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The effect of microbial oil, evening primrose oil, and borage oil on rumen ciliate populations in an artificial rumen (Rusitec)*

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ABSTRACT

Microbial (MO), borage (BO) and evening primrose (EPO) 5% (wt/wt) oils were supplemented to a diet consisting of meadow hay and ground barley (60:40) for six days following a five-day adaptation period in an artificial rumen (Rusitec) inoculated with sheep rumen fluid having an A-type ciliate population. After the adaptation period, the following rumen ciliate genera and species were established: *Entodinium* spp., *Dasytricha ruminantium, Eremoplastron bilobum, Diploplastron affine, Polyplastron multivesiculatum* and *Isotricha* spp. (*I. prostoma* and *I. intestinalis*). The total ciliate population as well as the population of *Eremoplastron* decreased ($P<0.05$ and $P<0.01$, respectively) in the group supplemented with BO. In contrast, the population of *Polyplastron* increased ($P<0.01$) following BO supplementation. The populations of *Dasytricha, Eremoplastron,* and *Isotricha* spp. decreased ($P<0.01$) in the group with EPO supplement, in contrast to the population of *Entodinium* spp., which increased ($P<0.05$). MO supplementation decreased the populations of *Eremoplastron* and *Isotricha* spp. ($P<0.01$). None of the oil supplements influenced the population of *Diploplastron.*

The results of this study showed that the examined species of rumen ciliates had no uniform response to the tested oils. Responses strongly depended on the composition of oils and the resultant concentration of the main fatty acid components.

KEY WORDS: microbial oil, evening primrose oil, borage oil, rumen ciliates, artificial rumen

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INTRODUCTION

Microbial oil (MO), evening primrose oil (EPO) and borage oil (BO) are oils with high polyunsaturated fatty acid contents, especially gamma linolenic acid ($C_{18:3}$, n-6), which participates in the metabolism of a wide variety of important metabolites regulating critical biological functions. Little information was found about the use of these oils in ruminants and none about their effects on protozoa, especially rumen ciliates. Increasing the content of polyunsaturated fatty acids in meat and milk is, however, a strategy to improve the quality of ruminant meat and dairy products due to their positive action on human health. In contrast to rumen bacteria, the levels of C 18:2 and C 18:3 fatty acids are higher in rumen ciliate protozoa (Viviani, 1970; Emmanuel, 1974; O'Kelly and Spiers, 1990). Enhancing the population of ciliate protozoa may therefore also prove to enhance the production of polyunsaturated fatty acids.

The aim of this work was to determine the effects of MO, EPO, and BO on the rumen ciliate population in an artificial rumen.

MATERIAL AND METHODS

The oil supplements and the general rumen simulation technique (Rusitec), its operation, and experimental design were described by Jalč et al. (2005). Briefly, MO (fermentation vessel 2), EPO (fermentation vessel 3), and BO (fermentation vessel 4) were supplemented at a rate of 5 % (wt/wt) of the diet consisting of meadow hay and ground barley (60:40) for six days following a five-day adaptation period in a Rusitec inoculated with sheep rumen fluid having an A-type ciliate population. Fermentation vessel 1 was not supplemented (control group). The chemical composition of the diet was presented by Jalč et al. (2005). Samples were collected on days 6-12 of Rusitec operation according to Kišidayová et al. (2001). After the adaptation period, the following rumen ciliate genera and species were established: *Entodinium* spp., *Dasytricha ruminantium, Eremoplastron bilobum, Diploplastron affine, Polyplastron multivesiculatum,* and *Isotricha* spp. (*Isotricha intestinalis* and *Isotricha prostoma*).

The results are given as arithmetic means ± standard error of means (SEM). The statistical significances of the differences between control and treatment values were assessed using one-way analysis of variance with Dunnet's post test (GraphPad Prism version 4.0 for Windows, GraphPad Software, Inc., San Diego, CA). Probability values of $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

In the present study the most numerous species of the *Entodinium* genus were: *Entodinium bursa, Entodinium caudatum, Entodinium longinucleatum, Entodinium furca monolobum,* and *Entodinium simplex.* The effects of oil supplements on the ciliate populations are summarized in Table 1. The total ciliate population

Table 1. Effect of microbial oil, evening primrose oil, and borage oil on ciliate populations in an artificial rumen

Fermentation vessel	Ciliate concentration, n/ml				Statistics
	V 1	V 2	V 3	V 4	
Treatment	none	microbial oil	evening primrose oil	borage oil	
Total concentration	8 065 ± 325	9 018 ± 471	8 846 ± 769	5 963 ± 168	V1 vs V4; P<0.05
Entodinium spp.	5 600 ± 318	7 050 ± 440	7 400 ± 751	4 125 ± 167	V1 vs V3; P<0.05
Dasytricha ruminantium	1 768 ± 43	1 575 ± 70	1 070 ± 58	1 568 ± 57	V1 vs V3; P<0.01
Eremoplastron bilobum	634 ± 31	373 ± 24	345 ± 22	196 ± 28	V1 vs V2-4; P<0.01
Diploplastron affine	24 ± 3	21 ± 4	29 ± 4	19 ± 4	NS
Polyplastron multivesiculatum	9 ± 8	0	0	38 ± 4	V1 vs V4; P<0.01
Isotricha spp.	31 ± 10	0	3 ± 3	15 ± 7	V1 vs V2-3; P<0.01

values are means ± SEM; NS, nonsignificant

as well as the population of *Eremoplastron bilobum* was decreased in the group supplemented by BO (P<0.05 and P<0.01, respectively) in comparison with the control group. In contrast, the population of *Polyplastron multivesiculatum* increased following BO supplementation (P<0.01). The populations of *Dasytricha ruminantium, Eremoplastron bilobum,* and *Isotricha* spp. decreased (P<0.01) in the group supplemented with EPO, in contrast to the *Entodinium* spp. population, which increased (P<0.05). MO supplementation decreased the populations of *Eremoplastron bilobum* and *Isotricha* spp., respectively (P<0.01). None of the oil supplements influenced the population of *Diploplastron affine.* It can be said that the most sensitive species was *Eremoplastron bilobum,* whose population decreased on all supplementation regimes.

As BO is the richest in gamma-linolenic acid (24%) among the tested oils, it seems that this fatty acid had an adverse effect on the total ciliate population. On the other hand, it was shown that linoleic acid had a stimulatory effect on the growth of *Entodinium caudatum* in *in vitro* culture (Kišidayová et al., 2005) which is in accordance with the present results. EPO, which contains 81% linoleic acid, had a stimulatory effect on the *Entodinium* spp. population.

CONCLUSIONS

The results of this study show that the examined species of rumen ciliates did not show a uniform response to the tested oils. The responses strongly depended on the composition of the oils and resultant concentration of the main fatty acid components.

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