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Meta-Analysis of Performance and Intestinal Histomorphology of Broilers Fed Diets Containing Fermented Tropical Seed Meals

[1,2]Ifeanyichukwu Princewill Ogbuewu, [1]Nnanyere Okwunna Aladi,
[2]Monnye Mabelebele and [2]Christian Anayo Mbajiorgu

[1]*Department of Animal Science and Technology, Federal University of Technology, Owerri, Imo State, Nigeria*
[2]*Department of Agriculture and Animal Health, University of South Africa, Florida Science Campus, Florida, South Africa*

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Corresponding Author:
Ifeanyichukwu Princewill Ogbuewu
Department of Animal Science and Technology, Federal University of Technology, Owerri, Imo State, Nigeria
Email: ifeanyi.ogbuewu@futo.edu.ng

Abstract: There are variable results on the effect of dietary Fermented Tropical Seed Meal (FTSM) intervention on the growth and intestinal histomorphological indices of broilers. This meta-analysis aimed to investigate the effects of FTSM intervention on the growth performance and intestinal histomorphology of broilers. A database of 31 peer-reviewed studies that assessed the effect of dietary FTSM intervention on growth performance [Feed Intake (FI), Average Daily (ADG), Feed Conversion Ratio (FCR)] and intestinal histomorphological parameters such as Villus Height (VH), Crypt Depth (CD) and VH/CD ratio of the small intestine of broilers was developed and used. Data on growth performance and intestinal histomorphology were pooled using a random effect model and results were presented as Standardized Mean Differences (SMDs) at 95% Confidence Intervals (CIs) for each study. Data extracted from the 31 studies utilized for the meta-analysis were analyzed using Open Meta-analyst for Ecology and Evolution (OpenMEE) and R statistical software. Results revealed that FTSM increased FI (SMD = 0.16; p = 0.015) and ADG (SMD = 0.15; p = 0.032) compared to the controls. Similarly, dietary FTSM increased the VH of the duodenum, jejunum, and ileum and reduced the CD of the ileum, but did not affect the CD of the duodenum and jejunum. Results also indicate that the VH/CD ratio of the duodenum, jejunum, and ileum of broilers fed FTSM-based diets was significantly ($p<0.05$) higher than the controls. The subgroup results suggest that Ross broilers fed fermented grape seed meal and soybean meal at an inclusion level of >20% during the finisher production phase had increased FI and ADG. There was evidence of significant ($p<0.05$) heterogeneity among the studies included in the meta-analysis and the meta-regression analysis results showed that seed type, feed class, and broiler strains explained most of the heterogeneity. Fermented tropical seed meal can be included at levels more than 20% to improve growth performance and enhance histomorphological indices of the small intestine of broilers. This study also established guidelines for standardized experimental designs on the use of dietary fermented tropical seed meals to enhance broiler performance.

Keywords: Feed Conversion, Feed Intake, Intestinal Histology, Meta-Regression, Weight Gain

Introduction

Poultry meat represents about 38.2% of global meat production and its demand is projected to grow by more than 38.7% in 2028 (O.E.C.D., 2019). To meet this demand, it is necessary to improve the productivity of broilers through the use of conventional and underutilized seed meals. Tropical climates, including sub-tropical climates, are endowed with rich plant diversity that produces seeds that have high nutritional value as livestock feed (Soumeh *et al*., 2019; Olasehinde and Aderemi, 2023). However, most of these seeds contain some Antinutritional Factors (ANFs) and toxic compounds like cyanogenic glycosides, lectins, tannins, phytate, oxalates, and trypsin inhibitors, that limit their use as feed resources in broiler diets (Ogbuewu *et al.*, 2017; Ashayerizadeh *et al.*, 2018; Abang *et al.*, 2018). According to Abang *et al.* (2018), some ANFs bind with divalent ions to form indigestible complexes, while others produce toxic compounds that interfere with nutrient bioavailability and utilization. Tropical seeds contain appreciable quantities of Non-Starch Polysaccharides (NSPs) that cannot be utilized by broilers due to their inability to produce endogenous enzymes needed to break down NSPs into their monomers. On the other hand, soluble NSP increases digesta viscosity and reduces nutrient digestibility (Sugiharto and Ranjitkar, 2019).

It has been reported that Solid-State Fermentation (SSF) and Submerged Fermentation (SmF) can mitigate these issues associated with the use of tropical and sub-tropical seed meals in broiler diets and at the same time, increasing crude protein and fat contents, while decreasing crude fiber content, thus leading to an increase in digestibility and nutrient bioavailability (Hu *et al.*, 2016; Soumeh *et al.*, 2019; Olasehinde and Aderemi, 2023). The acidic medium and desirable microbiota in the fermented feeds might improve gut health by promoting the growth of beneficial microbes while decreasing the number of harmful microbes through mechanisms similar to that of probiotics. Several studies have reported the potential of fermentation to enhance growth performance, immune responses, and meat quality in animals other than broilers (Canibe *et al.*, 2008; Drażbo *et al.* 2018; Dimidi *et al.*, 2019; Hu *et al.*, 2016; Ashayerizadeh *et al.*, 2018) observed that FTSM improved the intestinal morphology and growth of broilers. Chiang *et al.* (2009); Gao *et al.* (2020) also found enhanced growth and intestinal histomorphology in broilers fed FTSM. Similarly, (Jazi *et al.*, 2017) observed that FTSM in broiler diets increased FI and ADG and reduced FCR. In contrast, (Olisa *et al.*, 2010; Akinduro *et al.*, 2023) reported that FTSM reduced the growth indicator of broilers. However, Xu *et al.* (2012) reported that FTSM did not affect ADG and FCR in broilers. Therefore, the difference in the growth performance and intestinal histomorphology of broilers fed FTSM-based diets remains inconclusive. These conflicting results could be attributed to broiler age, broiler strains, type of seeds, fermentation techniques adopted, duration of fermentation and the quantity of FTSM included in the diets, duration of FTSM feeding, and several other factors.

The use of meta-analysis (a mathematical procedure that pools results of studies addressing the same objective) to resolve contradictory findings, uncover research gaps, and provide new insights among studies addressing the same research objectives has been reported (Ogbuewu and Mbajiorgu, 2022; Sierra-Galicia *et al.*, 2023; Hernández-García *et al.*, 2024). Understanding the impacts of dietary FTSM on broiler performance is critical in advancing the use of FTSM in the global broiler industry. Currently, there is no meta-analysis of the effect of FTSM on the performance of broilers. Therefore, the objective of this meta-analysis was to determine the effects of dietary FTSM intervention on the growth and histomorphology of broilers.

Materials and Methods

Search Strategy and Study Selection

The research question was formulated using the Population, Intervention, Comparison, and Outcomes (PICO) format as displayed in Table (1). Systematic searches were done on peer-reviewed studies on the topic using the following databases; PubMed, Scopus, Web of Science, Google Scholar, and ScienceDirect databases. The search was done on the five databases using the boolean logic operators (OR/AND) and the following keywords; “broilers”, “broiler chickens”, “fermentation”, “fermented seed meal”, “growth performance” and “intestine histomorphology”. Bibliographies of the identified articles were examined for other relevant studies that may have been missed by the search strategy. For an article to be added to the meta-analysis, the article must report the mean and number of broilers (sample size) on at least one of the response variables in broilers fed diets with and without FTSM. A systematic search of the five electronic databases yielded 1486 published research articles. Out of the 1486 articles, 1394 were excluded after reading the titles and abstract or because they were duplicates across two or more databases. After a detailed review of the 92 full-text studies, 61 were further removed. Thirty-one (31) research articles passed the pre-defined eligibility conditions as described in detail in The Preferred Reporting Items for Systematic Reviews and Meta-analyses flow chart in Fig. (1). Studies were reviewed by two independent reviewers and disagreements on whether or not to include a study were resolved through consensus.

Table 1: PICO strategy

	Search strategy	Exclusion criteria
Population	Broilers	Not reported in broilers
Intervention	Dietary FTSM	Not FTSM treatment
Comparison	Control group (diet without FTSM)	
Outcomes	Growth performance and intestinal histomorphology	

PICO Population, Intervention, Comparison, Outcomes; *FTSM* fermented tropical seed meal

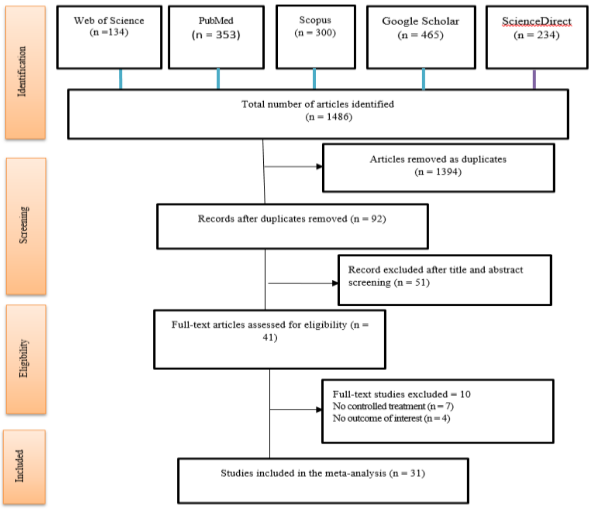


Fig. 1: The study selection flowchart

Data Abstraction and Synthesis

A database of 31 studies was built using information on (i) Study characteristics (author name and year of publication); (ii) Study location (Nigeria, Iran, China, Poland, Turkey, Korea, and Australia); (iii) Broiler strains (Anak, Ross, Marshall, Cobb, Yellow feathered and Arbor Acres); (iv) Fermentation techniques [Solid-State Fermentation (SSF) versus Submerged Fermentation (SmF)]; (v) Seed type (rapeseed, cotton, locust bean, soybean, roselle, castor, millet, prosopis, physic nut, grape, mango, sesame, sorghum, christmas bush, rubber, pigeon pea and African yam beans); (vi) Feed class (protein versus carbohydrates); (vii) Production phase [starter (d 1-28), finisher (d 28 -56) and overall (d 1-56)] and (viii) inclusion level (0.30-38.34%: ≤10%, 11-20% and >20%) extracted from each study. The inclusion level of FTSM in the diet and the production phase were categorized based on the values used by the authors whose studies were included in the meta-analysis. The measured outcomes included in this meta-analysis were FI, ADG, FCR, Villus Height (VH), Crypt Depth (CD), and VH/CD ratios of the duodenum, jejunum, and ileum. Information on the number of broilers and the means of the response variable in the experimental and control groups for each study that met the predefined eligibility criteria were also extracted.

In studies that reported Standard Error (SE) instead of Standard Deviation (SD), the SE value was converted to SD following the method of (Higgins *et al*., 2011). The extracted data were in an Excel workbook and thereafter converted to a Comma-Separated Value (CSV) file format, which is the acceptable file format for OpenMEE software used for the meta-analysis. Three authors were contacted to supply missing information on the broiler strain used. More than 60% of the articles used for the meta-analysis had missing information on fermentation conditions (temperature, humidity), duration of fermentation, and microbes used. Low statistical power was the reason the effect of fermentation conditions, duration of fermentation, and microbes used as covariates was not determined in the present meta-analysis.

Data Analysis

Data extracted from the 31 peer-reviewed that satisfied the predefined eligibility criteria were analyzed in OpenMEE software (Wallace *et al.*, 2017) and meta-for (Viechtbauer, 2010) packages available in the R statistical software (version 4.1.2). The Standardized Mean Difference (SMD) was employed to explore the impacts of FTSM-based diets on growth performance and intestinal histomorphology of broilers using a random-effect model. Results were presented at a 95% Confidence Interval (CI) for each study (Der-Simonian and Laird, 1986; Higgins and Thompson, 2002). The overall pooled result is considered statistically significant when the lower and upper limits of 95% CIs did not touch the line of null effect (SMD = 0) (Koricheva *et al*., 2013). Heterogeneity defined as the variation in study outcomes was detected and quantified using the Cochran (Q) test and I squared statistics. Publication bias defined as the failure to publish studies with non-significant findings was assessed using Egger and Begg tests (Begg and Mazumdar, 1994; Egger *et al*., 1997). Subgroups with less than three comparisons were excluded from this meta-analysis due to poor statistical power (Ogbuewu and Mbajiorgu, 2022). The effects of aspects of seed type (Christmas bush, rubber, roselle, physic nut, sorghum, sesame, mango, and Prosopis) on FI were not conducted due to the low sample size. Subgroup effects of aspects of seed type (i.e., rubber, roselle, sorghum, sesame, castor, and mango) and broiler strains (Anak, Marshall, and Cobb) on ADG were not analyzed due to low sample size. In addition, the influence of aspects of seed type (African yam bean, Christmas bush, rubber, roselle, sesame, Prosopis, and mango) and broiler strains (Marshall and Cobb) on FCR was not assessed due to low statistical power. Meta-regression and subgroup analysis were performed using broiler strains, fermentation techniques, seed type, feed class, production phase, and inclusion level of FTSM as covariates. Meta-regression and subgroup analyses of the effect of covariates on the histomorphological markers of the small intestine of broilers were not performed because the studies used for their analysis were less than ten (Borenstein *et al*., 2009). Meta-regression results were considered significant at a 5% probability level.

Results

Study Selection and Characteristics

The search yielded 1486 published research studies. Figure (1) shows the article selection process and the number of articles excluded. A total of 1455 studies were excluded due to duplication and not reported in response variables of interest. The characteristics of 31 studies included in the present study are displayed in Table (2).

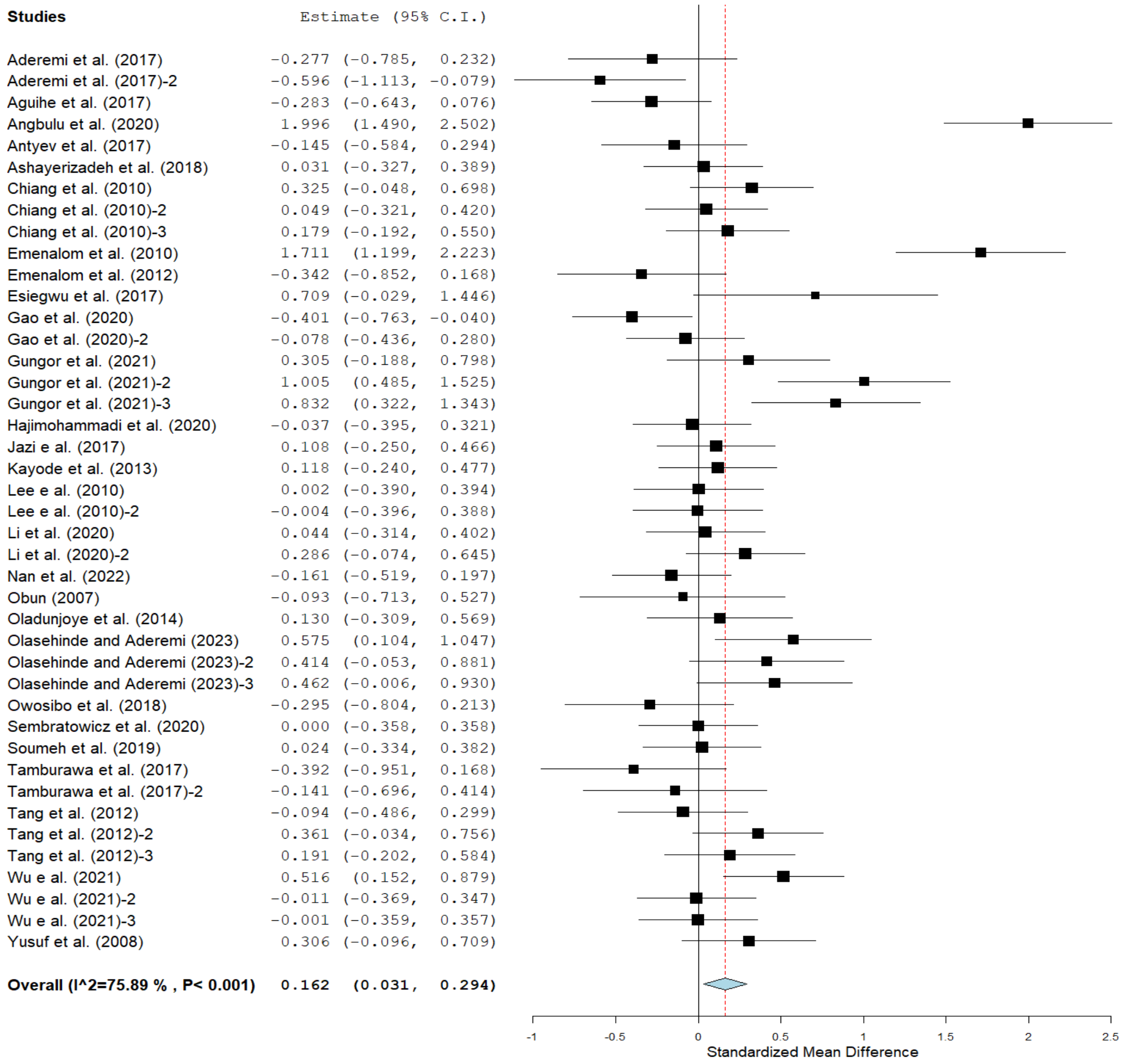
Table 2: Characteristics of studies used for the meta-analysis

		Covariates						
Authors	Country	strains	FT	ST	Feed class	PP (d)	Inclusion (%)	Response Variables
Feng *et al*. (2007)	China	Ross	SmF	Soybean	Protein	1-42	0, 28.25	4, 5, 7, 8, 10, 11
Obun (2007)	Nigeria	Anak	SSF	LB	Protein	1-56	0, 5.75-23	1, 2, 3
Yusuf *et al*. (2008)	Nigeria	Anak	SSF	Prosopis	Protein	7-56	0, 6.5-33.4	1, 2, 3
Chiang *et al*. (2009)	China	AA	SSF	Rapeseed	Protein	1-42	0, 10	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
Lee *et al*. (2010)	Korea	Ross	SSF	Soybean	Protein	1-35	0, 0.3-1.0	1, 2, 3
Emenalom *et al*. (2021)	Nigeria	Ross	SSF	CB	Protein	1-28	0, 10-20	1, 2, 3
Emenalom *et al*. (2021)	Nigeria	Anak	SSF	CB	Protein	1-35	0, 10	1, 2, 3
Tang *et al*. (2012)	China	YF	SSF	Cotton	Protein	1-42	0, 4-12	1, 2, 3
Kayode *et al*. (2010)	Nigeria		SSF	Mango	CHO	1-28	0, 20	1, 2, 3
Oladunjoye *et al*. (2014)	Nigeria	AA	SSF	Physic nut	Protein	1-28	0, 26.1-38.6	1, 2, 3
Hu *et al*. (2016)	China	AA	SSF	Rapeseed	Protein	1-42	0, 9.41	4, 5, 6, 7, 8, 9, 10, 11, 12
Aderemi *et al*. (2017)	Nigeria	AA	SSF	LB	Protein	1-56	0, 3.5-7	1, 2, 3
Aguihe *et al*. (2017)	Nigeria	AA	SSF	Rubber	Protein	1-42	0, 7.8	1, 2, 3
Antyev *et al*. (2017)	Nigeria	Anak	SSF	Physic nut	Protein	1-56	0, 10	1, 2, 3
Esiegwu (2021)	Nigeria	Marshall	SmF	Sorghum	CHO	1-28	0, 10-30	1, 2, 3
Jazi *et al*. (2017)	Iran	Ross	SSF	Cotton	Protein	1-42	0, 10-20	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
Tamburawa *et al*. (2017)	Nigeria	Marshall	SSF	LB	Protein	1-56	0, 7.5 -30	1, 2, 3
Olisa *et al*. (2010)	Nigeria		SSF	PnP/AYB	Protein	1-42	0, 19.17-38.34	1, 2, 3
Ashayerizadeh *et al*. (2018)	Iran	Cobb	SSF	Rapeseed	Protein	1-42	0, 13.17-26.33	1, 2, 3
Owosibo *et al*. (2020)	Nigeria		SSF	Roselle	Protein	22-42	0, 12.5	1, 2, 3
Soumeh *et al*. (2019)	Australia	Ross	SmF	Soybean	Protein	1-42	0, 34.5	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
Angbulu *et al*. (2020)	Nigeria	Ross	SSF	Roselle	Protein	1-56	0, 20	1, 2, 3
Gao *et al*. (2020)	Poland	Ross	SSF	Rapeseed	Protein	7-35	0, 15	1, 2, 3
Hajimohammadi *et al*. (2020)	Iran	Ross	SSF	Sesame	CHO	1-42	0, 15-25	1, 2, 3
Li *et al*. (2020)	China	Cobb	SSF	Soybean	Protein	1-35	0, 12.59-14.46	1, 2, 3
Sembratowicz *et al*. (2020)	Poland	Ross		Soybean	Protein	1-40	0, 3-6	1, 2, 3
Gungor *et al*. (2021)	Turkey	Ross	SSF	Grape	CHO	1-42	0, 0.5	1, 2, 3
Wu *et al*. (2022)	China	AA	SSF	Rapeseed	Protein	1-42	0, 5-15	1, 2, 3, 4, 5, 6, 7, 8, 9
Nan *et al*.(2022)	China	YF	SSF	Grape	CHO	14-56	0, 2-6	1, 2, 3
Ombugu *et al*. (2022)	Nigeria	Ross	SmF	Castor	Protein	1-28	0, 5.75-23	1, 2, 3
Olasehinde and Aderemi (2023)	Nigeria	AA	SmF	Millet	CHO	1-42	0, 14.81-42.03	1, 2, 3, 4, 5, 7, 8

FT fermentation techniques; *ST* Seed types; *PP* production phase; *FI* feed intake; *ADG* average daily gain; *FCR* feed conversion ratio; *VH* villus height carcass; *CD* crypt depth; *AA* Arbor Acres; *YF* Yellow feathered; *CHO* Carbohydrate; *SSF* solid-state fermentation; *SmF* submerged fermentation; *AYB* African yam bean; *PnP* pigeon pea; *CB* Christmas bush; *LB* Locust bean; 1- FI; 2-ADG; 3-FCR; 4-DVH; 5-DCD; 6-DVH/CD ratio; 7-JVH; 8-JCD; 9-JVH/CD ratio; 10-IVH; 11-ICD; 12-IVH/CD ratio

Feed Intake (FI)

The effect of FTSM on FI of broilers is described in Fig. (2). The pooled results indicated that broilers fed FTSM recorded higher FI than broilers fed the control diet (SMD = 0.16; p = 0.015). Table (3) indicates the effects of covariates on FI in broilers. Dietary FTSM increased FI in the Ross strain (SMD = 0.40; p = 0.027) but did not affect FI in arbor acres, anak, cobb, marshall, and yellow feathered strains. The type of fermentation (SmF vs SSF) had an impact on FI. Broilers from studies that used SmF had increased FI. Results indicate that FI was decreased in broilers fed fermented locust bean meal, but was increased in those that fed fermented millet meal (SMD = 0.48; p<0.001). The result shows that FI was not affected by the production phase. In contrast, FI was increased in broilers offered FTSM at >20%. There is evidence of significant heterogeneity across the 27 studies that assessed the impact of FTSM on FI (Q-statistic p<0.001, I^2-statistic = 76%; Table (4). Table (4) also revealed that Egger's regression asymmetry test and Begg's adjusted rank correlation were not significant for FI, implying no publication bias. Meta-regression analysis as displayed in Table (5) found a linear relationship between feed class and FI in broilers. Feed class (p = 0.016) explained 13% of the heterogeneity detected in FI. There was no relationship between FI and the other five studied covariates.



Studies	Estimate (95% C.I.)
Aderemi et al. (2017)	-0.277 (-0.785, 0.232)
Aderemi et al. (2017)-2	-0.596 (-1.113, -0.079)
Aguihe et al. (2017)	-0.283 (-0.643, 0.076)
Angbulu et al. (2020)	1.996 (1.490, 2.502)
Antyev et al. (2017)	-0.145 (-0.584, 0.294)
Ashayerizadeh et al. (2018)	0.031 (-0.327, 0.389)
Chiang et al. (2010)	0.325 (-0.048, 0.698)
Chiang et al. (2010)-2	0.049 (-0.321, 0.420)
Chiang et al. (2010)-3	0.179 (-0.192, 0.550)
Emenalom et al. (2010)	1.711 (1.199, 2.223)
Emenalom et al. (2012)	-0.342 (-0.852, 0.168)
Esiegwu et al. (2017)	0.709 (-0.029, 1.446)
Gao et al. (2020)	-0.401 (-0.763, -0.040)
Gao et al. (2020)-2	-0.078 (-0.436, 0.280)
Gungor et al. (2021)	0.305 (-0.188, 0.798)
Gungor et al. (2021)-2	1.005 (0.485, 1.525)
Gungor et al. (2021)-3	0.832 (0.322, 1.343)
Hajimohammadi et al. (2020)	-0.037 (-0.395, 0.321)
Jazi e al. (2017)	0.108 (-0.250, 0.466)
Kayode et al. (2013)	0.118 (-0.240, 0.477)
Lee e al. (2010)	0.002 (-0.390, 0.394)
Lee e al. (2010)-2	-0.004 (-0.396, 0.388)
Li et al. (2020)	0.044 (-0.314, 0.402)
Li et al. (2020)-2	0.286 (-0.074, 0.645)
Nan et al. (2022)	-0.161 (-0.519, 0.197)
Obun (2007)	-0.093 (-0.713, 0.527)
Oladunjoye et al. (2014)	0.130 (-0.309, 0.569)
Olasehinde and Aderemi (2023)	0.575 (0.104, 1.047)
Olasehinde and Aderemi (2023)-2	0.414 (-0.053, 0.881)
Olasehinde and Aderemi (2023)-3	0.462 (-0.006, 0.930)
Owosibo et al. (2018)	-0.295 (-0.804, 0.213)
Sembratowicz et al. (2020)	0.000 (-0.358, 0.358)
Soumeh et al. (2019)	0.024 (-0.334, 0.382)
Tamburawa et al. (2017)	-0.392 (-0.951, 0.168)
Tamburawa et al. (2017)-2	-0.141 (-0.696, 0.414)
Tang et al. (2012)	-0.094 (-0.486, 0.299)
Tang et al. (2012)-2	0.361 (-0.034, 0.756)
Tang et al. (2012)-3	0.191 (-0.202, 0.584)
Wu e al. (2021)	0.516 (0.152, 0.879)
Wu e al. (2021)-2	-0.011 (-0.369, 0.347)
Wu e al. (2021)-3	-0.001 (-0.359, 0.357)
Yusuf et al. (2008)	0.306 (-0.096, 0.709)
Overall (I^2=75.89 % , P< 0.001)	**0.162 (0.031, 0.294)**

Fig. 2: FI of broilers fed FTSM-based diets. The overall effect size is deemed significant when the lower and upper limits of 95% CIs (i.e., the lateral tips of the sky-blue diamond at the base of the forest plot) did not touch the line of null effect (SMD = 0) (Koricheva *et al*., 2013). The points to the left of the line of null effect, i.e., the thick vertical line indicate a decrease in FI, while the points to the right connote an increase in FI

Table 3: Subgroup effects of covariates on FI of broilers fed FTSM-based diets

Covariates	Subgroup	Dataset	SMD	(95% CI)	*P*-value	Heterogeneity	
						I^2 (%)	P value
Broiler strains	Arbor Acres	13	0.12	-0.06, 0.30	0.182	58	0.005
	Ross	13	0.40	0.05, 0.75	0.027	90	<0.001
	Anak	4	-0.04	-0.33, 0.25	0.789	32	0.219
	Cobb	3	0.12	-0.09, 0.33	0.257	0	0.541
	Marshall	3	0.01	-0.58, 0.61	0.962	64	0.061
	YF	4	0.07	-0.17, 0.31	0.586	36	0.194
FT	SSF	37	0.15	-0.01, 0.30	0.053	78	<0.001
	SmF	5	0.37	0.13, 0.61	0.003	24	0.261
Seed type	Locust bean	5	-0.32	-0.56, -0.07	0.012	0	0.718
	Rapeseed	9	0.07	-0.10, 0.23	0.445	48	0.050
	Grape	4	0.48	-0.08, 1.04	0.095	83	0.001
	Cotton	4	0.14	-0.05, 0.33	0.155	0	0.446
	Soybean	6	0.06	-0.09, 0.21	0.077	0	0.869
	Millet	3	0.48	0.21, 0.75	< 0.001	0	0.887
Feed class	Protein	30	0.06	-0.07, 0.20	0.369	70	<0.001
	Energy	12	0.45	0.14, 0.76	0.005	82	<0.001
Production phase	Starter	14	0.24	-0.02, 0.49	0.068	79	<0.001
	Finisher	9	0.31	-0.16, 0.77	0.193	89	<0.001
	Overall	19	0.04	-0.06, 0.14	0.435	23	0.176
Inclusion (%)	≤ 10	22	0.09	-0.05, 0.22	0.216	58	<0.001
	11-20	18	0.23	-0.06, 0.52	0.121	86	<0.001
	>20	4	0.33	0.07, 0.60	0.012	30	0.234

FI feed intake; *YF* yellow feathered; *FT* fermentation techniques; *SMD* standardized mean difference; *CI* confidence interval; *SSF* solid-state fermentation; I^2 Inconsistency index; *SmF* submerged fermentation; *p* probability

Table 4: Heterogeneity and publication bias analysis

Variables	N (nc)	Heterogeneity				Egger test	Begg test
		df	*Q*	I^2 (%)	P-value	p-value	p-value
Feed intake	25 (42)	41	170.062	76	< 0.001	0.621	0.483
Average daily gain	21 (34)	33	122.766	73	< 0.001	0.514	0.816
Feed conversion ratio	24 (40)	39	107.497	65	< 0.001	0.839	0.634

N number of publications; *nc* number of comparisons between fermented tropical seeds group and control group; *CI* confidence interval of SMD; p-value to Q-statistic of heterogeneity; I^2 heterogeneity; *a*n Egger's regression asymmetry test; *b* Begg's adjusted rank correlation; *df* degree of freedom

Table 5: Meta-regression comparing the relationship between growth performance and studied covariates

Variables	Covariates	Q_M	df	P-value	R^2 (%)
Feed intake	Broiler strains	3.77	5	0.583	0
	Fermentation technique	1.24	1	0.265	0.4
	Seed type	17.9	13	0.162	11
	Feed class	5.83	1	0.016	13
	Production phase	2.18	2	0.336	0.4
	Inclusion level	1.47	2	0.480	0
Average daily gain	Broiler strains	9.30	5	0.098	11
	Fermentation technique	2.01	1	0.156	0.7
	Seed type	82.30	10	<0.001	95
	Feed class	6.47	1	0.011	16
	Production phase	0.29	2	0.865	0
	Inclusion level	0.23	2	0.890	0
Feed conversion ratio	Broiler strains	25	5	<0.001	66
	Fermentation technique	2.98	1	0.085	10
	Seed type	44	15	<0.001	70
	Feed class	0.23	1	0.630	0
	Production phase	0.72	2	0.697	0
	Inclusion level	0.503	3	0.918	0

Q_M: Coefficient of moderators; Q_M is considered significant at $p<0.05$; *df* degree of freedom; R^2 the amount of heterogeneity accounted for

Average Daily Gain (ADG)

The effect of FTSM intervention on the ADG of broilers is displayed in Fig. (3). The ADG of broilers in FTSM treatment was significantly higher than those in the control treatment (SMD = 0.15; p = 0.032). Table (6) provides the effect of covariates on ADG in broilers. Dietary FTSM increased ADG in the Ross strain (SMD = 0.24; p = 0.034) but did not affect ADG values in arbor Acres, cobb, and Yellow feathered strains. Fermentation techniques (SmF vs SSF) affected ADG with broilers from studies that used SmF having increased ADG.

The results show that ADG was increased in broilers offered fermented grape meal (SMD = 0.80; p = 0.002) and fermented soybean meal (SMD = 0.27; p<0.001). However, ADG was not increased in broilers fed fermented locust bean meal (SMD = 0.22; p = 0.184), fermented rapeseed meal (SMD = -0.13; p = 0.062), fermented cotton Seed Meal (SMD = 0.12; p = 0.240) and fermented pearl meal (SMD = 0.17; p = 0.212). On the other hand, ADG was increased in broilers fed FTSM, which was rich in carbohydrates. The result shows that ADG was affected by the production phase. Broilers in the starter phase had a lower ADG than in the finisher phase. The result shows that ADG was increased in broilers that received FTSM at >20%. There is evidence of significant heterogeneity across the 21 studies that evaluated the effect of dietary FTSM intervention on ADG (Q-statistic p<0.001, I^2-statistic = 73%; table. Table (4)shows that Egger's regression asymmetry test and Begg's adjusted rank correlation were not significant for ADG, ruling out the possibility of publication bias. Table (5) shows the relationship between ADG and Fermentation Techniques (FT); Seed Types (ST); Production P#hase (PP); Feed Intake (FI); Average Daily Gain (ADG); Feed Conversion Ratio (FCR); Villus Height carcass (VH); Crypt Depth; (CD) Arbor Acres (AA) Yellow Feathered (YF) *CHO* Carbohydrate; Solid-State Fermentation (SSF) Submerged Fermentation (SmF) African Yam Bean (AYB) Pigeon Pea; (PnP) Christmas Bush (CB) Locust Bean (LB); 1-FI; 2-ADG; 3-FCR; 4-DVH; 5-DCD; 6-DVH/CD ratio; 7-JVH; 8-JCD; 9-JVH/CD ratio; 10-IVH; 11-ICD; 12-IVH/CD ratio.

Feed Intake (FI) Yellow Feathered (YF) Fermentation Techniques (FT) Standardised Mean Difference (SMD); Confidence Interval (CI); Solid-State Fermentation (SSF); Inconsistency Index (II); Submerged Fermentation (SmF); Probability (P).

Number (N) of publications; Number of Comparisons (NC) between fermented tropical seeds group and control group; Confidence Interval (CI) of SMD; P-value to Q-statistic of Heterogeneity Heterogeneity (HH); (a) Egger's regression asymmetry test; (b) Begg's adjusted rank correlation; Degree Of Freedom (DF).

Q_M: Coefficient of moderators; Q_M is considered significant at p<0.05; Degree of Freedom (DF); R^2 the amount of heterogeneity accounted for. Average Daily Gain (ADG) Yellow Feathered (YF) Fermentation Techniques (FT) Standardised Mean Difference (SMD); Confidence Interval (CI); Solid-State Fermentation (SSF) Inconsistency Index (II); Submerged Fermentation (SmF) Probability (P).

Covariates. There was a linear relationship between ADG and aspects of covariates (seed type and feed class) in broilers. Seed type (p<0.001) and feed class (p = 0.011) explained 95 and 16% of the heterogeneity detected in ADG. There was no relationship between ADG and the other three studied covariates.

Table 6: Subgroup effects of covariates on ADG of broilers fed FTSM-based diets

Covariates	Subgroup	Datasets	SMD (95% CI)		*P*-value	Heterogeneity	
						I^2 (%)	*P*-value
Broiler strains	Arbor Acres	11	0.37	-0.16, 0.89	0.386	39	0.091
	Cobb	3	0.04	-0.40, 0.47	0.867	77	0.031
	Ross	11	0.24	0.02, 0.46	0.034	70	<0.001
	YF	4	0.37	-0.16, 0.89	0.170	86	<0.001
FT	SSF	29	0.11	-0.04, 0.26	0.149	73	<0.001
	SmF	5	0.43	0.04, 0.85	0.048	73	0.005
Seed type	Locust bean	3	0.22	-0.10, 0.54	0.184	0	0.487
	Rapeseed	9	-0.13	-0.27, 0.01	0.062	21	0.257
	Grape	4	0.80	0.28, 1.31	0.002	79	0.003
	Cotton	4	0.12	-0.08, 0.31	0.240	0	0.967
	Soybean	6	0.27	0.12, 0.42	< 0.001	0	0.818
	Millet	4	0.17	-0.10, 0.44	0.212	0	0.655
Feed class	Protein	24	0.04	-0.07, 0.15	0.514	47	0.007
	Energy	10	0.47	0.08, 0.86	0.020	86	<0.001
Rearing duration	Starter	11	0.09	-0.15, 0.32	0.473	71	<0.001
	Finisher	9	0.58	0.46, 0.92	0.044	65	0.010
	Overall	16	0.17	-0.03, 0.38	0.101	78	<0.001
Inclusion (%)	≤ 10	18	0.17	-0.03, 0.37	0.100	79	<0.001
	11-20	12	0.10	-0.13, 0.33	0.402	72	<0.001
	>20	4	0.22	0.01, 0.43	0.049	0	0.763

ADG average daily gain; *YF* yellow feathered; *FT* fermentation techniques; *SMD* standardized mean difference; *CI* confidence interval; *SSF* solid-state fermentation; I^2 Inconsistency index; *SmF* submerged fermentation; *p* probability

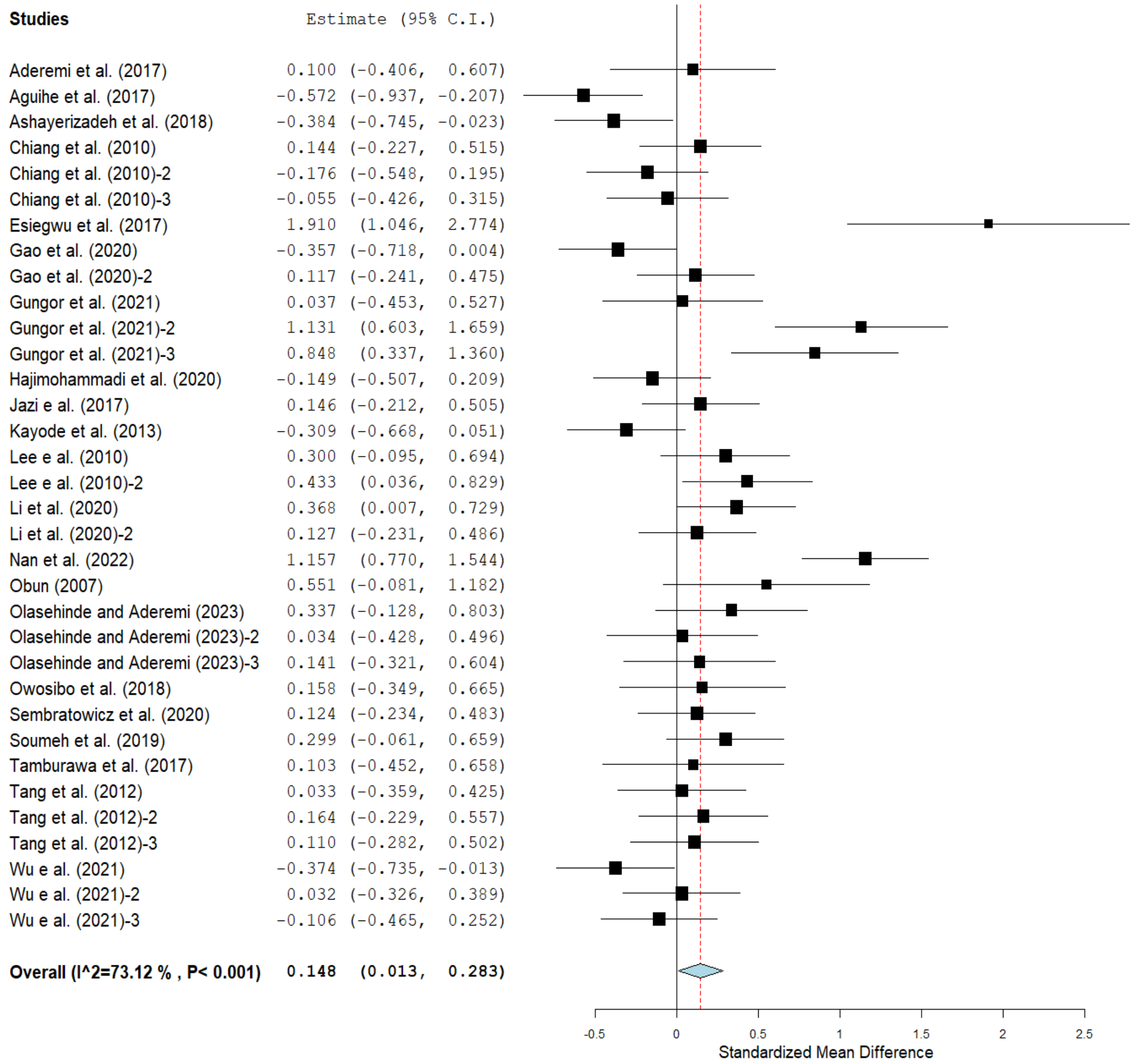


Fig. 3: ADG of broilers fed FTSM-based diets. The overall effect size is deemed significant when the lower and upper limits of 95% CIs (i.e., the lateral tips of the sky-blue diamond at the base of the forest plot) did not touch the line of null effect (SMD = 0) (Koricheva *et al*., 2013). The points to the left of the line of null effect, i.e., the thick vertical line indicate a decrease in ADG, while the points to the right connote an increase in ADG

Feed Conversion Ratio (FCR)

The forest plots of the influence of dietary FTSM treatment on the FCR of broilers are presented in Fig. (4). FCR (SMD = 0.11; p = 0.064) was not significantly different from controls. Table (7) presents the effect of studied covariates on FCR in broilers fed FTSM-based diets. The results indicate that Arbor Acres (SMD = 0.15; p = 0.033) and Cobb (SMD = 0.25; p = 0.019) fed FTSM-based diets had poor FCR. However, FTSM-based diets did not affect FCR in Anak (SMD = 0.56; p = 0.118), Yellow feathered (SMD = 0.05; p = 0.688), and Ross (SMD =-0.12; p = 0.067). In comparison to the control, broilers fed FTSM processed via the SSF method had poorer FCR. Broilers in the starter phase had a higher FCR. Results also indicate that broilers offered FTSM processed via the SmF method had similar. FCR with the controls. The results showed that FCR was poorer in broilers fed fermented rapeseed meals than in the controls. However, broilers that received fermented locust bean, grape, cotton, soybean, and millet had similar FCR with the control. The results showed that FCR was not affected by the feed class and inclusion levels. There was evidence of significant heterogeneity across the 23 studies that assessed the effect of dietary FTSM intervention on FCR (Q-statistic p<0.001, I^2-statistic = 65%; Table (4). Table (4) revealed that Egger's regression asymmetry test and Begg's adjusted rank correlation were not significant for FCR, ruling out the evidence of publication bias. Meta-regression results as displayed in Table (5) showed a very large effect for broilers and seed type as covariates and explained most of the sources of heterogeneity.

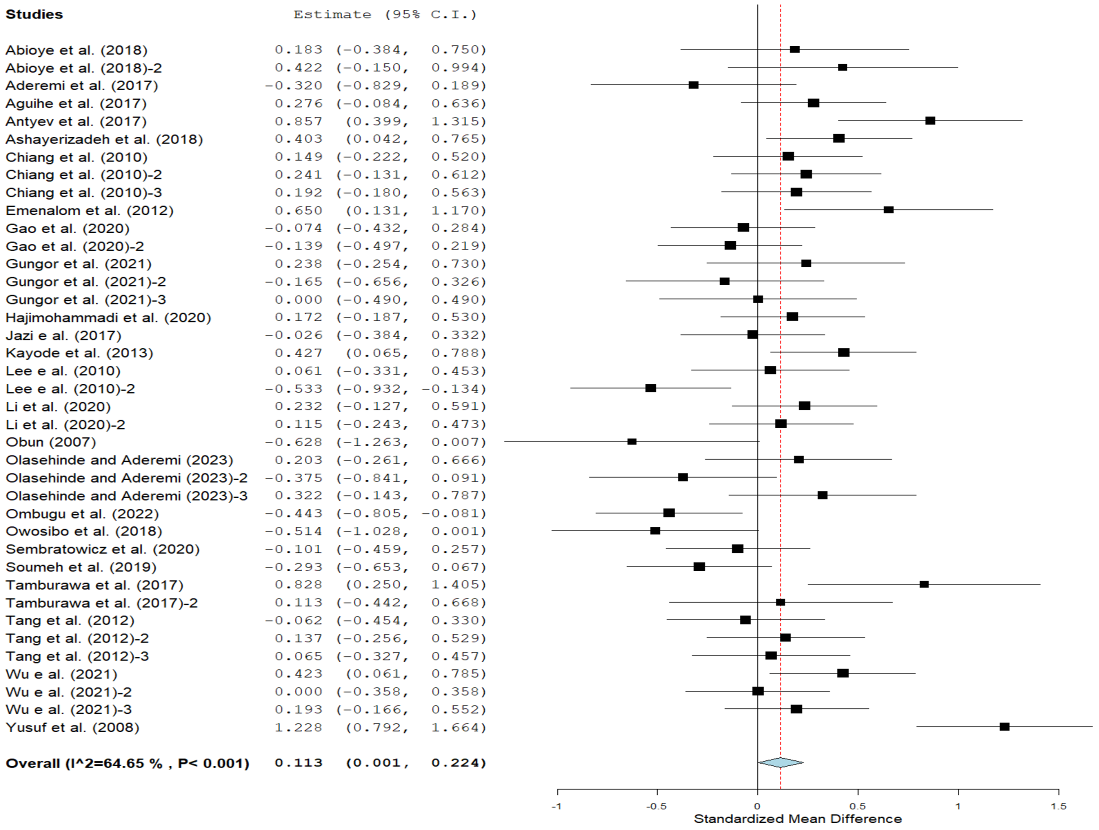


Fig. 4: FCR of broilers fed FTSM-based diets. The overall effect size is deemed significant when the lower and upper limits of 95% CIs (i.e., the lateral tips of the sky-blue diamond at the base of the forest plot) did not touch the line of null effect (SMD = 0) (Koricheva *et al*., 2013). The points to the left of the line of null effect, i.e., the thick vertical line indicate a decrease in FCR, while the points to the right connote an increase in FCR

Table 7: Subgroup effects of covariates on FCR of broilers fed FTSM-based diets

Covariates	Subgroup	Datasets	SMD (95% CI)		*P*-value	Heterogeneity	
						I^2 (%)	*P*-value
Broiler strains	Arbor Acres	11	0.15	0.01, 0.28	0.033	19	0.260
	Anak	4	0.56	-0.14, 1.25	0.118	87	<0.001
	Cobb	3	0.25	0.04, 0.46	0.019	00	0.536
	Ross	12	-0.12	-0.25, 0.01	0.067	22	0.229
	YF	3	0.05	-0.18, 0.27	0.688	00	0.778
FT	SSF	35	0.16	0.04, 0.28	0.009	63	<0.001
	SmF	5	-0.14	-0.44, 0.16	0.369	61	0.038
Seed type	Locust bean	4	0.01	-0.60, 0.60	0.996	77	0.004
	Rapeseed	9	0.15	0.02, 0.28	0.021	12	0.336
	Grape	3	0.02	-0.26, 0.31	0.868	00	0.520
	Cotton	4	0.03	-0.17, 0.22	0.792	00	0.893
	Soybean	6	-0.08	-0.30, 0.14	0.476	54	0.054
	Millet	3	0.05	-0.37, 0.47	0.815	59	0.085
Feed class	Protein	31	0.10	-0.04, 0.23	0.149	69	<0.001
	Energy	9	0.17	-0.02, 0.36	0.086	40	0.104
Production phase	Starter	12	0.31	0.07, 0.29	0.015	57	0.007
	Finisher	10	0.62	-0.32, -0.65	0.032	53	0.037
	Overall	19	0.15	-0.03, 0.33	0.100	72	<0.001
Inclusion (%)	≤ 10	22	0.12	-0.01, 0.25	0.077	50	0.007
	11-20	15	0.09	-0.13, 0.32	0.418	78	<0.001
	>20	3	0.05	-0.34, 0.45	0.791	61	0.077

FCR feed conversion ratio; *YF* yellow feathered; *FT* fermentation techniques; *SMD* standardized mean difference; *CI* confidence interval; *SSF* solid-state fermentation; I^2 Inconsistency index; *SmF* submerged fermentation; *p* probability

Table 8: Intestinal histomorphological parameters of broilers on dietary fermented seed meal

Variables	N (nc)	SMD (95% CI)	Heterogeneity				Egger test*	Begg test**
			df	Q	I^2 (%)	*P*-value	*P-value*	*P-value*
DVH	7 (16)	0.32 (-0.09, 0.73)	15	252.29	94.05	< 0.001	0.711	0.833
DCD	7 (16)	-0.40 (-0.70, 0.09)	15	143.53	89.549	< 0.001	0.218	0.616
DVH/CD ratio	5 (10)	0.97 (0.49, 1.46)	9	138.58	93.51	< 0.001	0.422	0.542
JVH	7 (16)	1.53 (0.82, 1.49)	15	155.15	90.33	< 0.001	0.444	0.872
JCD	7 (16)	-0.33 (-0.81, 1.44)	15	332.82	95.49	< 0.001	0.082	0.121
JVH/CD ratio	5 (10)	1.63 (1.22, 2.03)	9	85.59	89.48	< 0.001	0.076	0.286
IVH	5 (10)	0.65 (0.44, 0.85)	9	26.90	66.54	0.001	0.212	0.436
ICD	5 (9)	-0.72 (-1.01, -0.42)	8	44.78	82.13	< 0.001	0.316	0.873
IVH/CD ratio	4 (7)	1.41 (0.82, 2.01)	6	89.25	93.28	< 0.001	0.221	0.645

N number of publications; *nc* number of comparisons between fermented seeds group and control group; *SMD* standardized mean difference between broilers in fermented seeds group and control group; *CI* confidence interval of SMD; P-value to Q-statistic of heterogeneity; I^2 heterogeneity; *Egger's regression asymmetry test; **Begg's adjusted rank correlation; *df* degree of freedom; *DVH* duodenum villus height; *DCD* duodenum crypt depth; *DVH/CD* duodenum villus height/ crypt depth ratio; *JVH* jejunum villus height; *JCD* jejunum crypt depth; *JVH/CD* jejunum villus height/crypt depth ratio; *IVH* ileum villus height; *JCD* ileum crypt depth; *DVH/CD* ileum villus height/crypt depth ratio

Intestinal Histomorphology

Histomorphological indices of the intestine of broilers fed FTSM-based diets are shown in Table (8). The villus height in the duodenum, jejunum, and ileum of broilers fed FTSM-based diets was significantly ($p<0.05$) higher. Dietary FTSM significantly reduced the crypt depth in the ileum when compared to controls. In contrast, FTSM-based diets had no significant effect on the crypt depth of the duodenum and jejunum. Increased villus height-to-crypt depth ratios of the duodenum, jejunum, and ileum were recorded in broilers fed FTSM-based diets when compared to those fed diets without FTSM. Meta-regression was not conducted on intestinal histomorphological parameters because it is recommended by Ogbuewu and Mbajiorgu (2022); and Sierra-Galicia *et al.* (2023) that meta-regression analysis should not be applied to response variables that had less than ten studies due to low statistical power. Therefore, meta-regression was not applied to intestinal histomorphological measurements. Table (3) shows that Egger's regression asymmetry test and Begg's adjusted rank correlation were not significant for intestinal histomorphology measurements, so ruling out the existence of publication bias.

N number of publications; *nc* number of comparisons between fermented seeds group and control group; *SMD* standardized mean difference between broilers in fermented seeds group and control group; Confidence Interval (CI) of SMD; P-value to Q-statistic of heterogeneity; I^2 heterogeneity; *Egger's regression asymmetry test; **Begg's adjusted rank correlation; Degree of Freedom (DF); Duodenum Villus Height (DVH); Duodenum Crypt Depth (DCD); Duodenum Villus Height/ Crypt Depth ratio (DVH/CD); Jejunum Villus Height (JVH); Jejunum Crypt Depth (JCD); Jejunum Villus Height/Crypt Depth ratio (JVH/CD); Ileum Villus Height (IVH); Jileum Crypt Depth (JCD); ileum Villus Height/Crypt Depth ratio (DVH/CD)

Discussion

Growth Performance

The meta-analysis was designed to determine the growth performance and intestinal histomorphology of broilers fed FTSM-based diets. To the best of our knowledge, this is the first meta-analysis to assess the effects of FTSM-based diets on the growth performance and intestinal histomorphology of broilers. Broilers fed rations with FTSM gained more weight and had a higher feed intake, but did not change the FCR. Similar results were observed in broilers fed FTSM-based diets (Gungor *et al*., 2021) and fermented blends of cassava root meal and palm kernel cake (Chukwukaelo *et al*., 2018). This observation supports the findings of other researchers (Chen *et al*., 2013; Ashayerizadeh *et al*., 2017; Sugiharto and Ranjitkar, 2019), who discovered that fermented feeds lower gut pH, promote the proliferation of lactic acid bacteria through antagonistic behavior and competitive exclusion, increase the production of lactic acid and short-chain fatty acids and reduce the competition for readily available nutrients, leading to an increase in ADG and broiler productivity. Similarly, the metabolic activity of microbes during fermentation can increase the activities of digestive enzymes and other metabolites such as peptides, amino acids, and organic acids, which may improve gut health and function (Jazi *et al*., 2017). The increased ADG in broilers offered FTSM suggests a higher nutritive value and digestibility owing to the breakdown of soluble NSPs during fermentation (Sugiharto and Ranjitkar, 2019). Although the mechanism by which FTSM improves ADG in broilers is unclear, but may be attributed to the capacity of FTSM-based diets to modulate gut microbiota composition and enhance intestinal morphology.

Intestinal Histomorphology

Broiler growth performance and welfare depend on the proper functioning of the various anatomical segments of the small intestine. The intestinal mucosa performs key functions in feed digestion and nutrient uptake, shields the internal milieu from unfavorable luminal contents, and defends against pathogens and toxins (Sugiharto *et al.*, 2015). Studies have shown that VH, CD, and VH/CD ratio are important markers for the estimation of the absorptive potential of the small intestine, and absorption of dietary nutrients increases with increasing VH/CD ratio (Bai *et al.*, 2018; Li *et al.*, 2019). Higher VH and VH/CD ratios of the duodenum, ileum, and jejunum were observed in response to dietary FTSM. This indicates the high ability of the diet to improve the absorptive capacity of the small intestine. The observed higher VH and VH/CD ratio might be linked to fast epithelial cell regeneration in response to dietary FTSM intervention, which in turn results in the differentiation and growth of the crypts. The crypt is where enterocytes multiply and develop, enabling them to move and support villus formation (Li *et al.*, 2019). The enhanced intestinal morphology in the present meta-analysis may be partly responsible for the improved ADG in broilers fed FTSM-based diets.

Analysis of Moderators

Broiler Strains and Fermentation Techniques

Increased FI and ADG were seen in Ross broilers that received FTSM-based diets. The increased FI in Ross broilers suggests that the capacity to digest and utilize FTSM-based diets varies across broiler strains. Arbor Acres and Cobb strains had high FCR, suggesting a poor ability to utilize FTSM-based diets. However, this finding should be interpreted with caution especially for the Cobb strain because of the small sample size. These results support the earlier reports that chicken genetics affect its growth performance (Rondelli *et al.*, 2003; Sebola *et al.*, 2015). The significant linear relationship between FCR and broiler strains suggests that broiler strains are predictors of FCR in broilers and explain 66% of the observed heterogeneity. According to Sugiharto and Ranjitkar (2019), fermentation techniques are divided into two categories: Solid-State Fermentation (SSF) and Submerged Fermentation (SmF). Broilers fed seed meals processed using the SmF method, which involves the fermentation of solid substrates in free-flowing liquid, exhibited enhanced growth performance indicators in this meta-analysis, indicating that they can produce a variety of secondary metabolites (Sugiharto and Ranjitkar, 2019). However, the result should be interpreted with caution given that a limited number of studies were utilized for its computation.

Seed Type and Feed Class

Meta-regression showed a very large effect for seed type as a covariate and so more than 60% of the sources of variations on FCR and ADG are explained by seed type. This finding supports earlier reports that the positive effects of fermented feeds on broiler performance are linked to individual feed characteristics (Sun *et al.*, 2013; Sugiharto and Ranjitkar 2019; Nan *et al.*, 2022) reported that fermentation increased the concentration of crude protein and free amino acids in grape seed by 2+4% and 10%, respectively. In this study, broilers fed fermented grape seed meal gained weight at similar FI and FCR. The increased ADG in broilers fed fermented grape seed meal confirms the finding of (Nan *et al.*, 2022) that fermentation improves the amino acid contents of grape seed meal. The improved amino acid content of grape seed meal may result in an increase in the uptake of amino acids in the small intestine, which in turn promotes protein synthesis and muscle accretion. Soybean meal is an essential source of dietary protein in livestock feed and processed soybean meal constitutes about 20-30% of broiler feed (Chukwukaelo *et al.*, 2018; Ahiwe *et al.*, 2022). However, the presence of trypsin inhibitors in raw soybeans limits its usage in chicken feed (Zhang *et al.*, 2018; 2020; Yang *et al.*, 2018) and found that microbial fermentation improved the nutritional quality of soybean meal. The enhanced growth performance of broilers fed fermented soybean meal might be related to the increase in free amino acids that occurs in soybean meal after fermentation (Li *et al.*, 2020). Meta-regression showed a low effect for feed class as a covariate and so no more than 16% of the heterogeneity on ADG is explained by feed class. The improved ADG of broilers-fed fermented seeds used as a carbohydrate source over the control corroborates the earlier view that fermentation primarily targets carbohydrates via the action of microorganisms converting soluble carbohydrates to ethanol, lactic acid, and other organic acids in addition to carbon dioxide (Chukwukaelo *et al.*, 2018; Sharma *et al.*, 2020).

Inclusion Level and Production Phase

Growth performance indices were affected by inclusion levels, with the broilers that received FTSM at >20% inclusion level exhibiting higher FI and ADG than the control broilers. This suggests that the threshold level of FSTM for optimum growth performance in broilers is more than 20%. However, the observed result should be interpreted with caution given that a limited number of studies were utilized for its computation. Broilers fed FTSM at ≤10 and 11-20% had similar growth performance indicators, suggesting that these inclusion levels supported broiler productivity, which is similar to the findings of other researchers that inclusion of FTSM in broiler diets at 0.50-16.94% supported broiler production (Ashayerizadeh *et al.*, 2018; Li *et al.*, 2020; Gungor *et al.*, 2021). In the current meta-analysis, the production phase was not a significant predictor of growth performance parameters in broilers. However, FCR and ADG were enhanced more during the finisher production phase than during the starter production phase. This enhanced performance suggests the higher ability of finisher broilers to utilize FTSM-based diets than starter broilers. The mechanism for the poor growth performance of broilers during the starter production phase is not clear; however, it could be attributed in part to poor digestion and utilization of FTSM, as their digestive tracts were not fully developed to utilize the test diets. However, further studies are required to determine the inclusion level of FTSM that optimizes growth parameters in broiler chicks using an optimization model as well as to ascertain the influence of FTSM-based diets on the expression of genes that regulate growth performance in broilers; as such information is missing in the literature.

Conclusion

Fermented tropical seed meal can be included in a broiler diet at a level of more than 20% to increase feed intake and average daily gain at a similar feed conversion ratio. Pooled effect estimation indicated that dietary fermented tropical seed meal increased the villus height and villus height-to-crypt depth ratios of the duodenum, jejunum, and ileum of broilers but reduced the crypt depth of the ileum when compared to controls. The subgroup results indicate that broilers fed diets containing fermented grape seed meal and soybean meal at an inclusion level of >20% during the finisher production phase had increased FI and ADG. Meta-regression results indicate that seed type, feed class, and broiler strains accounted for the inconsistent performance results in broilers fed fermented tropical seed meal. The need for additional research on the effect of specific fermented seed types on the growth performance of broilers is highlighted by the present meta-analysis. The use of the optimization function to determine the inclusion levels of fermented tropical seed meals that support optimum growth performance in broilers is recommended.

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Author’s Contributions

All authors equally contributed to this study.

Ethics

The authors state that they have no competing interest to declare.

Data Availability

Data will be made available on reasonable request.

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