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Effect of *Vitex agnus-castus* on *in vitro* digestibility in ruminant

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The objective of this study was to evaluate the effects of different levels of *Fructus agni-casti* (FAC) on *in vitro* gas production (GP), organic matter digestibility (OMD) and net energy lactation (NEL) using an *in vitro* gas-production method. Two rumen-fistulated sheep were used in the experiment. The sheep were fed 60% alfalfa hay and 40% concentrate feed twice daily. Five different levels of FAC were added to the concentrate (CON). The volume of gas produced was recorded at 2, 4, 8, 12 and 24 h after incubation. In this study, the GP-improving effect occurred at 2 h of incubation in FAC_3 and FAC_4 groups. Degradation of the water-soluble carbohydrates promoted the concentrate feed with FAC (3 and 4% level) additions. However, the GP-reducing effect occurred from 8 to 12 h of incubation in all the treatments. It was shown that, the concentrate feed with FAC addition reduced degradation of storage polymers such as starch. In addition, GP (at the 24 h), OMD and NEL were not affected by supplementation with FAC.

Key words: *Vitex agnus-castus*, essential oil, feed digestibility, *in vitro* gas production

INTRODUCTION

The aim of manipulating rumen fermentation is to improve the feed efficiency for the production of meat, milk or wool from ruminant animals. The fermentation pattern can be selectively modified to maximize microbial protein synthesis and produce end-products of digestion. Antibiotic feed additives, such as ionophore and antibiotic monensin, have been widely used for this purpose (Nagaraja, 1995). However, the use of antibiotics as feed additives in intensive production systems was banned in the European Union because of the presence of residues in milk in meat (EC, 2003, Regulation 1831/2003/EC). This ban has created the need for suitable alternatives for these antibiotics.

One of these alternatives is the use of herbal extracts and their active substances such as essential oils (Greathead, 2003). The essential oils are drawing interest both in the industry and the scientific research due to their antibacterial and antifungal properties, which make them more useful as natural additives in feeds. Previous *in vitro* studies on different plant extracts and plant metabolites showed the potentials of some extracts, including saponins, anise oil, capsicum extract, eugenol

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and cinnamaldehyde, to modify ruminal microbial fermentation. Calsamiglia et al. (2005) indicated that the combination of additives with different mechanisms of action might result in synergistic effects that may enhance ruminal fermentation. Active components or mixtures of EO have the potentials to modify rumen N metabolism by reducing degradation of proteins and ammonia production in the rumen at low concentrations (McIntosh et al., 2003; Molero et al., 2004; Newbold et al., 2004; Busguet et al., 2005 a, b; Chaves et al., 2008).

Vitex agnus-castus L. (Verbenaceae) is a small tree or shrub, widely distributed along the Anatolian coastal lane (Davis, 1982). The fruits were formerly used as a substitute for pepper from Italy to Eastern Georgia (Stojkoviç et al., 2011). In Anatolian folk medicine, *V. agnus-castus* is used as diuretic, digestive, antifungal, anti-anxiety, early-birth and stomach-ache drugs (Baytop, 1999; Honda et al., 1996).

Nutritional quality of feeds are usually evaluated with *in vitro* techniques due to their convenciences, adaptability and efficiency (Getachew et al., 2005). The close association between rumen fermentation and gas production has long been recognized. Menke et al. (1979) developed *in vitro* gas measuring technique for feed evaluation. Feed fermentation is conducted in a 100 ml calibrated glass syringes containing the feedstuff and buffered rumen fluid. The gas measuring technique was reported had a high correlation between gas production *in vitro* and *in vivo* apparent digestibility (Menke et al., 1979). The gas produced in the gas technique is the direct gas produced as a result of fermentation (CO_2 and CH_4) and the indirect gas produced from the buffering of short chain fatty acids (CO_2 released from the bicarbonate buffer) (Getachew et al.,1998).

The objective of this study was to evaluate the effects of increasing doses of *V. agnus-castus* on *in vitro* gas production, organic matter digestibility and net energy lactation contents by using *in vitro* gas production method.

MATERIALS AND METHODS

Animal and experimental diets

Two rumen-fistulated rams (Tahirova breed and East Friesian 75% x Kivircik 25 %) were used. The rams were fed 60% alfalfa hay and 40% concentrate feed twice daily, as described by Steingass and Menke (1986). The feed material consists of fattening concentrate (CON) and *F. agni-casti* (FAC). Five different levels of FAC (6 replications per treatment) were added to the concentrate (CON) to produce 200 mg DM (CON- without FAC; 1 - 2 mg FAC + 198 mg CON; 2 - 4 mg FAC + 196 mg CON; 3 - 6 mg FAC + 194 mg CON; 4 - 8 mg FAC + 19 2 mg CON; 5 - 10 mg FAC + 190 mg CON). The results of the crude nutrient analysis of CON and FAC are presented in Table 1.

Chemical analysis

The concentrate feed and the aromatic plants were ground on a 1 mm screen in preparation for chemical analysis. The dry matter (DM), crude protein (CP), ether extract (EE), crude ash (CA) and crude fibre (CF) were analysed according to Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten, VDLUFA (Naumann and Bassler, 1993). The metabolisable energy (ME) was calculated based on the chemical composition (Anonymous, 1991).

The rumen fluid was collected from two fistulated ruminal rams before the morning-feeding. The estimates of gas production were obtained using the method of Menke and Steingass (1988). A buffer solution (macro and micro-minerals) was prepared on the day prior to the analysis, and incubated in a water bath at 39°C under a continuous CO_2 stream (DLG, 1981). Incubations were terminated after 24 h for the organic matter digestibility (OMD) and for net energy lactation (NEL) estimations of the concentrate and for the aromatic plant mixtures. The volume of gas produced was recorded at 2, 4, 8, 12 and 24 h after inoculation; the gas production (GP) results were applied in order to calculate OMD and NEL, using the following equations:

OMD (%) = 0.889 x GP + 0.448 x CP* + 0.651 x CA* + 14.88 (Menke and Huss, 1987). *in % DM.

NEL (MJ/kg DM) = 3.95 + 0.3305 x GP-0.0023 x GP^2 + 0.0535 x CP + 0.0132 x EE^2-0.0336 x CF -0.1073 x CA (Aiple, 1993).

GP: 24-h cumulative gas production in DM.

The essential oil from 10 g of dry fructus was extracted by hydro-distillation for 3 h using a Clavenger-type apparatus, according to the European Pharmacopoeia (1975), with three replications. The GC analyses were performed at the Medicinal Plants, Drugs and Scientific Research Center of Antolian University using a Shimadzu GC-9A gas chromatograph.

Statistical analysis

The data obtained were evaluated using the GLM procedure of SPSS V10 software. Duncan's test was employed for the comparison of the differences between the group averages (Efe et al., 2000).

RESULTS AND DISCUSSION

As shown in Figure 1A and Table 2, the highest and the lowest GP values were found after 2 h of incubation respectively, for FAC_3, FAC_4 and CON. The highest and the lowest GP values were found after 4 h of incubation, respectively, for CON and FAC_5 (Figure 1B).

Gas production mainly reflected carbohydrate degradation. Cone et al. (1997) and Cone and Van Gelder (1999) showed that initially, gas is produced from fermentation of the water-soluble components, such as sugars and protein. In this study, the GP-improving effect occurred at 2 h of incubation in FAC_3 and FAC_4 groups. Degradation of the water-soluble carbohydrates increased the concentrate feed with FAC (3 and 4% level) additions. However, gas produced at 4 h incubation was reduced by FAC_4 and FAC_5. The GP values determined after 8 h of incubation (Figure 1C) showed a significant difference between CON and all the treatments ($P<0.01$). After 12 h, the highest GP was found in CON (48.49±0.55), whereas

Table 1. The nutrient composition of CON and FAC, and essential oil ratio of VAC and the chemical content of essential oil.

Sample name	DM (%)	CA (%)	CP (%)	EE (%)	CF (%)	NFE (%)	ME (Kcal/kg)
CON	89.38	4. 8	15.15	4.39	6.27	58.03	2725
FAC	91.12	3.72	6.49	6.26	64.63	10.01	490
	EOR (% DM)	**1,8-cineole**	**Sabinen**	**α-Terpenyl acetate**	**α-Pinene**	**Unknown**	**Total compounds**
FAC	0.27	32.10	20.70	7.4	7.00	32.8	100

EOR: Essential oil ration, DM: dry matter, CA: crude ash, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: nitrogen free extract, ME: metabolic energy.

Table 2. GP, OMD and NEL contents of CON and FAC.

Samples	Gas production (ml/200 mg DM) incubation period					OMD (%)	NEL (MJ/kg DM)
	2-h	4-h	8-h	12-h	24-h		
CON	$13.12^{b}\pm0.18$	$22.44^{a}\pm0.31$	$39.81^{a}\pm0.44$	$48.49^{a}\pm0.55$	60.13±0.52	79.47±0.47	8.01±0.03
FAC1	$13.43^{b}\pm0.11$	$22.27^{a}\pm0.01$	$37.47^{bc}\pm0.01$	$46.84^{b}\pm0.01$	58.59±0.17	78.11±0.16	7.92±0.01
FAC2	$13.43^{b}\pm0.22$	$22.44^{a}\pm0.29$	$37.46^{bc}\pm0.39$	$46.91^{b}\pm0.45$	59.46±0.56	78.88±0.50	7.97±0.03
FAC3	$14.36^{a}\pm0.17$	$22.38^{a}\pm0.16$	$37.90^{b}\pm0.21$	$46.19^{b}\pm0.27$	60.28±0.40	79.61±0.34	8.02±0.02
FAC4	$14.27^{a}\pm0.18$	$22.11^{b}\pm0.29$	$37.19^{bc}\pm0.45$	$46.10^{b}\pm0.57$	60.10±0.78	79.45±0.69	8.00±0.04
FAC5	$13.46^{b}\pm0.09$	$21.31^{b}\pm0.17$	$36.29^{c}\pm0.25$	$44.49^{c}\pm0.25$	58.67±0.40	78.18±0.35	7.93±0.02
P	0.001	0.014	0.001	0.001	0.077	0.077	0.084

$^{abc:}$ Means with different letters in the same column are statistically significant (P<0.01). CON: Concentrate, FAC: *Fructus agni-casti*, 1, 2, 3, 4, 5: FAC dose in concentrate (200 mg DM).

the lowest value was found in FAC_5 (44.49±0.25). Gas produced at 6 h incubation was a good estimate of the extent of fermentation of non structural carbohydrate (estimated primarily as sugars, pectin and starches). Gas production level from 6 to 24 h of incubation was a good estimate of the amount of fermentation of structural carbohydrate that occur in cows at high feed intake levels (Orskov and McDonald 1979). The soluble carbohydrates are more rapidly digested by ruminal microorganism than the structural polysaccharides such as cellulose or the storage polymers such as starch (Stefanon at al., 1996). In this study, the GP-reducing effect occurred from 8 to 12 h of incubation in all the treatments (Figure 1C and D). It was shown that, the concentrate feed with FAC addition reduced degradation of storage polymers such as starch.

The highest GP (at the 24 h) value was found in the FAC_3 group at 60.28±0.40 mL/200 mg DM. However, FAC addition had slightly changed GP values as compared to CON group. Similarly, there was no significant difference found between OMD and NEL (P>0.01). Busquet et al. (2005b) found that cinnamaldehyde and garlic oil had no effect on DM, OM, NDF and ADF digestibility or on the total VFA concentration, and suggested that these additives cannot modify the overall diet fermentability. Castillejos et al. (2005) determined that 1.5 mg/L BEO (Crina ruminants) supplemented with high concentrate and forage rations (100 forage and 900 concentrate versus 600 forage and 400 concentrate) cannot affect DM, OM, NDF, ADF and CP digestion, though BEO increased the total VFA concentration (122.8 versus 116.2 mM). In another study, Newbold et al. (2004) observed a reduction in the *in situ* DM degradation of soya-bean meal after 8 and 16 h of incubation when 110 mg/d essential oil (a mixture of thymol, guaiacol and limonene) was added to the diet of sheep. However, the mixture had no effect on DM degradability of rapeseed meal and hay. Similarly, the digestibility of NDF and ADF was not affected in lactating dairy cows supplemented with a mixture of EO compounds according to Benchaar et al. (2003). Garcia et al. (2007) reported that the addition of carvacrol reduced *in vitro* DM, CP and neutral-detergent fibre (NDF) digestion. The effects induced by 250 mg/L carvacrol on DM digestion after 72 h of incubation were comparable to those of monensin, whereas a greater decrease was noticeable when carvacrol was added at a concentration of 500 mg/L. The researchers explained that the reduced CP potential degradability by addition was caused by the reduction of the slowly degradable fraction. Azar et al. (2011) evaluated the effects of three doses of *Zataria multiflora* water extract (0, 0.15 and 0.3 ml/30 ml buffered rumen fluid) on the short chain fatty acid, net energy, metobolizable energy and organic matter digestibility of canola meal using *in vitro* gas production technique and

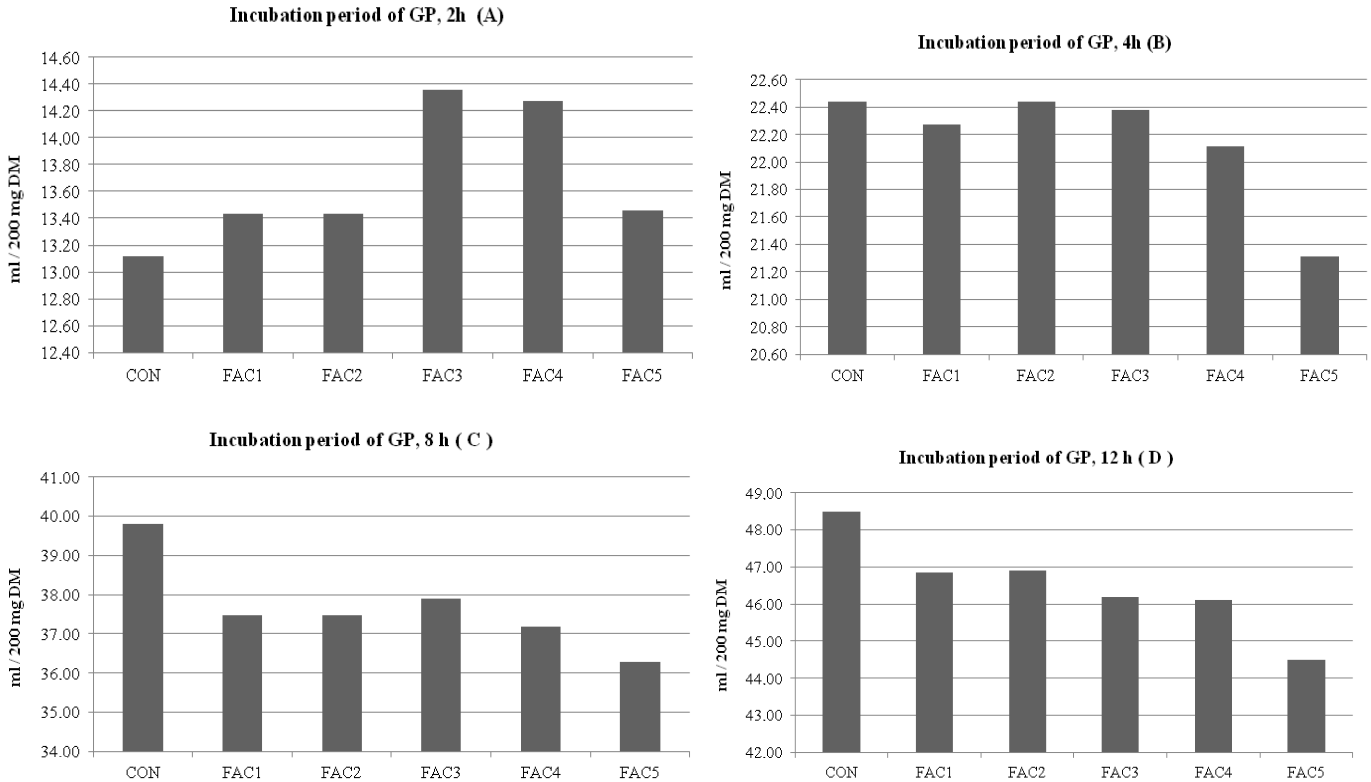

Figure 1. Incubation periods of GP at 2 h (A), 4 h (B), 8 h (C), 12 h (D).

reported that the amounts of organic matter digestibility, metabolizable energy, short chain fatty acid and net energy for lactation (NEL) of canola meal (79.46 g/ kg DM, 10.27 MJ/kg DM, 1.046 mmol and 5.28 MJ/kg DM respectively) were high as compared to *Z. Multiflora* water extract (0.3 ml/30 ml buffered rumen fluid) which were 41.85 g/kg DM, 3.63 MJ/kg DM, 1.047 mmol and 1.22 MJ/kg DM, respectively.

In the present study, it was found that GP (at the 24 h), OMD and NEL were not affected by addition of FAC. These findings corresponded with the findings of Busquet et al. (2005b), Castillejos et al. (2005) and Newbold et al. (2004). Essential oils have antimicrobial activities against Gram-negative and positive bacteria, which were related to a number of small terpenoid and phenolic compounds (Helander et al., 1998; Dorman and Deans, 2000). Pattnaik et al. (1997) stated that 1,8-cineole and camphor exhibit strong antimicrobial effects. Lis-Balchin and Deans (1997) showed that essential oils containing large amounts of 1,8-cineole are better anti-listerial agents than those without 1,8-cineole. Mourey and Canillac (2002) reported that 1,8-cineole is more bacteriocidal than β-pinene and are ineffective bacteriostatic at concentrations less than 0.062%. In another study, Hayouni et al. (2008) reported that the antimicrobial activity of *S. officinalis* essential oil is related to the 1,8-cineole, α/β-thujone and borneol content in the oil. Nagy and Tengerdy (1968) evaluated the sensitivity of ruminal microorganisms to the essential oil of *Artemisia tridentate* (main compound 1,8-cineole) because some evidence indicated that high intake of this plant can cause digestive problems in wild deer. In addition, Soycan-Onenc (2008) reported that varying levels of FAC addition to dry timothy grass and barley decreased the total GP and OMD, which was associated with a decrease or inhibition of the activities of amylolytic and sellulolitic bacteria in the rumen. Santoyo et al. (2005), Pattnaik et al. (1997) and Hayouni et al. (2008) reported that antimicrobial effect is related to borneol and camphor concentrations. In this study, FAC contains 0.8% essential oil (0.27% ground FAC in DM), which comprised of 32.10% 1.8-cineole and 20.70% sabinen (Table 1).

Antimicrobial activity of EO is dependent on ruminal pH and a more pronounced effect at a lower ruminal pH (Calsamiglia et al., 2007), whereas low pH can increase the influence of some active compounds of EO due to conformational changes in their structures and the higher sensibility of rumen bacteria to these compounds (Skandamis and Nychas, 2000). Also, most studies have been carried out on rumen fluids and diets of dairy cattle, but the generalized results that beef cattle consume high levels of concentrate can be misleading. This is because the effects of EO were indicated to be highly dependent on the diet and the ruminal pH (Cardozo et al., 2005;

Castillejos et al., 2005).

Conclusion

In this study, the GP-improving effect occurred at 2 h of incubation in FAC_3 and FAC_4 groups. Degradation of the water-soluble carbohydrates promoted the concentrate feed with FAC (3 and 4% level) additions. However, the GP-reducing effect occurred from 8 to 12 h of incubation in all the treatments. It was shown that, the concentrate feed with FAC addition reduced degradation of storage polymers such as starch. In addition, GP (at the 24 h), OMD and NEL were not affected by supplemented with FAC. The effects of FAC on GP, OMD and ME varied according to the feed used, the pH of the rumen fluid, the EO content of the aromatic plant and the amount supplemented.

Conflict of Interests

The authors have not declared any conflict of interests.

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