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Original Article

Does the Use of Emulsifier or Lipase Improve Weight Gain and Feed Conversion in Broilers? A Systematic Literature Review and Meta-Analysis

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ABSTRACT

Emulsifiers and exogenous lipases are feed additives used to increase lipid utilization in broiler diets. A meta-analysis was carried out to quantify the effects of the supplementation of emulsifiers and exogenous lipases on broiler weight gain (BWG, g/broiler/d) and feed conversion ratio (FCR, g/g) during the whole production cycle. Studies were obtained from the PubMed, Scielo, Science Direct, Scopus, and Web of Science databases. A total of 2669 studies were identified, 25 of which composed the database for the meta-analysis, representing data from a total of 14,643 broilers. These results demonstrate that there is evidence in the literature supporting that supplementation with emulsifiers or lipases improves broiler weight gain and feed conversion during the whole production cycle. However, the effect of the additives is influenced by bird sex, lipid source and concentration, type of additive and concentration, energy level of the feed, and bird strains. The isolated use of emulsifiers increases weight gain by 1.62g/day and reduces feed conversion by 0.04. However, there is no evidence that exogenous lipase use alone improves weight gain or feed conversion in broilers during the whole production cycle. The latter result may be due to the small number of studies with the additive in question, rather than its possible effects on weight gain and feed conversion. Therefore, further investigation should be conducted on this topic, especially studies on females fed diets supplemented with lipases.

INTRODUCTION

Dietary energy is essential for poultry nutrition, as it is an important component in diet costs and has a significant impact on animal performance (Wickramasuriya *et al.*, 2020a). Fats and oils are among the main sources of energy used in feed formulations; and with the increase in poultry production, controlling feed costs has become a difficult task, especially due to the volatility in prices for feed energy components (Ghazalah *et al.*, 2021a).

Given the importance of the complementation of energy sources in feed composition, the poultry industry has sought to optimize lipid digestion with the use of additives such as emulsifiers and exogenous lipases, so as to to reduce production costs and improve bird performance (Oliveira *et al.*, 2019).

Exogenous emulsifiers are additives used poultry nutrition with the aim of improving lipid absorption. They act by increasing lipib solubility through the creation of favorable conditions for the formation of micelles in the small intestine (Zhao & Kim, 2017). Exogenous lipases, on the other hand, have been used as a dietary tool to improve the utilization of fats that birds cannot digest, complementing endogenous enzyme activity. Given the immaturity of the digestive system and its lower bile and pancreatic lipase production capacity, Oliveira *et al.* (2019) suggested the use of exogenous lipases as a strategy to improve the efficiency of dietary energy use, mainly in the early stages of birds' life. However, at least in theory, we can see positive effects of the supplementation with exogenous emulsifiers and lipases throughout the entire broiler production cycle.

Despite their specific targets and modes of action, emulsifiers and exogenous lipases are used with the same objective in broiler diets, which is to maximize the use of lipids present in the feed. Even so, there are important gaps to fill regarding their use protocols, such as the incremental gains provided by each additive, and their interrelationships with the energy level and source of fat in the diet. In this context, the objective of this study was to quantify, through a systematic literature review and meta-analysis, the effect of emulsifiers and exogenous lipases on broiler weight gain and feed conversion throughout the complete cycle.

MATERIAL AND METHODS

Literature search and selection of studies

A systematic review of the literature was carried out through the following steps: formulation of the question to be answered, construction of the search strategy and definition of the bases to be consulted, investigation of relevant studies, critical evaluation of the studies, data collection and analysis, and interpretation of the results.

The search algorithm was built using formal descriptors from the DeCS and MeSH databases, alongside keywords clustered into concept blocks according to the PICO acronym (participants, intervention, comparison, and results) (Moher *et al.*, 2015). Several search strategies were designed and tested, with the search strategy that best suited the research question being: (chickens OR broiler OR "gallus gallus") AND (lipase OR "emulsifying agents" OR emulsifier). The search strategy was adjusted according to the premises of the scientific database used, always maintaining the defined descriptors. No automatic filter was applied, so as to access all published studies. When the database allowed, the search was limited to the title, abstract, and keywords. Searches were performed in the PubMed, Scielo, Science Direct, Scopus, and Web of Science databases, on the reference date January 26, 2022. An individual search for studies was also carried out directly in the archives of some local journals that are not indexed to the databases used.

The lists of primary studies obtained from the different databases were exported and compiled in a reference management software (Zotero). Duplicates (studies included in more than one database) were automatically eliminated, and the remaining studies were submitted to analysis by reading the title and abstract. In this stage, all those that clearly did not relate to the objectives of this research were eliminated.

After the previous step, a detailed analysis of the full text was carried out considering the following criteria: 1) studies with broilers, which presented lineage and sex, and which evaluated weight gain and feed conversion; 2) studies that evaluated emulsifier or lipase in isolation, not in combination; 3) studies that presented at least two different treatments (a control group without lipase or emulsifier and a group that included lipase or emulsifier); 4) studies that presented the composition of experimental diets and that reported the average results of the studied variables, measures of dispersion, and the number of repetitions of each treatment; 5) studies that compared treatments with the same energy content and the same type and concentration of lipid source; 6) studies that evaluated chickens in their complete cycle (i.e. those that evaluated birds for a minimum total period of 35 days and a maximum total period of 44 days). In this review, only studies that simultaneously m*et al*l these criteria were included.

Data extraction and management

All the included studies were analyzed by two independent researchers to identify possible data collection errors. Weight gain (BWG, g/broiler/d) and feed conversion (FCR, g/g) data were manually extracted, entered, and organized in electronic spreadsheets, along with their dispersion measurements, numbers of participants (repetitions per treatment), and number of birds per repetition. From each study, quantitative data were extracted from one or more control groups (without emulsifier or lipase) and compared with one or more intervention groups (with emulsifier or lipase). Along with these quantitative data, qualitative information was also extracted from the studies, such as year of publication, place of the experiment (country), strain of the birds, sex and age in days, lipid source and concentration in the diet, type of additive and concentration in the diet, and levels of energy (kcal/kg).

Transformations were performed on the data for statistical comparisons. Total weight gain was adjusted for daily weight gain; therefore, BWG is given in g/broiler/d. The measure of dispersion used for the comparisons was the standard deviation, transforming those that were not in this format. Levels of lipid sources and levels of additive use were transformed into percentages to equalize the comparisons. When the study presented more than one comparison for the control and intervention groups, each comparison was considered an observation within the meta-analysis.

Data analysis

The measure of the effect size of each variable was the mean difference (MD) between the comparison, control (non-supplemented group) and intervention (supplemented group) groups, as follows:

$$DM = \{(mean_{supplemented\ group}) - (mean_{non\text{–}meansupplemented\ group})\}$$

Different weights were assigned to the studies by the inverse of variance method, allowing for a balance between the individual contributions of the studies to the meta-analysis, based on their level of precision in the estimates (means) of the treatments. The significance of the overall mean difference (overall effect) was obtained by the Z test ($p<0.05$). Moreover, 95% confidence intervals were calculated for each observation.

The heterogeneity between studies was verified by the chi-square test (X^2) ($p<0.10$) and its magnitude was estimated by the inconsistency index ($I^2 = Ch^2 - DF/ Ch^2 \times 100$), where *DF* are the degrees of freedom of the Ch^2 test (Higgins *et al.*, 2003; Higgins & Thompson, 2002). Regardless of its significance, the heterogeneity between studies was incorporated into the meta-analysis, adopting a random effects model to assess overall effects and their statistical significance.

In the case of significant heterogeneity between studies, subgroup analyses were performed to explain at least part of its origin, considering the variations between studies in terms of sex of the animals, strain, lipid source and concentration in the diet (%), type of additive and concentration in the diet (%), and metabolizable energy in the feed (kcal/kg). In these cases, the quantitative data were grouped into ranges of values, allowing for better incorporation into the analysis. Thus, the concentration of the lipid source in the diet was organized into three distinct groups (‘up to 2%’, ‘2 to 3.5%’, and ‘above 3.5%’); the concentration of the additive in the diet was organized into four groups (‘up to 0.05%’, ‘0.051% to 0.1%’, ‘above 0.1%’ and ‘not informed’); and the metabolizable energy of the diet, into three groups (‘up to 3000kcal/kg’, ‘3001 to 3200kcal/kg’ and ‘above 3200kcal/kg’).

The robustness of the meta-analysis results was determined by a sensitivity analysis that consisted in detecting discrepant data and publication bias using a funnel plot. Studies with data outside the normality area of the funnel plot were temporarily excluded from the meta-analysis and were only reintroduced into the database if their exclusion had not significantly interfered with the estimation of the effect size and the value of the general test, as per the ‘fill and trim’ method. All the statistical procedures were performed in the software RevMan5 (RevMan, 2014).

RESULTS

Studies and participants

The application of the search algorithm in the different databases returned a total of 2629 studies. After the screening and selection process, a total of 25 studies met the inclusion criteria and formed the database for the meta-analysis. Figure 1 shows an illustration of the systematic review procedure and the reasons for rejection of studies that did not meet at least one of the criteria established in the eligibility phase.

Table 1 presents a summary of the main characteristics of the studies selected in the systematic literature review.

After filtering the variables and groups/treatments of interest, a total of 51 comparisons for weight gain and 51 for feed conversion remained. Of the 25 studies included, only one presented data from two separate experiments (Allahyari-Bake & Jahanian, 2017), with all others bringing results from a single experiment. In total, the data collected from the included studies involved 13,875 chickens (11,547 males, 480 females, 1848 in mixed flocks), distributed among 26 experiments.

In all the studies, the chicks were fed experimental diets containing emulsifier or lipase from day one of life, except for the studies of Wang *et al.* (2016) and Cho *et al.* (2012), who supplemented the birds’ diets from the second day of age.

Altogether, 80% of the studies included in the meta-analysis were carried out with male broilers, 4% with females, and 16% with non-sexed birds (mixed flocks). 64% of the studies retrieved from the databases were conducted with the Ross strain, 28% with Cobb, and 8% with Arbor Acres. The lipid source used was of vegetable origin in 52% of the studies (of these, 82% used soy oil, 6% used palm oil, 6% used palm fat, and 6% used soy free fatty acids), while in 36% of the studies, animal sources were used (of these, 64% used tallow, 27% chicken fat, and 9% yellow grease). In

Table 1 – Summary of the information from studies included in the meta-analysis database.

Study	Participants	Group control	Intervention group
Aguilar *et al.* (2013)	Ross 308 broilers, male, (n=320), evaluated from 1 to 42 days.	Diet without emulsifier; Lipid source of plant origin; EM level 2977kcal/kg	Control diet + emulsifier (0.05%)
Allahyari-Bake and Jahanian. (2017) (Experiments I and II)	Ross 308 broilers, mixed, (n=576 experiments I and II), evaluated from 1 to 42 days.	Diet without emulsifier; lipid source of plant origin; EM level 2957kcal/kg	Control diet + emulsifier (0.1%)
Arshad *et al.* (2020)	Cobb 500 broilers, mixed, (n=280), evaluated from 1 to 35 days.	Lipase-free diet; Lipid source of animal origin; EM level 2968kcal/kg	Control diet + Lipase (0.018%)
Bontempo *et al.* (2015)	Cobb 500 broilers, female, (n=480), evaluated from 1 to 34 days.	Diet without emulsifier; Lipid source of plant and animal origin; EM levels 3075kcal/kg	Control diet + emulsifier (0.07%)
Bontempo *et al.* (2018)	Ross 308 broilers, male, (n=600), evaluated from 1 to 44 days.	Diet without emulsifier; Lipid source of plant and animal origin; EM levels 3178 kcal/kg	Control diets + emulsifier (0.07%)
Castro and Kim. (2021)	Cobb 500 broilers, male, (n=480), evaluated from 1 to 42 days.	Lipase-free diet; Lipid source of plant origin; EM levels 3083 and 2983 kcal/kg	Control diet + Lipase (0.01%)
Cho *et al.* (2012)	Ross 308 broilers, male, (n=216), evaluated from 1 to 35 days.	Diet without emulsifier; Lipid source of animal origin; EM level 3070 kcal/kg	Control diet + emulsifier (0.05%)
Dabbou *et al.* (2019)	Ross 708 broilers, mixed, (n=224), evaluated from 1 to 35 days.	Diet without emulsifier; Lipid source of plant origin; EM level 3509 kcal/kg	Control diet + emulsifier (0.05%)
Haetinger *et al.* (2021)	Cobb 500 broilers, male, (n=1050), evaluated 1 to 42 days	Diet without emulsifier; Lipid source of plant origin; EM level 3142 kcal/kg	Control diet + emulsifier (0.05%)
Kaczmarek *et al.* (2015)	Ross 308 broilers, male, (n=384), evaluated from 1 to 35 days.	Diet without emulsifier; Lipid source of plant and animal origin; EM levels 3072 and 2972 kcal/kg	Control diet + emulsifier (0.04%)
Liu *et al.* (2020a)	Ross 308 broilers, male, (n= 480), evaluated from 1 to 35 days	Diet without emulsifier; Lipid source of animal origin; EM level 3179 kcal/kg	Control diet + emulsifier (0.1%)
Liu *et al.* (2020b)	Ross 308 broilers, male, (n=1024), evaluated from 1 to 35 days.	Diet without emulsifier; Lipid source of animal origin; EM levels 3169 and 3070 kcal/kg	Control diet + emulsifier (0.15%)
Majdolhosseini *et al.* (2019)	Ross 308 broilers, male, (n=800), evaluated from 1 to 42 days.	Diet without emulsifier; Lipid source of plant and animal origin; EM levels 3093 and 3017 kcal/kg	Control diet + emulsifier (0.1%)
Movagharnejad *et al.* (2020)	Ross 308 broilers, male, (n=120), evaluated from 1 to 38 days.	Diet without Lipase and/or Emulsifier; Lipid source of plant origin; EM level 2911 kcal/kg	Control diet + emulsifier (0.15%); lipase (ND)
Oliveira *et al.* 2019	Cobb 500 broilers, male, (n=840), evaluated from 1 to 37 days.	Diet without Lipase and/or Emulsifier; Lipid source of plant origin; EM level 3036 kcal/kg	Control diet + Lipase and/or emulsifier (0.1%)
Park *et al.* (2018)	Ross 308 broilers, male, (n=816), evaluated from 1 to 35 days.	Diet without emulsifier; Lipid source of animal origin; EM levels 3100 kcal/kg	Control diet + emulsifier (0,03; 0,06; 09%)
Saleh *et al.* (2020)	Ross 308 broilers, male, (n=200), evaluated from 1 to 35 days.	Diet without emulsifier; Lipid source of plant origin; EM level 2436 kcal/kg	Control diet + emulsifier (0.05%)
Shen *et al.* (2021)	Arbor Acres broilers, male, (n=192), evaluated from 1 to 42 days	Diet without emulsifier; Lipid source of plant origin; EM level 3000 kcal/kg	Control diet + emulsifier (0.01%)
Silva *et al.* (2018)	Cobb 500 broilers, mixed, (n=768) evaluated from 1 to 42 days.	Diet without emulsifier; Lipid source of plant origin; EM level 3067 kcal/kg	Control diet + emulsifier (0.025; 0.025; 0.035%)
Upadhaya *et al.* (2017a)	Ross 308 broilers, male, (n=384), evaluated from 1 to 35 days.	Diet without emulsifier; Lipid source of animal origin; EM levels 3120 kcal/kg	Control diet + emulsifier (0.075; 0.10; 0.15%)
Upadhaya *et al.* (2017b)	Ross 308 broilers, male, (n=768), evaluated from 1 to 35 days.	Diet without emulsifier; Lipid source of animal origin; EM levels 3010 kcal/kg	Control diet + emulsifier (0.05; 0.075; 0.010%)
Wang *et al.* (2016)	Ross broilers, male, (n=216), evaluated from 1 to 35 days.	Diet without emulsifier; Lipid source of animal origin; EM levels 3070 kcal/kg	Control diet + emulsifier (0.05%)
Wang *et al.* (2020)	Cobb 500 broilers, male, (n=640), evaluated from 1 to 42 days.	Diet without emulsifier; Lipid source of plant origin; EM level 3101 and 3026 kcal/kg	Diet with emulsifier (0.1%)
Zampiga *et al.* (2016)	Ross broilers, male, (n=1765), evaluated from 1 to 42 days.	Diet without emulsifier; Lipid source of plant origin; EM level 3131 kcal/kg	Control diet + emulsifier (0.15% and 0.1%)
Zhang *et al.* (2011)	Arbor Acres broilers, male, (n=252), evaluated from 1 to 42 days.	Diet without emulsifier; Lipid source of plant and animal origin; EM levels 4168 kcal/kg	Control diet + emulsifier (0.05%)

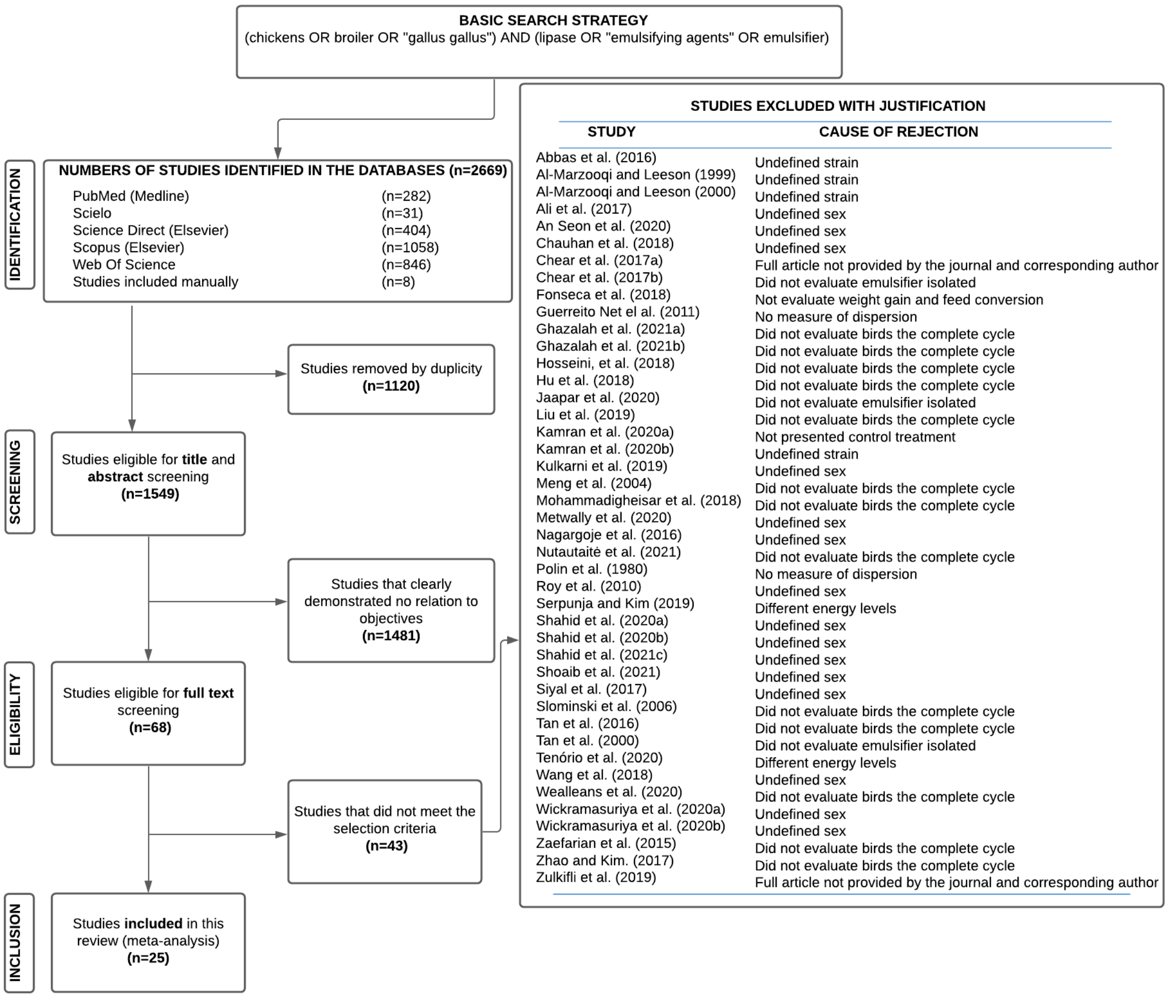

Figure 1 – Flowchart of the systematic review procedure.

the remaining 12% of the studies, mixed sources were used, including soybean oil, chicken fat, and lard.

The average inclusion of lipid sources in the diets was 3.10%, ranging between 0.88% (Saleh *et al.*, 2020) and 4.80% (Bontempo *et al.*, 2018). As for the type of additive used, 88% of the studies evaluated emulsifiers and 12%, exogenous lipases. The average concentration of additives (emulsifier or lipase) in the diets was 0.08%, with a variation of between 0.01% (Castro & Kim, 2021; Shen *et al.*, 2021) and 0.15% (Liu *et al.*, 2020 (b); Movagharnejad *et al.*, 2020; Upadhaya *et al.*, 2017 (a); Zampiga *et al.*, 2016). The average level of metabolizable energy of the diets was 3113 (kcal/kg), ranging from 2436kcal/kg (Saleh *et al.*, 2020) to 4168 kcal/kg (Zhang *et al.*, 2011).

Meta-analysis

Weight gain

The result for the general effect of the additives shows that there is evidence in the literature supporting that when animals' diets were supplemented with emulsifier or lipase, their daily weight gain increased by 1.59g/day, as compared to those in the non-supplemented group ($p<0.00001$). This data can be better observed in the forest plot in Figure 2.

Significant heterogeneity ($p<0.00001$; I2 =85%) was detected between the studies, and was explored through a subgroup analysis considering sex, broiler lineage, lipid source and its concentration in the diet, metabolizable energy, and type of additive and its concentration in the diet (Table 2).

Subgroup analysis showed that male broilers increased their weight gain by 1.46 g/day; ($p<0.00001$) when fed diets containing emulsifier or lipase. In the case of females, the increment was 9.10 g/day ($p<0.00001$). Under these conditions, however, only one comparison (one study) was used. In mixed batches, it was not possible to verify a significant effect of the additives ($p=0.10$).

Considering the influence of broiler strain as a subgroup on the effect of additives on the daily weight gain of broilers, there was evidence that the Ross strain

	SUPPLEMENTED			NOT SUPPLEMENTED				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Aguilar et al. 2013 (01)	56.44	0.64	8	55.7	0.64	8	2.8%	0.74 [0.11, 1.37]
Allahyari Bake and Jahanian 2017 (01)	55.9	1.84	4	54.6	1.87	4	1.6%	1.30 [-1.27, 3.87]
Allahyari Bake and Jahanian 2017 (02)	59.5	1.84	4	53.7	1.84	4	1.6%	5.80 [3.25, 8.35]
Allahyari Bake and Jahanian 2017 (03)	55.7	1.84	4	54.7	1.84	4	1.6%	1.00 [-1.55, 3.55]
Allahyari Bake and Jahanian 2017 (04)	56.9	3.68	4	56.5	3.68	4	0.6%	0.40 [-4.70, 5.50]
Allahyari Bake and Jahanian 2017 (05)	55.8	3.68	4	55.6	3.68	4	0.6%	0.20 [-4.90, 5.30]
Allahyari Bake and Jahanian 2017 (06)	54.5	3.68	4	53.5	3.68	4	0.6%	1.00 [-4.10, 6.10]
Arshad et al. 2020 (01)	65.2	1.44	5	68.18	1.44	5	2.1%	-2.98 [-4.77, -1.19]
Bontempo et al. 2015 (01)	60.5	2.45	24	51.4	2.45	24	2.4%	9.10 [7.71, 10.49]
Bontempo et al. 2018 (01)	78.06	2.44	15	76.6	2.44	15	2.1%	1.46 [-0.29, 3.21]
Castro and Kim 2021 (01)	79.55	1.52	6	76.21	1.52	6	2.1%	3.34 [1.62, 5.06]
Castro and Kim 2021 (02)	76.71	1.52	6	74.26	1.52	6	2.1%	2.45 [0.73, 4.17]
Cho et al. 2012 (01)	51.74	4.29	9	52.49	4.29	9	0.9%	-0.75 [-4.71, 3.21]
Dabbou et al. 2019 (01)	46.4	1.53	8	46.2	1.53	8	2.3%	0.20 [-1.30, 1.70]
De Oliveira et al. 2019 (01)	67.14	0.84	7	65.16	0.81	7	2.7%	1.98 [1.12, 2.84]
De Oliveira et al. 2019 (02)	67.11	0.84	7	65.16	0.81	7	2.7%	1.95 [1.09, 2.81]
Haetinger et al. 2021 (02)	84.81	0.64	7	82.83	0.64	7	2.8%	1.98 [1.31, 2.65]
Kaczmarek et al. 2015 (01)	58.29	0.89	8	58.29	0.89	8	2.7%	0.00 [-0.87, 0.87]
Kaczmarek et al. 2015 (02)	58.86	0.89	8	55.71	0.89	8	2.7%	3.15 [2.28, 4.02]
Liu et al. 2020 -A (01)	50.69	0.75	10	48.34	0.75	10	2.8%	2.35 [1.69, 3.01]
Liu et al. 2020 -A (02)	50.31	0.75	10	48.34	0.75	10	2.8%	1.97 [1.31, 2.63]
Liu et al. 2020 -B (01)	49.29	1.31	16	47.43	1.31	16	2.7%	1.86 [0.95, 2.77]
Liu et al. 2020 -B (02)	48.14	1.31	16	45.8	1.31	16	2.7%	2.34 [1.43, 3.25]
Majdolhosseini et al. 2019 (01)	60.79	2.1	5	59.71	2.1	5	1.5%	1.08 [-1.52, 3.68]
Majdolhosseini et al. 2019 (02)	61.35	2.1	5	59.19	2.1	5	1.5%	2.16 [-0.44, 4.76]
Majdolhosseini et al. 2019 (03)	57.49	2.1	5	57.62	2.1	5	1.5%	-0.13 [-2.73, 2.47]
Majdolhosseini et al. 2019 (04)	58.8	2.1	5	56.98	2.1	5	1.5%	1.82 [-0.78, 4.42]
Movagharnejad et al. 2020 (01)	65.66	0.88	4	82.31	1.2	4		Not estimable
Movagharnejad et al. 2020 (02)	61.79	0.88	4	82.31	1.2	4		Not estimable
Park et al. 2018 (01)	49.63	1.03	12	48.51	1.03	12	2.7%	1.12 [0.30, 1.94]
Park et al. 2018 (02)	50.06	1.03	12	48.51	1.03	12	2.7%	1.55 [0.73, 2.37]
Park et al. 2018 (03)	50.34	1.03	12	48.51	1.03	12	2.7%	1.83 [1.01, 2.65]
Saleh et al. 2020	50.31	0.34	4	34.97	1.17	4		Not estimable
Shen et al. 2021 (03)	47.18	1.42	6	47.45	1.42	6	2.2%	-0.27 [-1.88, 1.34]
Silva et al. 2018 (01)	65.19	2.05	8	64.24	2.02	8	1.9%	0.95 [-1.04, 2.94]
Silva et al. 2018 (02)	66.64	2.09	8	64.24	2.02	8	1.9%	2.40 [0.39, 4.41]
Silva et al. 2018 (03)	67.31	2.11	8	64.24	2.02	8	1.9%	3.07 [1.05, 5.09]
Upadhaya et al. 2017-A (01)	48.54	2.07	6	46.8	2.07	6	1.7%	1.74 [-0.60, 4.08]
Upadhaya et al. 2017-A (02)	48.86	2.07	6	46.8	2.07	6	1.7%	2.06 [-0.28, 4.40]
Upadhaya et al. 2017-A (03)	50.17	2.08	6	46.8	2.07	6	1.7%	3.37 [1.02, 5.72]
Upadhaya et al. 2017-B (01)	48.62	0.37	12	47.43	1.29	12	2.8%	1.19 [0.43, 1.95]
Upadhaya et al. 2017-B (02)	50	0.37	12	47.43	1.29	12	2.8%	2.57 [1.81, 3.33]
Upadhaya et al. 2017-B (03)	50.49	0.37	12	47.43	1.29	12	2.8%	3.06 [2.30, 3.82]
Wang et al. 2016 (01)	51.8	4.37	9	49.37	4.37	9	0.9%	2.43 [-1.61, 6.47]
Wang et al. 2020 (01)	59.86	1.49	8	62.05	1.49	8	2.3%	-2.19 [-3.65, -0.73]
Wang et al. 2020 (02)	71.43	1.58	8	60.02	1.58	8		Not estimable
Zampiga et al. 2016 (01)	61.9	1.08	9	61.3	1.08	9	2.6%	0.60 [-0.40, 1.60]
Zampiga et al. 2016 (02)	61.9	1.08	9	61.3	1.08	9	2.6%	0.60 [-0.40, 1.60]
Zhang et al. 2011 (01)	41.95	0.91	7	41.19	0.91	7	2.6%	0.76 [-0.19, 1.71]
Zhang et al. 2011 (02)	42.17	0.91	7	40.95	0.91	7	2.6%	1.22 [0.27, 2.17]
Zhang et al. 2011 (03)	41.5	0.91	7	42.64	0.91	7	2.6%	-1.14 [-2.09, -0.19]
Total (95% CI)			**390**			**390**	**100.0%**	**1.59 [1.13, 2.06]**

Heterogeneity: $Tau^2 = 1.87$; $Chi^2 = 307.35$, df = 46 ($P < 0.00001$); $I^2 = 85\%$
Test for overall effect: $Z = 6.73$ ($P < 0.00001$)

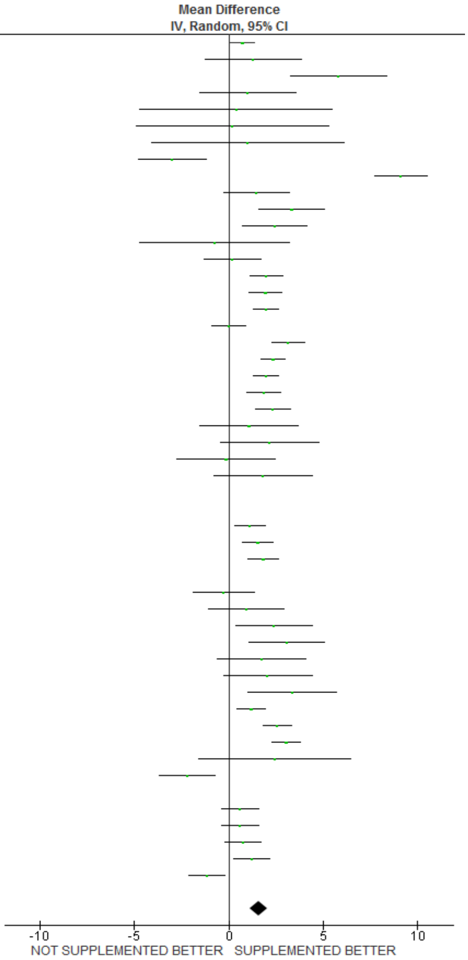

Figure 2 – *Forest plot* meta-analysis on the overall effect on weight gain, comparing supplemented versus non-supplemented groups (g/bird/day), for broilers supplemented with diets containing emulsifier or lipase.

has an increase of 1.65 g/bird/day (p<0.00001) when fed on diets with emulsifier or lipase. Likewise, Cobb broilers had an increase in daily weight gain of 2.02 g/bird/day (p<0.01). There was no significant evidence of improvement in the Arbor Acres strain (p=0.41). It should be noted that the number of comparisons in this group was more representative of the Ross (k=35) and Cobb (k=12) strains, while the number of comparisons with the Arbor Acres strain was relatively smaller (k=4).

When we evaluated the effect of the additives on diets with lipid sources of animal origin, we observed that emulsifiers or lipases promoted an increase of 1.52 g/bird/day in weight gain (p<0.00001), while in diets with plant sources, the positive effect of additives on weight gain was 1.25 g/bird/day (p<0.00001). In diets with mixed lipid sources, no significant effect of additives on bird weight gain was detected (p=0.06). The evidence observed in the literature also shows that, when lipid sources constituted up to 2% of the diet, birds fed on diets supplemented with emulsifier or lipase had an increase in weight gain of 2.36 g/bird/day (p<0.00001). When the lipid source constituted between 2% and 3.5% of the diet, there was a weight gain of 1.00 g/bird/day (p<0.0002); whereas when the inclusion of the lipid source in the diet was over 3.5%, supplementing the diet with emulsifiers or lipases led to an increase in weight gain of 2.52 g/bird/day (p<0.00001).

Supplementation of emulsifiers or lipases in diets with metabolizable energy levels between 3000 and 3200 kcal/kg promoted an increase of 1.88 g/bird/day in the daily weight gain of the birds (p<0.00001). However, in diets with levels lower than 3000 kcal/kg or higher than 3200 kcal/kg, supplementation with emulsifiers or lipases did not result in a significant change in animals' weight gain.

Regarding the inclusion of additives in the diet, emulsifiers or lipases promoted a significant increase in the daily weight gain of the birds, regardless of the supplemented concentration (*p<0.001).* However, when we analyzed the individual effect of each additive, we only found evidence of a significant benefit on the weight gain of birds for the emulsifiers. This increase in daily weight gain was estimated at 1.62 g/bird/day (p<0.00001).

Table 2 – Subgroup analysis to explore study heterogeneity within the meta-analysis for the effects of emulsifiers or lipases on the daily weight gain of broilers.

Subgroup		Mean Difference (MD) (g/broiler/day)		Heterogeneity		
Sex	k	IV, Random, 95% CI	*p*-value	Tau^2	*p*-value	I^2
Male	39	1.46 [1.07, 1.85]	<0.00001	0.91	<0.00001	78%
Female	1	9.10 [7.71, 10.49]	<0.00001	NE	NE	NE
Mixed	11	1.24 [-0.24, 2.73]	0.10	4.30	<0.0001	75%
Strain						
Arbor Acres	4	1.16 [-1.57, 3.90]	0.41	7.46	<0.00001	96%
Cobb	12	2.02 [0.45, 3.59]	0.01	6.41	<0.00001	94%
Ross	35	1.65 [1.26, 2.04]	<0.00001	0.64	<0.00001	66%
Lipid source						
Animal origin	20	1.52 [0.97, 2.07]	<0.00001	1.04	<0.00001	80%
vegetable origin	27	1.25 [0.70, 1.80]	<0.00001	0.97	<0.00001	68%
mixed origin	4	3.42 [-0.17, 7.01]	0.06	13.01	<0.00001	98%
Concentration of the lipid source in the diet (%)						
up to 2%	20	2.36 [1.56, 3.17]	<0.00001	0.00	0.91	0%
of 2 at 3.5%	27	1.00 [0.48, 1.52]	0.0002	1.31	<0.00001	76%
Above 3.5%	4	2.52 [1.71, 3.34]	<0.00001	2.01	<0.00001	89%
metabolizable energy of the feed (kcal/kg)						
Up to 3000 kcal/kg	14	1.22 [-0.10, 2.55]	0.07	3.35	<0.00001	83%
From 3001 to 3200 kcal/kg	33	1.88 [1.36, 2.40]	<0.00001	1.61	<0.00001	84%
Above 3200 kcal/kg	4	0.26 [-0.85, 1.38]	0.64	0.98	0.004	77%
Additive type						
emulsifier	46	1.62 [1.15, 2.10]	<0.00001	1.80	<0.00001	85%
lipase	5	1.23 [-1.12, 3.58]	0.31	5.12	<0.00001	90%
concentration of the additive in the diet (%)						
Up to 0.05%	20	1.04 [0.41, 1.66]	0.001	1.35	<0.00001	81%
From 0.051 to 0.1%	25	2.01 [1.29, 2.74]	<0.00001	2.30	<0.00001	86%
Above 0.1%	5	1.83 [0.87, 2.78]	0.0002	0.58	0.03	65%
Uninformed	1	NE	NE	NE	NE	NE

k= number of comparisons at each level of subgroups, IV= inverse variance, Random=random model, 95% CI= confidence interval for each MD, Tau^2 = between-study total variance (heterogeneity + sampling error), I^2 = proportion of variance due to heterogeneity; NE=not estimable.

Feed conversion

The general effect presented in the forest plot (Figure 3) shows that there is evidence in the literature that animals fed diets with emulsifier or lipase show a 4-point reduction in feed conversion (DM= -0.04; *p*<0.00001).

Differences between studies were identified based on the significance of heterogeneity (*p*<0.00001*; I*^{2} *=69%)*. Subgroups were used to improve observation of the heterogeneity in the analysis, as presented in the previous section. The data are presented in Table 3.

There is significant evidence of feed conversion reduction for male broilers (*p*<0.00001) and mixed flocks (*p*=0.02), with a 4-point reduction in feed conversion of both flocks when fed on diets with emulsifiers or lipase. No effect was observed for females showed no effect, but this data was especially impacted by the reduced number of comparisons. Ross broilers showed the greatest reduction in FCR (-0.05, *p*<0.00001), followed by Cobb broilers (-0.02, *p*=0.02) and Arbor Acres (-0.01), the latter showing no significant effect (*p*=0.41) for diets with emulsifier or lipase. The data for strain were potentially affected by the number of studies, and therefore comparisons.

The lipid source used in the diets influences the feed conversion of birds fed on diets with emulsifier or lipase. There is evidence that the use of lipids from animal sources reduces the feed conversion of broilers twice as much as lipids from plant sources (-0.06; *p*<0.00001 vs -0.03; *p*<0.00001). Diets with mixed sources, i.e. both animal and plant, do not show evidence of any changes in this variable. The best feed conversion reduction results were found with doses of up to 2% of lipid sources in the diet (-0.06; *p*=0.0005), but there are significant evidences at the 2% to 3.5% and above 3.5% levels, with a reduction in FCR by 4 points as compared to diets not supplemented with emulsifiers or lipase.

Feed conversion is influenced by the levels of metabolizable energy in broilers' diets that use emulsifier or lipase. There is evidence of a 4-point reduction in HRR in broilers fed on diets with levels

Study or Subgroup	SUPPLEMENTED Mean	SD	Total	NOT SUPPLEMENTED Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Aguilar et al. 2013 (01)	1.93	0.05	8	1.95	0.05	8	1.9%	-0.02 [-0.07, 0.03]
Allahyari Bake and Jahanian 2017 (01)	1.73	0.14	4	1.88	0.14	4	0.2%	-0.15 [-0.34, 0.04]
Allahyari Bake and Jahanian 2017 (02)	1.74	0.14	4	1.84	0.14	4	0.2%	-0.10 [-0.29, 0.09]
Allahyari Bake and Jahanian 2017 (03)	1.83	0.14	4	1.91	0.14	4	0.2%	-0.08 [-0.27, 0.11]
Allahyari Bake and Jahanian 2017 (04)	1.78	0.1	4	1.9	0.1	4	0.4%	-0.12 [-0.26, 0.02]
Allahyari Bake and Jahanian 2017 (05)	1.85	0.1	4	1.86	0.1	4	0.4%	-0.01 [-0.15, 0.13]
Allahyari Bake and Jahanian 2017 (06)	1.88	0.1	4	1.9	0.1	4	0.4%	-0.02 [-0.16, 0.12]
Arshad et al. 2020 (01)	1.5	0.02	5	1.48	0.02	5	2.9%	0.02 [-0.00, 0.04]
Bontempo et al. 2015 (01)	1.54	0.05	24	1.54	0.05	24	2.7%	0.00 [-0.03, 0.03]
Bontempo et al. 2018 (01)	1.55	0.03	15	1.57	0.03	15	3.0%	-0.02 [-0.04, 0.00]
Castro and Kim 2021 (01)	1.59	0.03	6	1.6	0.03	6	2.5%	-0.01 [-0.04, 0.02]
Castro and Kim 2021 (02)	1.65	0.03	6	1.7	0.03	6	2.5%	-0.05 [-0.08, -0.02]
Cho et al. 2012 (01)	1.71	0.12	9	1.72	0.12	9	0.6%	-0.01 [-0.12, 0.10]
Dabbou et al. 2019 (01)	1.55	0.06	8	1.59	0.06	8	1.5%	-0.04 [-0.10, 0.02]
De Oliveira et al. 2019 (01)	1.47	0.02	7	1.48	0.02	7	3.1%	-0.01 [-0.03, 0.01]
De Oliveira et al. 2019 (02)	1.46	0.02	7	1.48	0.02	7	3.1%	-0.02 [-0.04, 0.00]
Haetinger et al. 2021 (01)	1.42	0.02	7	1.46	0.02	7	3.1%	-0.04 [-0.06, -0.02]
Kaczmarek et al. 2015 (01)	1.49	0.02	8	1.52	0.02	8	3.1%	-0.03 [-0.05, -0.01]
Kaczmarek et al. 2015 (02)	1.5	0.02	8	1.56	0.02	8	3.1%	-0.06 [-0.08, -0.04]
Liu et al. 2020 -A (01)	1.58	0.03	10	1.61	0.03	10	2.8%	-0.03 [-0.06, -0.00]
Liu et al. 2020 -A (02)	1.57	0.03	10	1.61	0.03	10	2.8%	-0.04 [-0.07, -0.01]
Liu et al. 2020 -B (01)	1.53	0.04	16	1.58	0.04	16	2.8%	-0.05 [-0.08, -0.02]
Liu et al. 2020 -B (02)	1.58	0.04	16	1.66	0.04	16	2.8%	-0.08 [-0.11, -0.05]
Majdolhosseini et al. 2019 (01)	1.77	0.04	5	1.76	0.04	5	1.8%	0.01 [-0.04, 0.06]
Majdolhosseini et al. 2019 (02)	1.73	0.04	5	1.81	0.04	5	1.8%	-0.08 [-0.13, -0.03]
Majdolhosseini et al. 2019 (03)	1.87	0.04	5	1.88	0.04	5	1.8%	-0.01 [-0.06, 0.04]
Majdolhosseini et al. 2019 (04)	1.82	0.04	5	1.89	0.04	5	1.8%	-0.07 [-0.12, -0.02]
Movagharnejad et al. 2020 (01)	1.44	0.04	4	1.56	0.04	4	1.6%	-0.12 [-0.18, -0.06]
Movagharnejad et al. 2020 (02)	1.5	0.04	4	1.56	0.04	4	1.6%	-0.06 [-0.12, -0.00]
Park et al. 2018 (01)	1.61	0.03	12	1.65	0.03	12	2.9%	-0.04 [-0.06, -0.02]
Park et al. 2018 (02)	1.59	0.03	12	1.65	0.03	12	2.9%	-0.06 [-0.08, -0.04]
Park et al. 2018 (03)	1.58	0.03	12	1.65	0.03	12	2.9%	-0.07 [-0.09, -0.05]
Saleh et al. 2020	1.66	0.06	4	1.73	0.06	4	1.0%	-0.07 [-0.15, 0.01]
Shen et al. 2021 (03)	1.8	0.05	6	1.79	0.05	6	1.6%	0.01 [-0.05, 0.07]
Silva et al. 2018 (01)	1.7	0.06	8	1.72	0.06	8	1.5%	-0.02 [-0.08, 0.04]
Silva et al. 2018 (02)	1.69	0.05	8	1.72	0.05	8	1.9%	-0.03 [-0.08, 0.02]
Silva et al. 2018 (03)	1.67	0.05	8	1.72	0.05	8	1.9%	-0.05 [-0.10, -0.00]
Upadhaya et al. 2017-A (01)	1.59	0.05	6	1.65	0.05	6	1.6%	-0.06 [-0.12, -0.00]
Upadhaya et al. 2017-A (02)	1.57	0.05	6	1.65	0.05	6	1.6%	-0.08 [-0.14, -0.02]
Upadhaya et al. 2017-A (03)	1.53	0.05	6	1.65	0.05	6	1.6%	-0.12 [-0.18, -0.06]
Upadhaya et al. 2017-B (01)	1.56	0.04	12	1.6	0.04	12	2.6%	-0.04 [-0.07, -0.01]
Upadhaya et al. 2017-B (02)	1.5	0.04	12	1.6	0.04	12	2.6%	-0.10 [-0.13, -0.07]
Upadhaya et al. 2017-B (03)	1.48	0.04	12	1.6	0.04	12	2.6%	-0.12 [-0.15, -0.09]
Wang et al. 2016 (01)	1.75	0.13	9	1.75	0.13	9	0.5%	0.00 [-0.12, 0.12]
Wang et al. 2020 (01)	1.8	0.05	8	1.17	0.05	8		Not estimable
Wang et al. 2020 (02)	1.77	0.06	8	1.77	0.06	8	1.5%	0.00 [-0.06, 0.06]
Zampiga et al. 2016 (01)	1.87	0.03	9	1.91	0.03	9	2.8%	-0.04 [-0.07, -0.01]
Zampiga et al. 2016 (02)	1.88	0.03	9	1.91	0.03	9	2.8%	-0.03 [-0.06, -0.00]
Zhang et al. 2011 (01)	1.81	0.04	7	1.82	0.04	7	2.1%	-0.01 [-0.05, 0.03]
Zhang et al. 2011 (02)	1.95	0.04	7	1.94	0.04	7	2.1%	0.01 [-0.03, 0.05]
Zhang et al. 2011 (03)	1.82	0.04	7	1.88	0.04	7	2.1%	-0.06 [-0.10, -0.02]
Total (95% CI)			**402**			**402**	**100.0%**	**-0.04 [-0.05, -0.03]**

Heterogeneity: Tau² = 0.00; Chi² = 155.66, df = 49 (P < 0.00001); I² = 69%
Test for overall effect: Z = 8.47 (P < 0.00001)

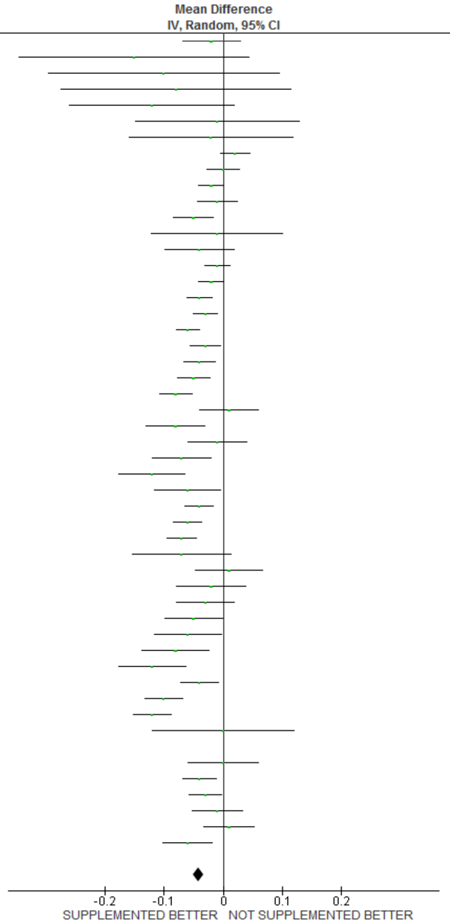

Figure 3 – Meta-analysis of the general effect of emulsifiers or lipases on broiler feed conversion (g/g/bird/day).

up to 3000 kcal/kg (p=0.003) and from 3000 to 3200 kcal/kg (p<0.00001). Above 3200 kcal/kg, there is evidence of a 2-point reduction, but there was no significant difference, probably due to the number of comparisons (k=4).

There was also evidence of a greater reduction in HRR with the use of higher doses of emulsifier additives or lipase in broilers' diets. With up to 0.05% dietary supplementation, there is evidence of a 3-point reduction in HRR (p<0.00001). With doses between 0.05% and 0.1% supplementation, the reduction is 4 points in HRR (p<0.0001), whereas with doses above 0.1%, there is evidence of a 7-point reduction in HRR (p<0.00001). Therefore, the data shows evidences of similar effects for the addition of emulsifiers or lipases (4 points vs 3 points); nevertheless, the emulsifier data are more consistent in terms of the number of studies and comparisons, and significance (p<0.00001) when compared to lipase data (p=0.29).

Sensitivity analysis

The entire database was subjected to sensitivity analysis through visual investigation of the funnel plot, and the mean differences obtained in each comparison group were plotted against their standard errors (Figures 4 and 5). Initially, we see a symmetrical distribution of the average differences obtained for weight gain and feed conversion around the central axis of the graphs (median), which indicates a lack of favoring of the publication of studies with positive or negative effects of the additives. This situation allows us to conclude that there is no publication bias.

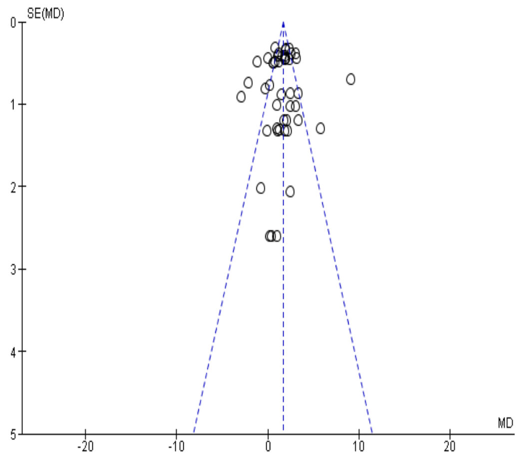

Figure 4 – Funnel plot of mean differences (MD) versus their inverse standard errors (SE) for weight gain (circles represent individual studies included in the meta-analysis) in studies with emulsifier or lipase.

Table 3 – Meta-analysis of feed conversion of broilers fed diets containing emulsifier or lipase, on the subgroups sex, strain, lipid source and concentration in the diet (%), metabolizable energy of the feed (kcal/kg), type and dietary concentration of the additive.

Subgroup		Mean Difference		Heterogeneity		
Sex	k	IV, Random, 95% CI	p-value	Tau²	p-value	I²
Male	39	-0.04 [-0.05,-0.03]	<0.00001	<0.00	<0.00001	69%
Female	1	0.00 [-0.03,0.03]	1.00	NA	NA	NA
Mixed	11	-0.04 [-0.07,-0.01]	0.02	<0.00	0.001	66%
Strain						
Arbor Acres	4	-0.01 [-0.05, -0.02]	0.41	<0.00	0.09	55%
Cobb	12	-0.02 [-0.03, -0.00]	0.02	<0.00	0.06	43%
Ross	35	-0.05 [-0.06, 0.04]	<0.00001	<0.00	<0.00001	59%
lipid source						
Animal origin	20	-0.06 [-0.07,-0.04]	<0.00001	<0.00	<0.00001	78%
vegetable origin	27	-0.03 [-0.04,-0.02]	<0.00001	<0.00	0.21	18%
mixed origin	4	0.00 [-0.01,0.01]	1.00	<0.00	1.00	0%
Concentration of the lipid source in the diet (%)						
up to 2%	20	-0.06 [-0.09, -0.03]	0.0005	<0.00	0.03	59%
From 2 to 3.5%	27	-0.04 [-0.05, -0.02]	<0.00001	<0.00	<0.00001	64%
More than 3.5%	4	-0.04 [-0.06,-0.03]	<0.00001	<0.00	<0.00001	76%
Feed metabolizable energy (kcal/kg)						
Up to 3000 kcal/kg	14	-0.04 [-0.07, -0.01]	0.003	<0.00	<0.0001	69%
From 3000 to 3200 kcal/kg	33	-0.04 [-0.05, -0.03]	<0.00001	<0.00	<0.00001	70%
More than 3200 kcal/kg	4	-0.02 [-0.06, 0.01]	0.15	<0.00	0.11	51%
Additive type						
emulsifier	46	-0.04 [-0.05, -0.04]	<0.00001	<0.00	<0.00001	63%
lipase	5	-0.03 [-0.03, 0.01]	0.29	<0.00	0.04	60%
Concentration of the additive in the diet (%)						
Up to 0.05%	20	-0.03 [-0.04, -0.02]	<0.00001	<0.00	0.005	50%
From 0.05 to 0.1%	25	-0.04 [-0.06, -0.03]	<0.00001	<0.00	<0.00001	73%
More than 0.1%	5	-0.07 [-0.10, -0.05]	<0.00001	<0.00	0.01	69%
Uninformed	1	-0.06 [-0.12, -0.00]	0.03	NE	NE	NE

k= number of comparisons at each level of subgroups, IV= inverse variance, Random=random model, 95% CI= confidence interval for each MD, Tau² = between-study total variance (heterogeneity + sampling error), I² = proportion of variance due to heterogeneity; NE=not estimable.

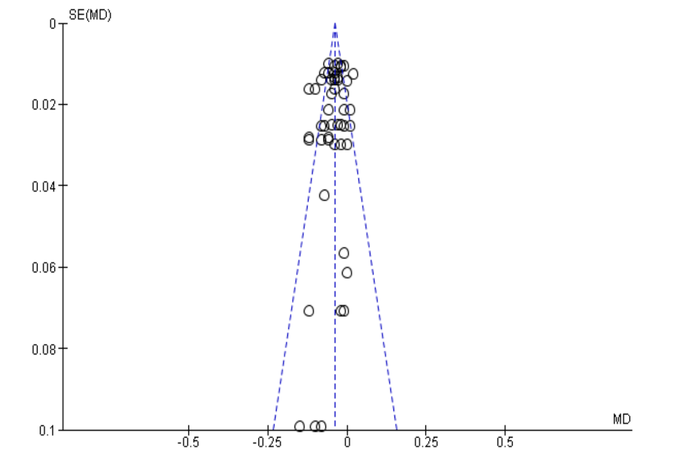

Figure 5 – Funnel plot of mean differences (MD) versus their inverse standard errors (SE) for feed conversion (circles represent individual studies included in the meta-analysis) in studies with emulsifier or lipase.

Furthermore, we also found that most of the average differences for both variables were found within the expected normality pyramid of the graph. Only a few studies showed mean difference (MD) values outside the normal range for the weight gain variable (Movagharnejad *et al.* (2020) (01); Movagharnejad *et al.* (2020) (02); Saleh *et al.* (2020); Wang *et al.* (2020) (02)) and in the feed conversion variable (Wang *et al.*, 2020 (01)). Such studies were considered outliers and, although they are present in the funnel plot, they were excluded from the meta-analysis calculations by assigning a zero value to their relative weight. Thus, they did not contribute to the estimation of the general effects or the Z test, that is, they did not compromise the robustness of the results of the meta-analyses presented here.

DISCUSSION

As shown in the Forest plot graphs (Figures 2 and 3), the results of this meta-analysis indicate that the overall effect of the supplementation with emulsifier or lipase is an improvement of broiler performance in the full cycle period, as compared to those that received a diet without this supplementation.

Subgroup analyses indicate possible interferences of sex, strain, lipid source and concentration (%), feed metabolizable energy (kcal/kg), and type and dietary concentration of the additive (%), on weight gain (Table 2) and feed conversion (Table 3).

In the sex subgroup, male and female broilers had better weight gain (Table 2), while for feed conversion, male and mixed broilers showed better weight gain (Table 3). The increase in weight gain for males that received emulsifier and lipase supplementation was 1.46g/day. For females, the gain was 9.10 g/day. Despite the literature showing evidences that these digestibility-enhancing additives have positive effects on female weight gain, this result should be further investigated, as it is not as robust as that of the other sexes, since the subgroup comprised only one study.

Supplementation with emulsifier or lipase did not influence the weight gain of mixed sex birds. This fact may be related to two aspects: firstly, the average differences between studies of mixed birds are smaller than those of sexed birds, which have a lower treatment effect; secondly, they present greater variability of the means. Both aspects can probably be related to the greater variability of the analyzed group of birds (differences in weights and nutritional requirements between males and females), which may have a greater contribution to the meta-analytic result.

The significant improvement in feed conversion for male or mixed broilers that received emulsifier or lipase supplementation was 4 points (0.04 g/g). The absence of a significant effect of additives on feed conversion in females may be related to digestibility and nutrient utilization, with males presenting greater weight gain and better feed conversion than females. It may also be related to the small number of studies within this subgroup, which implies less statistical power for more accurate estimates of effect sizes.

Positive performance effects were also observed in Cobb and Ross strains that received emulsifier or lipase supplementation during the full cycle period. Cobb broilers had an increase in weight gain of 2.02 g/day, while that of Ross broilers increased by 1.65 g/day (Table 2). Cobb and Ross broilers also showed reductions in feed conversion of 0.02 and 0.05 g/g, respectively, when fed on diets supplemented with emulsifiers or lipases (Table 3).

The non-detection of a significant effect of additives on weight gain and feed conversion in the Arbor Acres strain is probably related to the small number of studies identified with these animals (Zhang *et al.*, 2011; Shen *et al.*, 2021).

When birds received diets supplemented with a lipid source of animal origin and addition of emulsifier or lipase, they showed an increase in weight gain of 1.52 g/day (Table 2) and an improvement in feed conversion of 6 points (0.06 g/g) (Table 3). The same occurred for birds that received a diet with supplementation using a lipid source of vegetable origin: they showed greater weight gain, i.e., a significant gain of 1.25 g more per day (Table 2), with an improvement of 3 points (0.03 g/g) in feed conversion (Table 3).

This increase in weight gain and improvement in feed conversion are probably due to a greater efficiency in the digestion and absorption of lipids, as the additives improve the digestibility of fats and oils. However, it was harder to detect a significant effect of additives for weight gain and feed conversion when the lipid sources offered to the birds were of mixed origin. As discussed for the female subgroup, this is possibly more linked to the lower number of studies within this subgroup (Bontempo *et al.*, 2015; Kaczmarek *et al.*, 2015; Bontempo *et al.*, 2018) rather than to possible differences in the mode of action of the additives as a function of lipid source.

Regarding the concentration of lipid sources, at all levels of inclusion, there was a positive effect of the additives on weight gain (Table 2) and feed conversion (Table 3). Higher levels of lipid source concentration provided a greater benefit of additive supplementation on weight gain (2.52 g/day more). This superiority can be explained by the greater amount of substrate for the action of emulsifiers and lipases, providing a better use of the lipid components in the diet and, consequently, better availability of energy and fatty acids for animal metabolism.

In the subgroup of metabolizable energy levels, we observed a positive effect of the additives only when supplemented in diets with levels between 3000 and 3200 kcal/kg, with a significant increase of 1.88 g/day in the weight gain of the birds (Table 2). A similar behavior was observed in the feed conversion variable, and it was not possible to show a positive effect of the supplementation in diets with a metabolizable energy level above 3200 kcal/kg. Thus, intermediate levels of metabolizable energy can be recommended as ideal for future investigations.

When the additives were evaluated separately, it was found that diets with emulsifier provided a significant increase in weight gain of 1.62 g/day (Table 2), and an improvement of 4 points (0.04 g/g) in the feed conversion of the birds in the full cycle period (Table 3). When used alone in this subgroup, lipase was not effective in promoting bird weight gain and feed conversion.

Although some studies included in this meta-analysis comparison found a positive effect of lipase on weight gain and feed conversion (Oliveira *et al.*, 2019; Castro & Kim, 2021), it was not possible to find evidence that lipase, when used alone, improves broiler weight gain and feed conversion. This result should be analyzed more carefully, as the small number of studies with lipase may have impaired the statistical power of the meta-analysis in this subgroup.

As regards to the additive concentration level subgroup, it is worth mentioning that we observed positive effects on bird weight gain (Table 2) and feed conversion (Table 3) at all levels used, although a greater difference in weight gain was observed in the intermediate additive concentration level (0.05 to 0.1%). These findings show the efficiency of the additives under study, even at low concentrations in the diet. This an essential characteristic for any additive used in diets for broilers, given that the high nutritional densities of the formulations reduce the space available for the inclusion of any new component.

In general, the additives evaluated here proved to be effective in improving broiler weight gain and feed conversion. More assertive protocols for its use can be elaborated based on the results of subgroup analyses, identifying interesting opportunities to choose characteristics that favor their action based on the sex and strain of the animals, lipid source and level of metabolizable energy of the feed, or the type of additive.

It is noted, however, that the absence of significant effects in this meta-analysis absolutely does not imply the absence or limited action of the evaluated additives. In many of these situations, the interpretation of these findings was hindered by the small number of published studies with certain characteristics. This was clearly evidenced in the sex (few studies with female birds) and type of additive (few published studies with the lipase additive) subgroups. On the other hand, from the point of view of research and development, such situations clearly present opportunities for conducting new and more objective studies to overcome this lack of publications, as well as opening possibilities for the development of products with more specific characteristics and applications.

CONCLUSIONS

The results of this meta-analysis indicate that supplementing the feed of broilers with additives that improve lipid digestibility promotes greater weight gain and better feed conversion throughout the production cycle. However, while there is strong evidence of these positive effects with the use of emulsifiers alone, the same cannot be said for lipases. In addition, variables such as the sex and strain of the animals, source and concentration of the lipids, energy level of the feed, and the type and concentration of additives used, can significantly affect the action of these products, influencing the effect size estimates obtained in this study.

Thus, in order to generate more robust effect size estimates, we suggest a greater volume of publications on this topic, especially with female birds and with different types of lipases. More complete descriptions of the characteristics of the population evaluated, and the treatments tested, should also be presented in the studies, preventing them from being excluded from other meta-analyses in the future due to a simple lack of adequate qualitative information.

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