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Effects of Enzyme and Probiotic on the Growth Performance of Broiler Chickens: A Meta-Analysis

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ABSTRACT. A meta-analysis was conducted to determine the effects of enzyme and probiotic on the growth performance of broiler chickens. A collection of publications covering the years from 2009 to 2021 was compiled using several keywords, such as enzyme, growth performance, and probiotic. A total of 136 comparisons from 19 *in vivo* investigations were used to create the final dataset. The data were analyzed using the OpenMEE software, considering the feed additives (enzymes and probiotics) as fixed effects and individual studies as random effects. The result showed an insignificant difference (P>0.05) between enzymes and probiotics in terms of improving the body weight gain, feed intake, and feed conversion ratio (FCR) of broilers. In conclusion, both probiotics and enzymes improved broiler performances, such as body weight gain, feed intake and FCR. According to the meta-analysis, the ability of enzymes and probiotics to affect broiler performances was equal.

Keywords: broilers, enzyme, feed additives, meta-analysis, performance, probiotic

INTRODUCTION

Antibiotic growth promoter (AGP) has traditionally been used as a feed additive to maximize growth and maintain the health of broiler chickens for several decades. However, the use of AGP as a feed additive can have a negative impact on consumer health due to antibiotic residues in broiler chicken meat. This condition has prompted governments in most countries throughout the world to prohibit the use of AGP as a feed additive for broiler chickens (Anjum and Chaudhry, 2010; Sugiharto, 2016; Sapsuha *et al*., 2021). Apart from food safety issues, the AGP ban has had an impact on decreasing growth rates as well as increasing disease prevalence and mortality rates for broiler chickens. Considering the latter condition, poultry nutritionists and broiler producers currently are currently searching for safe substitutes for AGP that may effectively maintain broiler production by the genetic potential. Two AGP alternatives explored and applied in the broiler productions are enzymes and probiotics (Sugiharto, 2016).

Enzymes are protein-based chemical compounds that function as biocatalysts in all chemical reactions taking place in the body (Anjum and Chaudhry, 2010). Numerous studies showed that using enzymes can increase the growth rate of broiler chickens (Rahman *et al*., 2014; Polovinski-Horvatović, 2021). Exogenous enzymes with a large number of applications in broiler production include β-glucanase, xylanase, amylase, α-galactosidase, protease, lipase, and phytase (Sugiharto, 2016). Since most broiler feeds are formulated based on corn and soybean mealbased diets, which contain anti-nutritional compounds such as phytic acid (phytate), protease inhibitors and non-starch polysaccharides, exogenous enzymes have been beneficial in broiler nutrition. Indeed, the decomposition of anti-nutritional compounds by exogenous enzymes is crucial for increasing the nutrient digestibility of plant-based feed components (Velázquez-De *et al*., 2021).

Probiotics are single cultures or mixes of live microorganisms that, when given in sufficient quantities, enhance the balances of the host’s gut microbiota, boost intestinal resistance to infections, and strengthen the host's immune system. The most often utilized probiotics for broiler chicks are lactic acid bacteria and yeast, among other probiotic microorganisms that are accessible~~.~~ (Swiatkiewicz *et al*., 2014; Sugiharto, 2016). Probiotic supplementation generally supports the immune system, morphology, and intestinal ecology of broilers. Probiotic treatments have been associated with increased numbers of beneficial bacteria and the ratio of villous height to crypt depth in the small intestine. These improvements have been linked to better health and the ability of small intestine to digest and absorb nutrients, which improved broiler growth performance and feed efficiency (Sugiharto and Ranjitkar, 2019).

As alternatives to AGP for improving feed efficiency and growth performance of broiler chicken, both probiotics and enzymes offer advantages. However, poultry nutritionists are still

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unsure which supplement to use, either enzymes or probiotics. This study aimed to compare the growth rate, feed intake, and feed conversion ratio (FCR) of broiler chickens receiving either enzymes or probiotics during the rearing period using a meta-analysis.

MATERIALS AND METHODS

Ethical Approval

Ethical Approval for the study was not needed since there is no laboratory or animal subjects in this research. This research is based on a literature review.

Eligibility Criteria and Dataset Development

The literature search was conducted through a literature database, i.e., Science Direct (http://www.sciencedirect.com/) and Google Scholar (https://scholar.google.com/). The keywords used during the search were broiler, comparison, enzyme, FCR, feed intake, growth, and probiotic. Further, the following criteria were used for literature selection: (1) published in English as full-text articles; (2) peer-reviewed published journals; (3) indexed conference proceedings; (4) direct comparison between enzymes and probiotics; (5) study on broiler chickens; (6) measured body weight gain, feed intake, FCR and dry matter digestibility. The initial searches found 55 potential references. After screening, 36 references were eliminated due to nine studies indirectly comparing enzymes and probiotics and six studies not including the parameters of interest, such as body weight gain, feed intake, and FCR. Finally, only 19 articles were used for data coding and statistical analysis.

Table 1. List of comparison studies used in meta-analysis

No.	Period of rearing	Strains	Sex	Enzymes	Probiotics	N[1]	References
1	1-42	Ross	Male	Xylanase and phytase	*Lactobacillus salivarius*	12	Swiatkiewicz *et al*. (2014)
2	1-42	Ross	Male	Xylanase, β-glucanase, protease, α-amylase and phytase	*Saccharomyces cerevisiae* and *Bacillus subtilis*	16	Razuki *et al*. (2017)
3	1-42	Ross	Female	Xylanase, β-glucanase, protease, α-amylase and phytase	*S. cerevisiae* and *B. subtilis*	16	Razuki *et al*. (2017)
4	1-42	Ross	Male	Protease, cellulase, amylase, xylanase, endo-1,3; 1,4-beta glucanase, lipase and -glucosidase	*Streptococcus salivarius*, *Lactobacillus delbruckii*, *L. acidophilus*, *L. plantarum*, *L. rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Candida pintoloppesii* and *Asperigillus oryzae*	8	Kırkpınar *et al*. (2018)
5	1-35	Cobb	Mixed-Sex	Endo-1, 4-β-xylanase, α-amylase and protease	*Lactobacillus farciminis*	5	El-Kelawy *et al*. (2017)
6	1-42	NM	Not mentioned	Amylase, xylanase, pectinase, cellulase, glucanase and protease	*S. cerevisiae*, *L. acidophilus*, *Lactobacillus sporogenes* and *B. subtilis*	8	Thorat *et al*. (2015)
7	1-42	Ross	Mixed-Sex	Pectinase, protease, phytase, β-glucanase, xylanase, cellulase and amylase	*B. subtilis*	3	Kamel and Mohamed (2016)
8	1-42	Cobb	Mixed-Sex	Pectinase, protease, phytase, β-glucanase, xylanase, cellulase and amylase	*B. subtilis*	3	Kamel and Mohamed (2016)
9	1-56	Agrited strain	Mixed-Sex	Bio-enzyme	*S. cerevisiae*	3	Udeh *et al*. (2019)
10	1-42	Arbor Acres	Mixed-Sex	β-glucanase and phytase	*L. acidophilus* and *S. cerevisiae*	5	Agboola *et al*. (2021)
11	1-47	Ross	Male	β-glucanase, α-amylase, cellulase, protease and lipase	*L. acidophilus*, *L. casei*, *Bifidobacterium bifidum* and *E. faecium*	4	Momtazan *et al*. (2011)
12	1-35	Ross	Mixed-Sex	β-xylanase, α-amylase and protease	*Bacillus amyloliquefaciens*	4	Abdel Moati *et al*. (2022)
13	10-35	Cobb	Male	Xylanase	*Lactobacillus* sp.	6	Machado *et al*. (2020)
14	1-42	Ross	Mixed-Sex	Cellulase, xylanase, protease amylase, phytase, pactinase, invertase, hemicelulase, lipase and α-galactosidase	*L. acidophilus*, *Lactobacillus* bulgaricus, *L. plantarum*, *S. faecium*, *B. bifidus*, *B. subtilis*, *B. Licheniformis*, *Bacillus megaterum*,	8	Rahman *et al*. (2013)

					Bacillus mesentricus, Bacillus polymyxa and *Saccharomyces bourlrdii*		
15	1-42	Cobb	Mixed-Sex	Xylanase	*Bacillus* spp.	3	Nusairat and Wang (2020)
16	1-47	Arbor Acres	Male	Glucanase, cellulose and xylanase	*L. acidophilus, L. delbrueckii* subsp. bulgaricus, *Lactobacillus rhamnosus, Streptococcus salivarius* subsp. Thermophillus and *E. faecium*	4	Siddiqui *et al*. (2017)
17	22-42	Hubbard	Mixed-Sex	Amylase, protease, cellulase, beta-glucanase, xylanase, pectinase and phytase	*S. cerevisiae*	3	Kaushal *et al.* (2019)
18	1-42	Ross	Male	β-glucanase, α-amylase, cellulase, protease, and lipase	*L. acidophilus, L. casei, B. bifidum* and *E. faecium*	6	Seifi (2013)
19	1-42	NM	NM	Xylanase, cellulase, phytase, α-amylase, pectinase and β-glucanase	*L. acidophilus, Bifedobacteruim longhum, Bifedobacterium thermophilum, E. faecium* and *Lactobacillus planetarium*	4	Sherif (2009)

N^1: Number of comparisons; NM: not mentioned

Data Selection and Extraction

A dataset was constructed from articles published between 2009 and 2021 using several keywords such as enzyme, growth performance, and probiotic. The final dataset was developed from 19 *in vivo* studies comprising 136 comparisons. Some variables were used for the study's coding, including the period of rearing, strains of broilers, and sexes of chickens. The mean value and standard deviation from selected parameters, such as body weight gain, feed intake, FCR and dry matter digestibility were recorded and tabulated, and the units of measurement were homogenized for further data analysis. Dry matter digestibility was excluded from the parameters as there was a lack of available information (only four studies presented a direct comparison of dry matter digestibility between enzyme- and probiotic-treated broiler chickens). After this process, 19 eligible studies were included for meta-analysis as follows: Abdel Moati *et al*. (2022); Agboola *et al*. (2021); El-Kelawy *et al*. (2017); Kaushal *et al*. (2019); Kamel and Mohamed (2016); Kırkpınar *et al*. (2018); Machado *et al*. (2020); Momtazan *et al*. (2011); Nusairat and Wang (2020), Rahman *et al*. (2013); Seifi (2013); Siddiqui *et al*. (2017); Swiatkiewicz *et al*. (2014); Razuki *et al*. (2017); Thorat *et al*. (2015); Udeh *et al*. (2019).

Statistical Analysis

Coding and data analysis were carried out using OpenMEE 2.0. The probiotics group was pooled to form an experimental group (E), while the enzymes group was pooled as a control group (C). The effect size (d) was computed using the formula below:

$$d = \frac{(\dot{x}^E - \dot{x}^C)}{S} J$$

where the mean value from the experimental group is represented by X^E and the mean value from the control group by X^C. Consequently, when the effect size was positive, the measured parameter was higher in the enzyme group, and vice versa. *J* stands for the correction factor for small sample sizes, and its value is as follows:

$$J = 1 - \frac{3}{(4(N^C + N^E - 2) - 1)}$$

The pooled standard deviation, or *S*, may be written as follows:

$$S = \sqrt{\frac{(N^E - 1)(S^E)^2 + (N^C - 1)((S^C)^2)}{(N^E + N^C - 2)}}$$

where N^E is the sample size for the experimental group and N^C is the sample size for the control group, and S^E and S^C are the standard deviations of the experimental and control groups, respectively. Hedges' d (V_d) variance is described as follows:

$$V_d = \frac{(N^C + N^E)}{(N^C N^E)} + \frac{d^2}{(2(N^C + N^E))}$$

The computation of the cumulative effect size (d_{++}):

$$d_{++} = \frac{(\sum_{i=1}^{n} W_i d_1)}{(\sum_{i=1}^{n} W_i)}$$

where W_i is the inverse of the sampling variance: $W_i = {}^1/_{V_d}$. A 95% confidence interval (CI), represented by the phrase d ± (1.96 × sd) was used to describe the precision of the effect magnitude. The equations that were previously presented were obtained from the studies of Sanchez-Meca and Marin-Martinez (2010). If the estimated effect size differed from a null effect size, the result was statistically significant. In order to detect publication bias resulting from non-significant publications excluded from the analysis, a fail-safe number (Nfs) was computed. It was expected that a strong meta-analysis model would be represented by Nfs, which is greater than five times the study effect size that was used to compute the starting effect size (N) plus ten. The method of Rosenthal *et al*. (1979) was applied to determine Nfs. The smallest sample size accessible from the pertinent studies was utilized, along with N. Cohen's standards, as the reference points for impact size estimate. For small, medium, and high effect sizes, they were 0.2, 0.5, and 0.8, respectively.

RESULTS AND DISCUSSION

Enzymes as an alternative to AGP have been widely used by poultry nutritionists after AGP was banned in broiler chicken production (Rahman *et al*., 2014; Polovinski-Horvatović, 2021). The use of enzymes is reported to improve nutrient utilization, improve immune defense, and physiological conditions so that it has a positive impact on the growth and feed efficiency of broilers (Sugiharto, 2016). In addition, enzymes can also aid in the formulation of more balanced diets for broiler chickens by expanding the selection of ingredients, such as cereals and other agro-industrial byproducts, and by improving the digestibility of fiber materials. There are various kinds of enzymes available on the market that can be applied to broiler chickens. However, currently, there is a tendency to combine several types of enzymes to further increase the effectiveness of enzymes as feed additives for broiler chickens (Sugiharto, 2016). Although the role of enzymes in increasing feed utilization, growth, and as an alternative to AGP has been well reported, their quality, consistency and reproducibility are still questioned by many researchers (Anjum and Chaudhry, 2010).

Probiotics are live microorganism-based feed additives that, in certain levels, may improve the host's well-being and production. Like enzymes, probiotics are widely applied to broiler chickens as an alternative to AGP after the AGP prohibition (Sapsuha *et al*., 2021). The use of probiotics is basically aimed at improving the balance of the microbial population in the digestive tract, especially the small intestine. Improving the ecological conditions of the intestine then has a positive impact on the morphology and integrity of the intestine as well as its function in absorbing nutrients and immune function (Sugiharto, 2016). Hence, enhancing the ecological conditions of the intestine benefits the morphology, integrity, and immune system function of the intestine as well as its ability to absorb nutrients. There are many different types of probiotics for broiler chickens on the market right now. In practical application, probiotics can be administered singly or in combination (as a probiotic cocktail). The efficacy of probiotic cocktails and individual probiotics in broiler chickens has not yet been compared in any research. Apart from this latter point, many poultry nutritionists continue to question whether probiotics are an effective substitute for AGP (Sugiharto, 2016; Sapsuha *et al*., 2021).

Enzymes and probiotics are two types of feed additives that are widely used by broiler producers to replace AGP as growth-promoting agents. Both types of feed additives have their respective working mechanisms to improve broiler production performance. However, questions still often arise from poultry nutritionists regarding which additive is more effective in replacing the role of AGP for broiler chickens after the ban on AGP in various countries in the world. To deal with the question of which is more effective in replacing AGP as a feed additive, a meta-analysis has been carried out by directly comparing the use of enzymes and probiotics in several published articles. Various types of enzymes have been applied by poultry nutritionists, including xylenes, β-glucanase, protease, α-amylase, phytase, cellulose, pectinase, and multienzyme, which is a mixture of several types of enzymes that are applied together (Swiatkiewicz *et al*., 2014; Thorat *et al*., 2015; Kamel and Mohamed, 2016; Razuki *et al*., 2017; Kırkpınar *et al*., 2018). In this current study, we did not distinguish the types of enzymes used by each study as a large variation existed from one study to another. The same criteria also applied to probiotics, since the kinds of microorganisms utilized as probiotics varied widely among the studies.

During the meta-analysis, the effect size (d++) is represented in the forest plot (Figures 1, 2, and 3). When the horizontal interval line crosses the zero vertical line, then there is no significant difference ($P>0.05$) between enzymes and probiotic treatments. It was apparent from the meta-analysis that there is no significant difference between enzyme and probiotic treatments in terms of body weight gain, accumulative feed intake, and FCR of broiler chickens (Table 2).

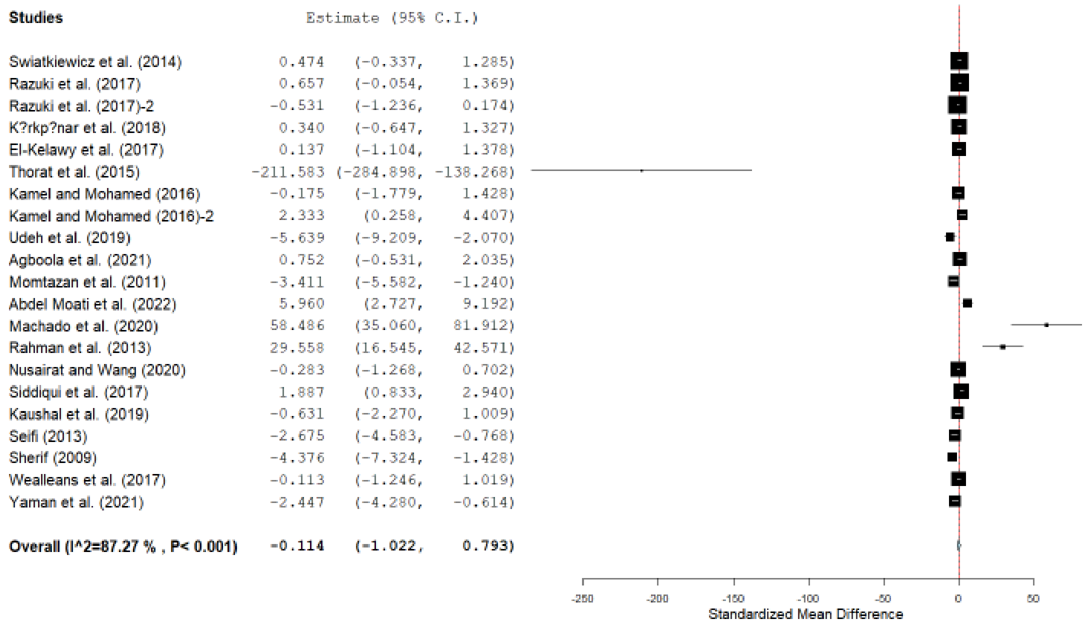

Figure 1. Forest plot of cumulative effect size (d_{++}) and 95% confidence interval (CI) of body weight gain of broilers comparing enzyme and probiotic treatments

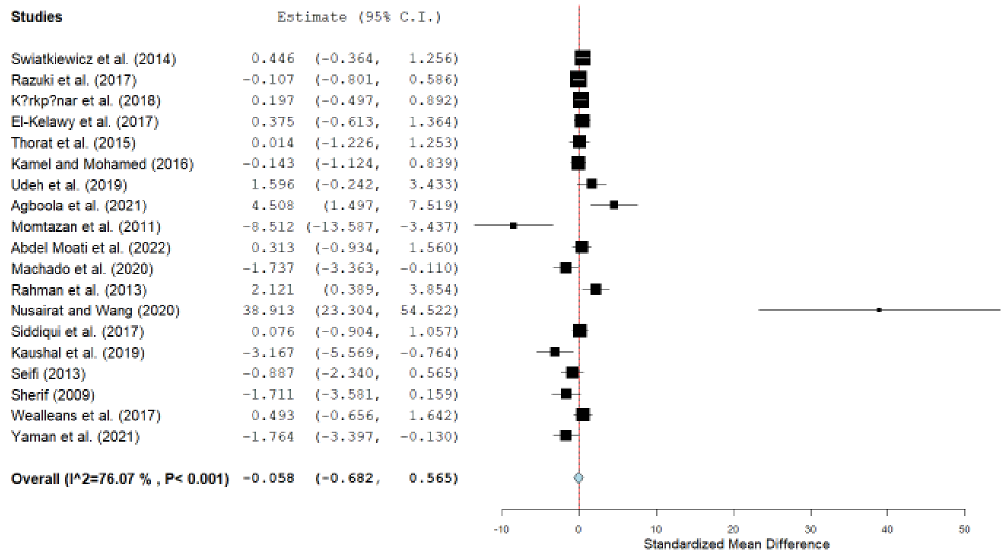

Figure 2. Forest plot of cumulative effect size (d_{++}) and 95% confidence interval (CI) of feed intake of broilers comparing enzyme and probiotic treatments

Table 2. Effect size, weighted mean value, and weighted *p*-value from all parameters measured

Parameters	Unit	N[1]	Cumulative effect size			SE	*p*-value	τ^2	Q	Het. *p*-value	I^2
			Estimate	Lower bound	Upper bound						
BWG	g	136	-0.114	-1.022	0.793	0.463	0.805	3.139	157.132	<0.001	87.272
FI	g	121	-0.058	-0.682	0.565	0.318	0.855	1.235	75.233	<0.001	76.074
FCR		121	0.309	-0.196	0.814	0.258	0.230	0.754	54.719	<0.001	67.105

N[1]= number of data, SE= standard error, τ^2=-variance of the effect size parameters across the study populations, Q= weighted sum of squared deviations, Het *p*-value= *p*-value for heterogeneity, I^2= heterogeneity level between studies, BWG= body weight gain, FI= feed intake, FCR= feed conversion ratio

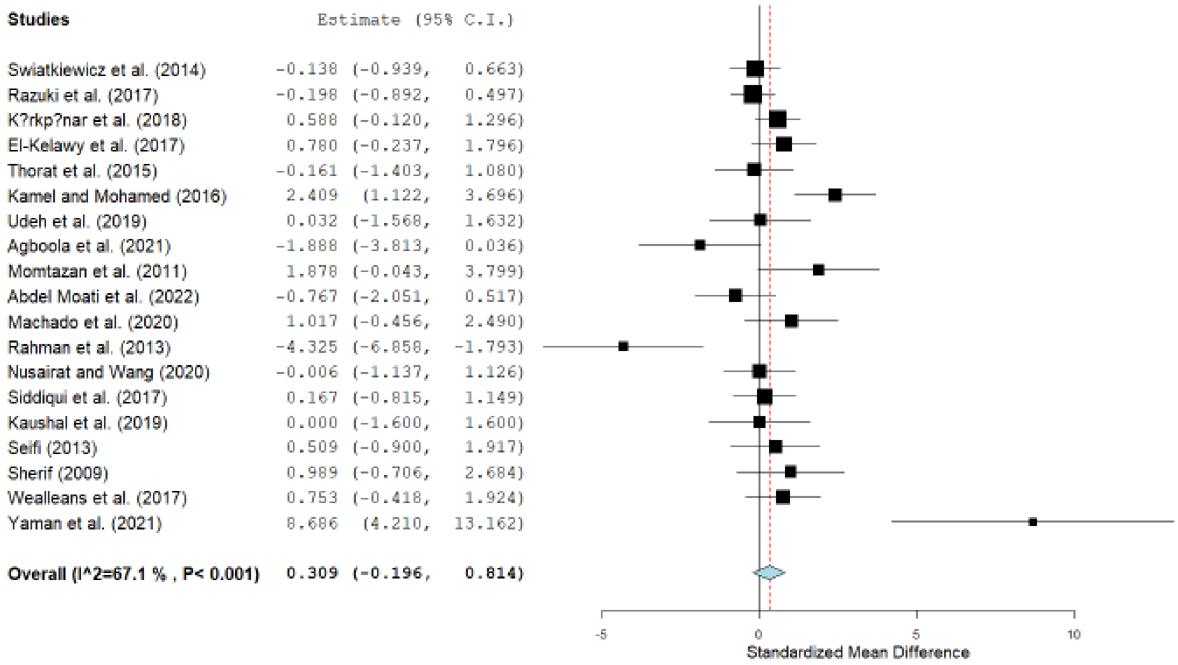

Figure 3. Forest plot of cumulative effect size (d_{++}) and 95% confidence interval (CI) of FCR of broilers comparing enzyme and probiotic treatments

This study compared the impact of using enzymes and probiotics on body weight gain, feed intake, and FCR in broiler chickens. The finding is crucial for poultry nutritionists seeking alternatives to AGP for broiler chickens. Previous studies have shown that both enzymes and probiotics had positive effects on broiler production parameters compared to control group) (chickens receiving no feed additive) (references in Table 1). Indeed, differences in the effects of enzymes and probiotics on body weight gain, feed intake, and FCR were not seen in this meta-analysis study. Thus, poultry nutritionists can choose either enzymes or probiotics as an alternative to AGP for broiler chickens. However, due to the insufficient data, this study did not investigate the differences in costs arising from the use of enzymes or probiotics as an alternative to AGP and the optimal level of use of enzymes or probiotics in broiler feed. To determine the most economical and effective levels of the enzymes and probiotics, further studies may therefore be necessary. Additionally, there is a scarcity of studies directly comparing single enzymes with single probiotic microorganisms as feed additives to replace AGP in broiler chickens. This gap in research makes it challenging to determine the most suitable enzyme or probiotic as an alternative to AGP.

CONCLUSIONS

Both enzymes and probiotics significantly improved broiler performances metrics, including body weight gain, feed intake and FCR. The meta-analysis revealed that probiotics and enzymes were equally effective in enhancing these performance parameters.

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