Flavonoids as natural plant extracts in the feed to stabilise rumen fermentation

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The increase in demand for livestock production is leading ruminant nutrition to a more intensive system. Grain diets (or concentrate rations) are replacing roughage as the main source of rumen fermentable carbohydrates in order to improve production efficiency.

However, the intensive use of concentrate rations promotes particular fermentation dysfunction; the risk of acidosis increases, as starch is rapidly broken down by α-amylases and glucose is fermented by rumen bacteria increasing production and concentration of VFA and lactate.

Antibiotic therapies are allowed for maintaining such systems by keeping fermentation dysfunctions at a latent stage.

However, massive widespread use of antibiotic substances, its associated potential cross-over resistance to human therapies, together with consumers demands on food quality and safety, has led to the elimination of dietary antibiotic administration in the EU.

Such reasons forced beef and dairy industries to search for alternatives acidosis-reduction strategies.

Therapies based on plant extracts have been proposed as an alternative to those based on antibiotics.

Flavonoids are naturally occurring benzo-l-pyrone derivatives widely distributed in plants and have recently received research interest because of their antimicrobial activity.

Antimicrobial activity

The antimicrobial activity of flavonoids rely either, in the blockage of the energetic transfer through cytoplasm membrane or in the microbial catabolism of the flavonoids into low-molecular-weight compounds that exert some specific toxicity on rumen microbes, such as 3,4-dihydroxyphenyl-acetic and fenyl-acetic acid.

Issue	Control	FLV	MSE	P value
pH	6.1	6.4	0.18	***
VFA (mol/l)†	63.4	73.3	2.57	*
Acetate (mol/100mol)	59.4	54.1	1.10	*
Propionate (mol/100 mol)	28.1	35.3	1.01	**
Butyrate (mol/100 mol)	7.9	7.6	0.34	ns
Acetate/Propionate	2.3	1.7	0.12	*
NH_3-N (mg/dl)	41.5	10.2	18.26	*

†Measured at 0, 2 and 4 hours after food supply ns: not significant; *: P<0.05; **: P<0.01; ***: P<0.005

Table 1. Mean values of volatile fatty acids (VFA) ammonia-N and ruminal pH as affected by Bioflavex RC (FLV) supplementation.

Bioflavex RC is a balanced mixture of flavonoids substances extracted from bitter orange and grapefruit. The aim of the study was to evaluate 'in vivo' the effect of that product on rumen fermentation in finishing cattle fed high grain diets.

Rumen fermentation

In the first trial, 16 rumen Fleckvieh cannulated heifers were used (live weight (LW): 362 SE 7.34kg). They were weighed on two consecutive days and randomly assigned to two feeding groups (eight heifer/group) receiving, respectively, the control corn-concentrate (CTR); or the same basal concentrate supplemented with the above mentioned mixture of flavonoids (FLV);). Both concentrates and barley straw were offered ad libitum.

After dietary changeover rumen content was sampled weekly (at 8:00, 10:00 and 12:00 hours, respectively) until heifers reached target slaughter weight of 402.1 SE 5.3kg. The average values of pH, volatile fatty (VFA) acid and ammonia nitrogen (NH_3-N) determined in the rumen liquor, are summarised in Table 1.

The presence of Bioflavex RC into the concentrate did affect rumen fermentation, those heifer eating FLV diets showed a better pH (6.4 vs 6.1) and a high concentration of VFA (P<0.05).

The proportion of different VFA was also altered, increasing propionate and reducing acetate (P<0.05), acetate/propionate ratio was significantly (P<0.05) lower in FLV supplemented heifers as a measure of an improvement in rumen fermentation efficiency.

Changes in pH evolution

In a second assay Bioflavex RC was tested as a stabilising factor of rumen fermentation under experimental acidosis induction, analysing simultaneously the influence of the flavonoids mixture in lactate concentration and in those specific rumen bacterial groups involved in lactate metabolism.

The experiment consisted of a cross-over design, using eight cannulated crossbreed heifers (LW 451.4 ± 14.32kg) for two periods. Each period lasted for 22 days.

From day one to day 20 animals were fed

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Table 2. Effect of Bioflavex RC (FLV) supplementation on rumen pH evolution.

pH	CTR	FLV	SEM	P value
Average	6.63	6.69	0.161	NS
Fasting (day 21)	7.08	7.07	0.023	NS
Acidosis induction	5.98	6.29	0.048	*
Minium	5.22	5.83	0.191	***
Area below pH<6 (h)	5.10	2.69	0.680	*
Area below pH<5.5 (h)	0.85	0.028	0.136	***

ns: no significant; *: P<0.05; **: P<0.01; ***: P<0.005

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ryegrass hay (circa 8kg fresh matter/day); on day 21 feed was removed for 24 hours, and on day 22 rumen acidosis was induced by supplying 5kg of grinded wheat through the rumen cannula.

The wheat was supplemented with Bioflavex RC (0.3%, to the FLV group) and not to CTR group. During the last three days (19-22) of each experimental period ruminal pH was continuously recorded and samples of ruminal content were collected after acidosis induction.

Rumen fluid was sampled for lactate and DNA extraction. Streptococcus bovis; Megasphaera elsdenii and Selenomonas ruminantium, titles were determined by real time (qPCR) using the specific pre-designed primers. Differences were recorded neither in the pH before acidosis induction nor during fasting period. However, pH decrease after wheat supplementation, but such decrease was less pronounced (P <0.05) in heifers that had received flavonoids than in CTR heifers (6.29±0.031 and 5.98± 0.029 for FLV and CTR, respectively).

	Treatment		Hour			
	CT	FLV	Initial	Pool	MSE	P value
Lactate concentration (mg/l)	84.7	71.1	36.6	122.6	6.76	ns ***
Relative quantification (CT)						
S. bovis	0.51	0.48	0.25	0.74	0.111	ns ***
S. ruminantium	0.51	0.53	0.36	0.68	0.113	ns **
M. elsdenii	1.08	1.46	1.18	1.35	0.242	* T
Biodiversity analyses (tRFs)	38.56	38.25	37.06	39.75	1.113	ns ns

ns: not significant; *: P<0.05; **: P<0.01; ***: P<0.005

Table 3. Effect of Bioflavex RC on lactate concentration (mg/l), ruminal populations of S. ruminantium, S. bovis and M. elsdenii and biodiversity-tRFLP analyses.

Flavonoids also significantly reduced the number of hours for which the pH level was below 6 (P<0.05) and 5.5 (P<0.005).

It has also been verified that the increase of lactate in the rumen was numerically less (P <0.09) in FLV (from 36.8-106.6mg/l) than CTR heifers (from 36.5-138.3mg/l).

The amount of Streptococcus bovis and Selenomonas ruminantium increased in both treatments after wheat supplementation, while Megaesphera elsdenii titers increased only in FLV supplemented heifers (P <0.05).

No effect of flavonoids was detected in biodiversity analyses.

The hypothetical mechanism of the antimicrobial action of flavonoids would be either:

- Decreasing starch digestion into the rumen and thus decreasing the acidification process trough VFA and lactate production.
- Assuming that lactate play a central role in the acidosis process, by altering the proportion between lactate producing/consuming bacteria. In this sense our results indicate that Bioflavex RC supplementation may be effective in reducing the acidosis process by modulating titers of lactate consuming micro-organisms such as M. elsdenii.

Implications

The addition of Bioflavex RC into the concentrate of growing cattle fed high grain rations significantly improved rumen acidity and also the proportion of propionate and acetate/propionate ratio in the rumen fluid.

Moreover, the presence of the flavonoids mixture under acidotic induced conditions alleviated pH reduction and also decreased significantly the number of hours below rumen pH 5.5 (when rumen had a pH bellow 5.5). Activity of Bioflavex RC may be partially explained by modulation of titers of lactate consuming micro-organisms such as M. elsdenii. ■

References are available from the author on request