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Effect of natural phytoestrogens on rumen microbial content in ewes

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ABSTRACT

Twelve Comisana ewes were fed two different diets based on subterranean clover or Italian ryegrass for about one year. Rumen samples were collected at slaughtering and total viable, cellulolytic and xylanolytic bacteria, fungi and protozoa were counted.

The effect of natural phytoestrogens on rumen microflora was revealed by a decrease in P in quantity and, consequently, an increase in bacteria growth.

KEY WORDS: rumen microbiology, ewes, phytoestrogens

INTRODUCTION

It is well known that forages with a high percentage of isoflavons (genistein, biochanin, formononetin) have a strong negative oestrogenic effect on the fertility of ruminants (Adams, 1990, 1995). Subterranean clover (SC) contains up to 5% of oestrogenic isoflavons and the detrimental effects are mainly due to the presence of a considerable amount of formononetin and its highly oestrogenic rumen metabolite equol (Dickinson et al., 1988).

In a previous experiment (Pace et al., 2000) some new lines of SC, selected for their very low content of formononetin (less than the 10% of total phytoestrogens), did not influence the fertility and fecundity of sheep, but increased body weight, when animals were fed fresh SC for about two months.

As no information on phytoestrogenic effects on rumen microrganism growth was found in the literature, the aim of this work was to examine potential changes in the microflora.

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MATERIAL AND METHODS

This work is part of a bigger trial investigating the general effect of SC phytoestrogen content on growth and reproduction of ewes in our Institute. The microbiological part of the trial was carried out on two groups of six Comisana ewes fed on SC (phytoestrogen content = 0.794 mg/g of dry matter, DM) or Italian ryegrass (IR) for about one year. *Ad libitum* forage was offered as fresh herbages for about 3 months and afterwards as hay. SC was a mixture of 4 varieties. The ratio was integrated with maize grains (300 g/d) and sunflower meal (250 g/d for SC and 300 to 350 g/d for IR) with a concentrate/forage = 45 to 50% of the total amount. Diets were iso-energetic (≅1.0 UFM) and iso-proteic (18.5% h/d during growing period, 11-12 to 15% during pregnancy).

The chemical composition of the forage is reported in Table 1. Phytoestrogen contents of SC is reported in Table 2. In IR no phytoestrogens were detected.

Total viable (TV), cellulolytic (CB) and xylanolytic bacteria (XB), fungi (F) and protozoa (P) were determined on samples of whole rumen content at slaughtering at 250 days of age.

The TV and XB were grown on Petri dishes on Leedle and Hespell solid media (1982) and counted after incubation at 39°C for 5 days. The CB were grown on Hungate liquid medium (Ogimoto and Imai, 1981), and the F were grown on Joblin medium (1981) and both were counted after incubation at 39°C for 15 days by the MPN procedure. The P number was determined by a light microscope by Warner's procedure (1962). The microbiological tests were all performed in an anaerobic glove box (CO_2 95% + H_2 5%).

The data were analysed using the General Linear Models procedure of least square means (LSM) and pooled standard error of the means were obtained. (SAS, 1982).

Table 1. Average chemical composition of subterranean clover (SC) and ryegrass (IR) (means of 5 determinations, % on dry matter) during the feeding period

	DM at 60°C	CP	Ash	NDF	ADF
SC	18.72	16.65	10.11	30.32	23.30
IR	26.45	10.48	8.67	46.67	29.17
SC hay	87.1	9.50	10.77	38.73	31.74
IR hay	88.7	6.77	8.29	70.22	45.04

Table 2. Average phytoestrogen concentrations of SC fresh herbage and hay (means ± s.d.)

	Phytoestrogen content, mg/g DM				
Forages	total	genistein	biochanin A	formononetin	F/G %
SC	0.794 ± 0.144	0.709 ± 0.137	0.023 ± 0.011	0.026 ± 0.023	3.60
SC hay	0.818 ± 0.112	0.780 ± 0.098	0.013 ± 0.009	0.016 ± 0.014	2.05

RESULTS AND DISCUSSION

Scored data are reported in Table 3, together with statistical analysis. TV, CB and F had higher values in SC compared to the IR group (Table 3), although only CB achieved a statistical difference between SC and IR groups. Results from the XB culture were random, and so they were not submitted to statistical analysis and therefore are not reported in the Table. On the contrary P were significantly higher in IR group. The negative effect of phytoestrogens on P growth would result in a reduced competition with other microganisms. Therefore, a decrease in P would indirectly improve bacteria development.

Bacteria constitute the main nitrogen source for animal growth, and a growth and reproduction trial reported differences in weights at the end of the growing period (9.7% higher in SC group) (Pace et al., 2004). Thus, we could hypothesize an increase in bacteria as the cause of the higher body weight of animals.

Table 3. Microbial counts (conc/g dry rumen) and statistical analysis

Microbial species	IR	SC
Fungi (F)	2.11×10^{4a}	2.26×10^{4a}
Total viable bacteria (TV)	1.43×10^{11a}	2.96×10^{11a}
Cellulolytic bacteria (CB)	$1.91 \times 10^{9\,a}$	$10.1 \times 10^{9b*}$
Protozoa (P)	$42.32 \times 10^{6b*}$	25.0×10^{6a}

* significance at 0.1%

CONCLUSIONS

The effect of natural phytoestrogens on rumen microflora was revealed by a decrease in protozoa. This led to an enhancement of rumen bacteria microflora which is presumably responsible for the body weight increase observed in ewes fed subterranean clover diet.

The random growth of xylanolytic bacteria needs to be investigated further.

REFERENCES

Adams N.R., 1990. Permanent infertility in ewes esposed to plant oestrogens. Aust. Vet. J. 67, 197-205

Adams N.R., 1995. Detection of the effect of phytoestrogens on sheep and cattle. J. Anim. Sci. 73, 1509-1515

Dickinson J.M., Smith G.R., Randel R.D., Pemberton I.J., 1988. In vitro metabolism of fomononetin and biochanin in a bovine rumen fluid. J. Anim. Sci. 66, 1969-1973

Joblin K.N., 1981. Isolation, enumeration and maintenance of rumen anaerobic fungi in roll tubes. Appl. Environ. Microbiol. 42, 1119

Leedle J.A., Hespell R.B., 1980. Differential carbohydrate media and anaerobic replica plating techniques in delineating carbohydrate-utilizing subgroups in rumen bacterial populations. Appl. Environ. Microbiol. 39, 709-719

Ogimoto K., Himai S., 1981. Atlas of Rumen Microbiology. Jpn. Sci. Soc. Press., Tokyo

Pace V, Settineri SD., Rassu S.P.G., 2000. Effetto della somministrazione di trifoglio sotterraneo sulla crescita e la riproduzione di agnelle di razza sarda. Nota 1. Proceeding of the XXXV International Symposium Società Italiana di Zootecnia, Ragusa (IT), pp. 257-265

Pace V., Carbone K., Spirito F., Terzano M.G., Verna M., Settineri D., 2004. Phytoestrogen content of subterranean clover related to ewe growth and reproduction. J. Anim. Feed Sci., Suppl. 1, 547-550

SAS User's Guide: Statistics. 1982. SAS Inst. Cary, NC

Warner A.C.I., 1962. Enumeration of rumen micro-organisms. J. Gen. Microbiol. 28, 119-128