Alfalfa hay does not negatively impact performance outcomes in broilers during coccidiosis challenge

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Summary and Implications
The use of alfalfa and similar forages in poultry products is often limited due to the detrimental effects of high-fiber ingredients on non-ruminant animal performance. Despite this, inclusion of alfalfa hay, specifically late-cutting hay, may confer health benefits that protect against the damaging effects of coccidiosis in poultry. The study objective was to examine performance outcomes in Ross 708 broilers fed 5% 5th-cutting alfalfa hay during a coccidiosis challenge. A total of 144 birds were assigned to a corn-soybean meal control or diet with 5% inclusion of ground 5th-cutting alfalfa hay for 42d divided into 14d starter, grower, and finisher periods. At 14d, half of the birds were orally inoculated with 10X Coccivac® B-52 vaccine with a 2x2 factorial treatment arrangement of diet and inoculation. Body weight (BW) and feed intake (FI) was recorded weekly to calculate average performance outcomes. Prior to inoculation, 5% alfalfa did not negatively impact performance but increased week 2 FI by 5.1% (P = 0.04). As expected, coccidiosis reduced performance in week 3 (P < 0.05). Alfalfa inclusion did not alter coccidiosis-induced performance outcomes; however, numerical differences observed in non-inoculated birds supports future research into the benefits of feeding late-cutting alfalfa and its products to poultry.

Introduction
Forages such as alfalfa in poultry diets have limited use due to the contributions of high fiber to depressed production performance. Forage fiber, however, provides a fermentation substrate for intestinal microbial communities that may have positive downstream benefits such as protection from damage related to diseases including coccidiosis caused by *Eimeria*. While high forage inclusion rates may reduce performance, health benefits may be conferred at lower inclusion levels without negatively impacting bird performance. One final consideration pertaining to feeding alfalfa is the variation in nutrient profiles between cuttings within a growing season, which has been anecdotally linked to improved animal health when later cuttings are fed. The objective of this study was to examine the performance effects of feeding 5% inclusion late-cutting alfalfa (5th cut) to broiler chickens during an *Eimeria* challenge.

Materials and Methods
All animal protocols were approved by the Iowa State University Institutional Animal Care and Use Committee. In total, 144 Ross 708 broilers were obtained from a commercial hatchery (Welp Hatchery, Bancroft, IA) and housed in 8 3’x3’ floor pens evenly distributed between two sides of a barn (18 birds/pen). Birds were randomly assigned to either a corn-soybean control or 5% 5th cutting alfalfa hay diet with ad libitum access to mash feed and water. The 42d study was divided evenly into 14d starter, grower, and finisher periods. On d14, half of the birds were orally inoculated with a 10X dose of Coccivac®-B52 (Merck, Kenilworth, NJ) vaccine. Healthy and inoculated birds were physically separated between the two rooms to reduce the spread of *Eimeria* to healthy animals. As a result of diet type and inoculation status, the treatments in this study were arranged in a 2x2 factorial arrangement with 4 treatments total (2 pens/treatment). Pen BW and feeder weights were taken weekly to calculate BW gain (BWG), FI, and feed conversion ratio (FCR). Throughout the post-inoculation challenge period, birds were removed from the study for tissue collection, therefore, the number of birds per pen reduced from 18 to approximately 13 by the end of the study. As a result, performance calculations were made based on the average BW and FI per bird within a pen. Data were analyzed using the MIXED procedure in SAS 9.4 with fixed effects of diet type, *Eimeria* status, and the interaction of diet×*Eimeria* and initial BW as a covariate. Data from the first 14d of the study excludes the effect of *Eimeria* from the model, as birds had not been inoculated at those timepoints. Results were considered statistically significant at P ≤ 0.05.

Results and Discussion
In the first 2 weeks of the study, inclusion of 5th cutting alfalfa had minimal impacts on bird performance, with a 5.1% increase in FI observed in week 2 (P = 0.04). Following inoculation, birds orally administered *Eimeria* showed a 14.1, 29.6, and 14.2% reduction in week 3 BW, BWG, and FI, respectively, compared to non-inoculated birds, resulting in a 17.9% less efficient FCR (P = 0.02, 0.005, 0.05, and 0.0003, respectively). This outcome is expected as coccidiosis is known to reduce broiler...
performance. Alfalfa inclusion numerically improved performance outcomes compared to the control diet in *Eimeria*-inoculated broilers during week 3. The main effect of *Eimeria* status was not determined to be significant in week 3, suggesting that disease-related performance losses were mediated by 7d post-inoculation in both control and alfalfa-fed birds.

For the remainder of the study, alfalfa, inoculation status, and their interaction did not impact bird performance (Table 1), with the only exception being a 14.2% more efficient FCR observed in the finisher period for healthy birds on hay diets compared to healthy birds on control diets (*P* = 0.04). Performance for the entire 42d trial was not significantly impacted, but numerical differences suggest that feeding late cutting alfalfa hay at a 5% inclusion in healthy broilers may offer slight performance improvements compared to the healthy control that requires further investigation (Table 2). Despite the improvements observed in healthy birds, performance in alfalfa-fed birds during *Eimeria* challenge was numerically worse than outcomes observed in their control-fed counterparts, suggesting that late-cutting alfalfa hay does not reduce the damaging effects of coccidiosis on our study setting.

Overall, outcomes suggest that feeding late-cutting alfalfa hay does not drastically alter broiler performance in healthy animals or protect from the negative outcomes associated with coccidiosis; however, slight performance improvements observed in healthy animals provides a foundation for future research into the effects of feeding late-cutting alfalfa and associated products to broiler chickens.

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Table 1: Weekly average bird performance in control and *Eimeria*-inoculated Ross 708 broilers fed diets ± 5% 5th cutting alfalfa

| Treatment         | SEM  | *P*-values | Diet | *Eimeria* | Diet*Eimeria |
|-------------------|------|------------|------|-----------|-------------|
| **d0 BW, kg²**     | 0.044| 0.043      | 0.0006| 0.766     | N/A         | N/A         |
| **Week 1**         |      |            |      |           |             |             |
| BW, kg            | 0.153| 0.147      | 0.005| 0.261     | N/A         | N/A         |
| BWG, kg           | 0.110| 0.104      | 0.004| 0.228     | N/A         | N/A         |
| FI, kg            | 0.121| 0.120      | 0.004| 0.762     | N/A         | N/A         |
| FCR               | 1.102| 1.158      | 0.026| 0.084     | N/A         | N/A         |
| **Week 2**         |      |            |      |           |             |             |
| BW, kg            | 0.344| 0.346      | 0.009| 0.867     | N/A         | N/A         |
| BWG, kg           | 0.191| 0.199      | 0.006| 0.221     | N/A         | N/A         |
| FI, kg            | 0.260| 0.274      | 0.005| 0.044     | N/A         | N/A         |
| FCR               | 1.362| 1.380      | 0.020| 0.408     | N/A         | N/A         |
| **Week 3³**        |      |            |      |           |             |             |
| BW, kg            | 0.719| 0.706      | 0.597| 0.627     | 0.032       | 0.750       | 0.022       | 0.416       |
| BWG, kg           | 0.378| 0.368      | 0.252| 0.274     | 0.021       | 0.707       | 0.005       | 0.342       |
| FI, kg            | 0.523| 0.533      | 0.442| 0.464     | 0.032       | 0.517       | 0.045       | 0.809       |
| FCR               | 1.389b| 1.454b     | 1.756a| 1.706a   | 0.034       | 0.668       | 0.0003      | 0.041       |
| **Week 4**         |      |            |      |           |             |             |
| BW, kg            | 1.152| 1.154      | 1.079| 1.107     | 0.087       | 0.767       | 0.364       | 0.900       |
| BWG, kg           | 0.433| 0.458      | 0.482| 0.481     | 0.056       | 0.783       | 0.429       | 0.768       |
| FI, kg            | 0.727| 0.722      | 0.697| 0.717     | 0.043       | 0.831       | 0.602       | 0.706       |
| FCR               | 1.712| 1.581      | 1.452| 1.486     | 0.078       | 0.638       | 0.154       | 0.442       |
| **Week 5**         |      |            |      |           |             |             |
| BW, kg            | 1.793| 1.742      | 1.690| 1.707     | 0.134       | 0.869       | 0.516       | 0.740       |
| BWG, kg           | 0.641| 0.579      | 0.611| 0.601     | 0.063       | 0.474       | 0.936       | 0.606       |
| FI, kg            | 0.953| 0.970      | 0.976| 0.987     | 0.079       | 0.819       | 0.744       | 0.952       |
| FCR               | 1.486| 1.681      | 1.599| 1.641     | 0.060       | 0.066       | 0.449       | 0.166       |

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|      | BW, kg  | BWG, kg | FI, kg  | FCR    |
|------|---------|---------|---------|--------|
| Mean | 2.291   | 0.498   | 1.051   | 2.113  |
| SD   | 0.237   | 0.121   | 0.150   | 0.139  |

1 Performance measurements on a per bird basis are based on the average number of birds per pen when BW and FI data were collected.
2 Effect of *Eimeria* excluded from the model, for weeks 1 and 2; n = 4 pens of birds / diet (control or + alfalfa hay).
3 Effect of *Eimeria* included in model following inoculation at d14; n = 2 pens of birds / treatment.
Table 2: Performance of control and *Eimeria*-inoculated Ross 708 broilers fed diets ± 5% 5th cutting alfalfa hay divided into 14-d starter, grower, and finisher periods on a per bird basis

| Treatment | SEM | P-values |  |
|-----------|-----|----------|---|
| Control   | Alfalfa Hay | Diet | *Eimeria* | Diet*Eimeria |
| d0 BW, kg | 0.044 | 0.043 | 0.0006 | 0.766 | N/A | N/A |
| Starter (d0-d14)² |  |
| BWG, kg | 0.300 | 0.302 | 0.009 | 0.882 | N/A | N/A |
| FI, kg | 0.381 | 0.394 | 0.008 | 0.163 | N/A | N/A |
| FCR | 1.267 | 1.304 | 0.016 | 0.067 | N/A | N/A |
| Grower (d14-d28)³ |  |
| BWG, kg | 0.811 | 0.825 | 0.732 | 0.755 | 0.074 | 0.754 | 0.248 | 0.938 |
| FI, kg | 1.250 | 1.255 | 1.139 | 1.181 | 0.071 | 0.672 | 0.157 | 0.738 |
| FCR | 1.558 | 1.523 | 1.555 | 1.568 | 0.077 | 0.857 | 0.724 | 0.687 |
| Finisher (d28-d42) |  |
| BWG, kg | 0.974 | 1.173 | 1.154 | 1.047 | 0.148 | 0.686 | 0.813 | 0.237 |
| FI, kg | 2.004 | 2.087 | 2.079 | 2.096 | 0.255 | 0.771 | 0.809 | 0.849 |
| FCR | 2.095a | 1.798b | 1.804ab | 2.004ab | 0.101 | 0.542 | 0.594 | 0.039 |
| Overall (d0-d42) |  |
| BWG, kg | 2.083 | 2.294 | 2.189 | 2.110 | 0.219 | 0.697 | 0.817 | 0.415 |
| FI, kg | 3.634 | 3.728 | 3.600 | 3.679 | 0.295 | 0.704 | 0.854 | 0.972 |
| FCR | 1.765 | 1.631 | 1.644 | 1.745 | 0.077 | 0.780 | 0.953 | 0.118 |

¹ Performance measurements on a per bird basis are based on the average number of birds per pen when BW and FI data were collected

² Effect of *Eimeria* excluded from the model, for the starter period n = 4 pens of birds / diet (control or alfalfa hay)

³ Effect of *Eimeria* included in model following inoculation at d14; n = 2 pens of birds / treatment