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The effect of various semi-arid native plant essential oils on *in vitro* acid load of a low forage diet

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Abstract

In ruminants, consumption of diets high in readily fermentable carbohydrates, such as starch, may result in a rapid increase in rumen acid load leading to a drop in ruminal acidosis. The aim of the present experiment was to determine the effect of various semi-arid native plant essential oils (EO) including thyme (TH) and peppermint (PE) leaves, and cumin (CU) and fennel (FE) seeds on *in vitro* medium pH, acidogenicity value (AV) and dry matter disappearance (DMD) of a low forage basal diet (BD) containing 20% alfalfa hay and 80% concentrate. Experimental treatments were BD, BD+TH, BD+CU, BD+FE and BD+PE. Approximately, 1 g of the basal diet was placed into a 125 ml bottle, as alone or combined with the EO evaluated, then 30 ml of buffered rumen liquor [60% diluted buffer, and 40% rumen liquor] was added (n=4). Each essential oil was applied as 1 ml/100 ml of the medium. The rumen fluid was collected, before the morning feeding, from three rumen cannulated sheep (body weigh; 45±2 kg) and filtered through four layers of cheesecloth. Animals were fed 1.4 kg alfalfa hay and 400 g concentrate (CP; 165 g kg⁻¹ of DM), twice daily (08.00 h and 20.00 h). Bottles were incubated for 24 hours under anaerobic condition in a water bath at 38.6 °C. After the incubation period, medium pH was measured immediately. Each bottle contents were filtered through a 45 µm filter, then unfiltered residual was dried using an air-forced oven at 65 °C for 48 h, then weighed to determine the dry disappearance. A sample (2 ml) was withdrawn from each bottle filtered content, then transferred to a tube containing 50 mg (excess) of CaCO₃ powder, then centrifuged at 4000 RPM for 10 min and supernatant was removed; next by means of atomic absorption device its calcium concentration was determined. The AV was calculated as the product of Ca concentration (from the analysis) and fluid volume (30 ml) divided by the sample weight (1 g). The experiment was carried out in a completely randomized design. Statistical analysis was done by SAS software using GLM procedure. Thyme leaf EO caused a significant (P<0.05) increase in the pH of the medium (6.13) and a significant decrease in the AV (1.1) compared with the BD as control (4.67 and 12.7, respectively). Therefore, the potential of this essential oil to optimize both pH and AV of a rich concentrate ruminant diet might be an important finding of the present study.

Keywords: essential oils, acidogenicity value, rumen

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Summary

In ruminants, consumption of diets high in readily fermentable carbohydrates, such as starch, may result in a rapid increase in rumen acid load leading to a drop in ruminal acidosis. The aim of the present experiment was to determine the effect of various semi-arid native plant essential oils (EO) including thyme (TH) and peppermint (PE) leaves, and cumin (CU) and fennel (FE) seeds on in vitro medium pH, acidogenicity value (AV) and dry matter disappearance (DMD) of a low forage basal diet (BD) containing 20% alfalfa hay and 80% concentrate. Experimental treatments were BD, BD+TH, BD+CU, BD+FE and BD+PE. Approximately, 1 g of the basal diet was placed into a 125 ml bottle, as alone or combined with the EO evaluated, then 30 ml of buffered rumen liquor [60% diluted buffer, and 40% rumen liquor] was added (n=4). Each essential oil was applied as 1 ml/100 ml of the medium. The rumen fluid was collected, before the morning feeding, from three rumen cannulated sheep (body weigh; 45±2 kg) and filtered through four layers of cheesecloth. Animals were fed 1.4 kg alfalfa hay and 400 g concentrate (CP; 165 g kg⁻¹ of DM), twice daily (0800 h and 2000 h). Bottles were incubated for 24 hours under anaerobic condition in a water bath at 38.6 °C. After the incubation period, medium pH was measured immediately. Each bottle contents were filtered through a 45 µm filter, then unfiltered residual was dried using an air-forced oven at 65 °C for 48 h, then weighed to determine the dry disappearance. A sample (2 ml) was withdrawn from each bottle filtered content, then transferred to a tube containing 50 mg (excess) of CaCO₃ powder, then centrifuged at 4000 RPM for 10 min and supernatant was removed; next by means of atomic absorption device its calcium concentration was determined. The AV was calculated as the product of Ca concentration (from the analysis) and fluid volume (30 ml) divided by the sample weight (1 g). The experiment was carried out in a completely randomized design. Statistical analysis was done by SAS software using GLM procedure. Thyme leaf EO caused a significant (P< 0.05) increase in the pH of the medium (6.13) and a significant decrease in the AV (1.1) compared with the BD as control (4.67 and 12.7, respectively). Therefore, the potential of this essential oil to optimize both pH and AV of a rich concentrate ruminant diet might be an important finding of the present study.

Key Words: essential oils, acidogenicity value, rumen

Introduction

Using high consent ration increases the possibility of some metabolic diseases like acidosis. As the concentrated in diet increases, ruminal pH decreases. If ruminal pH reaches an amount of less than 6, rumen acidosis occurs. Acidosis causes a decrease in appetite, milk production, and can even lead to death (Wadhwa et al., 2001). Recent studies have shown that extracts, essential oils (EO) and spices are safe, have antibacterial, antifungal, and antioxidant properties and can be used as natural additives in animal feeds, moreover, they can improve the process of ruminal fermentation (Cowan, 1999). The aim of this experiment was determine the acid load of diets containing 20% alfalfa and 80% concentrate in a medium containing enriched rumen fluid of sheep with thyme leaf, cumin seed, fennel seed and peppermint essential oils.

Materials and Methods

In this experiment thyme leaf, cumin seed, fennel seed and peppermint essential oils have been used. The basal diet contained %20 alfalfa (Crude protein (CP); 177 g kg⁻¹ of Dry matter (DM), Neutral Detergent Fiber (NDF); 457 g kg⁻¹ of DM) and %80 concentrate (CP; 180 g kg⁻¹ of DM and NDF 296 g kg⁻¹ of DM). The basal diet was milled through a 1.5 mm screen then placed in a 60°C oven to be dried out. Experimental treatments were basal diet (20% alfalfa and 80 % concentrate, control), basal diet plus thyme leaf EO, basal diet plus cumin seed EO, basal diet plus fennel seed EO and basal diet plus peppermint EO (4 replicate for each treatment). Each EO was used 1 ml per 100 ml of the medium. The acidogenicity value (AV) of the medium was determined by means of the method wadhwa (2001). In this method 1g of the basal diet (with or without EO) was placed in a 125 ml bottles and 30 ml of buffered rumen liquor[%60 diluted buffer suggested by Tilley and Terry (1963), and %40 rumen liquor] was added and Each bottle was incubated for 24 hours under anaerobic condition in 38.6°C. The rumen fluid was collected before the morning feeding from three fistulated male sheep (body weigh; 45±2kg) and filtered through four layers of cheesecloth. Animals were fed twice daily (0800 h and 2000 h) 1.4 kg alfalfa and 400 g concentrate (CP; 165 g kg⁻¹ of DM). After the incubation period, medium pH was measured immediately .Then, the medium was filtered through a 45 µm filter. Samples (2 ml) were withdrawn from each tube after 24 h and transferred to centrifuge tubes containing 50 mg (excess) of CaCO₃ powder. The mixture was shaken manually for 5 s and then centrifuged at 4000 g for 10 min and the upper liquid was removed; next by means of atomic absorption device its calcium concentration was determined. The AV was calculated as the product of Ca concentration (from the analysis) and fluid volume (30ml) divided by the sample weight (1 g). The remains of the filter were dried at 60°C to measure the rate of disappearance of dry matter. The experiment was carried out in a completely randomized design. Statistical analysis was done by SAS software via GLM procedure. The comparison experimental treatments with control (basal diet) was accomplished by using Dannel test (p<0/05).

Results and Discussion

The result of the effect of experimental treatments on the pH of medium, disappearance of DM and AV of the medium containing rumen fluid are shown in table1.

Table1. Effects of experimental treatments (thyme leaf, cumin seed, fennel seed and peppermint essential oils) on the pH of medium, disappearance of DM and AV of the medium.

	Experimental diet					¹ SEM	<i>P</i> -value
	control	fennel	cumin	peppermint	thyme		
pH(%)	4.67	4.46	4.44	4.48	6.13 ^a	0.07	< 0.001
Dis. Dm(%)	0.54	0.47	0.45	0.47	0.43	0.01	< 0.001
AV (mg Ca/g feed DM)	12.72	15.64	16.35	12.74	1.1 ^a	1.37	< 0.001

¹SEM = Standard error of means ; ^a: Means within a row with control different superscripts differ (*P* < 0.05).

Thyme leaf EO caused a significant increase in the pH of the medium and caused a significant decrease in the AV of the medium compared to the control (*p*<0/05). All essential oils used in this experiment significantly decreased the disappearance of the DM of the medium compared to the control (*p*<0/05). Fennel seed EO, Cumin seed EO and peppermint EO had no significant effect on the AV and PH of the medium (*p*<0/05).

It is reported that Thyme EO increase pH of medium but decrease feed digestion and volatile fatty acids production (Calsamiglia et al., 2007; Newbold et al., 2004; Spanghero et al., 2008). Ando et al (2003) showed that peppermint EO decrease fermentation but it has no effect on dry matter disappearance in medium. Kamra (2005) indicated that fennel EO decrease fermentation, without any effect on dry matter disappearance. In vitro experiments by patra (2005) showed that fennel EO can be reduced fermentation, methane production and dry matter disappearance. These results are agreed with our experimental.

Conclusion

These data indicate that the essential oils used in this experiment except thyme leaf had no significant effect on the AV of the medium. In addition, it can be concluded that thyme leaf essential oil had a potential to optimize both pH and AV of a rich concentrate ruminant diet.

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