The use of tannins as dietary supplements in dairy cattle nutrition

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1 Introduction

Tannins are phenolic compounds with a complex structure (Crozier, 2003; Naumann et al., 2017; Aboagye and Beauchemin, 2019; Mueller-Harvey et al., 2019; Kelln et al., 2021) and form an important group of secondary plant compounds that are prevalent in a number of plants. They are formed during plant metabolism and contribute to the plant's defence system among other functions (Swanson, 2003; Kelln et al., 2021). There are two classes of tannins that are determined by their difference in size and complexity, as well as degradability, known as

- larger-sized condensed tannins (CT); and
- hydrolysable tannins (HT).

Apart from the basic differences in molecular weight, there is also a large structural diversity within CT and HT. Of all secondary plant compounds, tannins have received the most attention in dairy cow nutrition research as they are considered to have potential beneficial effects in the diet at concentrations where possible adverse effects are either absent or less

important than the beneficial effects. The research on tannins and tanniferous feeds/supplements has been discussed in a number of review articles (e.g. Frutos et al., 2004; Patra and Saxena, 2011; Jayanegara et al., 2012a; Piluzza et al., 2013; Morales and Ungerfeld, 2015; Naumann et al., 2017; Min and Solaiman, 2018; Aboagye and Beauchemin, 2019; Mueller-Harvey et al., 2019; Nawab et al., 2020; Kelln et al., 2021). It should be noted that many other review articles include the effects of tannins when describing the influence of various diet supplements. Due to the plethora of information and reviews, this chapter focuses on results from *in vivo* studies. *In vitro* work is very important for screening and for researching modes of action, but the proof of principle and determination of the level of effect *in vivo* is the ultimate step prior to implementation in farm practice. As the number of *in vivo* studies carried out exclusively in dairy cows is limited, evidence from growing cattle and small ruminants is also considered. Emphasis is also placed on important, less well-researched aspects of tannin supplementation, such as the influence on emissions from the manure of tannin-supplemented animals or the level of transfer of such polyphenols into milk. As tannins are highly complex and largely differ in structure, their effect is difficult to predict and requires specific analytical and experimental techniques, which are aspects also considered in this chapter.

2   Sources of tannins for supplementation

Feed sources of tannins are often the entire above ground plant material (legumes and herbs) or their leaves (shrubs and trees), but tannins are also prevalent in roots, fruits, flowers, buds, wood and bark (Piluzza et al., 2013; Kelln et al., 2021). In order to identify sources of tannins with beneficial effects as feeds or supplements in ruminants, a clear programme has to be followed, for instance, by using the five-step procedure suggested by Flachowsky and Lebzien (2012). Hence, the key steps in tannin research would involve:

1 characterising the botanical and chemical composition of the sources;
2 analytical description of the tannins;
3 *in vitro* screening;
4 *in vivo* confirmation with recording of palatability and side effects; and
5 long-term studies confirming use and efficacy in farm practice.

Extensive *in vitro* screenings (Step 3), including the search for effective sources of tannins and tanniferous plants as supplements to ruminant diets, have been carried out by a number of researchers (e.g. Bodas et al., 2008; García-González et al., 2008; Kamra et al., 2008; Staerfl et al., 2010; Jayanegara et al., 2011, 2012b; Terranova et al., 2018; Kapp-Bitter et al., 2021b). Sources of tannins may

originate from the tropics or temperate climates. In this chapter, the most well-researched sources are presented, and their effects are described.

2.1 Tannin sources from the tropics

Two sources of tannins (especially CT), which are subject to a lot of *in vivo* studies, are extracts of the bark of an acacia species (*Acacia mearnsii*; mimosa; also called 'Black wattle') and of quebracho (*Schinopsis quebracho-colorado*). These extracts are produced in large amounts in South America for the industrial tanning of leather and are used in tannin research worldwide. In addition to these two sources, leaves from tropical shrubs rich in tannins (sometimes up to 40% of dry matter) have also been investigated and used as feeds in ruminant diets in the regions where these shrubs are grown as forage. Among them, the most widespread is *Leucaena leucocephala*, which has exceptional growth performance under good cultivation conditions (Tiemann et al., 2009b). Its high tannin concentration (similar to leaves from the related *Leucaena diversifolia*) does not seem to impair its feeding value as much as that of other tanniferous shrub species, like the leaves of *Calliandra calothyrsus* and *Flemingia macrophylla*. All of these plants are rich in CT and less so in HT. By-products from the food industry in arid and semi-arid countries, such as HT-rich pomegranate by-products, have recently also gained interest. Canul-Solis et al. (2020) and Nawab et al. (2020) specify further tropical feed resources rich in tannins, such as *Acacia nilotica*, *Acacia pennatula* and *Lespedeza cuneata*.

2.2 Tannin sources from temperate climates

Tannin sources originating from plants that only grow in tropical areas have to be imported when used in countries with a temperate climate. In these countries, the use of sources from on-farm cultivation would, therefore, be more sustainable. Tannin-rich (mainly CT) forages that can be grown on arable land under temperate climatic conditions include, in particular, the legumes sainfoin (*Onobrychis viciifolia*; Fig. 1) and birdsfoot trefoil (*Lotus corniculatus*), but big trefoil (*Lotus pedunculatus*) and sulla (*Hedysarum coronarium*) are also of interest. A number of herbs, among them small burnet (*Sanguisorba minor*), rosebay willow (*Epilobium angustifolium*) and wood avens (*Geum urbanum*), could also be future options as dietary tannin sources. Among the woody plants, leaves from hazel (*Corylus avellana*) and green grapevine (*Vitis vinifera*) are promising as dietary supplements, with hazel leaves containing both HT and CT. Other tannin sources from woody plants for ruminant nutrition, though not nutritious feeds, are HT-rich extracts from chestnut wood (*Castanea sativa*) and valonea oak (*Quercus valonea*). Interesting tanniferous pomaces from temperate climate plants that are by-products of the food industry include grape pomace.

Figure 1 Sainfoin (*Onobrychis viciifolia*), one of the most promising tanninferous forage plants growing under temperate climatic conditions. Photograph by Anna Scharenberg.

3 Effects of tannins in dairy cows

3.1 Effects on palatability, digestion and performance

The utility of tannins and tanniferous feeds in dairy cow nutrition depends on the presence or absence of adverse side effects of their dietary inclusion on performance. The key to maintaining high performance is a sufficient nutrient supply, which may be reduced by the low palatability of the diet and reduced digestibility of the nutrients. The majority of reports on the effects of tanniferous feeds point towards a lower or unchanged milk yield (e.g. Frutos et al., 2004).

Due to their astringent taste, tannins may substantially impair feed intake by reducing the palatability of the entire diet or part of the diet, forage or concentrate, that contains the tannins. An example of substantial intake decline was a diet supplemented with *C. calothyrsus* (Hindrichsen et al., 2004). To date, only a few studies have been carried out with dietary tanniferous supplements that have been specifically designed to test palatability, for instance, by offering choice situations (e.g. Terranova et al., 2020). Due to a lack of such studies, a decline in feed intake under both *ad libitum* and restricted feeding conditions is considered to be an indicator of reduced palatability. Attempts to avoid reduced nutrient supply include limiting dietary tannin concentration to below 5% and using palatable tannin sources, in addition to considering the nutritional value of the non-tannin constituents in these sources (Frutos et al., 2004). Adding molasses or other palatable supplements may also be useful. In addition, it is important to note that, when offered the choice, animals select their own diet and thus decide about the quantity of less palatable components, such as tanniferous supplements, they will ingest. For

instance, intake may be promoted when animals experience positive metabolic feedback, as is the case when combatting parasites or illness (Villalba et al., 2010). This points to situational responses in the palatability of tanniferous supplements. Accordingly, promising tanniferous supplements have to be tested for palatability under various conditions, including target animal species and production purpose (e.g. milk production by cows), dietary proportion and complementary dietary components.

Concerning digestion, inhibiting the growth of cellulolytic and proteolytic bacteria is known to be mediated via the formation of complexes of tannins and the bacterial cell wall (Bodas et al., 2012), resulting from their complex and large structures complete with side chains that have binding properties. However, the greater influence is probably due to the capacity of tannins to bind nutrients, making them inaccessible to microbes. For this reason, the tannin structure and side-chain composition determine the binding activity of tannins (Frutos et al., 2004; Tiemann et al., 2010a). At ruminal pH, binding by tannins is most pronounced with dietary protein, but nutrients such as fibre might also be bound or otherwise become inaccessible to rumen microbes (Vasta et al., 2019). Accordingly, Orlandi et al. (2015) found a substantially increased duodenal flux of amino acids in the presence of the *A. mearnsii* extract. The conditions in the lower gut, where pH substantially declines in the abomasum, might help cleave these bonds, but pH increases again in the small intestine. The extent to which protein–tannin complexes are actually cleaved in the lower gut determines how much metabolizable protein is generated from the amount of protein escaping ruminal degradation. Otherwise, only a shift of nitrogen (N) excretion from urine to faeces can be achieved, which is a goal in itself. Cortés et al. (2009) developed an *in vitro* model combining ruminal degradation with post-ruminal enzyme treatment and found highly variable post-ruminal soybean protein release levels when incubating the protein with pure CT extracts from tropical shrubs. This model was again applied by Tiemann et al. (2010b) who found effects of cultivation conditions of the shrubs on the level of post-ruminal protein release. Declines in ruminal protein degradability and increases in protein bypassing the ruminal degradation under simulated conditions of the lower gut were also found by Lavrenčič and Levart (2021) in the case of chestnut and quebracho tannins and by Abarghueia et al. (2021) with pomegranate peel, which indicates substantial cleaving activity. However, *in vivo* studies on the post-ruminal digestion of tanniferous diets are widely lacking, and there is no indirect evidence for a better metabolic protein supply of tanniferous diets, because no positive effects on milk N secretion or body N retention with tannin supplements have been reported (e.g. dairy cows: Grosse Brinkhaus et al., 2016; Gerlach et al., 2018; sheep: Hindrichsen et al., 2002, 2004; Carulla et al., 2005; Tiemann et al., 2008b). An exception is Pathak et al.

(2017), where lambs fed tropical tanniferous feeds exhibited a clearly higher body N retention. Lower protein digestibility and higher ruminal escape of protein often compensate each other (Naumann et al., 2017). Both outcomes, more metabolisable protein or lower urine N losses or both, are only helpful with diets containing excessive rumen-degraded protein. In tropical countries where diets are very often limited in rumen-degraded protein, the protein and energy supply is further reduced by tanniferous supplements, even when provided as high-crude protein–high-tannin legume shrub leaves (Tiemann et al., 2008b). The tannins still limit the amount of rumen-degraded protein, and a deficiency in degraded protein is particularly detrimental for fibre-degrading microbes. This hampers the protein supply from microbial matter and energy supply from volatile fatty acids.

The class of tannins gets important when provided at higher doses as, compared with CT, HT or its breakdown products may be absorbed to some extent and could be toxic (Reed, 1995). However, some bacteria are able to degrade HT not only to tannic acid but also to volatile fatty acids in further steps with the help of tannase (Bhat et al., 1998; Vasta et al., 2019); thus, the risk of toxicity could be lower than that assumed by Reed (1995). Different from the general assumption, Bhat et al. (1998) claimed that even CT might be degradable by certain microbes to a limited extent.

Overall, it seems that there are a few tannin sources that are highly palatable and have no or very low adverse effects on performance. Independent of the actual effects of tannins, their lack of (in tannin extracts), or low supply with, energy (in tanniferous plants) has to be balanced by high-energy ingredients when feeding tannins to high-yielding ruminants.

3.2 Use of tannins to mitigate environmentally relevant emissions

The two most important environmental concerns of dairy husbandry are emissions of N (as ammonia, nitrous oxides and nitrate) from manure and methane from the cow and its manure. In experimental research, N and methane emissions from the manure of tannin-fed cows are rarely measured. Indicators of potential N emissions from manure are the excretion of easily volatilised N from urine and, earlier in the digestive system, ruminal ammonia formation as a precursor of urine N. The milk urea content is another useful indicator of the N-emitting potential of manure. The considerations in Section 3.2.1 concerning N emissions mainly rely on these indicator traits, but the available experimental evidence on actual N emissions from manure is also described.

Tannins represent the most well-researched class of secondary plant compounds concerning urine N and methane mitigation. They are among the few dietary supplements that present a win–win situation in terms of mitigating

both types of emission simultaneously. An important, but not always reported, measure of the efficiency of tannins in mitigation is the emission intensity per unit of milk (protein). The efficiency may be small when tannins would impair feed intake and digestibility, and more animals are needed to secure food demand, ultimately leading to constant or even higher, rather than lower, system emissions.

3.2.1 Nitrogen emissions

The mode of action of tannins in reducing ammonia formation in the rumen has two components. The most important component is the binding of dietary protein, making it inaccessible to ruminal degradation; the other is the direct adverse effect of tannins against (certain) rumen microbes, as described in Section 3.1.

There is abundant evidence for the efficiency of various tannin sources to reduce the predicted N emission potential of manure, and one well-investigated source is the *A. mearnsii* extract. Carulla et al. (2005) fed 4% of this extract in the diet dry matter to growing sheep, which reduced urine N losses from 60% to 52% of N intake. In the study of Orlandi et al. (2015), feeding 0%, 0.9%, 1.8% and 2.7% *A. mearnsii* extract reduced urine N losses in growing steers from 67% to 46% of N intake. In these two studies, carried out with growing ruminants, the proportion of dietary N lost with urine was high compared with that expected from dairy cows. The urine N loss was reduced from 39% to 26% and 22% of N intake with 1% and 2% *A. mearnsii* tannins in the dairy cow study of Grainger et al. (2009). Gerlach et al. (2018) found no clear reduction of urinary N with 3% *A. mearnsii* extract fed to dairy cows despite trends towards a lower milk urea content. Combining quebracho tannins with chestnut tannins (0.75% each) was more efficient in reducing urine N (from 55 g/day to 43 g/day; an estimated decline from 26% to 20% of N intake) than supplementing 1.5% chestnut tannins alone (down to 51 g/day; 24% of N intake) (Aboagye et al., 2018), indicating the greater efficiency of quebracho tannins compared to chestnut tannins. There are also several *in vivo* studies investigating the effect of temperate climate forage legumes on potential N emissions. In the study by Grosse Brinkhaus et al. (2016), urine N excretion of dairy cows declined with 16% sainfoin in the diet from 21% to 18% of N intake but not with the same amount of birdsfoot trefoil, which may be explained by the 10-fold higher concentration of tannins analysed in the sainfoin compared to the birdsfoot trefoil. In the study by Huyen et al. (2016), a diet containing 30% sainfoin did not affect urine N excretion in dairy cows. In beef heifers fed exclusively sainfoin as a diet, absolute urine N losses decreased, but those relative to N intake were less clearly affected (Chung et al., 2013), illustrating the limited potential of temperate climate forage legumes in mitigating N emissions. A recent *in vitro* screening identified

several particularly promising temperate climate shrub and herb species that effectively mitigated ruminal ammonia formation (Terranova et al., 2018), of which four turned out to be highly palatable to dairy cows, namely, the leaves from hazel and green grapevine and the herbs rosebay willow and wood avens (Terranova et al., 2020). Hazel leaves were particularly efficient in reducing the urine N proportion of N intake, decreasing from 30% to 15% in sheep (Wang et al., 2018) and from 50% to 15% in dairy cows (Terranova et al., 2021) fed diets containing up to 50% hazel leaves. Part of the reduction may have resulted from certain concomitant declines in dietary N content. As in the study of Terranova et al. (2021) the milk yield of mid-to-late lactating cows remained unchanged, this mitigating effect also included urine N emission intensity. It should be noted that the maximum levels of hazel leaves tested were in a range not widely applicable in farm practice. Different from hazel leaves, vine leaves unexpectedly did not mitigate urine N proportion of intake in dairy cows (Birkinshaw et al., 2022). A certain but low efficiency in reducing urine N excretion was found for relatively low levels of chestnut extract by Wischer et al. (2014) as well as Aboagye et al. (2018 and 2019) and for valonea oak extract (Wischer et al. 2014). Highly tanniferous leaves of tropical plants also seem to be less efficient than hazel leaves in shifting N excretion from urine to faeces. For instance, *L. diversifolia* increased faecal N in sheep, but a decrease in urine N excretion was only found in one study (Hindrichsen et al., 2004) and not in the other (Hindrichsen et al., 2002). Similarly, *C. calothyrsus* and *F. macrophylla* enhanced faecal N excretion, but the reduction in urine N was not significant though numerically present (Tiemann et al., 2008b).

As outlined above, the proof of principle requires the actual measurement of N emissions from manure, which is not necessarily associated with urine N losses in a linear manner. Tannins may mitigate N emission from manure via two mechanisms. One is reducing the proportion of highly volatile urine N (as outlined above); the second is a direct effect of the tannins remaining undigested and therefore being present in manure. Indeed, in recently it was reported that adding 4% quebracho tannins or chestnut tannins directly to manure reduced nitrous oxide emissions (Min et al., 2022). This indicates that tannins act in a similar manner against microbial protein degradation in manure as they do in the rumen. However, the use of tannins in the diet of animals, other than adding them directly to manure, may also provide extra substrates, especially protein protected from digestion by tannins, which might get available to manure microbes and thus might promote N emissions. The few studies directly investigating N emissions from the manure of tannin-supplemented animals suggest that an effect either is dose dependent or may need time to be exhibited or both. In lambs, Śliwiński et al. (2002) found no effect of 0.1% and 0.2% of chestnut wood extract in the diet on urine N losses and ammonia emission from manure within 2 weeks of storage. In contrast,

Śliwiński et al. (2004) noted that gaseous N losses from the manure of cows fed a diet containing 0.5% of the same extract were reduced by half within 8 weeks of storage even though urine N losses had not been significantly lowered. An amount of 2.5% *A. mearnsii* extract in the diet of beef cattle had no clear effect on ammonia emission from manure in the study of Koenig et al. (2018), but the method used (horizontal flux technique with passive ammonia samplers from a few pens) might have added uncertainty to the measurement. It may also be important that, when inhibiting ammonia formation in manure, undigested tannins are likely to also impair the fertiliser value of manure. This was investigated by Tiemann et al. (2009a) feeding 15% and 30% of high-CT leaves of tropical shrubs (*C. calothyrsus* and *F. macrophylla*) in the diet to sheep. No clear effect on the N fertiliser value was found, but the soil used was probably not first limited by N as stated by the authors. Therefore, further studies are needed to clarify the conditions under which tannins excreted by cows are active in manure and may affect the fertiliser value of manure. It is also unclear if there is a difference in the efficacy between HT (chestnut) and CT. Hao et al. (2011) found no effect of a diet with 2.5% *A. mearnsii* tannins on nitrous oxide emissions during the composting of manure of growing cattle. By contrast, 15% of HT-containing pomegranate peel per kilogram of the diet of sheep reduced nitrous oxide emissions from manure (Yurtseven et al., 2018), which was estimated to be equivalent to about 1.5% HT when considering the values for pomegranate pomace made by Giller et al. (2022). Another approach was chosen by Duval et al. (2016), who measured total on-farm emissions when dairy cows were fed diets containing 0.45% and 1.8% of a 2:1 mix of quebracho and chestnut tannins. They found that emissions of ammonia and nitrous oxide were numerically lower per unit of milk than without tannin supplementation. The authors of one study (Clemensen et al., 2019) went even further and investigated whether grazing of a tannin-containing forage (sainfoin) influences soil N turnover and reported a reduction of soil nitrate concentration, pointing towards a mitigating effect of tannins against N leaching.

The experimental evidence illustrates that the most reliable environmental effect of tannins consists of mitigating urine N emissions and, thus ultimately, N emissions as ammonia, nitrous oxide and nitrate from their excreta during excretion, storage and distribution on the field.

3.2.2 Methane emissions

The mode of action of a tannin-caused mitigation of enteric (and manure-derived) methane has two components. One is the binding activity of the tannins, which also includes a reduction in ruminal fibre degradation (see Section 3.1). This phenomenon will also decrease the metabolic energy supply to the animals and thus only reduce absolute emissions and probably not

contribute to the more important mitigation of emission intensity. The second component is a direct action of tannins against ruminal microbes, which is explained by binding to microbes (especially by CT) as well as bacteriostatic effects or direct effects against methanogens (especially by HT) (Bodas et al., 2012; Aboagye and Beauchemin, 2019). Vasta et al. (2019) reported that the expenditure of hydrogen associated with ruminal degradation of HT to volatile fatty acids might provide another way of explaining the effect of HT against methanogenesis, but this offers no explanation for the frequently reported CT effects and seems to be too small in magnitude as a sole explanation.

Tannins appear to have an underlying general effect against methanogenesis. A meta-analysis demonstrated a clear dose–response relationship between tannin concentration and the level of *in vivo* methane mitigation (Jayanegara et al., 2012a). In addition to the general effect, there are clear differences between tannin sources in mitigation efficiency, with the majority of tannin sources remaining limited in their efficacy of methane mitigation. Among the sources currently investigated *in vivo*, the *A. mearnsii* extract is the most well researched and is one of the most efficient. This extract was found to be methane mitigating in sheep, growing cattle and dairy cows (Carulla et al., 2005; Grainger et al., 2009; Staerfl et al., 2012; Denninger et al., 2020). The level of methane mitigation obtained by 3% of the *A. mearnsii* extract ranges from 10% to 30%. Although rarely adversely affecting feed intake, digestion and performance, there is a tendency for lower values with *A. mearnsii*, and therefore, the efficiency to reduce methane emission intensity per kilogram of milk might be slightly lower than that of absolute emission. Less reliable than *A. mearnsii* in terms of methane mitigation in the animal, despite ample *in vitro* evidence, are extracts of chestnut (e.g. Śliwiński et al., 2002; Wischer et al., 2014; Aboagye et al., 2019), quebracho (e.g. Beauchemin et al., 2007; Aboagye et al., 2018) and valonia oak (Wischer et al., 2014). Leaves of the tropical shrubs *C. calothyrsus* and *F. macrophylla*, which are extremely rich in tannins, fed in dietary proportions of 30% reduced absolute methane emission by 20–25%, but not per unit of digested organic matter, an indicator of emission intensity (Tiemann et al., 2008b). This means that the decline in methane simply followed the lower level of available fermentable organic matter. Differing to these shrubs, beef cattle fed *L. leucocephala* emitted less methane and exhibited increased growth under subtropical and tropical conditions when pastures were enriched with this shrub (Harrison et al., 2015). For that study, open path lasers were applied to estimate methane emissions at herd scale in grazing cattle. Controlled *in vivo* studies to confirm the methane-suppressing activity of *L. leucocephala* are still necessary. In addition, other tanniferous parts of tropical plants could be of interest. As an example extract of *Terminalia chebula* seeds was reported to slightly reduce sheep methane emissions in the study by Patra et al. (2011), but *in vitro* results indicated a 90%

reduction of methane formation. Some tanniferous temperate climate forage legumes were shown to have potential in mitigating methane emission. After demonstrating this for big trefoil (Woodward et al., 2001) and sulla (Waghorn et al., 2002) in sheep, New Zealand researchers were probably the first to show a methane-declining effect of a tanniferous forage legume (birdsfoot trefoil) in dairy cows (Woodward et al., 2004). Huyen et al. (2016) described a similar effect of sainfoin in dairy cows. In both cases, methane yield per unit of feed intake was significantly reduced; however, the effects were minimal and Chung et al. (2013) did not find clear sainfoin effects in beef heifers. Small burnet (*Sanguisorba minor*) was found by Stewart et al. (2019) to be more efficient in mitigating methane emission in cows and heifers than sainfoin and birdsfoot trefoil, but digestibility was also impaired. Substantial methane-mitigating activity of other tanniferous herbs such as wood avens and rosebay willow which were effective *in vitro* (Terranova et al., 2020), may be possible in live animals. Effective woody plants from temperate climates include the leaves from hazel and green grapevine which are highly palatable (Terranova et al., 2020). Hazel leaves reduced methane emission in sheep by up to 35%; this high level of mitigation was achieved with a dietary hazel proportion of 50% (Wang et al., 2018). Lower dosages of hazel leaves, which are easier to implement in farm practice, were also effective, and in dairy cattle a close dose–response relationship was recently described (Terranova et al., 2021). Grapevine leaves, provided at 13% of dietary dry matter, reduced methane emission intensity per unit of milk yield by 12% in dairy cows (Birkinshaw et al., 2022). Grape pomace (marc) was found to be efficient in reducing methane emissions by 20% when added to the diet of low-yielding dairy cows at 25% (Moate et al., 2014), a level probably not applicable to diets of high-yielding cows. Caetano et al. (2019) found a 14% reduction in daily methane production with this feed, but emission intensity was probably less clearly mitigated due to the low energy value of the pomace. The use of pomegranate pomace to mitigate methane, as demonstrated *in vitro*, still awaits confirmation *in vivo* (Giller et al., 2022).

Concerning methane emission from manure, the same aspects are valid as for N emissions, namely that undigested tannins may inhibit methanogens whereas any extra undigested fibre might provide additional substrates for manure methanogens. The first was demonstrated by adding 4% quebracho tannins directly to manure, which caused a reduction in methane emissions, whereas 4% chestnut tannins remained ineffective (Min et al., 2022); however, the second component, extra fibre, also appears to be important. Indeed, the results to date would indicate a low (net) efficiency of fed tannins, if any, against methane formation in manure. Accordingly, including 2.5% *A. mearnsii* in the diet of growing cattle did not affect methane emissions during the composting of manure (Hao et al., 2011). Furthermore, adding 15% of pomegranate peel to the diet did not affect the methane emission from sheep manure (Yurtseven

et al., 2018). In this context, it is fortunate that most methane is formed in the rumen of the animal. Therefore, the absence or low tannin effects in manure are less detrimental than for N emissions, where tannin effects are restricted to those in manure. Accordingly, in the study of Staerfl et al. (2012), 3% of *A. mearnsii* tannin extract in the diet unexpectedly caused a non-significant increase in slurry-derived methane (+14% and +39% at storage temperatures of 14°C and 27°C, respectively). However, total system methane emissions (enteric and manure derived) were still substantially mitigated by tannin supplementation in that study. Supplementing 1.5% and 2.5% of CT from an *A. mimosa* extract mitigated absolute methane emission in adult cattle, but not methane yield per kilogram feed intake, despite a reduction of archaeal counts, and did not influence methane formation in biogas produced from their manure (Fagundes et al., 2020). The total on-farm methane emissions of dairy cows fed diets with 0.45% and 1.8% of a 2:1 mix of quebracho and chestnut tannins in the study of Duval et al. (2016) also numerically declined per unit of milk, like those of N emissions. It should be noted that the observed general lack of a clear methane-mitigating effect in the manure of tannin-fed cows would be beneficial when manure is used for biogas production.

In conclusion, there appears to be an underlying general tannin effect against methanogenesis, but only a few tannin sources exert substantial mitigating effects *in vivo*. It is, therefore, not possible to predict the efficiency of not well-researched sources simply by analogy.

3.3 Effects of tannins on milk quality

Tannins do not have a substantial effect on the gross composition of milk (e.g. Śliwiński et al., 2004; Woodward et al., 2004; Grainger et al., 2009; Huyen et al., 2016; Gerlach et al., 2018; Terranova et al., 2020, 2021). This would be different if tannins severely affected protein supply and/or the profile of volatile fatty acids. Then, changes in milk protein and fat content could not be excluded, but this would represent an indirect and no direct effect of tannins on rumen microbes.

3.3.1 Effects on ruminal biohydrogenation and milk fatty acid composition

Unlike the gross composition of milk, the fatty acid composition of milk fat can be substantially influenced by tannin supplementation (e.g. Buccioni et al., 2015; Morales and Ungerfeld, 2015; Girard et al., 2016a; Grosse Brinkhaus et al., 2016). Many microbial species are involved in the ruminal biohydrogenation of polyunsaturated fatty acids, such as linoleic acid and α-linolenic acid, but *Butyrivibrio fibrisolvens* is particularly important and

can be inhibited by tannins (Min and Solaiman, 2018). Tannins alter fatty acid composition by protecting distinct fatty acids, considered beneficial for human health, from ruminal biohydrogenation, which promotes their transfer to milk (Patra and Saxena, 2011). In addition, even an only partial biohydrogenation of fatty acids in the presence of tannins provides valuable intermediates such as C18:1 *trans*-11 and C18:2 *cis*-9, *trans*-11 (the latter being a conjugated linoleic acid and often generated from C18:1 *trans*-11 in the mammary gland). This effect again seems to depend on tannin source. For instance, Buccioni et al. (2015) found such effects using a quebracho extract but not with a chestnut extract. The *A. mearnsii* extract (Khiaosa-ard et al., 2009) and quebracho tannins (Vasta et al., 2010) were both shown to partially specifically inhibit the last step of ruminal biohydrogenation, whereas tanniferous sainfoin remained ineffective in this respect (Khiaosa-ard et al., 2009). It was, therefore, unexpected that the *A. mearnsii* extract did not change the fatty acid composition of the perirenal adipose tissue of beef cattle (Staerfl et al., 2011), whereas that of the intramuscular fat of lamb meat was observed to be richer in *n*-3 fatty acids when using sainfoin (Girard et al., 2016b). The same was found for sainfoin in dairy cows, where a 17% increase of the proportion of α-linolenic acid was recorded in both milk and cheese lipids when feeding sainfoin (Girard et al., 2016a). With birdsfoot trefoil, the increase in the proportion of α-linolenic acid was only 3%. *In vitro*, α-linolenic acid biohydrogenation was reduced with sainfoin, but it remained unclear if this was an effect of lower nutrient degradation or a tannin effect on ruminal biohydrogenation (Khiaosa-ard et al., 2009). Feeding vine leaves resulted in a trend towards higher proportions of C18:2 *cis*-9, *trans*-11 and α-linolenic acid in the milk fat of dairy cows (Birkinshaw et al., 2022). Overall, the results in terms of tannin effects on the fatty acids are highly variable and predictions are difficult, as many factors influence the outcome, including tannin dosage and source, other dietary ingredients, metabolic function of the body lipids and, possibly, animal species. Further studies are required to quantify the importance of the different factors (Vasta et al., 2019).

Accordingly, research evidence to date points towards a specific property of tannins that affects the fatty acid profile of foods from ruminants via their influence on ruminal biohydrogenation, but the effects are variable in terms of which fatty acids are influenced and they not very pronounced.

3.3.2 Transfer of tannins to milk

It would be of interest if bioactive substances such as tannins could also be transferred to human food, i.e. ruminant milk; however, whether or not this is beneficial needs to be clarified in individual cases. As CT are considered to be

widely indigestible, no substantial transfer would be expected, unless there is a not yet quantified partial degradation of CT molecules in the gut with some monomers being absorbed. A higher potential for transfer is given with HT, where the possibility of absorption (probably as breakdown products such as tannic acid) is demonstrated by their potential toxicity (Section 3.1). Studies specifically investigating the transfer of tannins into milk are scarce. Leparmarai et al. (2021) followed the recovery of total phenols from the diet in the milk of cattle and camels grazing tropical wood pastures with accessibility of the animals to various tanniferous shrub and tree leaves; elevated levels of phenols were indeed detected in milk. The transfer was, however, extremely low with around 0.1% of intake in cattle and not related to intake in camels. Camels seem to have developed coping mechanisms against tannins such as specific salivary binding properties. Feeding tanniferous hazel leaves (containing both CT and HT) to sheep resulted in an increased total antioxidant capacity of blood plasma which pointed towards the absorption of bioactive phenols, but unexpectedly total phenol concentrations in plasma were not elevated (Wang et al., 2019). In addition, hazel leaves did not significantly elevate the milk phenol concentration of dairy cows, whereas the tanniferous wood avens did but only by a small magnitude (Birkinshaw et al., 2020). Certain non-tannin phenols, like those from grapeseed extract, may be more efficiently transferred into milk as was shown for the milk of goats and sheep, but even in that case most phenols were excreted via urine (Leparmarai et al., 2019). Overall, limited data suggest that there is no great potential of tannins to beneficially elevate phenol concentrations of milk.

3.4 Anthelmintic properties of tannins

Intestinal parasites like nematodes mainly occur in small ruminants, but there are reports by veterinarians regarding an increasing prevalence in cattle, especially when grazing highly infected pastures. Anthelmintics are chemicals to which resistance is becoming more frequent (Barone et al., 2019) and, therefore, should be replaced by natural effective ingredients. Various studies point towards an anthelmintic activity of tannins and feeds rich in tannins. *In vivo* studies targeting infections with *Haemonchus contortus*, as a major nematode species, included the forage legumes birdsfoot trefoil (Lonngren et al., 2020) and sainfoin (Heckendorn et al., 2006, 2007; Azuhnwi et al., 2013b) as well as *Mimosa caesalpiniifolia* leaves (Brito et al., 2018) and *A. mearnsii* extract (Lima et al., 2019). Sulla is also among the tanniferous plants known for their antiparasitic properties (Ramírez-Restrepo and Barry, 2005). The structure of tannin plays an important role in antiparasitic function (Mueller-Harvey et al., 2019). Apart from varying efficacy among cultivars (Barone et al., 2019)

and sometimes a low *in vivo* recovery of *in vitro* results (e.g. Lonngren et al., 2020), in general, the complete extinction of nematode activity is unachievable. However, slowing down the life cycle may still be an important element of an on-farm strategy to combat nematode infections.

4 Factors influencing the effects of tannins in animals

4.1 Variability among plants and cultivars as well as within cultivars

One of the difficulties in recovering the effects of tannins on ruminants reported in previous studies is due to their variability, which concerns both the concentration and composition of the very complex tannin polymers. It is generally accepted that different plant species differ in their tannin properties as they may also vary in the proportions of CT and HT; cultivars may also differ in this respect [shown for birdsfoot trefoil by Barone et al. (2019) and for sainfoin by Azuhnwi et al. (2013a)]. This phenomenon got more even apparent from recent research triggered by the regained interest on temperate climate legumes for their usefulness as forage, with plant breeders searching for the most appropriate cultivars (Mueller-Harvey et al., 2019). Also the difficulty in efforts to reintroduce higher tannin levels in high-performance forage legumes (Kelln et al., 2021) emphasised this phenomenon. Therefore, expectations of reliable effects have concentrated on the same cultivar; however, environmental conditions during cultivation remain a major source of variability (Frutos et al., 2004; Aboagye and Beauchemin, 2019). This was experimentally demonstrated for the leaves of tropical shrubs where the same cultivar was grown in different countries (Hess et al., 2006) and on different soils with or without drought conditions and with or without mineral fertilisation (Tiemann et al., 2009b, 2010a,b). These studies revealed a repeatable pattern showing that tannin concentrations decline with improved plant growing conditions. It cannot be excluded that this is simply the consequence of a dilution of the tannins by non-tannin plant matter promoted by better environmental conditions. In addition, decreased environmental stress may lower the need to maintain the associated defence mechanisms. Even with this knowledge, it is difficult to predict tannin concentration and composition under defined cultivation conditions. Such differences in growing conditions may also affect the adoption of tanniferous plants by farmers. For instance, in Colombia, *C. calothyrsus* is considered to be low-quality forage, whereas it is called 'dairy shrub' in parts of Kenya, with this indigenous knowledge being confirmed by corresponding differences in quality found when obtaining the same cultivar of *C. calothyrsus* from the two countries (Hess et al., 2006). Another form of variability should also not be underestimated. Minimal concentrations seem to be needed to obtain

noticeable bioactivity, as concluded from the meta-analysis by Jayanegara et al. (2012a) where extreme variability in the antimethanogenic effects of tannins was found across *in vivo* studies at tannin concentrations below 2%, whereas results from studies with higher dosages were much nearer to the line of regression. For cost reasons, recommendations for the supply of commercial tannin supplements are often below this threshold, explaining the variable and inconsistent effects.

4.2 Importance of analytical and experimental techniques

The variability of tannin supplement effects is also caused by analytical limitations. Naumann et al. (2017) consider tannin diversity and analytical deficits as the main cause for minimal progress in knowledge regarding tannin–animal interactions. In particular, limitations concern the determination of structural properties such as molecular weight and subunit structure, which could be crucial for a tannin effect to be exhibited (Aboagye and Beauchemin, 2019; Mueller-Harvey et al., 2019), but even tannin concentrations have substantial analytical uncertainties. The standard method is outlined in the laboratory manual of Makkar (2003a), according to which the analysis of total tannins is performed by the Folin–Ciocalteu method and CT are analysed using the butanol–hydrochloric acid–iron method, sometimes complemented by distinguishing between free and bound tannins/CT. The HT are estimated from the difference between total tannins and CT, where the commonly different tannin standards used for total tannins and CT add to the already high uncertainty. The tannin standards used in these analyses may not even exist in the tannin supplement analysed. The limitations of this simple analysis are outlined by Ramsay et al. (2015). Few researchers create their own standards from the test plant species or cultivar via time-consuming extraction (Piluzza et al. 2013), as realised by Azuhnwi et al. (2013a). This would substantially improve the accuracy of quantitative values. Although considerable progress in analytical methods has been made (Ramsay et al., 2015; Mueller-Harvey et al., 2019), methods for tannin structural analysis are rarely applied [such as differentiation for delphinidin, prodelphinidin, cyanidin, procyanidin and pelargonidin (Tiemann et al., 2010a; Azuhnwi et al., 2013a)], and difficulty remains in predicting which structure will promote the desired effects of tannins in the animal or which have adverse effects. The bioactivity of tannins may, however, be assessed by other methods both in the animal and *in vitro*. The most widespread approach is to add polyethylene glycol (PEG) to diets (inert to the animal) containing tannin supplements, because it has been known since the 1970s that this compound widely inactivates tannins (Makkar 2003b). Tiemann et al. (2008a) reviewed issues associated with the PEG approach

including that the amount of PEG needed for neutralisation depends on tannin type (plant species) and should probably exceed a ratio of 1:1 of PEG:tannins. In this context, it remains unclear if the efficacy of PEG to inactivate tannins is different for purified tannins and tannin extracts compared with tannins still embedded in the plant matrix where they may have already formed bonds with protein. As tannins replace other nutrients in plant matter and their synthesis and deposition in the plant might be associated with changes in nutritional quality of the non-tannin matter, it remains unclear whether effects are (exclusively) from tannins or not. Tiemann et al. (2008c), therefore, developed a method to purify the fibre of tanniferous leaves of tropical shrubs and was able to separate fibre and tannin effects with this approach. Another laboratory-based indicator of tannin bioactivity (especially CT) is astringency, a measure of the protein-binding capacity of tannins (Tiemann et al., 2010a).

4.3 Verification of tannin effects on farms

The results to date suggest that the implementation of supplements of certain tannin sources on dairy farms is timely. The question remains, how can the effects of tannins be confirmed and controlled? A Swiss group of researchers identified and tested a set of variables to be quantified or sampled for analysis on farm and attempted to confirm their applicability with diets supplemented either with a chestnut tannin extract (Kapp-Bitter et al., 2020) or with sainfoin (Kapp-Bitter et al., 2021a). The focus was on N emission-related variables. Here, a particularly easily accessible variable is the routinely assessed milk urea content. Another approach is based on the analysis of faeces and urine spot samples using indicators for total faeces and urine volumes and N concentrations. Changes in milk protein content in cows fed tannin supplements would indicate variations in the supply of metabolic protein, resulting from concomitant changes in fermentable organic matter. Confirmation of the applicability of these indicators is still awaited as the variables investigated did not clearly respond to the two sources of tannins tested (Kapp-Bitter et al., 2020, 2021a); conversely, a decline in palatability was clearly obvious on farm (Kapp-Bitter et al., 2020). Milk is a type of matrix where samples are easily obtained on farm, allowing tannin effects on fat composition to be determined via gas chromatography.

5   Onset of tannin effects and long-term efficiency

An important aspect concerning the efficiency of tannin supplementation is the duration of the delay until effects begin and, more importantly, whether or not rumen microbes adapt with time to the tannins with time thus counteracting

their beneficial effects. Indeed, although tannins may inhibit the growth of many microbes, Bhat et al. (1998) outlined that there are also species (especially from the Streptococcus group; Patra and Saxena, 2011) that are resistant to tannins or have developed such resistance when exposed to tannins, mainly by degrading tannins. Smith et al. (2005) described several other resistance-enhancing mechanisms which bacteria might develop including the secretion of exo-polysaccharides forming a protective layer around the cell wall, or modification of the cell wall, or modes of dissociation of tannin–substrate complexes. If these resistant microbes represent key niches in the rumen and develop their ability under constant exposure to tannins, the tannin effects might be short-lived; hence, there are considerable concerns regarding the transiency of tannin effects. Studies are rare for both questions, i.e. the time for onset and long-term efficiency. It was recently shown that the effect of tannins seems to occur very rapidly, with only 20 minutes needed for significant methane mitigation to be exhibited when feeding 3% of an *A. mearnsii* tannin extract to dairy cows (Denninger et al., 2020). Birkinshaw et al. (2020; testing various shrubs and herbs) and Denninger et al. (2020; testing *A. mearnsii* extract) investigated how quickly tannin causes changes in the fatty acid composition of milk. They found modifications of the fatty acid profile after 3 days, but this included some fatty acids probably not affected in the longer term, and it seems that the key fatty acids of ruminal biohydrogenation were less or differently affected than would be expected with ongoing tannin supplementation. Concerning long-term efficiency, a breakthrough was the demonstration by Staerfl et al. (2012) of the sustained efficiency of the *A. mearnsii* extract at 3% in the diet of fattening bulls across more than 6 months (Fig. 2), a phenomenon that awaits confirmation for most other tannin sources. Wischer et al. (2014) also investigated the long-term
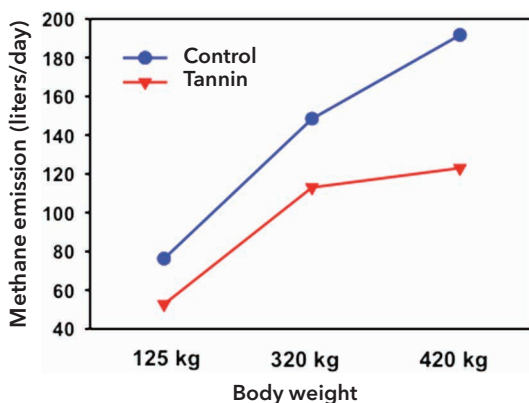
Figure 2 Long-term effect of *Acacia mearnsii* tannins against methane emission found in growing Limousin crossbred bulls. Adapted from Staerfl et al. (2012).

effects of tannins (chestnut and valonea extracts) in dairy cows. The shift from faecal to urinary N was still present 6 months after start of feeding at a level similar to that measured first approximately after 1 month of feeding. They also followed methane emission, but the tannin sources used had no effect on methane. The trend for lower total on-farm methane emissions in dairy cows fed a mix of quebracho and chestnut tannin extracts found by Duval et al. (2016) after 1.5 months of feeding was also recovered after 3 months of supplementation. Therefore, a limited number of studies suggest that, despite mechanisms of adaptation by ruminal microbes, tannin effects seem to be quite long-lasting in ruminants.

6   Strategies to maximise beneficial tannin effects

6.1 Optimal tannin dosage

The optimal dosage is one where the desired effects are achieved and adverse effects on performance and factors determining performance such as palatability, digestion and metabolic side effects are absent or clearly outweighed by the beneficial effects. However, this dosage is very difficult to define and often cannot be generalised as it not only depends on the basic effects of the respective tannins but also on the matrix they are embedded in and on other feed ingredients counteracting or promoting the tannin effects. Furthermore, the optimal dosage also depends on costs and cost-effectiveness, which is increased when, for instance, compensatory payments can be obtained balancing extra costs for supplements or performance declines or both. If tannin sources are homegrown, the farmer can determine the optimal and most cost-effective dosage. The thresholds of 2% tannins of dietary dry matter as minimal dosages for clear effects and 5% specified for the maximal dosage where no adverse effects are expected can probably not be generalised and should be determined separately for each tannin source.

6.2 Optimal form of tannin supplementation

Another decision to be made for farm practice is the form in which tannins should be supplemented. Basically, supplementation could consist of pure or highly purified compounds, extracts where tannins are enriched or entire tanniferous plants (or the respective tannin-rich plant parts). Pure compounds would offer the best repeatable effects, but they are too expensive to be applied on farm and costs are prohibitive even for experimental *in vivo* research. However, they are very helpful in determining the basic efficiency and mechanistic background of the effects in the rumen. They may even point towards the most effective monomers within the complex and large tannin

molecules, the abundance of which could be a criterion for selecting efficient tannin sources. Such *in vitro* research has, for instance, been carried out with various pure compounds from a chemical supplier including tannic acid (Sinz et al., 2018). With highly purified tannic acid (purity: 95%) and gallic acid (purity: 99%), Aboagye et al. (2019) carried out a beef cattle study. An approach where pure compounds are used also offers the opportunity to quantify interactions among tannins or of tannins with other bioactive compounds in a way that remains unbiased by other factors. An important restriction of this approach is that the effects of pure compounds may differ from those of extracts or whole plants, where a number of bioactive substances in various concentrations may be present and the efficiency of the tannins will also depend on how easily they are liberated from the plant matrix. The latter, along with the often large natural variation in tannin concentration and composition described in Section 4.1, make extracts possibly the best alternative as the technical effort is limited, compared with the synthesis of pure compounds, and the variation is considerably reduced compared with unprocessed plants. In research, extracts also allow quantifying interactions among secondary plant compounds. For instance, Sinz et al. (2019a) determined the interactions between extracts from *A. mearnsii* and from grapeseed in growing lambs. Nevertheless, when grown on their own land, farmers would prefer to use unprocessed tanniferous plants.

6.3 Combination with other tanniferous or non-tannin supplements

Research is widely in its infancy concerning the effects of combinations of bioactive compounds in dairy cattle nutrition. These effects could be additive or even associative (i.e. non-linear) but also compensatory. As there may be more interactions with the composition of the basal diet, such research is very complicated. Examples of *in vivo* research for interactions, in particular, include the studies by Canadian researchers where interactions of HT and CT were closely followed, one with extracts rich in either HT or CT (Aboagye et al., 2018) and the other with pure compounds (Aboagye et al., 2019). Williams et al. (2020) reported that tannins and fats act additively against methane in dairy cows, pointing towards different modes of action against methanogens. Instead, if the mechanisms for an effect are quite similar, combining extracts could be of lower efficiency as, for instance, shown by Sinz et al. (2019b), for some extracts concerning *in vitro* methane and ammonia formation. Other research has concentrated on alleviating the potentially adverse effects of the typically low energy content of tanniferous plants, which hampers introduction in farm practice even when no direct adverse effects of tannins would occur. Jayanegara et al. (2013) detected
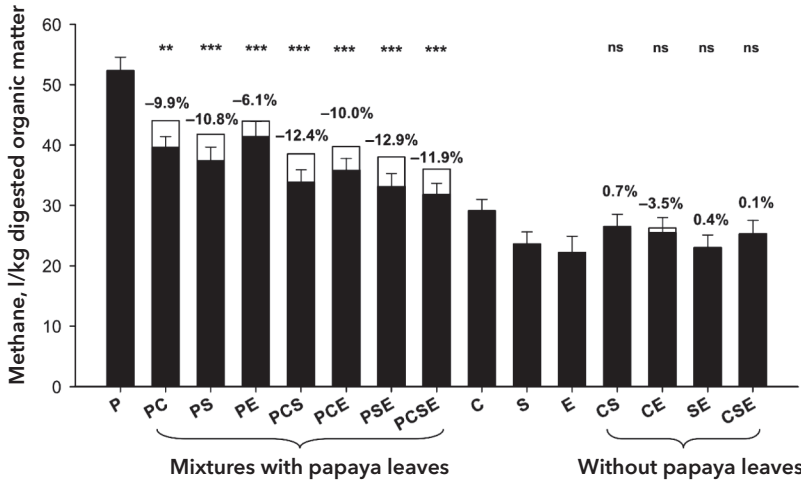
Figure 3 Positive associative effects of the combination of tanniferous tropical forage leaves (C, *Clidemia hirta*; S, *Swietenia mahagoni*; E, *Eugenia aquea*) and leaves from a high-quality forage (P, *Carica papaya*). The open parts of the columns illustrate the gap between methane emissions expected from the plants incubated alone (upper end) and that measured in the combinations (lower end of the open part). ***, significant at $P < 0.001$; **, significant at $P < 0.01$; n.s., not significant. Adapted from Jayanegara et al. (2013).

positive associative effects when combining various tanniferous forages with high-quality papaya leaves (Fig. 3). However, no corresponding positive associative effects were found when combining tanniferous hazel leaves with lucerne in dairy cow diets, where effects were linear or even of a negative associative nature (Terranova et al., 2021). All this points to considerable demand for further research.

7 Conclusion and future trends

This chapter demonstrates that feeding tannins to dairy cows can be strategically used to fulfil goals related to environmental sustainability and quality of the food produced. Several tannin sources have been intensively investigated for their effects, with respect to a number of aspects. Figure 4 illustrates the beneficial effects an optimal tannin supplementation to dairy cows should have and which supplements might be useful to reach the corresponding goals. The research evidence generated to date clearly shows that there is a delicate balance between avoiding adverse effects on performance and being able to profit from the beneficial effects. In addition, the presence and extent of beneficial effects is highly variable; therefore, considerations for the best trade-off are necessary. In this context, Grainger and Beauchemin (2011) asked the
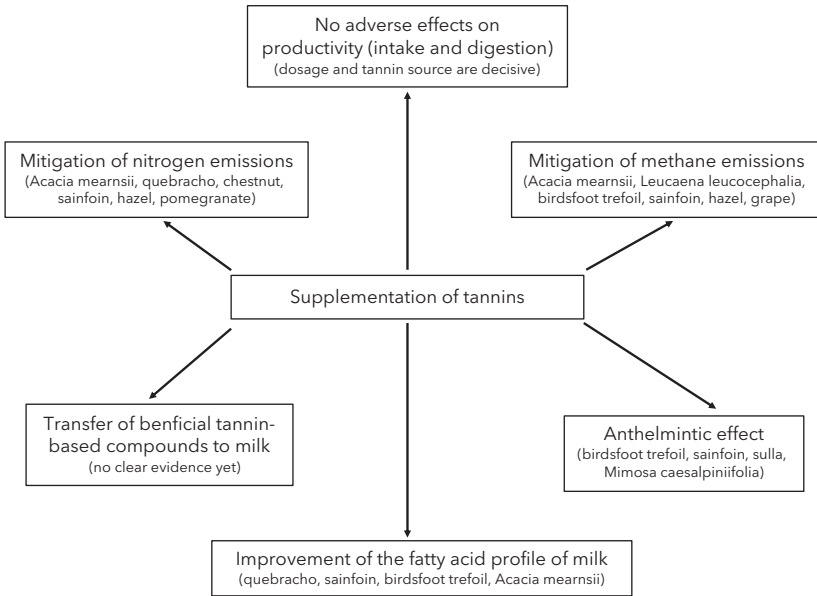
Figure 4 Anticipated effects of the optimal tannin supplementation strategy in dairy cows and tannin sources the use of which at least occasionally exhibits such effects *in vivo*.

question whether enteric methane emissions from ruminants can be mitigated at all without lowering their productivity and concluded that it is possible when certain boundary conditions are considered. An important issue is also the costs associated with feeding tannins. The economic result is influenced by the costs of purchasing the sources and by the indirect costs resulting from growing plants with lower yield and lower energetic value. To compensate for this, these plants either have to be combined with costly higher quality feeds or a lower income from a lower performance of the animals has to be accepted, or both. Growing the tannin sources on farm would be preferable, but only when the effect is as high as that of currently imported sources. Future political and food industrial developments such as compensation payments, fines and high-price labelling of foods produced with environmental and product quality benefits will also be decisive for the question of whether or not tannin supplementation becomes affordable and attractive to the individual farmer. Further research is needed in parallel to the promotion of on-farm implementation of tannin supplements. Large gaps in knowledge include proper tannin analytics and the relation of their structural properties to the recovery of desired tannin effects. This could lead to indicators that allow the quick determination (best *in vitro*) of the suitability of batches of tannin sources before their use on farm.

8   Where to look for further information

In 2006, Mueller-Harvey described the chemistry of the tannins and their role in animal nutrition in more detail than it was possible in this chapter (see: Unravelling the conundrum of tannins in animal nutrition and health, *J. Sci. Food Agric.* 86 (10), 2010–37). A recent article [Arndt et al. (2022). Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5°C target by 2030 but not 2050, *PNAS* 119, e2111294119] compares the efficiency of dietary measures, including the use of tannins, against methane emissions and their potential quantitative role in assisting to be able to meet the current climate change mitigation targets.

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