

Use of Coconut Oil to Protect Arginine from Rumen Degradation

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Abstract

Tall fescue toxicosis is responsible for millions of dollars of economic losses to the U.S. cattle industry. Animals grazing endophyte infected tall fescue have been shown to experience vasoconstriction as a result of decreased levels of nitrate/nitrite in vascular tissues. It was hypothesized that arginine, a substrate for nitric oxide formation, could lower the incidence of vasoconstriction if protected from rumen degradation by encapsulation in coconut oil. This project involved creating seven supplements; Treatment One: 100% arginine; Treatment Two: Tennessee Formula 56% soybean hulls, 22% arginine, 22% coconut oil; Treatment Three: Kentucky Formula 40% ground corn, 10% zein, 20% arginine, 30% coconut oil; Treatment Four: Food Science Formula 54% water, 0.27% sodium hydroxide, 5.4% casein, 21% soy flour, 5% arginine, 13% coconut oil with soy lecithin, 4% acetaldehyde; Treatment Five: Tennessee Formula containing no arginine: 70% soy hulls, 30% coconut oil; Treatment Six: Kentucky Formula containing no arginine 50% ground corn, 12.5% zein, 37.5% coconut oil; Treatment Seven: Food Science formula containing no arginine: 55% water, 0.27% sodium hydroxide, 5.5% casein, 21% soy flour, 13% coconut oil with lecithin, 4% acetaldehyde.

Samples of each treatment were digested *in vitro* in rumen fluid and buffers. The residues of the samples were analyzed after *in vitro* digestion for nitrogen, and the degree of arginine protection was determined. Results indicated that none of the treatments

provided significant protection to prevent arginine from being degraded by rumen bacteria.

Objectives

- To produce treatments containing combinations of coconut oil and L-arginine to protect the amino acid from degradation by rumen bacteria
- To use an *in vitro* procedure to determine the degree of amino acid protection present in the treatments.

Justification

The annual economic losses to the U.S. cattle industry associated with tall fescue toxicosis exceed \$600 million (Allen and Segarra 2001). This toxicosis is caused by animals ingesting tall fescue infected with the endophytic fungus *Neotyphodium coenophialum*. One of the major physiological responses in animals grazing endophyte infected (E+) tall fescue is vasoconstriction of the peripheral blood vessels, inhibiting the animals' ability to dissipate body heat. This vasoconstriction may be related to decreased levels of nitrate/nitrite in vascular tissues. Steers grazing endophyte-free tall fescue pastures had almost twice as much nitrate/nitrite per g of tissue compared to animals grazing E+ pastures. The cause of the decrease in nitric oxide (NO) formation in E+ steers may be a relative lack of tissue arginine, the substrate for NO formation (Oliver et al. 2001). Blood profiles of cattle grazing E+ indicated that those animals had decreased levels of serum arginine (Oliver et al. 2000). Al-Tamimi and Spiers (2002) investigated the role of nitroglycerin (N+) as a NO donor and a potential vasodilator of peripheral vasculature. These investigators determined that treatment with N+ reduced the

persistent vasoconstrictive effects of E+ in steers. In common supplements, arginine is degraded by rumen bacteria, consequently the animal does not benefit from added arginine. Therefore, arginine must be protected from ruminal degradation in order to reach the small intestine for absorption.

Ruminally protected lysine and methionine are commercially available because these two amino acids are frequently limiting in the diets of high-producing ruminants; however, a protected source of arginine is unavailable. One method of protecting amino acids involves encapsulation to provide a physical or chemical barrier to bacterial attack. Previous work reported by Davenport and Boling (1995) indicated that supplemental arginine could be protected from degradation in the rumen when it was included in a coconut oil carrier matrix. Providing supplemental arginine to cattle grazing E+ tall fescue may supply the animal with NO and result in a reduction of the vasoconstriction associated with tall fescue toxicosis.

Materials and Methods

The following seven treatments containing arginine were formulated in the Animal Science and Food Science laboratories:

- Supplement One: 100% arginine
- Supplement Two: Tennessee Formula – 56% soybean hulls, 22% arginine, 22% coconut oil
- Supplement Three: Kentucky Formula – 40% ground corn, 10% zein, 20% arginine, 30% coconut oil

- Supplement Four: Food Science Formula – 52% water, 0.27% sodium hydroxide, 5.4% casein, 21% soy flour, 5% arginine, 13% coconut oil with soy lecithin, 4% acetaldehyde
- Supplement Five: Tennessee Formula containing no arginine: 70% soy hulls, 30% coconut oil
- Supplement Six: Kentucky Formula containing no arginine: 50% ground corn, 12.5% zein, 37.5% coconut oil
- Supplement Seven: Food Science Formula containing no arginine: 56% water, 0.27% sodium hydroxide, 5.5% casein, 21% soy flour, 13% coconut oil with lecithin, 4% acetaldehyde

An *in vitro* digestibility procedure was used to evaluate the degree of protection provided by the oil used in the treatment. The procedure consisted of placing treatment samples weighing 0.25 g in F57 filter bags that do not contain ash or nitrogen (F57, 4 cm x 5 cm, 30- μ m pore size, ANKOM Technology, Fairport, NY). The bags containing the samples were incubated *in vitro* in rumen fluid and buffers in a 39° C water bath under anaerobic conditions for 0, 6, 12, 18, and 24 hours (DAISYII200/220, ANKOM Technology, Fairport, NY). The residue remaining after digestion was analyzed for nitrogen content (LECO Nitrogen Analyzer St. Joseph, MI), and the rates of degradation were determined. Four trial runs were conducted. Of those four, three runs yielded usable data and are labeled as runs 2, 3, and 4.

Results and Discussion

Figures 1-7 show the percentage of nitrogen remaining in the samples versus digestion time for the formulas containing arginine. Figures 5-7 display the percentage nitrogen remaining in the samples for the formulas without arginine.

Arginine was rapidly degraded in treatments 1-4 indicating that protection from degradation from rumen microbes was not accomplished. Changes in Formulas 5, 6, and 7, the formulas containing no arginine, indicated that the rumen microbes degraded some of the nonarginine containing protein present in those formulas. The peak of nitrogen for the 12-hour digestion time in run 3 can be attributed to several samples becoming lodged in the top of the digestion jar resulting in poor digestion of the substrates.

Figure 8 was generated by subtracting the nitrogen level for Treatment Five from Treatment Two. This is an indicator of the degradation of arginine in Treatment Two.

Figure 9 was generated by subtracting the nitrogen level from Treatment Six from Treatment Three. This is an indicator of the degradation of arginine in Treatment Three.

Figure 10 was generated by subtracting the nitrogen level from Treatment Seven from Treatment Four. This is an indicator of the degradation of arginine in Treatment Four.

As illustrated in Table 1 and Figures 1-10, none of the treatments created in this experiment were successful in protecting arginine. One possibility for providing more adequate amino acid protection could involve repeating the experiment and extruding the formulated supplements under a higher pressure.

Conclusions

Based on the information collected during these experiments, none of the formulas protected arginine from rumen degradation. However, the possibility of using

oils to protect arginine still holds promise as a means to offset the economic impact of tall fescue toxicosis, and other methods of encapsulation must be explored in order to sufficiently transport arginine through the rumen.

Literature Cited

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Table 1. Mean percent nitrogen remaining after incubation

Supplement	Time After Digestion, h				
	0	6	12	18	24
1	12.41	0.08	0.13	0.12	0.12
2	3.47	0.59	0.66	0.53	0.51
3	3.60	1.04	1.31	1.32	1.27
4	4.19	2.29	2.58	2.34	2.28
5	0.74	0.67	0.87	0.59	0.58
6	1.10	1.32	1.76	1.53	1.72
7	2.97	2.77	3.53	2.14	2.19

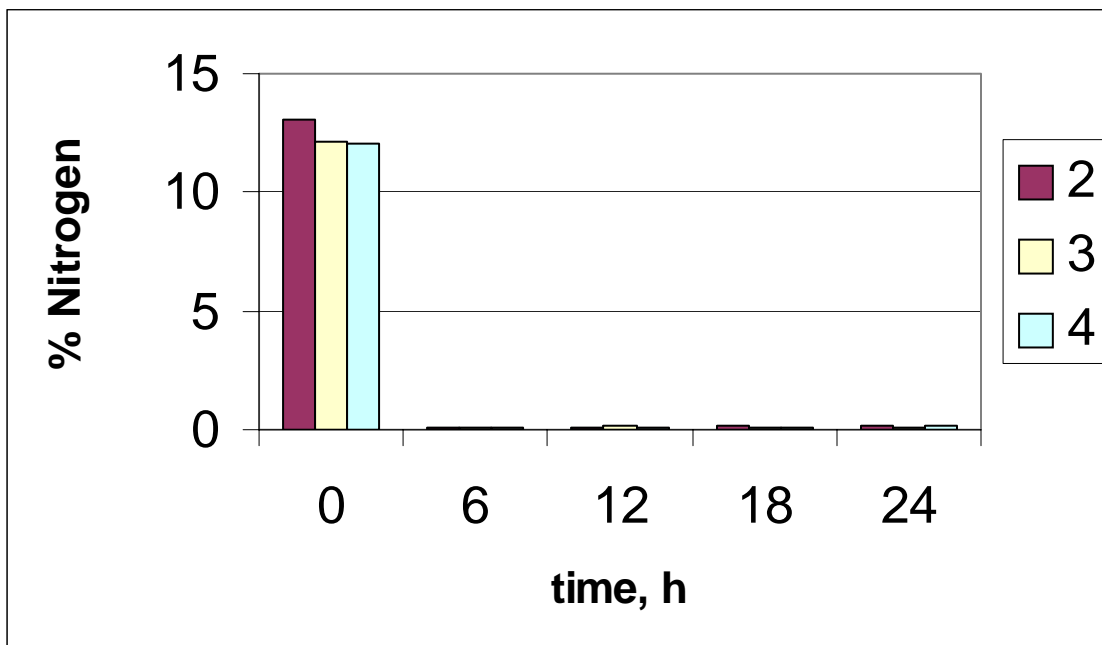


Figure 1. Percent Nitrogen present in F57 bags after digestion in rumen fluid *in vitro* for Runs 2, 3, and 4 for Formula One

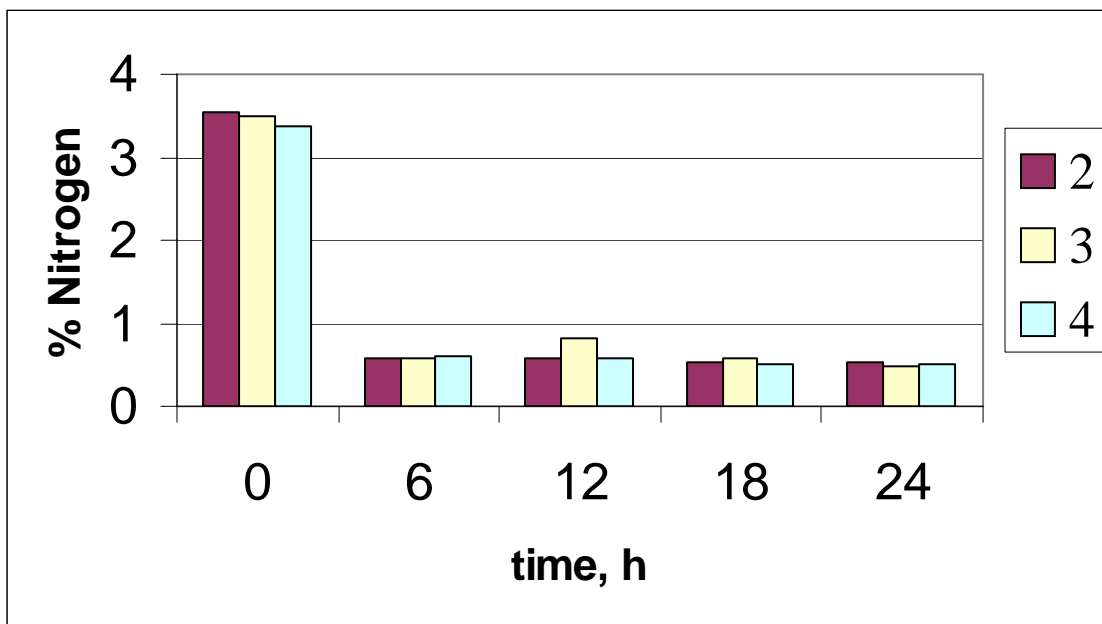


Figure 2. Percent Nitrogen present in F57 bags after digestion in rumen fluid *in vitro* for Runs 2, 3, and 4 for Formula Two

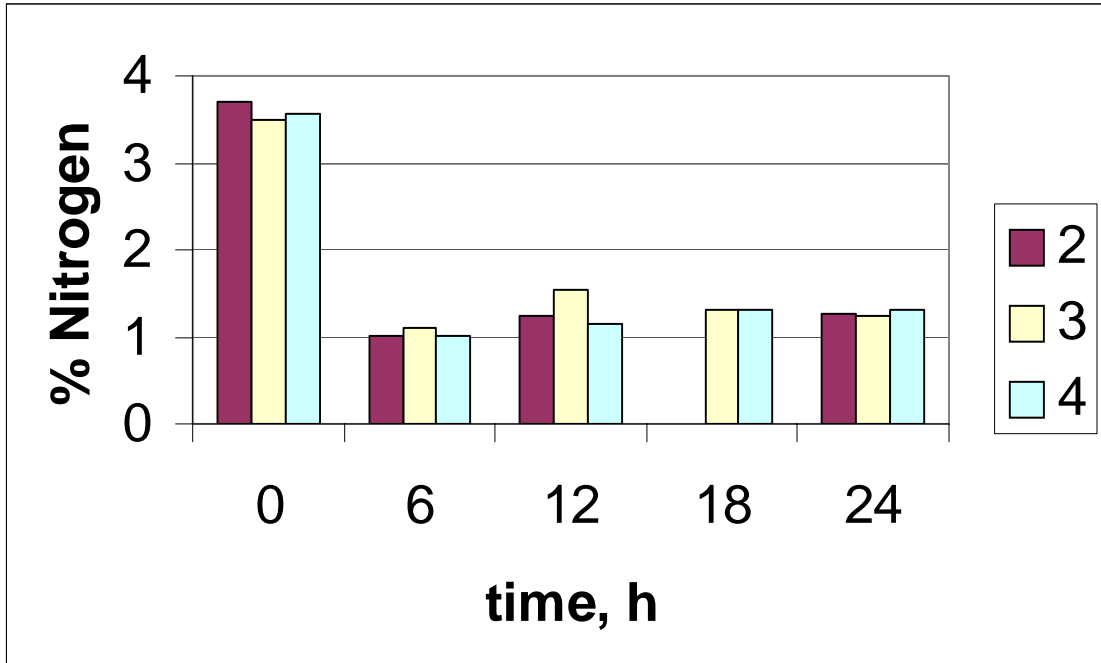


Figure 3. Percent Nitrogen present in F57 bags after digestion in rumen fluid *in vitro* for Runs 2, 3, and 4 for Formula Three

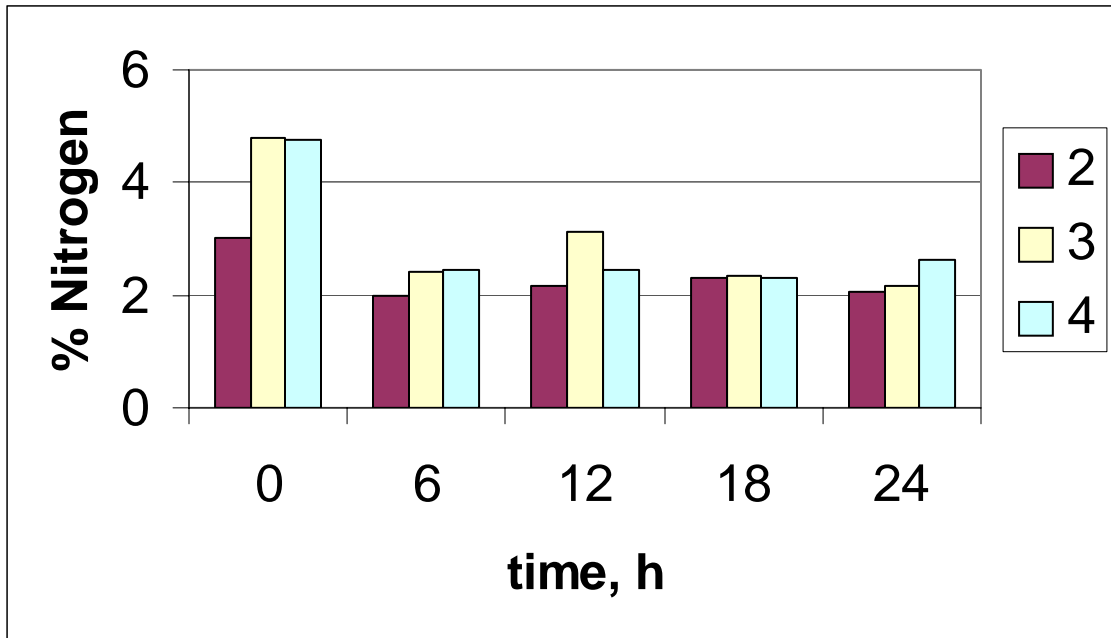


Figure 4. Percent Nitrogen present in F57 bags after digestion in rumen fluid *in vitro* for Runs 2, 3, and 4 for Formula Four

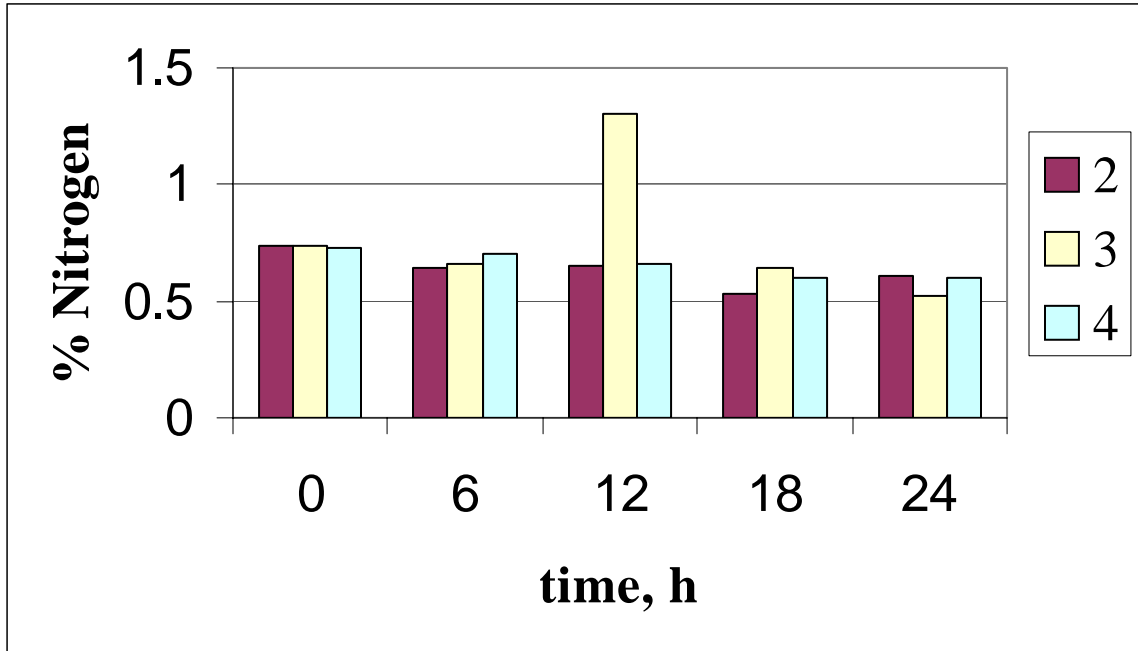


Figure 5. Percent Nitrogen present in F57 bags after digestion in rumen fluid *in vitro* for Runs 2, 3, and 4 for Formula Five

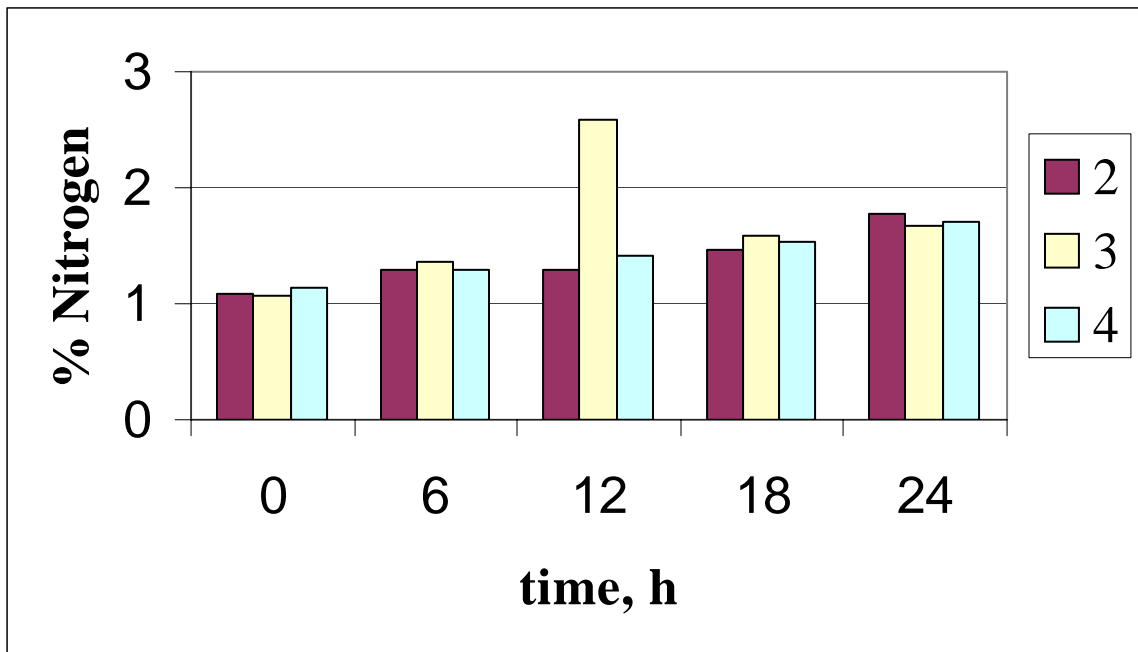


Figure 6. Percent Nitrogen present in F57 bags after digestion in rumen fluid *in vitro* for Runs 2, 3, and 4 for Formula Six

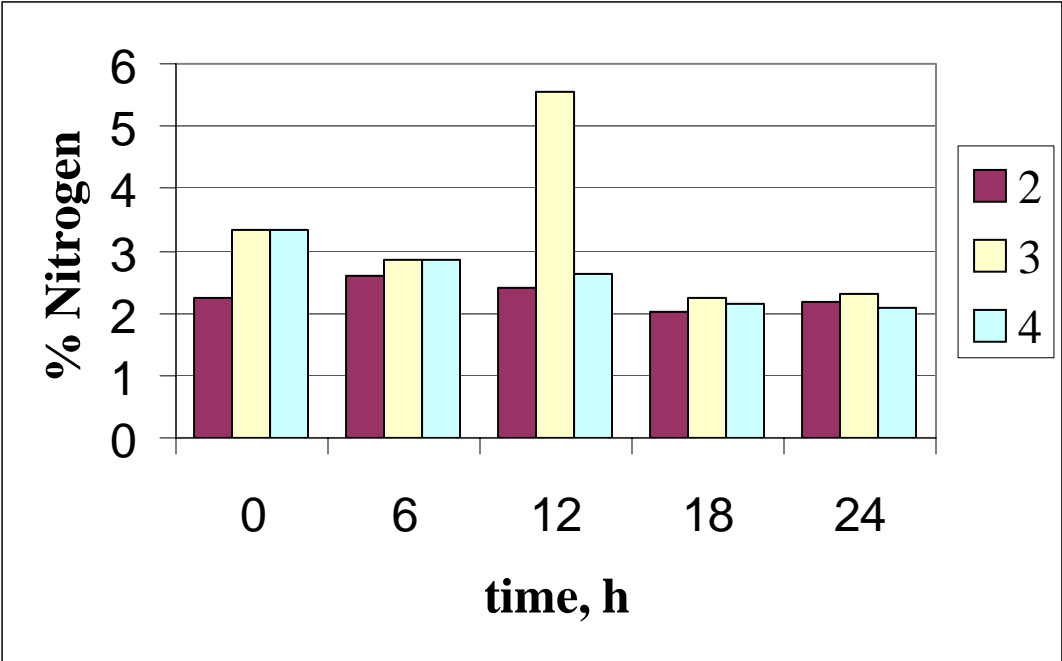


Figure 7: Percent Nitrogen present in F57 bags after digestion in rumen fluid *in vitro* for Runs 2, 3, and 4 for Formula Seven.

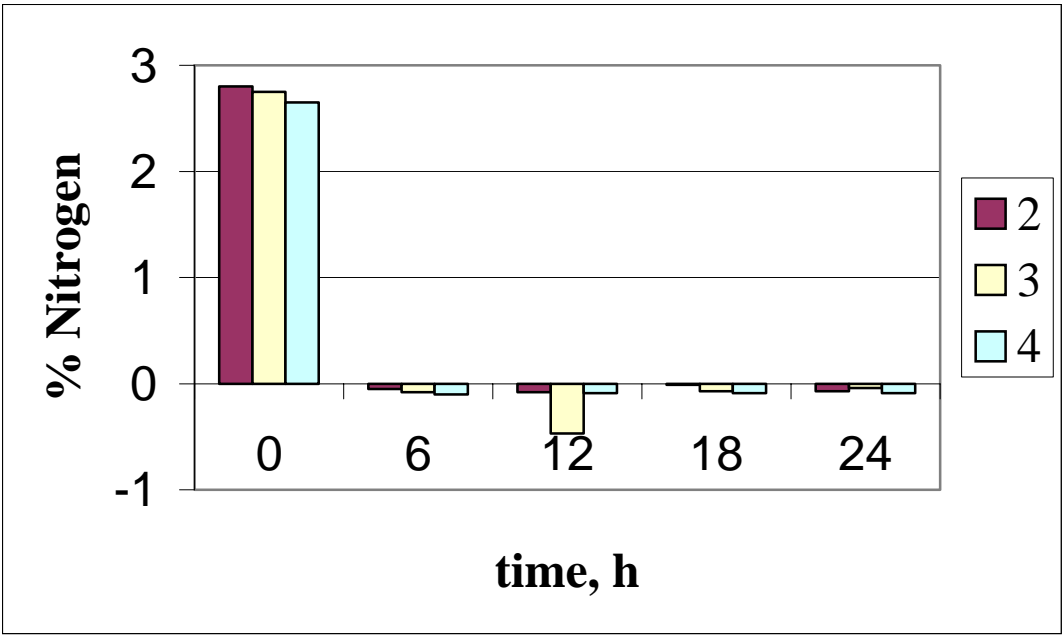


Figure 8. Formula Five Subtracted from Formula Two indicating the degradation of Arginine in Formula Two

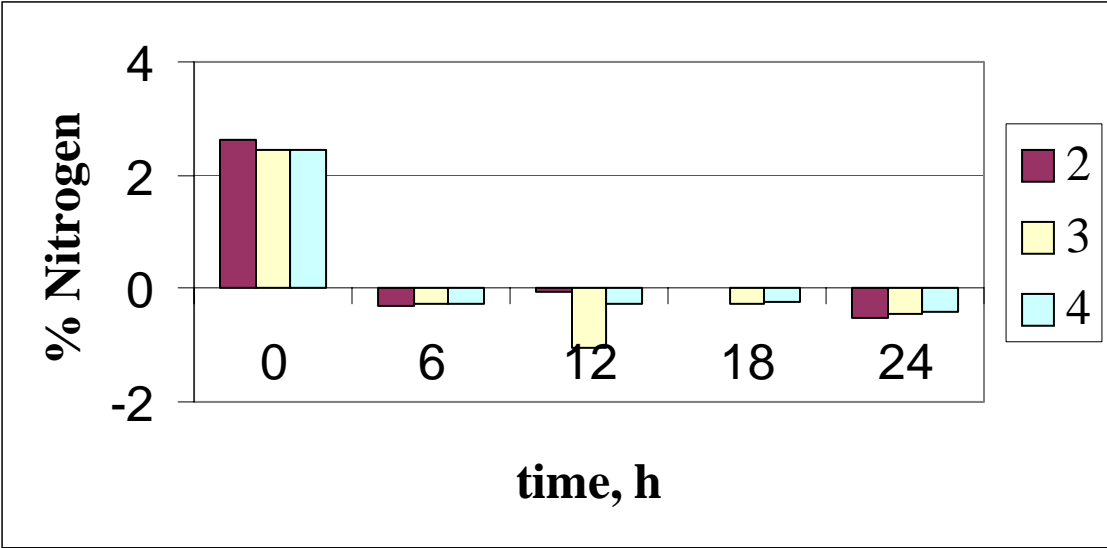


Figure 9. Formula Six Subtracted from Formula Three indicating the degradation of Arginine in Formula Three

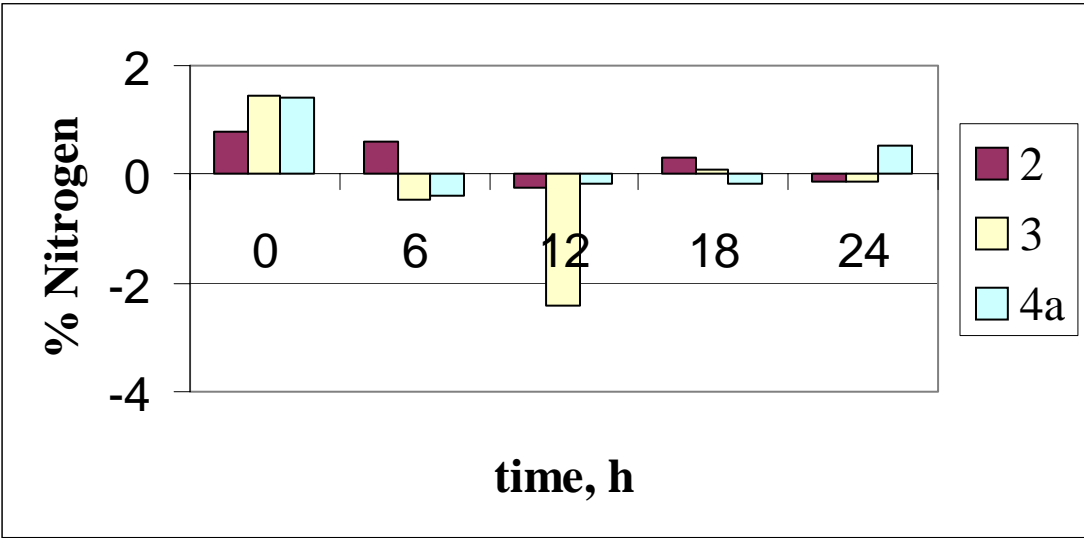


Figure 10. Formula Seven Subtracted from Formula Four indicating the degradation of Arginine in Formula Four