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Evaluation of Agolin® Ruminant, an Essential Oil Blend, as a Feed Additive for Cows at Two Levels of Production

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Abstract

Agolin® Ruminant (Agolin) is a commercially available blend of essential oils which has been demonstrated to reduce greenhouse gas emissions in dairy cows and improve energy corrected milk and feed efficiency. Further trials are needed with large numbers of cows to confirm the magnitude of effect of this feed additive on milk production under differing feeding conditions and stages of lactation. Information that can be quantified from newer on-farm systems of measurement is likewise needed. This study was conducted to determine the effects of this additive on high producing (48 kg of milk/day average) just past peak lactation and medium producing dairy cows (43 kg of milk/day average) further along in lactation under commercial conditions that would typically occur in the Pacific Northwest USA. Four pens of approximately 400 Holstein cows/pen (two high producing pens and two medium producing pens) were available for this side-by side study. Performance results were determined using data derived from a subscription standardized monitoring system (High Desert Dairy Laboratory, Inc, Nampa, ID, USA) that provided results for milk yield, fat percentage and protein percentage. As well daily in-stall electronic monitoring of milk only was available. All pens received a common total mixed ration typical of rations fed in the Pacific Northwest, USA. The Agolin feed additive was dispensed through the bulk mineral supply with control cows receiving the normal minerals and the treatment cows receiving the normal minerals plus Agolin. The trial began on August 24, 2021, with treatment cows provided with 1 g/head/day for the duration of the 8-week long study. Dry matter intakes were determined daily for the week before the trial was conducted and the last week of the trial. Treatment results were compared using a general linear model that considered pretrial milk, fat yield and protein yield, days in milk and lactation number.

There were 678 high producing cows available for the duration of the study. Based on the standardized testing results, milk yield was greater (1.12 kg/cow/day; $P < 0.05$) for the cows in the treatment group. There was a tendency for fat corrected milk (FCM) and energy corrected milk (ECM) to be greater for the cows receiving the Agolin feed additive (0.96 and 0.86 kg/cow/day for FCM and ECM respectively, $P < 0.10$). There were 646 medium producing cows that participated in the trial. There was no treatment effect upon milk production ($P = 0.27$). There was a significant ($P < 0.05$) increase in milk fat yield and no change ($P = 0.33$) in protein yield for the treatment cows in this test group, resulting in greater FCM and ECM (1.12 and 0.95 kg/cow/day; $P < 0.05$). Daily milk monitoring resulted in a reduced decline in milk yield from the week before to the final week of the trial revealing greater persistency of milk for Agolin-fed cows in both high and medium production pens (1.58 and 2.13 kg/cow/day; $P < 0.01$). Likewise, overall feed efficiency was improved by 5.3% ($P < 0.05$) with the test product.

Keywords

Greenhouse Gas Mitigation, Methane, Feed Efficiency, Lactating Dairy Cows, Agolin

1. Introduction

Globally, greenhouse gas (GHG) emissions resulting from the rearing of farm livestock are estimated to be approximately 13% of human-produced emissions [1]. Approximately 40% of these emissions are in the form of methane (CH4) from enteric fermentation and manure [2]. Of the GHG sources, CH4 offers the greatest opportunity to reduce atmospheric warming and achieve net zero emissions, and as such animal agriculture provides a unique opportunity to contribute to the abatement of climate change. Reduction of enteric CH4 production has been the subject of many recent research projects.

While this may be the case, forward movement is impeded as most proven technologies that reduce enteric CH4 production are costly to apply, without benefit to productivity [3]. Some less costly additives pose unique problems. For example, nitrates require special care to avoid toxicity to livestock or rumen microbes [4]. Vegetable oils can reduce total CH4 output, but, depending on the diet, may also reduce milk fat yield [5]. Each of these scenarios burdens the dairy with not only the added cost of the product, but also the additional risk of revenue loss. Such risks do not encourage voluntary participation in reducing CH4 output.

Agolin® Ruminant (Agolin SA, Bière, Switzerland, Agolin) is a commercially available blend of essential oils that has been demonstrated to reduce CH4 in vitro [6] [7] [8] and *in vivo* in lactating dairy cows [9] [10] [11]. Agolin has been shown to divert hydrogen from methane to propionic acid in the rumen, without the often powerful antibacterial effect found with some methane-inhibiting feed additives [6], thereby not reducing rumen digestibility. Instead, the product produces a desirable shift between rumen bacterial and archeal communities [11]. Unique to this technology, several trials have indicated that Agolin also improves milk yield [12] [13] [14] and feed efficiency [12] [13]. Belanche *et al.*, [13] is a meta-analysis of 23 trials.

Some types of feeding situations and levels of milk production may be more conducive to altered yields than others. Tests involving a range of conditions are needed to determine the magnitude of change in milk yield and feed efficiency when this technology is applied.

This experiment was conducted to evaluate the inclusion of Agolin in dairy diets on milk production and milk composition in high and medium producing cows receiving high energy diets typical of the Pacific Northwest, USA. A secondary purpose of this study was to evaluate results obtained from on-farm automated milk measurements and determine if these can be used in future evaluations of feed and feed additives.

2. Materials and Methods

2.1. Animals and Treatments

This trial was conducted in the Pacific Northwest region, USA. The farm maintains approximately 6,500 Holstein dairy cows in pens of approximately 400 cows each. Two pens of high producing cows and two pens of medium producing cows matched for production, stage of lactation and average lactation within each level were selected for inclusion in the study (**Table 1**). Test pens of animals were treated according to normal farm practice. To not impede these practices cows were permitted be moved into and out of pens as necessary but only cows that remained in the original treatment pens for the full duration of the trial were included as being enrolled in the study.

The selection of pens for test and control was determined by farm staff based on level of production and average days in milk (DIM). Within each level of production, a coin toss was used to determine treatment.

Cows were milked 3 times/day. Cows were individually equipped with an identification collar used to automatically capture and record milk weights from computerized flow meters (DeLaval MPC680 milk point controller, DeLaval Holding, Tumba, Sweden) from each milking.

The ingredient composition of the total mixed ration is given in **Table 2**. Fresh

Table 1. Classification of cows available at the start of the trial (means ± SD).

Parameter	High Pens	Average Pens
Number of cows	804	732
Milk Production, Kg/d	48.2 ± 6.5	42.7 ± 6.0
Days in milk	127 ± 51	215 ± 67
Lactation	3.61 ± 1.55	3.31 ± 1.45

Table 2. Ingredient composition of the diet provided to the cows.

Ingredient	% of dry matter
Alfalfa hay	21.82
Corn silage	23.64
Triticale silage	5.46
Wheat straw	0.91
Corn grain	22.28
Beet pulp	6.36
Corn distillers' grains	8.64
Solvent extracted soybean meal	5.46
Bypass fat	1.36
Micronutrients	4.07

feed was issued twice daily. Sufficient feed was provided to allow for 2% to 3% orts by pen, based on the previous day's consumption, and orts were removed and weighed daily before the morning feeding. A common diet was provided to all pens included in the study. The diets were formulated using a commercial feed formulation program (AMTS Cattle Pro, Ag Modeling Systems, Groton, NY, USA) based on CNCPS Technology [15]. The Agolin feed additive was provided at the rate of 1 g/cow/day for the duration of the 8-week long feeding period and was dispensed through the bulk mineral supply with control cows receiving the normal minerals and the treatment cows receiving the same minerals plus Agolin.

2.2. Analyses

Milk samples were collected before the trial began and at approximately 4 week intervals by High Desert Dairy Laboratory, Inc, Nampa, ID, USA. Milk weights were recorded at each milking for each cow over a 24 hour period by a certified technician. Aliquots of milk were collected, and fresh samples were analyzed for fat and protein percentages. The laboratory report was electronically transmitted to the farm's record keeping system (DairyCOMP 305, Valley Ag Software, Tulare, CA, USA). Values by cow for DIM, lactation number, milk yield, fat percentage and protein percentage were extracted from the DairyCOMP 305 program. Component yields were calculated by cow and date of analysis by multiplying milk yield by the component percentage values. Fat corrected milk (FCM) and energy corrected milk (ECM) was likewise calculated by cow using the equations given by Erdman [16]

$$\text{FCM} = 0.432 * \text{milk yield} + 16.23 * \text{fat yield} \quad (1)$$

$$\text{ECM} = 0.327 * \text{milk yield} + 12.95 * \text{fat yield} + 7.65 * \text{true protein yield} \quad (2)$$

Additionally, daily milk yield values for each cow as recorded on farm for the one-week period immediately before the start of the trial and for the final week of the trial were also used to assess the effects of treatment. Pen averages for the same time periods, along with pen based dry matter intakes were used to assess feed efficiency (milk yield/dry matter intake).

2.3. Statistical Analyses

Data were analyzed using Minitab 16 statistical software (Minitab Inc., State College. PA, USA). The general linear model considered the effects of treatment accounting for pretrial milk yield, fat yield, protein yield, days in milk and lactation number, with cows within pens serving as replicates. Feed efficiency was analyzed using pens as the experimental unit with days used for replication. Differences were declared significant when the probability (P) of a different result was found to be less than 5% ($P < 0.05$) and were declared a tendency when the P value was less than 10% ($P < 0.10$).

3. Results and Discussion

3.1. Nutrient Analyses

As Table 2 shows, the diet used over the course of the study was a high energy diet based on corn silage and alfalfa haylage and is one that would be typical in this region. Nutrient analyses are given in Table 3. These results further show that the nutrient specifications are within the range that would normally be expected for high producing dairy cows.

3.2. Standardized Testing Results

The initial pen selection was based on production parameters before the trial began. These values were recalculated removing cows that did not remain in the pens consistently to provide an accurate covariate period. Of the cows available at the start of the trial 678 and 646 remained available for the duration of the feeding period (Table 4). The high production cows averaged were over 100

Table 3. Calculated nutrient composition of the diet provided to all pens for the duration of the study.

Nutrient	% of dry matter
Dry matter	61.4
Crude protein	17.1
Acid detergent fiber	19.6
Neutral detergent fiber	30.4
Water soluble carbohydrates	7.1
Starch	24.8
Ether extract	3.14
Calcium	0.99
Phosphorus	0.35

DIM earlier in their lactation cycle than the medium production group, providing meaningful differences between the two experimental groups.

Treatment comparisons for the high producing cows are given in **Table 5**. Milk production was greater (1.12 kg/cow/day; $P < 0.05$) for the test relative to the control cows. The treatment cows produced slightly less protein ($P < 0.05$) on a percentage, but not on a yield basis, and milk fat was unaffected by the feeding regimen for these cows. Milk fat percentage and yield were not affected by the feed additive for this production group of cows ($P > 0.05$) Milk FCM and ECM persistency, defined as change in milk over time, favored the treatment cows (0.96 and 0.86 kg/cow/day; $P = 0.070$ and 0.086 for FCM and ECM change, respectively, $P < 0.10$). The difference between FCM and ECM is reflective of the inclusion of protein yield in the calculation of ECM, and is important in some markets.

Outcomes differed somewhat for the medium production levels cows that were in the later stage of lactation (**Table 6**). Although there were no differences in milk volume ($P = 0.27$) milk fat percentage and yield were significantly greater (0.1% and 0.13 kg/cow/day; $P < 0.05$) for the cows receiving Agolin. This resulted in greater FCM and ECM (1.12 and 0.94 kg/cow/day, $P < 0.05$) as well as

Table 4. Classification of cows available at the start of the trial (means ± SD).

Parameter	High Pens	Average Pens
Number of cows	678	646
Milk Production, Kg/d	48.1 ± 7.2	42.7 ± 6.1
Days in milk	118 ± 73	224.6 ± 73
Lactation	3.54 ± 1.28	3.27 ± 1.33

Table 5. Least squares means results at the end of the trial for the high production cows without (control) or with (test) the feed additive Agolin.

Variable	Treatment			P Value
	Control	Test	SEM	
Milk, kg/day	44.57	45.69	0.296	0.009
Fat, %	3.70	3.67	0.023	0.462
Protein, %	3.11	3.08	0.013	0.009
Fat, kg/day	1.59	1.61	0.012	0.238
Protein, Kg/day	1.33	1.35	0.008	0.295
FCM, kg	45.75	46.71	0.296	0.070
ECM, kg	46.22	47.08	0.281	0.086
Milk, Change	−3.06	−1.94	0.203	0.001
FCM, Change	−1.29	−0.33	0.270	0.070
ECM, Change	−1.00	−0.14	0.247	0.086

Table 6. Least Squares means results at the end of the trial for the average production cows without (control) or with (test) the feed additive Agolin.

Variable	Treatment			P Value
	Control	Test	SEM	
Milk, kg/day	39.72	40.11	0.272	0.274
Fat, %	3.88	3.99	0.024	0.015
Protein, %	3.35	3.32	0.012	0.334
Fat, kg/day	1.49	1.55	0.011	0.006
Protein, Kg/day	1.29	1.30	0.008	0.529
FCM, kg	41.63	42.75	0.275	0.015
ECM, kg	42.49	43.43	0.267	0.029
Milk, Change	−3.42	−3.03	0.161	0.274
FCM, Change	−1.44	−0.32	0.216	0.015
ECM, Change	−1.12	−0.17	0.203	0.029

FCM and ECM persistency (1.12 kg and 0.95 kg/cow/day; $P < 0.05$) for this production group. This relationship between stage of lactation and change in performance was previously not known.

In a similarly designed recent trial with high producing cows past peak lactation [12], FCM and ECM yields were both 1.5 kg/cow/day greater for the test cows. Cows averaged over 45 kg of milk and were 145 DIM at the start of the trial. While similar in magnitude to the medium production cows in the current study (Table 6) the nature of the improvement in FCM and ECM were not the same. Unlike the current trial, protein yield was improved with the inclusion of the additive, a contributing factor to the greater ECM. The reason for the lack of increase in protein percent in either of the two current evaluations is not obvious, and more information regarding the mode of action of Agolin as it relates to diet and rumen fermentation are needed to better predict outcome.

3.3. On Farm Results and Feed Efficiency

Table 7 provides the results by treatment group for milk yield and change in milk yield, based on findings from the on-farm automated milk recording system. Unlike the external testing results, which are one determined from values obtained on a single day before the trial began, and values again determined by cow at the end of the 8-week feeding period, the daily milk values are averages of 7 daily values for the week ending August 23, 2021 and 7 daily values/cow for the final week of the feeding trial for every individual cow that was present for the experimental period. The expectation would be for the farm generated values to be more accurate. Test cows in both the high and medium groups showed greater persistency than control (1.58 kg/cow/day and 2.13kg/cow/day; $P < 0.05$).

In a recent survey Tse et al. [17] determined that many dairy producers

Table 7. Least squares means results for the daily milk for the final week of the as determined by inline milk recording as well and overall feed efficiency cows without (control) or with (test) the feed additive Agolin.

Item	Control	Test	SEM	P Value
High Production Pens				
Milk, Kg	43.4	44.9	0.709	0.136
Change in milk, Kg	−3.84	−2.26	0.508	0.001
Medium Production Pens				
Milk, Kg	37.9	40.0	0.648	0.001
Change in milk, Kg	−3.47	−1.34	0.424	0.015
Feed Efficiency				
Milk/Feed	1.51	1.59	0.023	0.040

adopting automated milking technologies stop subscribing to milk testing services. The on-farm acquisition of data is less intrusive and is simply part of the normal routine. The additional advantage is the lack of need for scheduling milk testing to coincide with testing periods and the continuous availability of data. Further studies providing results from fully automated systems would be beneficial to gain greater insight into the extent of change in yield by length of time the additive is provided. Feed intake by pen and milk yield by pen were used to determine feed efficiency, with 2 pens/treatment for the final 7 days of the study. These variables were based on all cows in the pen, as opposed to only cows that were available for the duration of the trial. Using these values to compute milk/feed resulted in improved feed efficiency for cows fed Agolin (5.3%; $P < 0.05$). Feed intakes were not different ($P > 0.01$) between the two treatment groups.

The exact mode of action of Agolin has not been determined. Belanche et al [13], in a meta-analysis comparison of results from earlier feeding trials found two consistent changes: a reduction in CH_4 production and improved feed efficiency. As **Table 7** shows, there was a significant improvement in feed efficiency at this farm (+5.3%; $P < 0.05$). This is consistent with findings from another more recent study [12]. The consistent improvement in feed efficiency suggests that energy is captured in usable substrates rather than being lost as CH_4. Such an improvement in this parameter would offset the cost of the application of this technology on dairy farms such as this one and render CH_4 mitigation a viable option.

4. Conclusion

This feeding evaluation study was conducted on a large working dairy, that is typical of many dairy farms in the Pacific Northwest region. The trial involved large numbers of cows, which should contribute to the accuracy of conclusions regarding the use of this rumen modifier in this type of farming situation. ECM was increased in both high producing dairy cows, and medium producing dairy cows. Once dairy cows are past peak production, milk production gradually declines. Many technologies and feeding regimens target early lactation to have cows peak higher, as it is more difficult to improve production after this critical peak period. These findings indicate that the rate of decline in ECM production may be lessened with the use of Agolin. The medium producing cows averaged 224 days in milk at the start of the feeding period and were more capable of maintaining their level of production than the control cows that did not receive the feed additive. This product may help dairy producers to reduce CH4 production while increasing their profits.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper. Barry Callebaut® and Nestle® provided partial funding for this trial as a component of their support for sustainable technology, but had no direct involvement in this trial. Dr. Jorge Noricumbo-Saenz works for Feedworks USA Ltd. who markets Agolin® in the United States but was not involved in running the trial or the analysis of the data.

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