

ORIGINAL ARTICLE

Evaluation Effect of Thyme Extract on Degradability of Soybean Meal with Gas Product Technique

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ABSTRACT

In this study, an in vitro rumen gas production technique was utilized to evaluate the effect of thyme methanolic extract (0 and 1 ml/30ml buffered rumen fluid) on degradability of soybean meal. The feed sample (200mg soybean meal plus extract) were incubated with rumen liquor taken from three fistulated taresh cow at 2,4,6,8,12,24,48,72 and 96h. result show that gas production was reduced ($p<0/05$) on addition extract of Thyme. Result showed that extract of thyme decreased water soluble fraction (a), the slow degradable fraction (b), the potential degradability (a+b) and increased degradation rate of the slow fraction(c). This study suggested that thyme extract have the potential to affect fermentation efficiency.

Key words: Thyme, soybean meal, gas product test, fermentation efficiency

INTRODUCTION

A great number of plant species contain various chemical substances exhibiting health benefit properties, anti-oxidative, anti-inflammatory and antimicrobial effects. The anti-inflammatory effect of thyme has been employed in traditional medicine. The main active ingredients of the essential oil (EO) are thymol and carvacrol, with anti-oxidative, antimicrobial and antifungal effects [10]. Feeding ruminant in intensive production systems, especially for dairy production, requires supplies of high levels energy and protein. Animals are thus fed on rations rich in starch and high quality protein, which are fermented very rapidly. It is well know that the rapid degradation of starch tend to cause ruminal acidosis. The rapid breakdown of dietary protein to ammonia increases nitrogenous excretions rather than contributing directly to the animals nutrient requirements. Ruminants establish a symbiotic relationship with rumen microorganisms by which the animal provides nutrients and optimal environmental conditions for the fermentation of feeds, and microorganisms degrade fiber and synthesize microbial protein as an energy and protein supply for the animal, respectively. However, this symbiotic relationship has energy losses of methane and protein losses of ammonia (N) inefficiencies [12]. Ruminal fermentation of hexoses and amino acids is accompanied by losses of energy and amino nitrogen, respectively [3]. In fact, 8 to 12 percent of the digestible energy ingested by ruminants is lost in the rumen as methane, whereas from 75 to 85 percent of the nitrogen consumed by dairy cows is excreted in feces and urine [11, 3]. In order to delay ruminal protein degradation, dietary protein was 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 leaves harmful residues in the food chain and promote the spreading of resistance genes. These led to its prohibition in European Union in 2006 in animal feeding [1]. Accordingly there is greater interest in using plants and plant extract as alternatives to feed antibiotics to manipulate ruminal fermentation, improve feed efficiency and animal productivity [2, 8, and 9]. The purposes of this study were to evaluate effects of thyme methanolic extract on degradability kinetics, of soybean meal using *in vitro* gas production technique.

MATERIALS AND METHODS

Sample preparation and treatment

Thyme (shirazian thyme) and soybean meal samples were obtained from commercial sources in Iran.

Procedure of plant extracts preparation

The Thyme extract were prepared according to patra et al (2006) with some modifications. The plant materials were dried at 50°C and ground in mills to pass a1mm sieve and 100g placed in 1000 ml of methanol solvent (%95). The bottle of all the solvents were stoppered and agitated with a magnetic stirrer for 24h at room temperature. Then the solutions were filtered through Whatman 1 filter paper. The residue was reextracted with 500ml of methanol for 24h staying at room temperature and be filtered through Whatman 1 filter paper. Extracts were combined. Methanol was evaporated from the solution at approximately 45°C by using a rotary evaporator. The concentrated extract was stored at 4°C for further use.

Treatments and experimental design

Two level(0 and 1ml/30 ml buffered rumen fluid) of plant extract in a syringe (100ml) containing 200mg of milled (1mm) soybean meals were added to the syringe.

In vitro gas production

The buffer and rumen liquor were prepared as described by menke and steingass [5]. Three ruminal cannulated iranian Taleshi native male cow with weighing approximately 450 kg with a diet containing alfaalfa hay (60%) and concentrate (40%) were chosen for taken rumen ligure. The samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass [5, 6] as follows. 200 mg dry weight of the sample was weighed in triplicate into calibrated glass syringes of 100 ml in the absence and presence of 1ml of methanolic thyme extract. The syringes were pre-warmed at 39°C before injecting 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C. The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Gas production was measured as the volume of gas in the calibrated syringes and was recorded before incubation 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours after incubation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions for soybean meal samples were determined by subtracting the volume of gas produced in the blanks. Cumulative gas production data were fitted to the model of Ørskov and McDonald [7, 4]

$$P = a + b (1 - e^{-ct})$$

Where P is the gas production at time t, a the gas production from soluble fraction (ml/200 mg DM), b the gas production from insoluble fraction (ml/200 mg DM), c the gas production rate constant (ml/h), a + b the potential gas production (ml/200 mg DM) and t is the incubation time (h).

RESULT AND DISCUSSION

Gas production volume and gas production parameters a, b, c and a+b calculated amounts of Soybean meal, thyme methanolic extract (0 and 1 ml/30 ml buffered rumen fluid) are presented in Table 1 and 2. The gas volumes for soybean meal in different incubation times were higher than that of thyme methanolic extract (1 ml/30ml buffered rumen fluid).

Table 1. *In vitro* gas production volume (ml/200mg DM) of soybean meal at different incubation times.

Treatments	Incubation time								
	2	4	6	8	12	24	48	72	96
No additive	13/130	27/750	33/500	40/620	51/620	65/370	71/240	72/240	74/360
Thyme ₁	2/470	4/833	5/833	6/500	7/080	7/747	8/580	9/163	9/243

Table2. The estimated parameters from the gas production were soybean meal, thyme methanolic extracts (0 and 1 ml/30 ml buffered rumen fluid)

Treatments	Estimated parameters			
	a	b	a +b	C
No additive	1/767	70/880	72/647	0/100
Thyme ₁	0/577	8/669	9/276	0/190

a: the gas production from soluble fraction (ml/200 mg DM), b: the gas production from insoluble fraction (ml/200mg DM), c: rate constant of gas production during incubation (ml/h), (a + b): the potential gas production (ml/200mgDM).

Result show that gas production was reduced on addition extract of thyme. Extract of thyme caused decreased water soluble fraction (a) , the slow degradable fraction(b) , the potential degradability(a+b) and increased degradation rate of the slow fraction(c).

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 Blend Essential Oil (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. This study suggested that the plant extracts have the potential to affect ruminal fermentation efficiency.

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