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Use of methylglyoxal as a tool in predicting ruminal nitrogen status

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The Story in Brief

- Ruminal nitrogen (N) is most commonly measured via ruminal ammonia, but because of constant inputs and outputs of nitrogen from the diet and the animal, ruminal ammonia may not give an accurate picture of nitrogen available for microbial protein synthesis by the rumen microbes. Other methods for assessment of ruminal nitrogen are needed.
- Methylglyoxal (MG) is a toxic compound produced as a by-product of glycolysis.
  - Glycolysis is the process by which feed is converted to glucose, which is then converted to pyruvate, then volatile fatty acids, which are then used by the animal for energy.
- When a nutrient imbalance is established, the glycolytic process may branch, producing MG.
- Methylglyoxal production is a form of energy spilling utilized by the ruminal bacteria as a survival mechanism, when a nutrient imbalance is present.
  - Energy spilling is based around the ATP pool. When ATP levels exceed what is needed for anabolic reactions such a protein synthesis, ATP is spilled and not utilized by the bacteria.
  - Ruminal microbes produce MG in hopes that nutrient balance will be regained, however if it is not, MG production will result in microbial death, thus greatly reducing VFA production and microbial protein flow to the small intestine.

The Problem

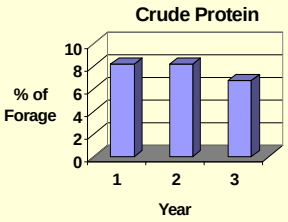
- Ruminants may produce MG when consuming low protein dormant forage.
  - This creates conditions in the rumen with excess carbohydrate and inadequate protein (N) in relation to the needs of the microbial population.
  - In addition to production of MG via glycolysis, this toxin may result from amino acid breakdown and/or metabolism of fatty acids.
  - •The hypothesis for this experiment is methylglyoxal may be a more sensitive marker for evaluating ruminal nutrient balance.

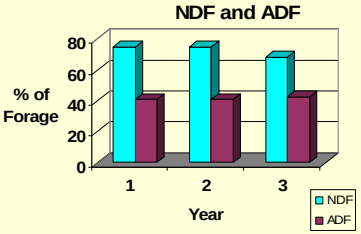
Objective

A three year study was conducted to determine the effects of protein supplementation to cattle grazing dormant winter forage on ruminal production of methylglyoxal.

Approach

- Six ruminally fistulated English crossbred cows (BW 1404 ± 110 lbs) were allotted randomly to one of two treatments.
- Treatments consisted of two feeding regimes:
  1. 36% CP cottonseed meal supplement fed 3x • week (1.98 lbs/head/feeding)
  2. No supplement (salt and mineral only).
- Cows on both treatments were assigned to separate pastures and were allowed free choice access to forage and water.





- Free choice salt and mineral mix was available in the pastures.
- Samples were collected at 3 week intervals beginning in mid-December and ending in mid-February each year from 2004 through 2007.
- Ruminal fluid was collected with a suction strainer and aliquoted for analysis of VFA, ammonia, methylglyoxal, and pH.
- Data were analyzed using computerized statistical software, in which P values of less than 0.05 were considered significant.

Results

Table 1: Effect of protein supplementation on rumen parameters by year

Item	Year			SE
	1	2	3	
Ammonia (mM)	3.18[a]	1.37[b]	1.22[b]	0.46
Methylglyoxal (mM)	0.65[a]	1.45[b]	1.26[b]	0.12
pH	5.89[a]	6.33[b]	5.59[c]	0.09

[abc] Unlike superscripts in rows differ at P < 0.05.

Table 2: Effect of protein supplementation on rumen parameters by day

Item	Day					SE
	1	2	3	4	5	
Ammonia (mM)	2.44[a]	2.10[a]	1.55[a]	1.41[a]	2.10[a]	0.59
Methylglyoxal (mM)	0.87[a]	0.80[a]	0.72[a]	0.94[a]	2.27[b]	0.17
pH	0.04[a]	5.99[a]	5.83[a]	6.04[a]	5.79[a]	0.11

[ab] Unlike superscripts in rows differ at P < 0.05

- Ruminal pH values were below the level where fiber digestion may be inhibited. A pH of less than 6.7 has been reported to inhibit fiber digestion.
- Ruminal ammonia values were below 3 mM which has been reported to be required for maximal microbial protein synthesis.
  - Only in yr 1 were ammonia levels adequate (3.18 ± 0.42 mM) for microbial protein synthesis. Supplementation 3x • week may not have been sufficient to correct nutrient imbalances or provide adequate N for optimal microbial protein synthesis.
- Methylglyoxal showed a difference for year (P < 0.01), with yr 1 being different from yr 2 (P < 0.01) and 3 (P < 0.01), while yr 2 and 3 were similar (P = 0.32). Average values of MG ranged from 0.65 mM – 2.27 mM.
  - Prior research indicates 1.0 mM of MG inhibits growth and decreases viability in ruminal bacteria.
    - Ruminal MG was 55.6% higher (P < 0.01) in yr 2 than yr 1 and 48.8% higher (P < 0.01) in yr 3 compared to yr 1. Methylglyoxal production differed depending on sample day (P < 0.01), no change occurred amongst sample dates 1 through 4 (P > 0.10), but sample date 5 was different from all others (P < 0.01).
- Results obtained from this experiment, were in part due to precipitation patterns. Although yr 1 received 19.13 in. of precipitation based off of a 14.98 in. annual avg. Precipitation timing and frequency was less than favorable. Crude protein levels in yr 1 were greater (8.2%) than in yrs 2 and 3 (6.7%). Nutrient composition of the forage in yr 1 better fit the needs of rumen bacteria than in yrs 2 or 3. This is indicated by lower levels of MG (0.65 ± 0.12 mM) in yr 1.
- Ammonia values were not different across sample day, and suggest no change in diet quality. Methylglyoxal values suggest otherwise. The difference associated with MG for sample day suggests that as the winter progressed and forage quality decreased, the result was a greater nutrient imbalance. From this potentially broadened imbalance of protein and carbohydrate, MG production increased. Change in MG by sample date is suggests it to be more sensitive in measuring available N for ruminal microbes than ammonia alone.

Potential Application

- These results indicate MG production is associated with a nutrient imbalance of carbohydrate and protein.
- More information regarding the conditions conducive to MG production and MG improved sensitivity, may potentially lead to development of a cost effective, producer friendly method of MG quantification.
- Development of such a technique would allow the operator to better assess and manage supplementation, in an effort to minimize inputs and optimize net returns.