Plant Extracts; a New Rumen Moderator in Ruminant Diets

A. Tekeli L. Çelik H. R. Kutlu

Çukurova Üniversitesi Ziraat Fakültesi Zootekni Bölümü, Adana

Since the use of antibiotics in animal feeds has been banned, researches on alternative natural products that modulate ruminal fermentation have been intensified. Natural compounds such as plant extracts have been considered to be replace antibiotics as safe and sustainable alternatives.

Plant extracts in the rumen form complexes with dietary proteins and thus protect them from microbial fermentation. Plant extracts, especially including saponins, have strong antiprotozoal activity. The elimination of protozoa from the rumen provides some benefits to overcome the loss of available protein. Plant extracts also have appetizing properties, which promotes greater feed intake, better performance and secretion of endogenous enzymes and digestive juices, leading to improve nutrient digestibility. Plant extracts have also potential to provide ammonia control and nitrogen binding activity; which contributes to form cleaner and healthier environment.

Key words: Plant extract, antibiotic, ruminant, saponin, protozoa

Bitkisel Ekstraktlar: Ruminant Rasyonlarında Yeni Bir Rumen Düzenleyici

Hayvan yemlerinde antibiyotiklerin kullanımının yasaklanması nedeniyle rumen fermantasyonunu düzenleyici alternatif ürünlerin araştırılmasına yönelik çalışmalar büyük yoğunluk kazanmıştır. Bu bağlamda, doğal, güvenilebilir ve sürdürülebilir ürün özelliği taşıyan bitkisel ekstraktların antibiyotiklerin yerini alabileceği düşünülmektedir.

Bitkisel ekstraktlar rasyonun proteinleriyle kompleks oluşturmakta ve onları mikrobiyal fermantasyondan korumaktadır. Bitkisel ekstraktlar özellikle saponin içerenler güçlü antiprotozoal aktiviteye sahiptir. Rumenden protozoaların eleminasyonu mevcut protein kaybının önlenmesinde oldukça etkilidir. Bitkisel ekstraktlar ayrıca iştah açıcı özellikleri nedeniyle yem tüketimini arttırmakta, performansı yükseltmekte, sindirim enzimi ve salgılarını uyarmaları nedeniyle de besin madde sindirilebilirliğini iyileştirmektedirler. Bitkisel ekstraktlar nitrojeni bağlaması ve amonyak kontrolü nedeniyle daha temiz ve sağlıklı çevre oluşumuna da katkı sağlamaktadır.

Anahtar Kelimeler: Bitkisel ekstrakt, antibiyotik, ruminant, saponin, protozoa

Introduction

Feeding ruminants in intensive production systems, especially for dairy production, requires supplies of high levels of energy and protein. Animals are thus fed on rations rich in starch and high quality protein, which are fermented very rapidly. It is well known that the rapid degradation of starch tends to cause ruminal acidosis. The rapid breakdown of dietary protein to ammonia increases nitrogenous excretions rather than contributing directly to the animals´ nutrient requirements. In order to delay ruminal protein degradation, dietary proteins were denatured by treatment with formaldehyde or more controversially, antibiotics were used to suppress the bacterial populations responsible for the rapid protein fermentation. But the use of such compounds has been criticized, as they may leave harmful residues in the food chain and promote the spreading of resistance genes (Hoffmann et al., 2003). Antibiotics which had been used as growth promoters in animal feeds, has been banned by European Union's Agricultural ministry since the first of January 2006 (Anonymous, 2005).

It has been considered that, those additives will be replaced by natural compounds. Plant extracts form dietary protein complexes that also protect them from microbial fermentation. Once they bypass the rumen, the complexes dissociate under the acidic conditions in the abomasum and proteins become available to the host animal. Plant extracts (especially saponins) destroy rumen fungi along with the protozoa and have foam-stabilizing properties that may enhance bloat, especially under high protein feeding regimes (Hoffman et al., 2003).

It has been suggested that one of the reasons for the beneficial effects of plant extracts is related to the its influence on microbial fermentation. The beneficial effects have also been attributed to the binding of ammonia to saponins. Besides the traditional use of plant extracts as flavour enhancers in foods may also used as antimicrobial and antioxidant that various novel applications have recently been proposed: (i) to decrease the ammonia level in the atmosphere, (ii) to suppress or stimulate microbial growth, (iii) to increase binding of ammonia during urea ammoniation of straw and binding of ammonia in the soil, (iv) to reduce odors from cattle manure in dairy barns (Makkar et al., 1998).

One of the reasons for the lower ammonia levels could be the higher incorporation of ammonia, peptide or amino acids into microbial protein (Makkar et al., 1998). The other reason, inhibition of bacterial lysis, is probably partially responsible for decreased ruminal ammonia concentrations observed in defaunated animals (Hristov et al., 1999).

Saponins are one of the most important active substances found in plant extracts. The detergent action of saponins kills rumen protozoa. The susceptibility of ruminal ciliate protozoans to saponins is probably expected due to the presence of cholesterol in eukaryotic (including protozoa) cells membranes, but not in prokaryotic bacterial cells, as saponins exhibit an affinity towards cholesterol (Klita et al., 1996). Suppression or elimination of protozoa may enhance the flow of microbial protein from the rumen, increase the efficiency of feed utilization, and improve the nutrition of the animal, provided that the loss of protozoa does not impair the fiber breakdown (Newbold et al., 1997).

Recently, a considerable number of research projects has been dedicated to the screening of spices and medicinal herbs for effects on the digestive physiology of both ruminant and monogastric animals.

Essential Oils

Plants have evolved a wide range of low molecular weight secondary metabolites. Essential oils are plant secondary metabolites responsible for odour and colour of plants (Helander et al., 1998; Wenk, 2000). Essential oils are the volatile components responsible for the characteristic aroma of spices. As active components in many spices and preservatives, essential oils have historically been used to inhibit bacterial growth (Hirasa and Takemasa, 1998). All essential oils have aromatic properties. They have also shown biological activities, such as antioksidative, fungicidal and antimicrobial activity, Furthermore they have found to have psychological and physical effects, which is why they have found their application in aromatherapy. Essential oils are the most concentrated form of phytobiotics, which are increasingly used in the animal feed industry (Biomin, 2005).

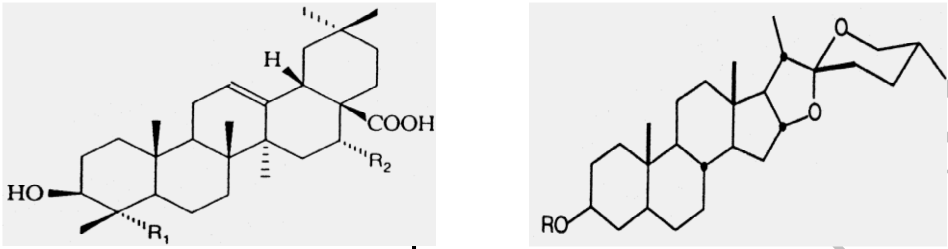
Essential oils are complex mixtures containing many different chemical substances. Each of the individual chemical compounds that can be found in an oil contributes to the overall character, i.e. it is the composition of the substances which will determine the aromatic properties and biological activity of an essential oil. Depending on the chemical composition the oil will have a characteristic flavour, which will stimulate taste and olfactory senses. Thus essential oils with particularly pleasing flavour can help to stimulate appetite. The scent of certain essential oils can stimulate areas of the brain influencing emotions and the nervous system (Biomin, 2005). Generally these compounds enable the plant to interact with the environment and may act in defense against physiological and environmental stress as well as predators or pathogenes. Some plant metabolites are toxic to animals, however several have been reported to provide benefical effects in food products and also in mammalian metabolism (Wenk, 2000).

Saponins

Saponins are a group of naturally occurring compounds which have properties resembling soaps and detergents. They are complex and chemically diverse group of compounds, mainly of plant origin, but also accruing in a number of marine animals. Their physiological effects are as diverse as their chemical structures and properties. Saponins have a number of common and characteristic properties which include;

1. Bitter taste,
2. Formation of stable foams in aqueous solution,
3. Hemoloysis of red blood cells,
4. Toxicity to cold-blooded animals such as fish, snails, insect, etc.
5. An ability to interact with bile acids, cholesterol or other 3-β-hydroxy steroids in aqueous or alcoholic solutions to form mixed micelles or coprecipitates (Cheeke, 1995).

Chemical structure of saponins are given in figure 1.



a **b**

Figure 1. Chemical structure of saponins (a: triterpenoid, b: steroid) (Kutlu, 1999).

Mode of Action of Essential oils and Saponins

Herbs are active initially in animal feeds as flavours, and can therefore influence eating patterns, secretion of digestive fluids and total feed intake. The primary site of activity is the digestive tract. Herbs or the phytochemicals can selectively influence the intestinal microflora by either antimicrobial activity or by favourably promoting eubiosis of the microflora resulting in better nutrient utilization and absorption, or stimulation of the immune system. Finally, herbs can contribute to nutrient requirements, stimulate the endocrine system and affect intermediate nutrient metabolism (Wang et al., 1998; Wenk, 2000).

Phenols are a group of active substances including plant extracts. Antimicrobial effects of phenols have been known for more than a century. Their main antimicrobial activity is against the bacterial cell wall, by denaturing and coagulating the protein within the cell wall structure. Phenols interact with the cytoplasmic membrane by changing its permeability for cations, like H^+, K^+. The dissipation of ion gradients leads to impairment of essential process in the cell, allows leakage of cellular constituents, resulting in water unbalance, collapse of the membrane potential and inhibition of ATP synthesis, and finally cell death (Gill, 2001; Giannenas et al., 2003). Oregano extract has been reported to accelerate the renewal rate of mature enterocytes at the surface of the villi of the intestine. This would reduce pathogen contamination of enterocytes and improve nutrient absorption capacity (Gill, 2001).

Plant extracts improved efficiency of utilization of nitrogen (N) in the rumen is attributed to the binding of ammonia by plant extracts when the ruminal ammonia concentration is high and release of the bound ammonia when its concentration is low in the rumen. Thus, modulating diurnal fluctuations in ruminal ammonia concentrations to provide a continuously adequate of ammonia for microbial metabolism. Higher microbial mass, lower proportion to short chain fatty acid (SCFA) and lower gas production in the *in vitro* fermentation system lead to the same or higher true digestibility of substrate in the presence plant extracts (Makkar et al., 1998).

Plant extracts, especially including saponins, have strong antiprotozoal activity. The elimination of protozoa from the rumen is a mean of overcoming the loss of available protein due to protozoal turnover (Jouany, 1996). Ruminant animals have a large population of rumen protozoa. The rumen protozoa reduce the efficiency of fermentation in the rumen and the increases in animal performance often occur when the protozoa are removed. On the other hand, high doses of saponin are known to cause damage to the villi tips of the intestinal epithelium (Gee et al., 1996); this would impair the digestive capability of the animal as the absorptive area is decreased and mucosal enzyme production is suppressed. This could potentially lead to lower feed efficiency and weight gain (Ilsley et al., 2005).

Some botanical additives that contain high concentration of thymol may have significant effects on protein metabolism, particularly increased nitrogen retention. This has positive *in vivo* effects such as increased lean meat percentage (Gill, 2001).

The Plant Resources of Active Substances and Their Concentrations

Botanical feed additives-even those extracted from a single species- are complex mixtures of many chemicals, more than one of which may be an "active ingredient" (Gill, 2001). The plant resources of active substances and their concentrations are given Table 1.

Table 1: The Plant source and Active Substance Concentrations of Some Active Substances

Active Substance	Plant Source and Active Substance Concentrations (in parantheses)
Anethole	Green anise (85), fennel (32)
Borneol	Ceylan citronnelle (7), Dalmatian sage (6), romarin (3), thyme (15)
Carvon	Spearmint (65), carvi (59), aneth (35)
Caryophyllene	Clove (10), pepper (4), true pepper (7), romarin (3), sarriette (5), Sage (5)
Cinnamaldehyde	Cinnamon (90)
Cineole (Eucalyptol)	Eucalyptus (80), laurel (50), romarin (3), cardomome (30)
Cuminaldehyde	Cumin (30)
Estragole	Estragon (78), basil (5), fennel (4), chervil (4)
Eugenol	Clove (80), true pepper (70), cinnamon leaf (70)
Geraniol	Formosa citronnele (20), Clean citronelle (15)
Linalol	Basil (48), thyme (60), orange flower (45), sage (15), coriander (60), laurel (10)
Allicin	Garlic
Sabinene	Macis (25), pepper (25), nutmeg (20), carrot (10), cardomome (5), persil(<5)
Terpineol	Orange flower (10), marjory (6), romarin (2)
Thymol	Thyme (41), oregano (10), Savory (10)
Carvacrol	Oregano (60)

(Kamel, 2000)

Some Aromatic Plant's Including Active Substances and Particularities

Many plant extracts have multiple activities directly linked to their active substance composition. Some aromatic plants which are used obtain essential oils including active substances and their effect manners given Table 2.

Some Plant's Levels of Saponin

Saponins are present in many hundreds of plant species. Plant food staples which contain significant levels of saponin are given Table 3.

Table 2: Aromatic Plant`s Active Substance Contents and Effects

Vegetal Form	Utilised Parts	Main Compounds	Reported Properties
Nutmeg	Seed	Sabinene	Digestion stimulate, antidiarrhoeic
Cinnamon	Bark	Cinnamaldehyde	Appetite and digestion stimulate, antiseptic
Clove	Flower buds	Eugenol	Appetite and digestion stimulate, antiseptic, antibacterial, antifungal, antioksidant, antiviral, larvicidal and vermifuge activity
Cardamom	Seed	Cineole	Appetite and digestion stimulant
Coriander	Leaf, seed	Linalol	Digestion stimulant
Cumin	Seed	Cuminaldehyde	Digestive, carminatif, galactague
Anise	Fruit	Anethole	Digestion stimulant, galactagogue
Celery	Fruit, leaf	Phtalides	Appetite and digestion stimulant
Parsley	Leaf	Apiol	Appetite and digestion stimulant, antiseptic
Fenugreek	Seed	Trigonelline	Appetite stimulant
Capsicum	Fruit	Capsaicin	Antidiarrhoeic, anti-inflammatory, stimulant, tonic
Pepper	Fruit	Piperine	Digestion stimulant
Horseradish	Root	Allyl isothiocyanate	Appatite stimulant
Mustard	Seed	Allyl isothiocyanate	Digestion stimulant
Ginger	Rhizome	Zingerole	Gastric stimulant
Garlic	Bulb	Allicin	Digestion stimulant, antiseptic
Raosemary	Leaf	Cineole	Digestion stimulant, antiseptic, antioksidant
Thyme	Whole plant	Thymol	Digestion stimulant, antiseptic, antioksidant
Sage	Leaf	Cineole	Digestion stimulant, antiseptic, carminatif
Oreganum	Leaf and Flowers	Carvacrol and Thymol	Antimicrobial and antifungal activity
Bay laurel	Leaf	Cineole	Appetite and digestion stimulant, antiseptic
Peppermint	Leaf	Menthol	Appetite and digestion stimulant, antiseptic

(Giannenas, 2003; Anonymous, 2004b; Kamel, 2000)

Table 3: Plant food staples which contain significant levels of saponin

Plant	Saponin Content (g/kg)
Chick-pea *(Cicer arietinum)*	2.3-60
Soybean *(Glycine Max)*	5.6-56
Navy bean *(Phaseolus vulgaris)*	4.5-21
Mung bean *(P.mungo)*	0.5-5.7
Mung bean shoots	27
Broad bean *(Vicia faba)*	3.5
Green pea *(Pisium sativum)*	1.8-1.1
Peanut *(Arachis hypogyea)*	16
Spinach *(Spinacea oleracea)*	15
Silver beet *(Beta vulgaris)*	47
Eggplant *(Solanum melongena)*	58
Yucca (Yucca schidigera)	80

(Cheeke, 1995; Özkaya, 2005)

Studies on Plant Extracts in Ruminant Diets

Thalib et al. (1995) found that when a capsule containing methanol-extracted *Sapindus rarak* fruit was given orally to sheep, protozoal numbers decreased by 57% and bacterial concentrations increased by 69% in the rumen fluid.

Fernandez et al. (1997) showed that a commercial product of blended essential oil compounds (BEO) inhibited the degradation of protein in the rumen, thus potentially increasing the protein supply to the post-ruminal tract.

Makkar et al. (1998) investigated effects of *Yucca schidigera (YS), Quillaja saponaria,* and *Acacia auriculofformis* saponin containing on rumen fermentation. Ammonia levels and protozoal counts at 24 h in an *in vitro* rumen fermentation system were also reduced by saponins; decreases were as high as 30 and 63%, respectively.

Hristov et al., (1999) investigated the effect of YS on ruminal fermentation and nutrient digestion in heifers. Doses of 0 (control), 20 or, 60 g/d were given to the animals. Acidity, concentrations of reducing sugars, free amino acids, and peptides in the rumen were not affected ($P>0.05$) by YS. Relative to control, ruminal ammonia concentration was reduced ($P<0.05$) 2 h after YS dosing.

Evans and Martin (2000) observed that thymol, a primary component of some essential oils, modified the concentration of volatile fatty acids (VFA) *in vitro* incubations of ruminal fluid. When thymol was added to ruminal fluid at the level of 400μg/ml, final pH and acetate to propionate ratio increased but the concentration of methane, acetate, propionate and lactate were decreased.

McIntosh et al. (2000) determined that essential oils (EO; CRINA) inhibited deamination of amino acids measured *in vitro* by 25%. Castillejos et al. (2005) notified that the addition of monensin to an *in vitro* 24 h incubation system resulted in a decrease in the deaminative activity of ruminal fluid that was larger than that from the addition of BEO. It was suggested that the microbial species affected by the essential oils were the same as those affected by monensin. This decrease in ammonia production was associated with a reduction in a number of group bacteria called hyper-ammonia producing (HAP) bacteria. McIntosh et al. (2003) observed that all of HAP species are sensitive to monensin. BEO inhibited some HAP bacteria (*Clostridium sticklandii* and *Peptostreptecoccus anaerobius*) while the other HAP species (*Clostridium aminophilum*) were not affected.

Wang et al. (2000) found that including YS (0.5 mg/ml) in the buffer of a rumen simulation system (RESITEC) did not affect the total bacterial numbers.

Sliwinski et al. (2002) investigated rumen fermentation and N balance of lambs fed diets containing plant extracts rich in tannins and saponins. Tannins were added to experimental diets at levels of 1 and 2 g/kg dry matter (DM) (*Castanea sativa wood extract*) and saponin at 2 and 30 mg/kg DM *Yucca Schidigera extract* (YSE). The low tannin dose significantly decreased bacteria count compared to the high saponin dose ($P<0.05$). Saponin supplementation and the high tannin dose showed some potential to reduce ruminal ammonia concentration. Methane release was increased by the low tannin dose compared to the unsupplemented control. N utilisation (N retention/N intake) was numerically higher with both extracts given at the highest dose.

Eryavuz and Dehority (2004) investigated the effects of YSE on pH and the concentration of protozoa, bacteria, and fungi in sheep rumen contents. YSE was added to experimental diets at levels of 5, 10, 20 and 30 g/head/day, respectively. Animals fed YSE at a level of 30 g/head/day had higher rumen protozoal concentrations ($P<0.015$) and pH ($P<0.01$) than when fed the basal diet.

Newbold et al. (2004) investigated the effects of specific blend of essential oil compounds (BEO, Crina Ruminants); (the major components are thymol, guajacol and limonene) on rumen fermentation in sheep. Deamination of amino acids measured *in vitro* in rumen fluid removed from the sheep decreased by 25% ($P<0.05$). However, total VFA and ammonia concentrations were unaffected. Similarly, Wallace et al. (2002) supplemented ruminally fistulated sheep with 100 mg/d of the same BEO and did not observe effects on total VFA concentrations.

Castillejos et al (2005) investigated the effects of a specific blend of essential oil compounds (BEO, Crina Ruminants) on rumen microbial fermentation and nutrient flow. Main factors were type of diet (10:90 forage:concentrate versus 60:40 forage:concentrate) and the addition of BEO (0 or 1.5 mg/I). There were no interactions between diet type and the addition of BEO, and no effects of diet type on DM, organic matter (OM), neutral detergent fibre (NDF), acid detergent fiber (ADF) and crude protein (CP) digestion found, but BEO increased the concentration of total VFA (122.8mM versus 116.2mM) without affecting individual VFA proportions or N metabolism.

Soliva et al. (2005) chemical composition and energetic value unextracted (Moringa leaves, ML) and ethanol/acetone-extracted Moringa (*Moringa oleifera* Lamarck, EML) leaves, and their effect on ruminal N turnover and other ruminal fermentation traits investigated in comparison with soybean meal and rapeseed meal, using Hohenheim Gas Test (HGT) and the Rumen Simulation Technique. *In vitro* degradability of OM and fibre of complete ML and EML diets were similar or even higher as compared with those containing soybean meal or rapeseed meal. Daily methane emission was 17% lower (P<0.05) with the complete EML diet as compared with the diets containing soybean meal or rapeseed meal. According to Hohenheim Gas Test; methane emission was found at the highest rate with soybean meal, lowest with ML and intermediate with rapeseed meal and EML (P<0.05). The calculated metabolic energy (ME) and net energy lactation (NEL) concentrations were highest for soybean meal, intermediate for EML and lowest for rapeseed meal and ML (P<0.05).

Busquet et al. (2006) an experiment were conducted to determine the effects of some plant extracts *in vitro* on rumen microbial fermentation. They were used different doses (3, 30, 300 and 3.000 mg/L) of plant extracts (anise oil, cade oil, capsicum oil, cinnamon oil, clove bud oil, dill oil, fenugreek oil, garlic oil, ginger oil, oregano oil, tea tree oil and yucca) and secondary plant metabolites (anethol, benzyl salicylate, carvacrol, carvone, cinnamaldehyde and eugenol). Plant extracts and secondary plant metabolites were incubated for 24 h in diluted ruminal fluid with a 50:50 forage : concentrate diet. Garlic oil (300 and 3000 mg/L) and benzyl salicylate (300 and 3.000 mg/L) reduced acetate and increased propionate and butyrate proportions, suggesting that methane production was inhibited. At 3.000 mg/L, capsicum oil, cinnamon oil, clove bud oil, oregano oil, carvacrol, carvone, cinnamaldehyde, eugenol, fenugreek resulted in a 30 to 50 % reduction in N concentration. Anethol, carvone, anise oil and tea tree oil decreased the proportion of acetate and propionate, which suggests these compounds may not be nutritionally beneficial to dairy cattle.

Wina et al. (2006), investigated the short (7 days) and long (105 days) term effects of an extract of *Sapindus rarak* saponins (SE) on the rumen fibrolytic enzyme activity and the major fibrolytic microorganisms. SE significantly depressed rumen xylanase activity in both trials and carboxymethylcellulase activity in long term trial (P<0.01). *Fibrobacter sp*. were not affected by the SE in both trials. Protozoal counts were decreased only in the long term trial with sheep.

Conclusion

The main reasons for the wide use of antibiotic feed additives are beneficial effects on health status, performance, and nutrient and energy utilization. The trend toward more ‘natural’ animal production systems has led to an increasingly critical attitude on the part of consumers about in-feed antimicrobial agents. It is a common misconception that all plant extracts made from plants are safe because they are natural or organic. An example of a dangerous plant extract is Ephedra. It has been known to cause raised blood pressure, nerve damage, muscle injury and even death. Whether herbs, spices or botanicals (eg. plant essential oils) are appropriate must be considered in each practical application. There are evidences that some essential oils reduce the rate of deamination of amino acids, rate of ammonia production and the number of ammonia producing bacteria. Therefore, natural plant extracts can be used to manipulate ruminal fermentation by selective modulation of certain microbial species. Considering these hypotheses, further research is required to identify optimal dose, and the effect of time of adaptation of BEO on N metabolism of rumen microorganisms. The possible use of natural plant products as a productivity enhancer provides cheaper, safer, sustainable and more consumer- acceptable alternatives to synthetic compounds.

References

Anonymous, 2004b. Eulara. http:www.eulara.com/shop/content.php?pgid=160 (03.12.2005).

Anonymous 2005. http://www.wfan.org/newsletters/vol6no3.pdf (03.12.2005).

Biomin, 2005. Biomin. http://www.biomin.net/homepage/biomin.htm (03.12.2005).

Busquet, M., Calsamiglia, S., Ferret, A. and Kamel, C. 2006. Plant Extracts Affect *in vitro* Rumen Microbial Fermentation. J. Dairy Sci. 89: 761-771.

Castillejous, L., Calsamigkia, S., Ferret, A. and Losa, R. 2005. Effects of a Spesific Blend of Essential Oil Compounds and Type of Diet on Rumen Microbial Fermentation and Nutrient Flow from a Contuous Culture system. Anim. Feed Sci. Technol 119: 29-41.

Cheeke, P. R. 1995. Toxicants of Plant Origin. Volume II Glycosides. CRC Press, Inc. Boca Raton, Florida.

Eryavuz, A. and Dehority, B.A. 2004. Effect of *Yucca schidigera* Extract on the Concentration of Rumen Microorganisms in Sheep. Anim. Feed Sci. Technol. 117, 215-222.

Evans, J.D., Martin, S. A., 2000. Effects of Thymol on Ruminal Microorganisms. Curr. Microbiol. 41: 336-340.

Fernandez, M., Serrano, E., Frutos, P., Giraldez, F.J., Mantecon, A.R., Liach, J.R., 1997. Effect of Crina HC Supplement upon the Rumen Degradative Activitiy in Sheep. ITEA 18 (vol.extra): 160-162.

Gee, J.M., J.M.Wal, K.Miller, H. Atkinson, F. Grigoriadou, M.V.W. Wijnands, A.H. Penniks, G. Wortley and I.T. Johnson 1996. Effect of Saponin on the Transmucosal Passage of β-lacto-globulin across the Proximal small Intestine of Normal and β-lacto-globulin-sentized Rats. Toxicology 117: 219-228.

Giannenas, I., Florou-Paneri, P., Papazahariadou, M., Cheristaki, E., Botsoglou, Na., Spais, A.B., 2003. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenel.* J. British Poult. Sci. 57: 99-106.

Gill, C. 2001. “Safe and Sustainable” Feed Ingredients. Feed International March. pp. 40-44.

Helander, I.M., Alakomi, H-L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E.J., Gorris, L.G.M., Von Wright,A. (1998). Characterizationof the action of selected essential oil components on gram-negative bacteria. J. Agric. Food Chem. 46, 3590-3595.

Hirasa, K., Takemassa, M., 1998. Spice Science Technology. Marcel Dekker Inc., New York, USA.

Hofmann, E.M., Muetzel, S. and Becker, K. 2003. Efects of *Moringa Oleifera* Seed Extract on Rumen Fermantation In Vitro. Arch. Anim. Nutr. 57: 65-81.

Hristov, A.N., McAllister, TA., Van Herk, F.H., Cheng, K.-J., Newbold, C.J., Cheeke, P.R., 1999. Effect of *Yucca schidigera* on Ruminal Fermentation and Nutrient Digestion in Heifers. J. Anim. Sci. 77: 2554-2563.

Ilsley, S.E., Miller, H.M. and Kamel, C. 2005. Effects of Dietary Quillaja Saponin and Curcumin on the Performance and Immune Status of Weaned Piglets. J. Anim. Sci. 83 (1): 82-88

Jouany, J. P. 1996. Effect of Rumen Protozoa on Nitrogen Utilization by Ruminants. J. Nutr. 126: 1335-1346.

Kamel , C., 2000. A Novel Look at A Classic Approach of Plant Extracts. Feed Mix, Special-2000, pp: 19-21.

Klita, P.T., Mathison, G.W., Fenton, T.W., Hardin, R.T., 1996. Effects of Alfalfa Root Saponins on Digestive Function in Sheep. J. Anim. Sci. 74: 1144-1156.

Kutlu, H.R. 1999. The Importance of *Yucca Schidigera* Extract on Poultry Nutrition. Feed Industry Seminar Report. 3 June 1999. Tüyap Fair and Lecture Central, İstanbul.

Makkar, H.P.S., Sen, S., Blümmel, M., Becker, K. 1998. Effects of Fractions Containing Saponins from *Yucca shidigera*, *Quillaja saponaria*, and *Acacia auriculoformis* on Rumen Fermantation. J. Agric. Food Chem. 46: 4324-4328.

McIntosh, F.M., Newbold, C.J., Losa, R., Williams, P., Wallace, R.J. 2000. Effects of Essential Oil on Rumen Fermentation. Reprod. Nurt. Dev. 40 (Suppl.2): 221-222 (abstract).

McIntosh, F.M. Williams, P., Losa, R., Wallace, R.J., Beever, D.A., Newbold, C.J. 2003. Effects of Essential Oils on Ruminant Microorganisms and Their Protein Metabolism. App. Environ. Microbiol. 69: 5011-5014.

Newbold, C.J., El Hasan, S.M., Wang,J., Ortega, M.E., Wallace, R.J. 1997. Influence of Foliage from African Multipurpose Tress on Activity of Rumen Protozoa and Bacteria. Br. J. Nutr. 78: 237-249.

Newbold, C., McIntosh, F.M., Williams, P., Losa,R. and Wallace, R. J. 2004. Effects of a Spesific Blend of Essential Oil Compounds on Rumen Fermentation. Anim. Feed Sci. Technol. 114: 105-112.

Özkaya, H. 2005. Effect of *Yucca schidigera* Extract (DK 35 Powder) on Broiler Performance. University of Mustafa Kemal. Institue of Natural Andapplied Sciences. Department of Animal Science. MSc Thesis. p 27.

Sliwinski, B. J., Kreuzer, M., Wettstein, H.R. and Andrea Machmüller. 2002. Rumen Fermentation and Nitrogen Balance of Lambs Fed Diets Containing Plant Extracts Rich in Tannins and Saponins, and Associated Emissions of Nitrogen and Methane. Arch. Anim. Nutr. 56: 379-392.

Soliva, C.R., Kreuzer, M., Foidl, N., Foidl, G., Machmüler, A. and Hess, H.D. 2005. Feeding Value of Whole and Extracted *Moringa oleifera* Leaves for Ruminants and Their Effects on Ruminal Fermentation in Vitro. Anim Feed Sci. Technol. 118: 47-62.

Thalib, A., Widiawati, Y., Hamid, H., Suherman, D., Sabrani, M. 1995. The Effects of Saponin from *Sapindus rarak* Fruit on Rumen Microbes and Host Animal Growth. Ann Zootech. 44 (suppl): 161.

Wang, Y., McAllister, T.A., Newbold, C.J., Rode, L. M., Cheeke, P.R. and Cheng, K-J. 1998. Effects of *Yucca schidigera* Extract on Fermantation and Degradation of Steroidal Saponins in the Rumen Simulation Techniqiue (RUSITEC). Anim. Feed Sci. Technol. 74: 143-153.

Wang, Y., McAllister, T.A., Yanke, L.J., Cheeke, P.R. 2000. Effect of steroidal Saponin from *Yucca schidigera* Extract on Ruminal Microbes. J. App. Microbiol. 88: 887-896.

Wenk, C., 2000. Why all Discussion about Herbs? Biotechn. In the Feed Industry. Proc. of Alltech`s 16th Annu. Symp. Alltech Technical Publications, Nottginham Univ. Press. Nicholasvile, KY. pp: 79-96.

Wina, E., Muetzel, S. and Becker, K. 2006. The Dynamics of Major Fibroloytic Microbes and Enzyme Activity in the Rumen in Response to short-and long-term Feeding of *Sapindus rarak* Saponins. J. App. Microbiol. 100, 114-122.