Evaluation of Agolin®, an Essential Oil Blend, as a Feed Additive for High Producing Cows

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Abstract
There is a growing demand for feed additives that can not only reduce dairy enteric methane emissions but also increase milk production and feed efficiency. Just one product is currently commercially available which accomplishes both of these goals. The purpose of this study was to confirm the performance benefits of the product (Agolin®, Agolin SA, Biere, Switzerland) in high producing mid-lactation dairy cows under United States feeding conditions. Four matched pens of approximately 150 mid-lactation cows/pen and averaging over 50 kg of milk/cow/day were enrolled in a side-by-side study. All pens received a common total mixed diet ad libitum, and the essential oil blend was administered via a concentrated farm pack to provide 1 g/cow/day to cows in the 2 test pens. Milk weights were determined, and samples were collected for compositional analysis over the last 2 days of the pretrial (May 11 and 12, 2020) and end of the trial (July 18 and 19, 2020) periods. Dry matter intake was measured by pen daily for the last 10 days of each feeding period. Milk fat and milk protein yields were greater (P < 0.05) for cows receiving added Agolin. There was a tendency (P = 0.06) for energy corrected milk/dry matter intake to be greater for cows receiving the Agolin (1.88) relative to the control diet (1.76). The trial showed that Agolin assisted in improving production parameters of economic importance to dairy producers.

Keywords
Lactating Dairy Cows, Methane, Essential Oils, Feed Efficiency

1. Introduction
Cattle and other ruminants produce highly digestible human food from materials that are largely inedible by the human population, including grasses, milling...
residues, oilseed crushing byproducts, etc. This is accomplished through rumen microbial fermentation, which produces usable energy compounds and excellent quality protein. This miraculous anaerobic fermentation process, however, contributes to the production of unwanted greenhouse gases (GHG), in particular methane (CH₄) which can trap atmospheric heat.

Many GHG mitigation strategies have been studied, with the goal of reducing GHGs and CH₄. While reducing GHG production can be readily accomplished, doing so and maintaining production and feed efficiency has been much more difficult to achieve. Roque et al. [1] determined that seaweed (Asparagopsis) reduced CH₄ production by 67% when fed at 1% of the diet, but this substance also reduced intake and milk production. In the study of van Zijderfeld et al. [2] nitrate reduced CH₄ production by 40% but has the potential to be toxic.

Plant bioactive compounds have been extensively studied, but most lack consistent results, or have unwanted side effects. Tannins, as an example, have been demonstrated to reduce CH₄ production, but also bind feed proteins, reducing intestinal digestibility [3]. Goel and Makkar [4] reviewed saponins as compounds to reduce CH₄ production and concluded that these reduce protozoa, but this may or may not translate into reduced GHG. Many essential oil compounds have demonstrated potential in vitro, but limited in vivo testing has been much less promising [5].

Agolin, an essential oil blend has been shown to alter the rumen biota leading to greater energy efficiency as well as reductions in CH₄ and NH₃ output. A meta-analysis based on 23 studies demonstrated a 4.1% improvement in energy corrected milk yield after a four-week adaptation period, with no change in dry matter intake. In addition, methane intensity (g/kg ECM) was reduced by 9.9% on average and CH₄ output was reduced in all trials in which it was measured [6]. A subsequent feeding experiment [7] further confirmed the methane intensity reducing potential for Agolin at 11.1%, along with a reduction in NH₃ intensity of 16.0%.

The purpose of this study was to evaluate the essential oil blend Agolin in a high producing herd that would be representative of high concentrate feeding and management practices and determine if improvements in yield and efficiency were measurable.

2. Materials and Methods

2.1. Animals and Treatments

Holstein cows from a commercial dairy farm in the Finger Lakes region of New York, USA, were used in this evaluation. The farm housed over 2000 cows in pens of approximately 150 cows/pen. Four pens of multiparous cows were selected for this study based on similarity for average days in milk, and milk production.

Test pens of animals were treated according to normal farm practice. To not impede these practices cows could be moved out of the pens and cows could be
moved into the pens, but only cows that remained in the original pens for the full duration of the trial were used in the analysis.

Feed was issued twice daily and consisted of a common total mixed ration (TMR) provided to all 4 test pens. The diet was formulated to meet nutrient requirements using the feed formulation platform NDS (RUM&N Sas, Reggio Emilia, Italy). Cows were given sufficient feed to allow for 2% - 3% orts. Feed issued and orts were measured daily. The essential oil product was added to the TMR through a concentrated farm pack and was calculated to provide 1 g/cow/day.

2.2. Analyses

Milk samples were collected over the last 2 days of the pretrial (May 11 and 12, 2020) and end of the trial (July 18 and 19, 2020) during the regularly scheduled monthly DHIA collection visit. Fresh milk samples obtained by Dairy Herd Improvement Association (DHIA) personnel were sent to the Ithaca New York, USA DHIA Laboratory for the analysis of fat and true protein.

DHIA returned values for milk yield, percentage fat (w/w) and percentage protein (w/w). Component yields were calculated by cow. Fat corrected (FCM) milk and energy corrected milk (ECM) were likewise computed by cow using the equations described by Erdman [8]. Data were analyzed using Minitab 16 statistical software (Minitab Inc., State College PA, USA), the linear model analysis of variance accounting for the effects of treatment, and pen using pretrial values as covariates by cow. Feed intake was analyzed using pen as the experimental unit (n = 4) with days used for replication. Differences were declared significant when the probability (P) of a different result was calculated to be less than 5% (P < 0.05) and were declared a tendency when P was less than 10% (P < 0.10).

3. Results and Discussion

The ingredient composition of the diet used during the study period is given in Table 1. The diet contained 59.6% forage with 70% of the forage as corn silage, with a grain content of approximately 40% of the total dry matter.

The nutrient composition of the diet is shown in Table 2. The composition clearly shows that the diet was a high concentrate diet and would be expected to represent the type of diet typical for high producing dairy cows in the United States.

Pen selection was based on the level of production and average days in milk for all cows available in the pen at the time that the trial was started. While the numbers of animals remained consistent within the pen, some cows were removed, and others were placed in the pens. There were 184 control and 146 test cows that were in the same pens, receiving the correct diets for the full duration of the trial (Table 3). The pre-trial data for these cows showed that they remained well matched across treatments.

Results for milk yield and milk composition were based on cows included in
the analysis (Table 4). Milk yield did not change with the inclusion of Agolin, but fat and protein percentages were elevated (P < 0.05). There was a significant improvement in fat yield per cow/day (P < 0.05) resulting in a tendency for FCM and ECM to be greater for the treatment group (P < 0.10). Increased milk fat yield was also found in a California Agolin feeding trial [9] involving very high producing cows receiving high energy diets.

Efficiency was calculated by pen. There was a tendency (P < 0.10) for both FCM and ECM to be improved when the additive was included in the diet. This is in also agreement with the California Agolin feeding trial [9].

Table 1. Ingredient composition of the common diet used in the study.

| Ingredient                                      | % of Dry matter |
|------------------------------------------------|-----------------|
| Legume haylage                                 | 17.54           |
| Corn Silage                                    | 42.09           |
| Corn Grain, ground                             | 17.36           |
| Wheat shorts                                   | 4.84            |
| Citrus pulp                                    | 2               |
| Canola meal                                    | 4.68            |
| Soybean meal, solvent extracted                | 2.02            |
| Soybean meal, expeller extracted               | 2.36            |
| Porcine blood meal                             | 0.83            |
| Palm fatty acids                               | 1.24            |
| Sodium bicarbonate                             | 0.71            |
| Calcium carbonate                              | 0.7             |
| Sodium chloride                                | 0.44            |
| Micronutrient premix                           | 1.38            |
| Yeast culture                                  | 0.18            |
| Urea                                           | 0.13            |
| Cane molasses                                  | 1.5             |

Table 2. Nutrient composition of the common diet used in the study.

| Nutrient                                | % of dry matter |
|-----------------------------------------|-----------------|
| Dry Matter                              | 41.45           |
| Crude protein                           | 15.6            |
| Acid detergent fiber                    | 18.24           |
| Neutral detergent fiber                 | 27.55           |
| Water soluble carbohydrates             | 4.72            |
| Starch                                  | 28.55           |
| Ether extract                           | 5.24            |
| Calcium                                 | 0.91            |
| Phosphorus                              | 0.38            |
Table 3. Comparison of pretrial allocation of animals and final enrollment.

| All cows¹ | Participants² |
|-----------|---------------|
|           | Control | Test | Control | Test |
| Number of cows | 303     | 267   | 184     | 146   |
| Days in milk   | 143     | 145   | 146     | 151   |
| Milk yield, kg | 50.1    | 50.9   | 53.0    | 53.4   |

¹Values for all cows in pens when pen allocations were made; ²Pretrial values for cows that remained in the pens for the duration of the trial were used in the statistical analysis.

Table 4. Effects of the feed additive Agolin on production parameters in high producing mid-lactation cows¹.

| Item                  | Control | Test  | P Value |
|-----------------------|---------|-------|---------|
| Milk, kg              | 45.76   | 46.21 | 0.567   |
| Fat %                 | 3.65    | 3.71  | 0.232   |
| Protein %             | 2.92    | 2.97  | 0.027   |
| Fat yield, kg         | 1.63    | 1.72  | 0.023   |
| Protein yield, kg     | 1.33    | 1.36  | 0.181   |
| Fat corrected milk (FCM), kg | 46.31 | 47.85 | 0.061   |
| Energy Corrected milk (ECM), kg | 46.31 | 47.81 | 0.083   |
| FCM/DMI               | 1.78    | 1.91  | 0.060   |
| ECM/DMI               | 1.76    | 1.88  | 0.060   |

¹P < 0.05 differences are significantly different; P < 0.10 there is a tendency for differences to be significantly different.

Agolin has been demonstrated to significantly increase FCM and ECM in mid-lactation cows producing more average levels of milk of approximately 30 kg [10] [11] but prior to conducting the current experiment, it was questioned if the product could improve performance in very high producing cows. During ruminal methanogenesis, CO₂ and H⁺ react to form methane, representing a loss in energy to the host animal. If methanogenesis is inhibited and H⁺ accumulates, rumen pH declines, and fiber digestion can be reduced. Reducing CH₄ production in the rumen without reducing organic matter digestibility requires the H⁺ to be utilized in the production of volatile fatty acids, mainly propionic acid. This results in the energy being available to the host to use for productive purposes.

Knapp et al. [12] categorized CH₄ mitigation strategies into three categories: 1) Feeding diets to reduce the acetate/propionate ratio, to decrease H⁺ to be used in the formation of CH₄. 2) Improving animal productivity to dilute maintenance and improve feed efficiency. 3) Provide rumen fermentation modifiers. All three strategies were evidently in practice for the test pens of animals as reported here.
4. Conclusion

This feeding study compared the lactational performances of high producing cows receiving the rumen modifier Agolin® to cows receiving the same diet without the feed additive. The results provide further evidence that Agolin has the potential to increase fat and protein corrected milk in high producing dairy cows. Other trials have also demonstrated the ability of Agolin to reduce enteric methane production. With a combination of improved energy corrected milk and feed efficiency in addition to a decrease in enteric methane emissions Agolin Ruminant shows potential to be of benefit not only to dairy profitability but also to the growing demand for reducing the carbon footprint of dairying.

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Conflict of Interest

Mr. John Clark and Mr. Peter Williams market Agolin in the United States of America. Both were involved in the design but not the analysis of data or interpretation of results.

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