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DIETARY MODULATION OF RUMEN METABOLISM: A KEY FACTOR TO ENHANCING RUMINANT PRODUCTION [1, 2]

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Abstract

Owing to microbial hydrolysis of nutrients in the rumen, ruminants are relatively efficient animals for livestock production considering that they can utilize low-quality feeds including industrial by-products. On the other hand, however, they also contribute to global warming because of their methane emission from the enteric fermentation. The methane emission not only affects the environment but also represents the loss of consumed energy. Thus, mitigating enteric methane emission may improve animal production efficiency as well as contribute to alleviate the impact of ruminants on climate change. This review focuses on various achievements of the last years which have expanded our knowledge regarding mitigation of enteric methanogenesis and its potential contribution in improving cattle production efficiency. The article also discusses the role of nutrition and rumen metabolism on gut and host's health status as well as on enhancement of product quality of cattle products. The improvement of quality of ruminant products relies mainly on improving their fatty acids composition toward health-promoting polyunsaturated fatty acid profile such as omega-3 fatty acids and conjugated linolenic acids. Plant secondary compounds appear to possess multi-beneficial effects that when fed to ruminants can beneficially modify rumen metabolism, contributing to methane mitigation, gut health, food safety and quality. However, identification of active compounds for use as feed additives in ruminant production and understanding their mode of specific actions remain a big challenge for research.

Key words: rumen metabolism, enteric methane, feed efficiency, milk quality/

1. Introduction

It used to be much simpler: humans used to rear and feed ruminants to produce milk and meat, and less attention was paid to animal emissions or even the quality and safety of the products. This time is already gone and likely will never come again. During the recent years both emissions and food safety and quality have become an important issue. Ruminants are viewed as the major contributors of enteric methane emissions and political pressure to decrease both methane and other pollutant excrements from livestock is steadily increasing worldwide. In addition, intensive animal production systems often raise public criticism for their unfavorable effects on the environment, animal welfare, and food safety [1]. Moreover, consumers have negative perceptions of animal origin products, especially those of ruminants, in terms of health aspects because of their cholesterol and fat contents and proportionally high contents of saturated fatty acids. As a consequence, nowadays animal nutritionists and animal nutrition science in general have been faced to multiple and more complicated challenges for innovations to achieve sustainable improvement in animal productivity without negative consequences on animal health, and at the same time to improve safety and quality of the animal products. Achieving such goals is very challenging because there appears to be direct conflicts among them. For instance, high production level is negatively associated to health and fertility traits [2]. Further, potentially pathogenic bacteria harboring the gastrointestinal tract of animals and their toxins are regarded as a major issue of food safety. It appears that energy-rich feeding can maximize growth efficiency of cattle but this feeding strategy may induce inflammation [3], and increase shedding of pathogenic bacteria including *Escherichia coli* O157:H7 [4], being a major threat of food safety. This article emphasizes the improvement in cattle production, both past success and future trends, based on scientific findings and its considerable role in food security and food safety issues. Last but not least, this

[1] Work presented at BOKU Symposium Tierernährung in Vienna on April 19, 2012: "*Tierische Produktion als Schlüssel zur nachhaltigen Ernährungssicherheit am Beispiel Rind*, R. Khiaosa-ard, C. Iben, Q. Zebeli".

[2] This work is dedicated to Prof. Thanas Piu for his long-standing contribution to developing, teaching, and spreading the science of animal nutrition and production in Albania.

article concludes the critical role that feeding of ruminants plays on rumen health and metabolism, as well as on subsequent production efficiency, food quality and safety.

2. Lowering *E. coli* shedding to improve food safety

The intensive management systems of ruminants encourage dietary inclusion of large amounts of cereal grains or easily degradable by-products to support high milk yields or rapid weight gains [5]. Although these feeding practices seem to enhance production in a short-term, they do not cope with cattle's digestive physiology. The most important consequence thereof is the impaired ecosystem of the gastrointestinal tract with major consequences for gut and also systemic host health [5]. Apparently healthy animals suffering from sub-clinic and chronic disorders have lower production efficiency [5]. Moreover, a poor health status is not only responsible for greater susceptibility of other diseases, but also these animals have a higher likelihood to serve as a reservoir and spread pathogens. According to the estimation of Centers for Disease Control and Prevention (CDC), in 2009 over 100,000 illnesses were caused by Shiga toxin-producing *E. coli* (both O157 and non O157) in the United States [6]. In Europe 3,744 cases of Shiga toxin-producing *E. coli* O104:H4 have been reported within 2 months since the start of the outbreak in May 2011 [7]. Many pathogenic bacteria, such as *E. coli* O157:H7, are confined to the gastrointestinal tract of cattle, and the cattle remain non-symptomatic while shedding these pathogenic bacteria into the environment [8]. According to [9], approximately 30% of feedlot cattle shed *E. coli* O157:H7. Thus, for many years the focus on improving the safety of meat products has been laid on post slaughter managements, including antimicrobial treatment and HACCP in slaughter plants. However, the illness due to consumption of contaminated meat products still remains. As a consequence, recently there has been a great interest in finding strategies to reduce the pathogenic microbial population in living animal before slaughter. These pre-harvest intervention strategies include dietary and management strategies as well as probacterial and antipathogenic strategies [10].

Management practices can affect the *E. coli* O157:H7 shedding level in cattle. Cray et al. [11] showed that dietary stress led to an increase in *E. coli* O157:H7 shedding of calves. Interestingly, the diet-stressed calves were more susceptible to infection by *E. coli* O157:H7 than their well-fed counterparts. This is because fasting or starvation results in decreased concentration of gut volatile fatty acids (VFA) which can limit generation of *E. coli* O157:H7 [10]. Diet composition can affect *E. coli* shedding in cattle too. It appeared that grain-fed cattle had higher *E. coli* population than in forage-fed cattle, however depending on the grain type [9]. Abruptly switching from high-grain diets to hay diet could induce *E. coli* O157:H7-negative animals [12]. However, long-term forage feeding showed no promising result [13]. In addition to dietary effects, animal physiology and management seems to play a role. For instance, the study of Fitzgerald et al. [14] indicated that lactating cows had higher *E. coli* O157:H7 shedding compared to non-lactating cows. Stress could induce cattle to metabolic and infectious diseases and in turn the animals are more susceptible to pathogenic bacteria and subsequent *E. coli* shedding may occur.

The use of high-throughput metabolomic technologies during the recent years has provided interesting insights on bovine gastrointestinal health. Accordingly, the study of Ametaj et al. [15] showed that feeding cows with highly degradable diets (e.g. diets rich in barley grain) was associated with a subacute rumen acidosis and major perturbations in the metabolomic profile in the rumen. For example, compared with cows fed 15% grain those fed 60% grain in the diet showed about 14-fold increases of endotoxin, and above 20-fold greater toxic compounds in the rumen fluid such as biogenic amines and N-nitrisodimethylamine [15]. Since these compounds can be translocated into the bloodstream [16], it is probable that these toxic compounds might go into the food chain with consequences for food safety, hence emphasizing the major role of nutrition on gut health and subsequently on food safety.

Feeding of fiber-rich diets and the use of probiotics, antibiotics, competitive exclusion, ionophores, bacteriophage and vaccination have been introduced and employed to decrease incidences of foodborne pathogenic bacteria entering the slaughter plants [10]. However, more studies are still needed because the outcome of these methods appears to be inconsistent, some methods are impractical, and there are possible negative side effects. For instance, ionophores primarily inhibit gram-positive bacteria but many food borne pathogens including *E. coli* and *Salmonella* are gram-negative and ionophores have no effect on these pathogens [17]. Some antibiotics like neomycin could significantly reduce *E. coli* O157:H7 shedding [18], but antimicrobial resistance is concerned.

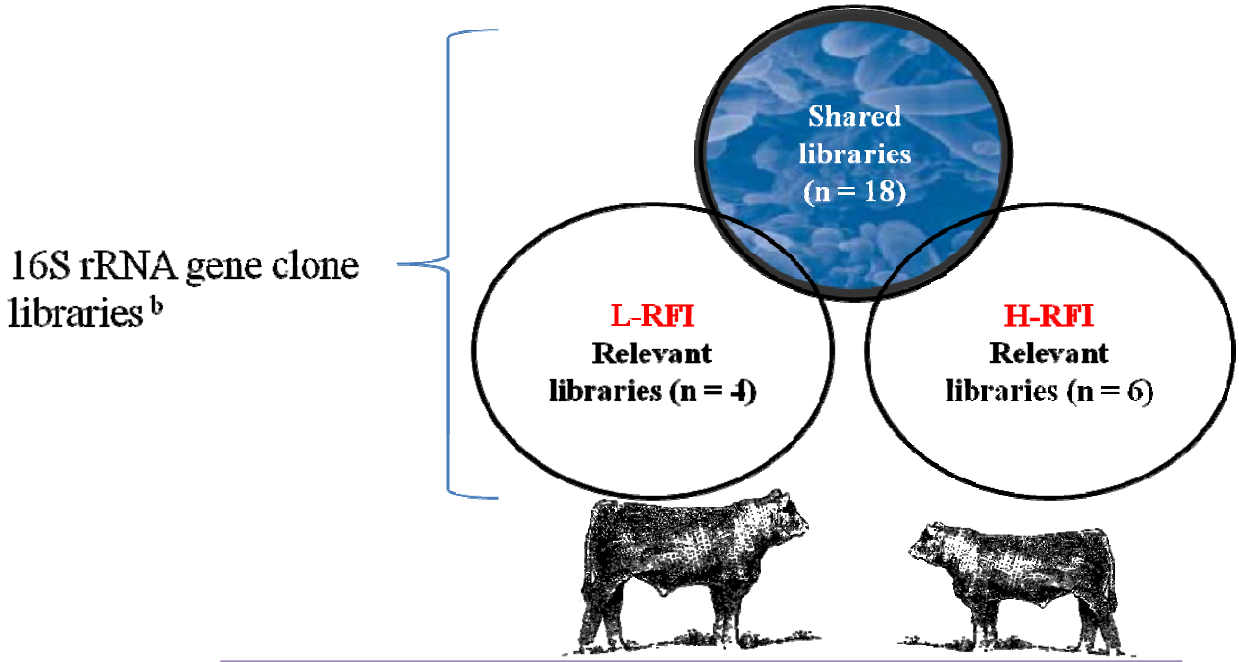
As mentioned earlier, forage feeding could influence *E. coli* shedding, yet there are inconsistencies found between studies as different forages were used. Callaway et al. [9], who intensively reviewed dietary effects on *E. coli* shedding in cattle, proposed that some intrinsic factors of forages are responsible, to some extent, to the variable results. The recent study by Berard et al. [19] showed that feeding phytochemical-rich forage sainfoin (*Onobrychis viciifolia*) to steers over 9-week period reduced generic *E. coli* number in fresh feces and no adaptation occurred. The mode of action is believed to be via the combined action of phenolic and flavonol glycosides, which have antimicrobial activity. In a good agreement, tannins were shown to have bactericidal effect on *E. coli* O157:H7 in the earlier *in vitro* studies [20]. To date, number of studies on the effects of plant secondary compounds is still limited and therefore further research is required in order to find promising natural feed additives that can replace the use of traditional antibiotics, which has been banned in the Europe Union.

3. Lowering methane may increase production efficiency

Methanogenesis is carried out by rumen methanogens and serves as a major hydrogen sink in the rumen. Methanogens form methane from the major substrates (CO_2 and H_2) derived from nutrient degradation in the rumen. On one hand, methanogenesis helps preventing the accumulation of H_2, which otherwise can result in a decline in pH and subsequent inhibition of many rumen microorganisms which are essential for nutrient degradation, especially for fiber. On the other hand, methanogenesis has been estimated to contribute to the loss of 6-10% of gross energy intake or 8-14% of digestible energy intake of ruminants [21]. Therefore, mitigating rumen enteric methane without altering overall rumen fermentation is one of the key roles to improve production efficiency in cattle. In addition, the decreased methane emission will also positively contribute to the environment.

Current methane mitigation strategies are nutritional strategies (i.e. concentrate level, forage quality) and rumen modification strategies (defaunation, ionophores, oils, dicarboxylic acids, methane analogues; [22]. However, many of these strategies cannot hold promise for long-term effects due to possible microbial adaptation. Some methods are costly, while some may harm animal health and limit rumen digestion. Because of these limitations there is a need for new approaches for mitigating methane production in cattle. Iqbal et al. [22] proposed that integrated research investigating animal, plant, microbe, and nutrient level strategies might offer a long term solution of methane emission in livestock production.

At the animal level, it involves animal genetic selection as a major approach. Variations among cattle in feed efficiency exist. The feed efficiency can be determined by residual feed intake (RFI). Residual feed intake or net feed efficiency is the difference between an animal´s actual feed intake and its expected feed requirements for maintenance of body weight and production (growth in beef cattle or milk production in dairy cattle; [23]. Efficient animals consume less than expected and have low-RFI, while inefficient animals have high-RFI. Residual feed intake has been shown to have moderate heritability and is genetically and phenotypically correlated with feed conversion ratio, indicating that genetic improvement in feed efficiency can be achieved through selection lines for low and high RFI [23]. It was shown that low-RFI Angus steers had lower (-40%) dry matter intake than the high-RFI steers to attain the similar average daily gain and thus gain:feed ratio of the low-RFI was drastically lower (Figure 1, [24]. Theoretically, efficient animals that consume fewer feeds should produce less methane than do inefficient animals. This has been confirmed by several studies [24]. However, the role of methane producers in such difference remains unexplored. By using molecular technology, the recent study by Zhou et al. [25] was able to reveal the differences in methanogenic communities between efficient and inefficient cattle. In fact, both cattle groups had similar quantities of the methanogens but inefficient cattle had more diverse methanogenic communities and higher prevalence of *Methanosphaera stadtmanae* and *Methanobrevibacter* sp. compared to efficient cattle ones (Figure 1; [25]. Further, they observed that the differences at the strain and genotype levels were associated with feed efficiency in the host, suggesting that the methanogenic ecology at the species, strain, and/or genotype level in the rumen may play important roles in contributing to the methanogenesis between cattle with different RFI.



	Low residual feed intake	High residual feed intake
Dry matter intake (kg/d)[a]	8.38	14.13
Average daily gain (kg)[a]	1.126	1.229
Methane (g/d)[a]	142.5	190.2
Total methanogens (10^6)[b]	2.12	2.52
M. Stadtmanae (× 10^6)[b]	1.33	2.48

Figure 1: Differences in production efficiency, methane production and methanogenic communities between cattle with different residual feed intake (RFI; adapted from [24, 25].

Besides methanogen community, there appears to be variations in bacterial community in the rumen of animals differing in productivity as well [26], and particular bacteria phenotype (*Succinivibrio* sp., *Robinsoniella* sp.) contributes to such differences in feed efficiency of host animal. At the microbial level, vaccination and probiotics have been shown to be effective, though the results are quite inconsistent and more work is required to confirm their effects [22]. Recently great emphasis has been placed on the investigation of natural plant components which have antimicrobial properties, and thus may decrease methane production in ruminants. Phenols such as tannins can possess a direct inhibition of methanogen number [27] and/or their activity [28]. Tannins can also indirectly inhibit methane production by reducing H_2 supply for methanogenesis [29] most possibly due to a lower nutrient digestibility [30][31] reported that all fractions of phenols, i.e. non-tannins, condensed-tannins and hydrolysable tannins, in tropical plants were associated to suppressing methane formation *in vitro*, however not all phenolic fractions of alpine plants showed the positive methane inhibition effect [32]. The generally lower phenol contents of the alpine plants compared to the tropical plant ones may explain the unclear effect. Further study from the same group [31] indicated that combining phenol-rich plants with high quality forage plants (low in phenols) could provide additional benefits as the synergistic associative effect was found. Depending on dose and structure, tannins may favorably modulate rumen fermentation such decreasing rumen protein degradation, prevention of bloat and therefore may improve body weight and wool growth, milk yields and reproductive performance [33]. Other natural additives such as essential oils and their main components have been repeatedly shown to lowering methane formation *in vitro* [34, 35] but this has not always occurred *in vivo* [36]. Opposite to tannins, there is a lack of data on effects of essential oils on animal performance, except for Benchaar et al. [37] who found no major effect of feeding essential oils on beef cattle performance. Indeed, there are potential ways either at the animal level or the microbial level to mitigate methane production and improve animal feed efficiency. However, as mentioned before, in the future there is the necessity of the integrated research. It can be expected that intensive information regarding identification of specific methanogen and bacterial species associated with host feed efficiency could help to direct to the more efficient and reliable strategies at the microbial level.

4. Increasing health-promoting fatty acids to improve ruminant product quality

Two important groups of polyunsaturated fatty acids (PUFA) are considered in terms of human health, i.e. omega-3 fatty acids and conjugated linoleic acids (CLA). Omega-3 fatty acids, such as α-linolenic acid (*c*9,*c*12,*c*15-18:3), have been recognized as "desired" fatty acids particularly due to cardiovascular effects of these fatty acids [38][39]. Ratio of omega-6:omega-3 around 4 or lower is associated with reducing the risk of many of chronic diseases [40]. Conjugated linoleic acids became of interest since the discovery of their anticarcinogenic properties [41]. In contrast to PUFA, saturated fatty acids (SFA) are considered "unhealthy" and this led to a negative image of animal products perceived by the consumers and a consequent decline in dairy products (milk and cream) in recent years [42]. For many years there has been a focus in livestock production research on producing healthier foods for consumers. The goal is to decrease SFA content and increase PUFA especially of omega-3 fatty acids and CLA in animal source foods.

Among natural-source diets for human, ruminant lipids are the major source of CLA, with rumenic acid (*c*9,*t*11-18:2) being a major isomer [42]. Furthermore, ruminant products are one of the few regular consumed foods containing the healthy omega-6:omega-3 ratio [43]. However, in comparison to SFA, these favorable fatty acids are represented only in small proportions, although cattle may consume large amount of PUFA including α-linolenic acid. Such loss of ingested PUFA and the occurrence of CLA begin in the rumen and are caused by a microbial process called biohydrogenation. In addition, it has been confirmed that major proportion of milk rumenic acid is endogenously synthesized from vaccenic acid (*t*11-18:1), which is formed during biohydrogenation process [44]. Therefore, extent of ruminal biohydrogenation importantly determines fatty acid composition in ruminant lipids. Theoretically, in order to promote the health-beneficial α-linolenic acid and CLA in ruminant derived products there should be an increase of content of these fatty acids and also vaccenic acid (for further endogenous synthesis of CLA) leaving the rumen. To do so, there are three fundamental strategies which are i) increasing PUFA supply, ii) protecting dietary lipids from biohydrogenation and iii) altering lipolysis and biohydrogenation steps.

Based on these three strategies, several methods have been investigated but their success can be expected only to some extent, and some methods may have side effects on digestibility and animal health. In spite of possible adverse effect of dietary oils on fiber degradation, increasing PUFA supply by feeding oils and oilseeds rich in either α-linolenic acid or linoleic acid (*c*9,*c*12-18:2) can increase milk rumenic acid content [45] and, to lesser extent, content of the respective PUFA in milk fat. Protection of dietary PUFA can decrease biohydrogenation extent but it appeared to have only minimal effects on milk PUFA composition, except for formaldehyde treatment which, however, has not gained wide acceptance [46]. Because biohydrogenation is driven by rumen microbes a measure that alters rumen environment (e.g. pH, passage rate) and therewith the microbes may manipulate biohydrogenation. Starchy concentrate diets favoring a low rumial pH can limit biohydrogenation extent but also shift isomer of the intermediates toward *t*10 derivatives in the rumen [47]. However, these *t*10 derivatives were shown to have rather undesirable effects, i.e. eliciting milk fat depression [48] and increasing blood risk factors for cardiovascular diseases [49]. When comparing to grass silage, legume silages such as clovers can increase transfer of α-linolenic acid to the milk because of faster passage rate through the rumen (red clover) or a lower lipolysis in the rumen (white clover, [50]. But the positive effect of legume silages on transfer of α-linolenic acid into milk was not always observed [51].

Some plant secondary compounds have antimicrobial properties [52] and they are believed to be able to alter biohydrogenation of fatty acids. For instance, plant secondary compounds were hypothesized to be associated to the increased

Table 1 Investigations about the use of tannins as an option for modification of biohydrogenation process in the rumen and promoting beneficial fatty acids[1]

Type and form of active components	Dose (% of dry matter)	Effect			Study	Reference
		ALA	VA	SA		
Acacia CT extract	10.0	No data	+	-	Batch culture	[53]
Acacia CT extract	7.9	0	+	-	Rusitec	[29]
Tannin extract (acacia+ carob+ quebracho)	9.5	+	+	0	Batch culture	[54]
Tannin extract (acacia+ carob+ quebracho)	15.8	0	+	-	Batch culture	[54]
Quebracho CT extract	0.5	0	0	0	Cattle (milk)	[55]
CT plant (sainfoin)	7.9	+	0	-	Rusitec	[29]
CT plant (sulla)	2.7	+	-	0	Sheep (milk)	[56]

[1] CT = condensed tannins, ALA = linolenic acid (18:3 n-3); VA = vaccenic acid (18:1 t-11, the major precursur of c9,t11 CLA, SA = stearic acid (18:0); Effect: 0 = unchanged; + = increased; - = decreased, compared to their respective controls.

α-linolenic acid in milk of the cows grazing on alpine pasture [57]. To our knowledge, except for few indications (e.g. [56] which showed the increased α-linolenic acid effect, plant secondary compounds often resulted in the specific inhibition of the last biohydrogenation step leading to the increase of ruminal vaccenic acid [29, 42, 54, 58]. Among the tested compounds, tannins are the most effective compounds as a vaccenic acid enhancer in the rumen (Table 1).

The inhibitory effect of tannins appears to be caused by a direct inhibition of rumen bacteria responsible for the last step of biohydrogenation, i.e. converting vaccenic to stearic acid [59]. Tanniniferous forages have been shown to improve product quality such as increase CLA in meat and improve color stability and sensorial profiles [60]. The contributions of dietary tannins to animal production and product quality are, however, not always observed [60, 61], and structure and concentration of tannins present in the feeds seem to play an important role [60]. Likewise, Benchaar and Chouinard [55] reported no effect of condensed tannins on milk fatty acid composition probably due to the dosage used. Other candidate substances are essential oils and their active components which have the potential to modify rumen fermentation but so far less is known for their effects on rumen biohydrogenation as well as animal product quality [34]. However, their effects on modification of rumen metabolism also depend on nature of the active components, dosage use, type of basal diet, and adaptation period of rumen microbes to the presence of the compounds [34].

5. Conclusions

The goal of ruminant production has changed drastically during the last decades from merely producing milk and meat efficiently to more complicated goals, i.e. additional focus on reducing emissions, greater safety as well as quality of the products. The advanced research at the animal genetic level as well as at rumen microbial level has expanded the knowledge of methanogenesis. This could lead to a discovery of the more potential (long term) strategy for methane mitigation. *E. coli* shedding in cattle is one of the most concerns in terms of food safety of animal source foods. The management regarding *E. coli* shedding has changed from post slaughter to pre slaughter interventions. These include dietary and management strategies as well as probacterial and antipathogenic strategies. The improvement of quality of ruminant source foods relies mainly on improving their fatty acids composition toward health-promoting PUFA such as omega-3 fatty acids and CLA. Plant secondary compounds appear to possess multi-beneficial effects that can contribute to food security and food safety and quality, i.e. decreasing *E. coli* shedding, mitigating enteric methane emission, and enriching PUFA in ruminant lipids. However, identification of active compounds for use as efficient feed additives in ruminant production remains a big challenge for livestock research.

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