage:concentrate ratio. Degradation of orally supplemented folic acid in the rumen seems to be very high (about 97 %), as supplementation of folic acid hardly increases folate concentrations in the digesta at the duodenum. However, it must be considered that dietary supplements of folic acid higher than 0·5 mg/kg body weight increased serum folate concentrations in all available studies and milk folate concentrations in most studies. Additionally, milk production tended to be increased in some studies. Therefore, degradation of folic acid in the rumen may be overestimated as folates can be absorbed at the proximal duodenum. For future research it is necessary to consider the whole flow and the metabolic pathways of folates from the rumen to duodenum, blood, tissue, milk and transfer to calf to declare requirement values for cows. Consequently, the present review discusses current knowledge and emphasises areas for future research.

Folic acid: Dairy cows: Folate: Tetrahydrofolate

In general, it is assumed that B-vitamin requirements for ruminants can be met by microbial synthesis in the rumen, even when the animals are fed a diet providing very small amounts of those vitamins. This hypothesis was already established in 1928 by Bechdel *et al.* [1]. However, since that time average milk and milk component yields have increased drastically (already by about 33 % in the last 15 years in the USA), whereas the increase of average DM intake was considerably lower (only about 15 %)[2]. Furthermore, feeding strategies changed to support the increase in milk production and milk component yields. Changes in diet composition (from less forage towards more concentrate) influence the microbial population in the rumen, so it is a moot point whether the B-vitamin requirements of dairy cows are still being met.

Folic acid is very important during lactation and for DNA synthesis of fetal and placental tissue during pregnancy[3], therefore a suboptimal supply should be avoided. In agricultural practice in dairy cows, gestation and lactation are concomitant during several months per year, so the avoidance of progressive folate deficiency must be a priority.

Up to now the folate content of feed is rarely analysed and values on microbial folate synthesis are scarce. So it is very difficult to estimate a cow's actual supply with folates[4]. The National Research Council[4] tried to estimate requirement values of folates for cows, but they had to extrapolate the cows' requirements from data of swine and average vitamin contents found in cows' milk. They estimated a daily folate requirement of 33 mg/d for tissue and 2 mg/d for milk for a dairy cow with a body weight (BW) of 650 kg and a milk production of 35 kg fat-corrected (4 %) milk per d. However, final evidence in the form of studies on cows' requirements is still lacking, as the number of appropriate studies is limited. Therefore this review will present current knowledge of folate metabolism and the influence of folic acid supplementation on ruminal variables, folate absorption and performance, especially for dairy cows. Areas on which future research should focus will be highlighted.

Chemical structure

The vitamin folic acid (chemical name pteroylglutamic acid) consists of three parts: a pteridine nucleus, *para*-aminobenzoic acid and glutamic acid[5]. The name folic acid is deduced from *folium*, the Latin word for leaf, because native forms of folic acid were originally isolated from spinach leaves[6]. In chemistry the name folic acid is only used for the synthetic form. It is a stable compound and the basal structure of a wide family of vitamin coenzymes[7]. In nature, more than 100 compounds, with the basal structure of folic acid, feature a common vitamin activity. These pteroylglutamate forms of

Abbreviations: BW, body weight; i.m., intramuscular; THF, tetrahydrofolate.

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folic acid are generally called folates[5,8,9]. Native folates vary in three chemical characteristics from folic acid: first, in the level of reduction of the pteridine nucleus (dihydrofolate or tetrahydrofolate (THF)); second, in the character of the one-carbon substituent linked to the N atoms N-5 and N-10 (for example, formyl, formimino, methyl, methylene or methenyl residues); third, in the chain length of the glutamyl residues which can be linked to the γ-carboxyl group of the glutamate via peptide linkages[5,10].

Absorption and biochemical functions

There are several excellent reviews on absorption and biochemical functions of folates[11–14]. Derived from studies with non-ruminant animals, two mechanisms of folate absorption from the intestinal tract seem to exist: an active saturable process and a non-saturable passive process. In fact, the relative importance of passive absorption changes according to folate supply, increasing with the amounts of folates available[13,15]. However, folates are perhaps degraded, converted and synthesised in the forestomachs of ruminants[16], and even absorbed on a small scale[17]. Unfortunately the forms and the availability of the forms present in rumen contents and duodenal digesta are unknown.

In bovine blood, mainly 5-methyl-THF is found[18]. Cells take up this compound and demethylate it to THF. To retain THF in cells it must be converted by folylpolyglutamate synthase to polyglutamate THF, the coenzymic form of folates[14]. Polyglutamate THF is involved in several biochemical pathways in mammals[19]. Mainly, folates are donors and acceptors of one-carbon units[8,20]. Thus they are involved in the remethylation of homocysteine to methionine, as an essential part of the methylation cycle. This reaction is also vitamin B_{12} dependent, as the catalysing enzyme methionine synthase needs vitamin B_{12}[11]. Furthermore, the transfer of one carbon unit involves folates in the synthesis of purines and pyrimidines and thereby in DNA synthesis and cell proliferation[14]. THF is regenerated after these catalytic reactions[14]. However, folate disappears through urinary excretion and through bile, although a very effective reabsorption by the enterohepatic cycle exists[15]. Up to now only one study reported on the urinary excretion of folates in dairy cows (nine animals) after intramuscular (i.m.) injection of 0·3 mg folic acid per kg BW[21]. The authors found an excretion of the injected dose of 35·1 % after 8 h and 44·2 % after 48 h.

A deficit of folates can lead to a decrease in *S*-adenosylmethionine levels and to an abnormal DNA precursor metabolism resulting in faulty DNA synthesis and a decrease in NAD[22], as a decrease in NAD levels is consistent with an increase in DNA repair activity[23]. An indirect lack of folates can be caused by a vitamin B_{12} deficit. This results in an accumulation of 5-methyl-THF called a methyl-trap, as 5-methyl-THF cannot be regenerated to THF[15]. If so, cells are unable to conjugate absorbed folate monoglutamates, resulting in a decreased intracellular folate polyglutamate level. Additionally, intracellular folate accumulation declines as only polyglutamates can be retained in cells[24].

As folates influence DNA synthesis and the methionine cycle, they are involved in the metabolic pathways of reproduction and milk protein synthesis; therefore they are very important especially in gestating and lactating cows. An additional special situation for cows is that they have a very high demand for methyl groups in early lactation. Concurrently some precursors for methylated compounds (for example, serine and glycine) are also needed for gluconeogenesis, as the amounts of glucose reaching the small intestine through the digestive system are generally low. So, coincident demand for precursors of methylated compounds leads to competition between different metabolic pathways, for example, gluconeogenesis, lecithin synthesis, DNA synthesis and remethylation of methionine[25,26].

Sources and stability of folates

The following section gives a survey of approximate folate concentrations in some feeds and foods. Different folate contents are given in the literature for the same feedstuffs (Table 1) and for most feeds they are not analysed at all, so only few data are available. It must be considered that as compilations were used in Table 1, the number of samples analysed is not known. Additionally, it must be pointed out

Table 1. Folate content of several feeds given in the literature

Feed	Range of folate content (mg/kg DM)	References
Lucerne meal	1·65–5·55	Anonymous (1996)[59], National Research Council (1998)[60]
Lucerne hay	2·60–3·40	Schwab *et al.* (2006)[44], Anonymous (1996)[59]
Barley, whole grain	0·07–0·68	Anonymous (1996)[59], National Research Council (1998)[60], Souci *et al.* [61], LfL[62]
Maize, whole grain	0·03–0·45	Anonymous (1996)[59], National Research Council (1998)[60], Souci *et al.* [61], LfL[62]
Maize silage	0·48	Schwab *et al.* (2006)[44]
Grass hay	0·52	Schwab *et al.* (2006)[44]
Oats, whole grain	0·04–0·58	Anonymous (1996)[59], National Research Council (1998)[60], Souci *et al.* [61], LfL[62]
Peas	0·17–0·66	Anonymous (1996)[59], National Research Council (1998)[60], Souci *et al.* [61], LfL[62]
Rapeseed meal, solvent extracted	0·22–2·4	Anonymous (1996)[59], LfL[62]
Rye, whole grain	0·17–0·81	Anonymous (1996)[59], National Research Council (1998)[60], Souci *et al.* [61]
Soyabean meal, solvent extracted	0·50–4·18	Schwab *et al.* (2006)[44], Anonymous (1996)[59], National Research Council (1998)[60], LfL[62]
Wheat, whole grain	0·10–0·56	Anonymous (1996)[59], National Research Council (1998)[60], Souci *et al.* [61], LfL[62]
Whey powder	0·01–0·97	Anonymous (1996)[59], National Research Council (1998)[60], Souci *et al.* [61]

LfL, Bayerische Landesanstalt für Landwirtschaft.

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that the native folate concentrations in feeds vary due to influences of climate, species, vegetation stage, habitat and fertiliser[27]. Furthermore, most naturally occurring folates are chemically relatively unstable. Thus folates exhibit a significant loss of activity during harvesting, storage and processing, but measured folate concentrations are also highly influenced by the method used for sample preparation[28]. The synthetic form, folic acid, is more resistant to chemical oxidation[11].

The figures generally show very low folate contents in feedstuffs. However, quantities are not the only decisive factor; the folates in the feed must also be available for absorption[10]. The so-called bioavailability describes the proportion of an orally administered dose which is available in plasma after absorption[29]. It is difficult to consider the bioavailability of native folates because unknown numbers and amounts of folate metabolites exist in every plant species or feedstuff. For ruminants the assessment of folate bioavailability is more difficult as their micro-organisms in the rumen synthesise, but also degrade, ingested folates. The synthesis and degradation of folates in the rumen is crucial for the amount absorbed from the small intestine of ruminants. Up until now the number of studies on rumen folate synthesis and degradation has been very low; therefore for ruminants no values of folate bioavailability from feedstuffs are available. Furthermore the bioavailability of native folates is influenced by different physico-chemical properties and certain feed constituents. For example, polyglutamyl folates have a lower bioavailability than monoglutamyl folates, as polyglutamyl folates must be hydrolysed to monoglutamates before absorption[30]. Additionally, the actual amount available for each individual animal varies depending on differences in intestinal pH or general living conditions[10].

Microbial synthesis, degradation and absorption of folates in the gastrointestinal tract of ruminants

It is well known that the microbial activity and the ruminal population are influenced by the level of concentrates in the diet and the type of feed[31]. As some bacterial species are able to synthesise folates, and some others need them[32], different amounts of folates can be synthesised and used in the rumen depending on the feed composition. For steers, Hayes *et al.* [33] and Girard *et al.* [34] described a relationship between the proportion of concentrates in the diet and the amount of folates in the rumen. High-concentrate diets resulted in an increase of folates (Table 2). The authors hypothesised that this increase is due to an enhanced microbial activity in the rumen, caused by rapidly degradable carbohydrates. But it must also be considered that concentrations are not necessarily representative of the total amount synthesised in the rumen, as digesta passage and rumen volume could vary between treatments, for example, due to fibre differing greatly in amount and length. Santschi *et al.* [35] could not corroborate this hypothesis for cows, because they found no difference in the amount of folates in the liquid fraction of ruminal content between the high-forage (58 % forage) and low-forage (37 % forage) diets (Table 2). However, the concentrate:forage ratio of the two diets used in the study of Santschi *et al.* [35] was not as extreme as in the studies with the steers[33,34] and additionally Santschi *et al.* [35] used diets with more ingredients than Girard *et al.* [34] and Hayes *et al.* [33] (Table 2). Furthermore the steers had a BW of 340 kg[33] and 352 (SE 27) kg[34], whereas primiparous and multiparous cows weighed 582 (SE 17) kg and 692 (SE 17) kg[35], respectively, which resulted in a different DM intake between cows and steers (Table 2). It should be noted that in all three studies folate concentrations in ruminal fluid were very different. One reason for this could possibly be the different diet composition used in the three studies (Table 2). Additionally, Hayes *et al.* [33], who found the highest values, used a different sample preparation method from Girard *et al.* [34] and Santschi *et al.* [35]. The higher values found by Hayes *et al.* [33] in the supernatant fraction could result from bacterial content, as the samples were centrifuged after freezing and thawing, which could have destroyed bacterial cells. Contrary to this, Girard *et al.* [34] and Santschi *et al.* [35] centrifuged their samples before freezing. Furthermore, Hayes *et al.* [33] used a microbiological assay (*Streptococcus faecalis*) to analyse folate concentration in ruminal samples. In contrast, Girard *et al.* [34] and Santschi *et al.* [35] analysed their samples by radioassay.

An *in vitro* study by Hall *et al.* [36] showed that the degradation of fibrous materials by rumen micro-organisms increases (42 %) with supplementation of folic acid (100 μg/20 ml medium). So it seems that cellulolytic micro-organisms require folates, which would endorse the findings of Hayes *et al.* [33] and Girard *et al.* [34], who found decreased folate concentrations with high-forage diets. Studies on folate requirements of micro-organisms are rare. Some strains of *Ruminococcus flavefaciens*, a cellulolytic rod, seem to require either folic acid, THF or *p*-aminobenzoic acid[37–40] (which is a part of folic acid). Also two strains of *Ruminococcus albus* require folic acid[40]. Furthermore Hayes *et al.* [33] observed that the level of folates in the ruminal fluid correlated negatively with pH. This corroborates the previous findings, because *R. flavefaciens* is sensitive to acid[41] and ruminal pH increases with diets rich in fibre. Since these studies were conducted, feeding strategies, genetics and keeping conditions have changed, and it would be interesting to see if similar and if possible even more detailed results relating to species of micro-organisms could be obtained today.

Consequent to folic acid supplementation to steers, Girard *et al.* [34] observed an increase in folate concentration in the solid and liquid fractions of the rumen contents compared with a supplement-free diet ($P=0{\cdot}0001$ for both fractions; Table 2). However, different from supplement-free diets, the concentrate:forage ratio had no influence on the ruminal folate concentration. Furthermore, they observed that neither folic acid supplementation nor the nature of the diet made a difference to the quantity of protein synthesised per unit of microbial mass[34]. Chiquette *et al.* [42] analysed the effect of folic acid supplementation on digestibility and ruminal fermentation in growing steers. They noticed a tendency ($P=0{\cdot}08$) of pH to decline 4–8 h after feeding a diet consisting of 70 % rolled barley, 30 % timothy hay and a supplementation of 2 mg folic acid per kg BW as compared with the unsupplemented diet. The results showed that the concentration of ruminal acetate and butyrate did not change due to folic acid supplementation, whereas ruminal propionate concentrations increased ($P\leq 0{\cdot}05$) after feeding, and the acetate:propionate ratio was numerically higher during the 24 h of observation due to folic acid supplementation. The apparent

Table 2. Folate content of ruminal material and body weight (BW) (at the beginning of the trial) of steers and cows
(Mean values with their standard errors)

						Folates in				
		BW (kg)		DM intake (kg/d)		Solid fraction (mg/kg DM)		Liquid fraction (ng/ml)		
Diet	Animals (*n*)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Reference
6·3 kg flaked maize* + 1·1 kg soyabean meal with minerals*†	8	340						186[a]		Hayes *et al.* [33]
6·6 kg ground maize* + 1·1 kg soyabean meal with minerals*†	8	340						180[a]		
6·9 kg flaked maize* + 1·8 kg long lucerne hay* + 0·7 kg soyabean meal with minerals*†	8	340						80[b]		
7·5 kg ground maize* + 1·8 kg long lucerne hay* + 0·7 kg soyabean meal with minerals*†	8	340						83[b]		
7·5 kg flaked maize* + 1·8 kg ground lucerne hay* + 0·7 kg soyabean meal with minerals*†	8	340						91[b]		
7·4 kg ground maize* + 1·7 kg ground lucerne hay* + 0·7 kg soyabean meal with minerals*†	8	340						144[c]		
70 % rolled barley + 30 % timothy hay† + 2 mg folic acid per kg BW†	8	352	27	8·09	0·64	0·81[a]	0·03	53·0[a]	3·8	Girard *et al.* [34]
	8			7·88	1·35	5·84[c]‡		613·5[c]‡		
30 % rolled barley + 70 % timothy hay† + 2 mg folic acid per kg BW†	8	352	27	7·67	1·11	0·66[b]	0·01	40·9[b]	3·0	
	8			8·09	0·77	5·84[c]‡		613·5[c]‡		
58 % forage (hay silage, maize silage) + 42 % concentrate (cracked maize, soyabean meal, protein supplement, minerals)	8§	582§	17	24·5[a]§	1·0			4·18[a]§	0·32	Santschi *et al.* [35]
	8‖	692‖	17	29·4[b]‖	1·0			3·52[a]‖	0·32	
37 % forage (hay silage, maize silage) + 63 % concentrate (cracked maize, soyabean meal, protein supplement, minerals)	8§	582§	17	24·5[a]§	1·0			4·05[a]§	0·32	
	8‖	692‖	17	29·4[b]‖	1·0			3·60[a]‖	0·32	

[a,b,c] Mean values within a trial with unlike superscript letters were significantly different ($P \leq 0{\cdot}05$).
* In these studies no DM values of feed were given; therefore fresh matter values are presented here.
† This study was conducted with steers.
‡ In this study values of the solid and liquid fraction of the ruminal content were given as area under the curve; concentrations presented here are calculated on this basis.
§ Data from primiparous cows.
‖ Data from multiparous cows.

digestibility of DM, fibre fractions and crude protein was not influenced by the addition of folic acid[42]. So it seems that folic acid has no major influence on digestibility and ruminal fermentation, but until today these processes have only been tested once (with eight steers), comparing few different diet compositions, so it is difficult to extrapolate these data to other experimental conditions.

Santschi *et al.* [43] and Schwab *et al.* [44] determined the daily apparent ruminal folate synthesis for lactating cows without supplementation of folic acid, whereas Zinn *et al.* [16] provided data for growing steers (Table 3). On average, Schwab *et al.* [44] calculated 16·2 mg daily apparent folate synthesis and Santschi *et al.* [43] 20·0 mg. Strikingly opposing these results, Zinn *et al.* [16] on average calculated a negative daily apparent ruminal folate synthesis of −0·1 mg for growing steers (194 kg BW). These results could be due to the fact that growing male animals which were used in their experiment had a much lower organic matter intake (3·44 kg organic matter/d) than the adult female animals of Santschi *et al.* [43] (18·4 kg organic matter/d; calculated from DM intake and ash content of the diet) and Schwab *et al.* [44] (18·7 kg organic matter/d) and due to the differences in the ruminal passage rate. Additionally, the negative values of Zinn *et al.* [16] could also result from a dietary effect, as Zinn *et al.* [16] fed a diet with a very high concentration of maize grain, in contrast to diets used in the studies of Santschi *et al.* [43] and Schwab *et al.* [44] (Table 3).

It must be pointed out that disappearance rates, expressed as the amount of folates appearing at the duodenum relative to the quantity given, were very high in all experiments with dietary supplements of folic acid (about 97 %)[16,43]. Therefore it is arguable whether unprotected folic acid can be supplemented effectively. However, one has to keep in mind that disappearance could either be caused by degradation or absorption[16]. Indeed, a net flux across the rumen wall was only found if high amounts of folic acid were present in the rumen. So it seems that the rumen wall is able to absorb folic acid. However, the efficiency is very low, so net flux across the rumen wall into the blood circulation can be neglected[45,46]. On the other hand, folates are absorbed at the proximal intestine[46], and therefore they could already be absorbed at the proximal duodenum before the cannula, hence ruminal disappearance of folic acid could be overestimated[43].

Table 3 gives a survey of the amount of folates found in the duodenum. The apparent intestinal disappearance (between duodenal and ileal cannula) seems to be very low[43]. Without supplementation of folic acid, the duodenal flow of folates was lower than the ileal flow, and it rose above the ileal flow only by supplementation of folic acid (Table 3). So it seems that there is no apparent intestinal disappearance of folates without supplementation of folic acid and with supplementation the apparent intestinal disappearance approximates 4 %[43]. Santschi *et al.* [43] hypothesised that the apparent intestinal absorption of folates is underestimated, as folates are extensively recycled by the enterohepatic cycle and then released between the duodenal and ileal cannula – thus explaining the higher values of folates in the ileal flow.

Generally, Girard *et al.* [45] reason that for dairy cows, folate absorption in the gastrointestinal tract is an active saturable process. With dietary supplements of 2·6 g folic acid/d this process was already saturated, as higher supplementations could not effectively increase the amount of folate reaching the blood circulation. Due to the destruction of folates by micro-organisms, and the low importance of passive absorption, they deduced the minor efficiency of folate absorption in ruminants (< 5 %)[45] *v.* humans (10–46 %)[47]. In general it should be considered that at present no studies are available comparing the amount of folates in the ruminal content and the amount in the duodenum, hence no statement can be made on the coherence between the amount of folates in the ruminal content and the amount of folates in the digesta at the duodenum.

Folate concentrations in blood, milk and liver

Blood

Table 4 shows serum folate concentrations in different feeding studies with cows. Without supplementation of folic acid serum folate levels varied between 13·6 and 17·2 ng/ml. In plasma Santschi *et al.* [35] found significantly ($P=0·005$) lower folate concentration for primiparous cows (12·7 (SE 1·6) ng/ml) compared with multiparous cows (18·4 (SE 1·6) ng/ml). These observations were not affected by the composition of the diet (58 or 37 % forage).

Without supplementation, serum and plasma folate concentrations increase after parturition, as the maternal–fetal complex no longer requires folates (Fig. 1). However, starting on the day of parturition, the cow requires folates for milk production, but it seems that this demand is lower than that for the maternal–fetal complex[48,49]. From mating (about 2 months after previous parturition) to parturition Girard *et al.* [50] found a decrease of total serum folates of 40 %, indicating that the maternal–fetal complex has an increasing demand for folate. Also, Girard & Matte[21] discovered a higher demand for folates in the tissues of lactating and gestating cows than in lactating non-gestating cows. Serum clearance was significantly slower ($P=0·04$) in non-gestating cows after an intravenous injection of 50 μg folic acid. However, in another study, Girard & Matte[49] could not find a difference in serum folate concentrations between gestating and non-gestating cows.

Oral supplementation (Table 4) or i.m. injection of folic acid significantly increased serum folate concentrations[34,45,49–54]. Actually, oral doses higher than 0·5 mg folic acid/kg BW are required to produce a noticeable effect in serum folate concentration, as concluded from a dose–response study with heifers (150 kg BW)[51]. However, the heifers used in the present study were very young and therefore full ruminal function may not have been developed. Comparable investigations have not been carried out again and in all further studies supplementations higher then 0·5 mg folic acid/kg BW were used. As shown in Fig. 1, folic acid supplementation increased serum folate concentrations in cows from 4 weeks before calving until calving. The increase intensified with higher dietary supplementation[49]. Unfortunately no further measurements were performed between the initial sampling time 4 weeks before calving and at the time of calving, so it is not evident if serum folate levels had already increased before calving due to dietary supplementation. After calving, supplemented cows had decreasing serum folate concentrations. The more folic acid was added to the diet, the sharper the decline, reaching a plateau value 16 weeks after parturition[49]. As well as the influence of folic acid supplementation, Girard & Matte[49] ($P=0·0001$) and

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Table 3. Folate intake, duodenal flow, ileal flow, apparent synthesis (AS) and body weight (at the beginning of the trial) of steers and cows
(Mean values with their standard errors)

Diet	Number of animals	Body weight (kg)		Folate intake (mg/d)		Duodenal flow (mg/d)		Ileal flow (mg/d)		AS* (mg/d)		Literature
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
35 % lucerne hay + 10 % Sudan grass + 45 % maize + 6 % molasses + 4 % fat												Zinn *et al.* [16]
Without folic acid supplementation	3 s.	194		1·2	0·3	1·1†	0·3			−0·1‡		
+ 10 mg of folic acid per d	3 s.	194		11·2	0·3	1·2†	0·3			−10‡		
+ 100 mg folic acid per d	3 s.	194		101·2	0·3	3·8†	0·3			−97·4‡		
44 % grass-legume silage + 15 % maize silage + 34 % high-moisture maize + 5 % protein supplement + 2 % minerals												Santschi *et al.* [43]
Without folic acid supplementation	4 m.			7·0		27·0	2·0	40·0	3·0	20‡		
+ 2600 mg folic acid per d	4 m.			2607		106·0	2·0	102·0	16·0	−2501‡		
35 % forage§ + 65 % concentrate with soyabean hulls, beet pulp, soyabean meal (30 % NFC)	4 p. and 4 m.	574 p. 651 m.	59 67	13·7	0·9‖	28·9¶**	2·4‖			15·2¶**	2·0‖	Schwab *et al.* [44]
35 % forage§ + 65 % concentrate with maize, barley, soyabean hulls, beet pulp, soyabean meal (40 % NFC)	4 p. and 4 m.	574 p. 651 m.	59 67	12·2	0·9‖	32·4¶**	2·4‖			20·2¶**	2·0‖	
60 % forage§ + 40 % concentrate with soyabean hulls, beet pulp, soyabean meal (30 % NFC)	4 p. and 4 m.	574 p. 651 m.	59 67	12·4	0·9‖	25·4¶**	2·4‖			13·0¶**	2·0‖	
60 % forage§ + 40 % concentrate with maize, barley, soyabean hulls, beet pulp, soyabean meal (40 % NFC)	4 p. and 4 m.	574 p. 651 m.	59 67	12·6	0·9‖	29·0¶**	2·4‖			16·4¶**	2·0‖	

NFC, non-fibre carbohydrates; s., steers; m., multiparous cows; p., primiparous cows.
* Apparent synthesis = duodenal flow minus intake.
† Values did not differ significantly.
‡ In Zinn *et al.* [17] and Santschi *et al.* [44] apparent ruminal synthesis was not calculated by the authors, but daily intake and duodenal flows were given. Furthermore, Santschi *et al.* [44] declare no levels of significance at all, therefore it was not possible to characterise significance in these studies.
§ Forage = 50 % maize silage, 33 % lucerne hay, 17 % grass hay.
‖ SEM, not SE, was used.
¶ Significant effects of NFC ($P \leq 0{\cdot}05$).
** Significant effects of forage ($P \leq 0{\cdot}05$).

Table 4. Average serum folate concentration (ng/ml) of cows with and without dietary supplementation of folic acid (Mean values with their standard errors)

		Primiparous		Multiparous		Primiparous + multiparous		
Supplementation	Number of cows	Mean	SE	Mean	SE	Mean	SE	Literature
None	14			13·6*	1·0			Girard *et al.* [50]†
None	56			23·3‡	0·6			
None	8 p. and 12 m.	14·5*§	0·7	17·2*§	0·7			Girard *et al.* [54]†
None	8 p. and 12 m.	14·3‡§	0·8	16·8‡§	0·8			
None	10 p. and 11 m.					16·7	1·4	Girard & Matte[52]
2 mg/kg BW	12 p. and 9 m.					25·3‖	1·4	
4 mg/kg BW	10 p. and 11 m.					35·5‖	1·4	
None	18			15·8¶	1·6			Girard *et al.* [53]
3 mg/kg BW	16			20·6‖¶	1·7			
6 mg/kg BW	16			23·0‖¶	1·7			

p., Primiparous cows; m., multiparous cows; BW, body weight.
* Concentration determined in serum from cows before parturition.
† Girard *et al.* [50] and Girard *et al.* [54] declare no levels of significance between cows before parturition and after parturition; therefore it was not possible to characterise significance in these studies.
‡ Concentration determined in serum from cows after parturition.
§ Significant effect between primiparous and multiparous cows.
‖ Significant differences ($P \leq 0{\cdot}05$) between control and folate groups.
¶ Concentrations determined in serum from cows 18 weeks after calving.

Girard *et al.* [53] ($P=0{\cdot}02$) showed a significant time effect, as the gain in serum folate concentration due to dietary supplementation was greater in the first 8 weeks of lactation than later in lactation (Fig. 1). It seems that increasing serum folate concentrations during early lactation could result from a decreased ability of the cells to retain and use folates[53]. A reason for this may be generally lower serum vitamin B_{12} levels (181 pg/ml) at early lactation compared with 252 pg/ml in the later lactation[53]; therefore folates can get into the methyl-trap mentioned above.

Three studies are available dealing with an influence of folic acid supplementation on packed cell volume and Hb concentrations in blood. In one study with an oral supplementation of 4 mg folic acid/kg BW an increase ($P \leq 0{\cdot}05$) in packed cell volume and Hb in primiparous cows was found 16 weeks after parturition compared with non-supplemented primiparous cows[49]. Oral supplementation of folic acid had no effect on these variables in multiparous cows[49,53]. These effects could be explained by generally lower vitamin B_{12} levels in primiparous compared with multiparous cows, as folates and vitamin B_{12} are both required for DNA synthesis (as described in the 'Absorption and biochemical functions' section)[49]. Hence a lack of each individual vitamin or of both vitamins together can delay the maturation of blood cells[55]. Folic acid supplemented to primiparous cows, which have low vitamin B_{12} levels, may decrease the deficit in DNA synthesis that results in higher packed cell volume and blood Hb values. However, it must be pointed out that changes of packed cell volume and blood Hb due to folic acid supplementation are smaller than natural changes taking place during lactation[49,54]. No effects on these parameters were found in either primiparous or multiparous cows with i.m. injections of 160 mg folic acid once per week[54].

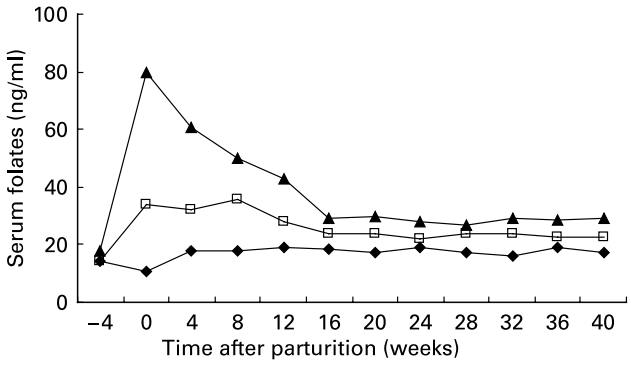

Fig. 1. Serum folate concentration of cows fed different daily folic acid supplements (according to Girard & Matte[49], modified). (−◆−), Unsupplemented; (−□−), 2 mg folic acid/kg body weight; (−▲−), 4 mg folic acid/kg body weight.

Up to now Graulet *et al.* [48] are the only authors studying the influence of folic acid on plasma concentrations of amino acids and glucose. Between week 3 before calving and week 8 after calving, supplementation of folic acid significantly increased plasma concentrations of alanine, glycine, serine, threonine and total sulfur amino acids. Concurrently, plasma concentrations of glucose and aspartate significantly decreased[48]. As aspartate is one of the main N-donors during purine biosynthesis, a decrease in plasma aspartate levels due to folic acid supplementation could be based on an increased DNA formation. A higher availability of glycine and serine could induce an increase in 1-C-donors for synthesis of methyl-THF[48]. So far these are the only explanations for the observed effects. Therefore it would be interesting to conduct further studies on the influence of folic acid supplementation on plasma concentrations of amino acids and glucose.

As the few available studies show increasing folate concentrations in serum and plasma, it is important to study the influence of folic acid supplementation on blood variables and thus on whole-animal metabolism.

Milk

Supplementation of folic acid does not influence feed intake[48,52,53]. However, the effects of folic acid supplementation

on milk production of cows vary (Table 5). For gestating primiparous and multiparous cows, Girard *et al.* [54] found a non-significant increase in milk production of 14 % in the last part of lactation due to an i.m. injection of 160 mg folic acid once per week. However, they could not find an effect on milk production immediately after calving. In contrast, Girard & Matte[52] found an increased milk production of 6 % during the first 100 d of lactation ($P=0{\cdot}06$) for multiparous cows receiving 4 mg folic acid per kg BW and a 10 % increase from day 100 to day 200 ($P=0{\cdot}05$). For primiparous cows, however, milk production decreased in the first 100 d of lactation ($P\leq 0{\cdot}08$) with a supplementation of 2 and 4 mg folic acid per kg BW; in the following lactation no effect could be noticed. Graulet *et al.* [48] only studied the first 56 d of lactation; during this time cows fed a supplement of 2·6 g folic acid per d showed a significant ($P=0{\cdot}01$) increase in milk production (Table 5). The effects on multiparous cows could result from folate body stores depleted by several lactations and gestations. The effects on primiparous cows were explained by their generally lower vitamin B_{12} levels compared with multiparous cows[52,54]. Graulet *et al.* [48] established the hypothesis that higher milk production during folic acid supplementation results from an improved synthesis of purines and pyrimidines which are necessary for DNA replication. This hypothesis could be supported by the decreased plasma aspartate levels mentioned earlier. These different hypotheses and observations make clear that more studies are necessary to find an explanation for the effects and to discover under which conditions the effects can be reproduced, as Girard *et al.* [53] could not find any effect on the milk production of multiparous cows by either supplementing 3 or 6 mg folic acid per kg BW (Table 5).

Folate occurs in cows' milk mainly as 5-methyl-THF, whereas approximately half of it exists as mono- and the other as polyglutamates[56]. Almost all folate in cows' milk is bound to specific folate-binding proteins. Generally, the highest milk folate concentrations are found in the colostrum. Starting at parturition, folate concentrations in milk decrease until 4 weeks after parturition when folate concentrations reach a plateau which is stable until the end of lactation[54]. All studies with different levels of oral folic acid supplementations showed significantly increased milk folate concentrations[48,49,52,53] (Table 5). In humans the transfer of folates into milk is controlled by an active-transport mechanism in the mammary glands. The transfer is linked to the secretion of folate-binding proteins. Once the binding capacity is saturated no further folates can be transferred into the milk[57]. The same seems to be true for cows, as supplementations higher than 3 mg/kg BW could not increase milk folate concentrations while serum folate concentration increased[49,52,53] (Tables 4 and 5). As observed for serum, the response of milk concentrations of folates to oral supplementation of folic acid was greater during the first 8 weeks after calving then later in lactation[49]. However, i.m. injection of folic acid could not influence milk folate concentrations during the first part of lactation[50,54]. Furthermore, in contrast to the results after an oral supplementation of folic acid, i.m. injection of folic acid tended to increase the folate content only in the colostrum and during progressed lactation[54].

At present, only four studies are available dealing with milk components. In these four studies an influence of folic acid on milk protein and casein was detected for multiparous cows. It seems that i.m. injections and oral supplementations of folic acid increase milk protein and casein concentrations or yields[48,52–54] (see Table 5). The authors explained these effects by depleted folate body stores and generally higher folate requirements because of higher milk production and heavier calves of multiparous cows. Additionally, they hypothesised that the effects on milk protein, similar to the effect on milk production, arise from either an increased synthesis of purines and pyrimidines for DNA synthesis, from an increased secretory capacity of the cells, or from amino acid interconversion which perhaps results in a greater supply of essential amino acids.

It becomes apparent that more studies on supplementation of folic acid are needed to examine the influence on milk production and milk components and its causes.

Liver

After a single supplementation of 2·6 g folic acid Girard *et al.* [45] could not find a significant increase in the amount of folates taken up by the liver during a 24 h period (calculated from folate flow through portal-drained viscera and total splanchnic tissue). Before the supplementation approximately 50 % of the portal blood folates were extracted by the liver; after supplementation only approximately 30 % were extracted (calculated from averaged net flux per h). Graulet *et al.* [48] studied the concentration of folates in liver biopsies. Cows receiving a daily supplementation of 2·6 g folic acid had significantly ($P=0{\cdot}0001$) increased liver folate concentrations of 2·56 μg/g DNA compared with control cows with 1·50 μg/g DNA during the first 8 weeks of lactation. The results of these two studies led to the assumption that folic acid supplementation increases the liver folate concentration but decreases the percentage of extraction from arterial blood into the liver. Lower percentages of extraction reflect that more folates are available for post-splanchnic tissues, as for example the mammary glands. This fact was confirmed by the results of Girard *et al.* [45] who found 71 % of folates from arterial blood in post-splanchnic tissues after supplementation of 2·6 g folic acid and only 50 % without supplementation. Beside the increase in liver folate concentration Graulet *et al.* [48] found higher values of total lipids, TAG and cholesterol in the liver during the first 2 weeks of lactation following a daily supplementation of 2·6 g folic acid. They explained these higher values with an increased mobilisation of body reserves during the first weeks of lactation which is necessary to meet the requirements for the above-mentioned increases in milk production and milk protein yield. Another explanation for an increase in TAG could be an inhibition of the β-oxidation of fatty acids in the liver, caused by a lack of vitamin B_{12}, as cows receiving folic acid and vitamin B_{12} had no increase in TAG[48]. If it can be proven that folic acid supplementations increase lipid values in the liver during the first weeks of lactation, a time when the risk of fatty liver is high, supplementation of folic acid during this time would be questionable. Therefore further studies with a higher number of animals are needed, as up to now only twenty-four multiparous cows have been tested.

Future research directions

For ruminants future research should focus on the determination of the demand for folates. Up until now only

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Table 5. Influence of oral folic acid supplementation on milk production and composition
(Mean values and standard errors)

Feed ration	Number of cows	BW (kg)		Milk (kg/d)		Folate content (ng/ml milk)		Protein (g/kg)		Fat (g/kg)		Lactose (g/kg)		Literature
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
22 % grass legume silage + 15 % maize silage + 18 % barley + 18 % high-moisture maize + 10 % soyabeans + 15 % distillers dried grains + 3 % minerals + 1 kg grass legume hay per d														Girard & Matte[52]
Without folic acid supplementation	11 m.	682*†	126	27·4†‡	1·6	37·9†	3·6			38·1†	0·11	43·0†	0·04	
	10 p.	572*§	6	27·1‡§	0·7	42·5§	3·6			37·5§	0·11	43·8§	0·04	
+ 2 mg folic acid/kg BW daily	9 m.	682*†	126	28·3†‡	1·3	56·1†‖	3·6			37·7†	0·11	42·4†	0·04	
	12 p.	572*§	6	25·3‡§	0·8	46·0§‖	3·6			38·7§	0·11	43·3§	0·04	
+ 4 mg folic acid/kg BW daily	11 m.	682*†	126	29·6†‡	0·6	48·2†‖	3·6			38·0†	0·11	43·0†	0·04	
	10 p.	572*§	6	25·5‡§	0·9	46·6§‖	3·6			37·1§	0·11	43·3§	0·04	
20 % grass silage + 20 % maize silage + 13 % high-moisture maize + 19 % barley + 6 % wheat + 2 % soyabean hulls + 4 % soyabean meal + 4 % protected soyabean meal + 4 % extruded soyabeans + 8 % minerals														Girard *et al.*[53]
Without folic acid supplementation	9	694*	11	36·2	1·6	39·3	3·7	31·9	0·8	32·7	1·8	48·3	0·7	
+ 3 mg folic acid/kg BW daily	8	694*	11	33·7	1·6	54·6‖	3·7	33·0	0·8	35·1	1·8	46·5	0·7	
+ 6 mg folic acid/kg BW daily	8	694*	11	33·9	1·6	43·1‖	3·7	32·4	0·8	35·5	1·8	46·5	0·7	
7 % grass hay + 27 % legume-grass silage + 18 % maize silage + 32 % cracked maize + 9 % soyabean meal + 4 % maize, wheat, rapeseed products + 3 % minerals														Graulet *et al.*[48]
Without folic acid supplementation	6	735¶	25	39·5**	1·3	45·6**	6·2	30·3**	0·6	35·9**	1·4	45·1**	1·2	
+ 2600 mg folic acid/d	5	755¶	27	41·8‖**	1·6	71·7‖**	6·8	29·5**	0·7	39·2**	1·6	43·5**	1·0	

BW, body weight; m., multiparous cows; p., primiparous cows.
* BW measured at the beginning of the trial, 1 month before calving.
† Data from multiparous cows.
‡ This value was calculated and shows the average daily milk production for the whole lactation period.
§ Data from primiparous cows.
‖ Significant effect between control and folate groups.
¶ BW measured at the beginning of the trial, 3 weeks before calving.
** This value is the mean from data determined in the first 8 weeks of lactation.

requirement values for tissue and milk have been estimated, but they were derived from swine experiments and folate concentrations in cows' milk. Therefore it is necessary to examine the influence of different amounts of folic acid supplementation under different feeding regimens on rumen variables (for example, pH, volatile fatty acids, microbial population, degradation and synthesis of folates by micro-organisms) and available quantity and forms of folates for absorption at the intestinal tract. Additionally, understanding of mechanisms and sites of folate absorption in ruminants is insufficient; some authors mentioned a possible absorption of folates before the duodenal cannula[16,43], for example, at the beginning of the duodenum or in the abomasum. Furthermore, knowledge on the passage of folates from the intestine to blood and their following distribution to tissues and milk is important. The influences of an oral folic acid supplementation on amino acids and glucose concentrations in blood were tested only once and significant differences were found, but so far no explanations exist[48]. The present review shows that very often controversial results exist, for example, the influence of folic acid supplementation on milk production or the influence of feed ration on folic acid availability in the rumen, therefore surveys should be conducted to reassess the variability of previous studies. Also, maximum and minimum daily intake limits are neither available for folic acid, nor for any other B vitamins[58]. Indeed, no toxic reactions appeared in any of the experiments mentioned above. As the present review shows, there are many unanswered questions regarding the effects of folates for cows. Therefore the following list points out desirable research areas concerning folates:

(1) Studies with different feeding regimens, with and without folic acid supplementation, should be conducted to assess the influence of the diet on folate degradation, synthesis and absorption in the rumen and the duodenum on the one hand and the influence on digestibility and ruminal fermentation on the other hand.
(2) *In vitro* studies with ruminal micro-organisms would be helpful to characterise their folate requirements and synthesis.
(3) Research on the mechanism and the sites of folate absorption in ruminants is necessary.
(4) Experiments on the interactions of physiological stage and folate metabolism in dairy cows are essential.
(5) Surveys should be conducted to explain the available effects of folic acid supplementation on concentrations of amino acids and glucose in blood.
(6) Studies to ascertain the whole flow of folates through the body and implications of folic acid supplementation on the whole organism of dairy cows are crucial.
(7) Further studies should focus on the effects of folic acid supplementation on liver metabolism and milk.
(8) Further determinations of folate concentrations in feedstuffs are required to calculate the folate intake.

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