Original Research

Journal of Advanced Veterinary Research
(2025) Volume 15, Issue 1, 101-105

Meta-Analysis of the effect of alfalfa (*Medicago sativa* L.) supplementation on broiler growth performance

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ARTICLE INFO

Recieved: 01 October 2024

Accepted: 29 December 2024

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Keywords:

Fiber, Growth performance, Fabaceae, Poultry, Feed ingredient

ABSTRACT

Alfalfa (*Medicago sativa* L.) is a leguminous plant rich in amino acids and micronutrients, making it a potential feed supplement for poultry. However, its high fiber content may limit its efficacy. This meta-analysis aimed to evaluate the effects of alfalfa supplementation on broiler performance, including feed intake, weight gain, and feed conversion ratio (FCR). Articles were selected from Scopus, PubMed Central, and Google Scholar based on eligibility criteria developed with reference to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Written information in English that includes age of animal, form of alfalfa, growth phase, supplementation level, and evaluation of animal performance (consumption, weight gain, and feed conversion of broilers). The effect size of the alfalfa supplementation treatment was calculated to estimate the standardized mean difference (SMD) at 95% Confident Interval (95% CI) using a random effects model (REM). The meta-analysis included 8 research papers that were eligible. Open Meta-Analyst for Ecology and Evolution (OpenMEE) software was used for all analyses. The collected results showed that afalfa supplementation decreased feed consumption (SMD -0.98; P<0.001), decreased body weight (SMD -1.4; P<0.001), but increased feed conversion value (SMD 0.83; P<0.001). The conclusion of the meta-analysis indicates that alfalfa supplementation as a feed protein-fibre additive can reduce broiler performance.

Introduction

Feed costs constitute 60-70% of poultry production expenses, and optimizing feed composition is crucial for maximizing performance This is an alternative feed being implemented to reduce production costs, and provide nutrients for animals (Sánchez-Quinche *et al*., 2022). Fibrous protein can be obtained from leguminous products such as alfalfa (*Medicago sativa* L.) commonly used as feed in various animal species (Suwignyo and Sasongko, 2019; Suwignyo *et al*., 2020; 2021; 2022; 2023).

Alfalfa is a leguminous with complete nutrient content and can be consumed by non-ruminants, especially poultry. Protein content was reported to be 15.3-32.27%, high complete amino acids L- glutamic acid 15.650 mg/kg and L- leucine 1.02-1.29% contains macrominerals (Ca, P, Na, and K) and microminerals (Zn and Fe), and secondary metabolites; flavonoids (1.99%), saponins (5.1%), and tannins 4.28% (Suwignyo *et al*., 2020; Suwignyo *et al*., 2023). Alfalfa is a promising feed ingredient due to its high protein content, amino acids, and micronutrients. However, its high fiber content and associated anti-nutritional factors may limit its usefulness in poultry diets. However, the crude fiber content of alfalfa is still a limiting factor in feed quality for broilers (Jiang *et al*., 2017; Gulizia and Downs, 2020; Fries-Craft *et al*., 2023).

Several studies on alfalfa supplementation in broiler diets have been conducted on productivity. However, in several studies, the responses and needs of different animals resulted in different and ambiguous conclusions. Therefore, through the statistical approach of using meta-analysis methods by synthesizing and combining the results of different research data to detect trends, resolve ambiguities, identify the impact size or effect size, and develop new perspectives. Consequently, the study used meta-analysis to examine the impact of alfalfa supplementation on broiler performance.

Materials and methods

The database was collected and developed from literature studies using alfalfa (*Medicago sativa* L.) as a feed supplement for broiler performance. Some types of literature were checked using computerized scientific platforms including Scopus (https://www.scopus.com), PubMed Central (https://pubmed.ncbi.nlm.nih.gov), and Google Scholars (https://scholar.google.com). The search used the keywords "alfalfa", "broiler" and "performance". The meta-analysis followed PRISMA guidelines, systematically reviewing studies from databases such as Scopus, PubMed, and Google Scholar. Studies were included if they provided control groups, used alfalfa supplementation in broiler diets, and measured feed intake, body weight gain, and FCR (Liberati *et al*., 2009).

All literature were compiled into a database during the data tabulation process. The tabulation of data is the organization of data from the relevant literature according to the inclusion criteria to guide the calculation of the desired analysis. All literature titles relevant with the keywords were collected in the database. Scopus had 31 references, PubMed Central had 11 references, and Google scholars had 19,100 references. Each literature was examined using meta-analysis, as shown in Table 1.

Figure 1 illustrates the procedure for selecting relevant articles matching the inclusion criteria for inclusion in the database. Following the application of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol, 8 publications were identified. A flowchart of the process of selecting studies used for meta-analysis can be seen in Figure 1.

Data extraction

Meta-analysis refers to the systematic use of statistical methods to analyze effect sizes. The concept of effect size is concerned with quantifying and summarizing the impact resulting from observations of the

ISSN: 2090-6277/2090-6269/

Table 1. Studies considered in the meta-analysis.

Reference	Broiler strain	Alfalfa tropic	Level (g/kg)	Period
Gulizia and Downs (2020)	Cobb 700	Meal	73	Starter Grower Finisher
Sánchez-Quinche *et al.* (2022)	Cobb 500	Meal	10, 20, 30, and 40	Starter Grower Finisher
Jiang *et al.* (2017)	Chinese yellow-feathered	Meal	40 and 80	Finisher
Varzaru *et al.* (2020)	Cobb 500	Meal	50	Grower Finisher
Vlaicu *et al.* (2021)	Cobb 500	Meal	50	Grower Finisher
Pleger *et al.* (2020)	Hubbard JA-757	Hay	50, 100, 150, and 200	Starter Grower Finisher
Fries-Craft *et al.* (2023)	Ross 708	Hay	50	Starter Grower Finisher
Shirzadegan and Taheri (2017)	Ross 308	Meal	30 and 60	Finisher

particular phenomenon being investigated (Albarki *et al.*, 2024). Alfalfa supplementation on broiler performance Broiler strains, form and level of alfalfa supplementation, and broiler period and inclusion criteria were collected from all articles. Articles contained mean values and measures of variance (Standard deviation or Standard error) of the outcome variables of interest from the treatment and control groups. Standard error (SE) values were converted to standard deviation (SD) using the Higgins and Deeks method (Higgings and Deeks, 2008) SD= SE ×√n, N is the number of animals assigned to each treatment group.

The experiments expressed the supplementation level in percentage (%) and if it was not in grams per kilogram (g/kg), then the percentage value was converted to grams per kilogram (g/kg) using the formula: 1% =10 g/kg. The data collected from the 8 studies that passed the selection criteria were transferred to Microsoft Excel 2021 comma-separated value (CSV) file format, which is a file format suitable for statistical analysis.

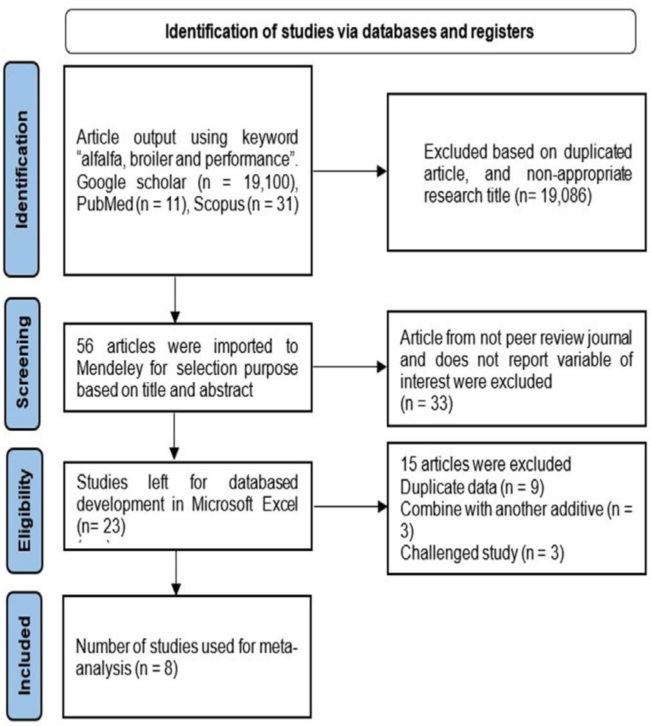

Figure 1. Flow charts of the studies selection process utilized for the meta-analysis.

Statistical analysis

Data were extracted and standardized mean differences (SMD) were calculated using OpenMEE software (Wallace *et al.*, 2017). Variable data is given as standardized mean difference between alfalfa supplementation treatment and control with a 95% confidence interval (CI). Inclusion criteria data were included in the multilevel analysis, broiler strain, alfalfa level and form, treatment period. The effect size was estimated as the difference between the means of the experimental and treatment groups divided by the overall standard deviation of the selected treatment group. The Der Simonian and Lard test (Chi-square (Q) - statistic) and the Inconsistency index (I2) - Higgins statistic were used to analyse heterogeneity (Higgins *et al.*, 2003). The I2 statistic is the percentage of variance in a meta-analysis that is due to study heterogeneity. Because heterogeneities existed in different levels in every combined study, a random-effects model (REM) was adopted for the meta-analysis. P-value <0.05 is considered significant, if there is an effect of alfalfa supplementation level on broiler performance, it shows a significant effect (p<0.05), thus in predicting the effect of the study, a visualization of the impact of alfalfa supplementation level on the difference in broiler performance (%) between control and treatment.

Results

Feed intake

The result of combined effect estimation showed that alfalfa (*Medicago sativa* L.) supplementation decreased broiler feed intake (SMD = -0.98; 95% CI = -1.57 to 0.38; P = 0.001), with I2 = 83.8% (P < 0.001). The supplementation form of alfalfa decreased feed intake in broilers, although the alfalfa meal form was not significant (P = 0.207). The level of alfalfa (*Medicago sativa* L.) supplementation decreased feed intake, even though the level of 10-50 g/kg was not significant (P=0.640). The data analysis of alfalfa supplementation on feed consumption in broilers showed in Table 2.

Body weight gain

The results of the treatment estimation assessment (Table 3) showed that alfalfa (*Medicago sativa* L.) supplementation had the effect of reducing the overall body weight gain of broilers (SMD = -1.4; 95% CI = -2.1 to 0.78; P = 0.001). Alfalfa supplementation showed a heterogeneity of 85.5% (P < 0.001). Alfalfa supplementation decreased body weight gain of broilers in both hay and meal form, in all growth phases, and at all levels of alfalfa supplementation. Although in the grower phase and levels of 10 - 50 g/kg did not significantly reduce body weight gain of broilers. The data analysis of alfalfa (*Medicago sativa* L.) supplementation on broiler body weight gain showed in Table 3.

Table 2. Subgroup analysis of the effect of alfalfa (*Medicago sativa* L.) supplementation on feed intake in broiler.

Covariates	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	45	-0.98	-1.57	-0.38	0.3	0.00	83.8	<0.001
Form								
Hay	14	-2.9	-4.67	-1.14	0.9	0.00	90.4	<0.001
Meal	31	-0.28	-0.71	0.15	0.2	0.21	62.7	<0.001
Growth phase								
Starter	15	-0.69	-1.47	0.09	0.4	0.08	75.4	<0.001
Grower	9	-0.8	-2.42	0.81	0.83	0.33	85.8	<0.001
Finisher	21	-1.3	-2.29	-0.31	0.51	0.01	87.3	<0.001
Inclusion rate								
10-50 g/kg	31	-0.14	-0.74	0.46	0.31	0.64	78	<0.001
60-100 g/kg	9	-2.05	-3.17	-0.93	0.57	<0.001	74.2	<0.001
>100 g/kg	5	-4.3	-5.8	-2.8	0.77	<0.001	61.9	0.03

Note: SMD and I2 were considered significant at p < 0.05; N = number of comparisons; SMD = standardized mean differences between the alfalfa supplementation treatment and controls; CI = confidence interval; p-value = probability value; SE = standard error; I2 = heterogeneity level of the meta-analysis model.

Table 3. Subgroup analysis of the effect of alfalfa (*Medicago sativa* L.) supplementation on body weight gain in broiler.

Covariates	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	45	-1.4	-2.1	-0.78	0.33	<0.001	85.5	<0.001
Form								
Hay	14	-5.47	-8.05	-2.88	1.32	<0.001	91.6	<0.001
Meal	31	-0.41	-0.77	-0.04	0.19	0.03	51	<0.001
Growth phase								
Starter	15	-0.86	-1.65	-0.06	0.41	0.03	75.8	<0.001
Grower	9	-0.5	-1.3	0.3	0.41	0.22	65.2	0.00
Finisher	21	-2.77	-4.2	-1.33	0.73	<0.001	90.7	<0.001
Inclusion rate								
10-50 g/kg	31	-0.48	-1.1	0.15	0.32	0.14	80.1	<0.001
60-100 g/kg	9	-2.1	-3.49	-0.69	0.71	0.00	84.7	<0.001
>100 g/kg	5	-8.7	-10.7	-6.7	1.03	<0.001	30.7	0.22

Note: SMD and I2 were considered significant at p < 0.05; N = number of comparisons; SMD = standardized mean differences between the alfalfa supplementation treatment and controls; CI = confidence interval; p-value = probability value; SE = standard error; I2 = heterogeneity level of the meta-analysis model.

Table 4. Subgroup analysis of the effect of alfalfa (*Medicago sativa* L.) supplementation on feed conversion ratio in broiler.

Covariates	N	SMD	CI 95%		SE	*p*-value	Heterogeneity	
			Lower	Upper			I^2	*p*-value
Overall	45	0.83	0.39	1.27	0.23	<0.001	76	<0.001
Form								
Hay	14	1.15	0.35	1.94	0.41	0.01	81.2	<0.001
Meal	31	0.68	0.15	1.22	0.27	0.01	73.6	<0.001
Growth phase								
Starter	15	0.87	0.07	1.67	0.41	0.03	76.4	<0.001
Grower	9	-0.04	-1.32	1.24	0.65	0.95	83.1	<0.001
Finisher	21	1.11	0.54	1.67	0.29	<0.001	71.9	<0.001
Inclusion rate								
10-50 g/kg	31	0.68	0.26	1.1	0.22	0.00	63.7	<0.001
60-100 g/kg	9	0.09	-1.2	1.4	0.65	0.89	85.2	<0.001
>100 g/kg	5	3	1.5	4.5	0.76	<0.001	74.1	0.00

Note: SMD and I2 were considered significant at p < 0.05; N = number of comparisons; SMD = standardized mean differences between the alfalfa supplementation treatment and controls; CI = confidence interval; p-value = probability value; SE = standard error; I2 = heterogeneity level of the meta-analysis model.

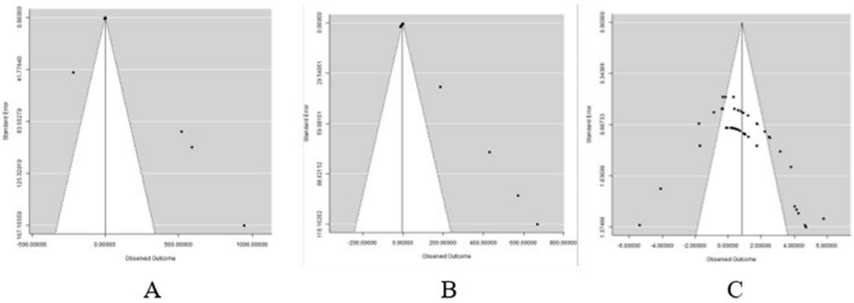

Figure 2. Fannel plot of effect of alfalfa (*Medicago sativa* L.) supplementation on the feed Intake (A), body weight gain (B), and feed conversion ratio (C) in broiler.

Feed conversion ratio

The study on the effect of alfalfa supplementation on the feed conversion ratio (FCR) of broilers showed that alfalfa (*Medicago sativa* L.) supplementation increased the overall FCR value of broilers by 0.83 (95% CI = 0.39 to 1.27; P < 0.001). The supplementation form and level of alfalfa significantly increased FCR values (P < 0.001). However, alfalfa supplementation in the grower phase of broilers showed a decrease in FCR values, although not significant (P = 0.945) within high heterogeneity (I2 = 83.1% and Q = <0.001). The data analysis of alfalfa (*Medicago sativa* L.) supplementation on feed conversion ratio in broilers showed in Table 4.

Publication Bias

Graphic fennel plot alfalfa (*Medicago sativa* L.) supplementation on feed intake, body weight, and feed conversion ratio in broiler can be seen at Figure 2.

Discussion

The study was investigated using a meta-analysis of published studies on alfalfa (*Medicago sativa* L.) supplementation on broiler performance. The results of the meta-analysis investigation showed that alfalfa supplementation in broilers, both in the form of hay and meal, feeding level, and growth phase, decreased broiler feed intake (Table 2). The decrease in feed intake may be influenced by the palatability factor of feed supplemented with alfalfa. Pleger *et al*. (2020) demonstrated that alfalfa feeding levels in broilers showed that the higher the level of alfalfa fed, the lower the feed consumption. It is related with the feed palatability factor. Oleszek *et al*. (1992) explained that the content of secondary metabolites in alfalfa such as saponins has a bitter taste that causes low feed consumption in animals.

The reduction in feed intake observed in this study may be attributed to the lower palatability of alfalfa, which contains saponins and other bitter secondary metabolites. Reduced nutrient intake likely explains the decrease in body weight gain. Furthermore, the increased FCR is consistent with the high fiber content of alfalfa, which can hinder digestion and nutrient absorption. While alfalfa contains beneficial micronutrients, its anti-nutritional factors, such as non-starch polysaccharides, appear to limit its efficacy in broiler diets (Suwignyo and Sasongko, 2019; Suwignyo *et al*., 2021; Murod *et al*., 2025). This may be related to the type of feed ingredients used. Increasingly, alfalfa supplementation tends to contain high levels of non-starch polysaccharides (NSP), which become a limiting factor for poultry, thus reducing body weight (Shirzadegan and Taheri, 2017; Hanif *et al*., 2023). The feed containing a high percentage of indigestible fibre leads to bulky conditions in the digestive tract and the bulkiness effect has the potential to negatively impact broiler performance (Gulizia and Downs, 2020).

The implication of decreased feed consumption and body weight in broilers will increase the feed conversion rate. The results of the meta-analysis investigation showed that alfalfa supplementation in broilers showed a significant improvement in feed conversion rate in broilers. However, the subgroup found in the grower phase reduced the feed conversion rate of broilers by -0.04 (Table 4). The low feed conversion rate is highly desirable because it implies that the addition of a particular quantity of feed produces a greater percentage of body weight gain in broilers (Albarki *et al*., 2024). Although the study found an increased overall FCR value. The increased conversion value of alfalfa-supplemented feed may be influenced by the antinutrient factors existing in alfalfa flour (Varzaru *et al*., 2020; Murod *et al*., 2025).

The antinutrient content contained in alfalfa is in its secondary metabolites. Alfalfa contains various secondary metabolites such as polysaccharides, saponins, leaf protein concentrate, flavonoids, and tocopherols in large quantities (Suwignyo *et al*., 2023). Despite having antinutrient effects, these secondary metabolites also have great benefits for animals. Rafińska *et al*. (2017) and Suwignyo *et al*. (2022; 2023) explained that the biological function of alfalfa extract plays an active role as antibacterial, anti-inflammatory, antioxidant, lowering cholesterol levels. These bioactive components will impact the digestive health of the poultry digestive tract (Murod *et al*., 2025). Therefore, alfalfa is one of the potential poultry feed ingredients for further implementation in different poultry species.

Alfalfa supplementation as a broiler feed has the potential to be developed and considered. Feeding protein-fibre feed sources in poultry has a tolerance limit that becomes the focus of nutrient balance. Tolerance in broilers approximately 4-6% (Badan Standar Nasional Indonesia, 2017). In addition to tolerance limits, fibre feeding in poultry needs to be considered in the age, breed, kind and type of fibre. Jiang *et al*. (2012); Suwignyo *et al*. (2021) and Murod *et al*. (2025) demonstrated that alfalfa as a feed supplement in hybrid ducks with different levels resulted in different performance responses.

Publication Bias

The funnel plot shown in Figure 2 has a distinct asymmetric pattern. The presence of asymmetrical findings indicates the possibility of publishing bias. Besides, Hanif *et al*. (2024) concluded that the presence of asymmetrical shapes in the funnel plot is an indication of publication bias. Publication bias may occur due to variations in the levels of alfalfa supplementation, broiler strains and housing management used in different studies.

Conclusion

Meta-analysis demonstrates that alfalfa supplementation in broiler diets negatively impacts feed intake, body weight gain, and FCR, particularly at higher levels. While alfalfa provides valuable nutrients, its high fiber content and anti-nutritional factors limit its overall effectiveness as a poultry feed additive. Future research should focus on optimizing supplementation levels and investigating methods to mitigate the adverse effects of fiber contents.

Acknowledgments

The authors would like to express their appreciation to colleagues for suggesting data references during the process of drafting the study. The help enabled the author to achieve the time to complete the research.

Conflict of interest

The authors have no conflict of interest to declare.

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