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Effect of multicarbohydrase enzymes containing α-galactosidase on the growth and apparent metabolizable energy digestibility of broiler chickens: a meta-analysis

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ABSTRACT

Exogenous enzyme supplementation is a valid strategy to improve nutrient availability and growth performance in broilers. Traditional carbohydrases, such as xylanase and β-glucanase, are well-researched solutions to increase the nutritional value of cereal grains, whilst reducing any negative impact of associated viscous polysaccharides. The feeding value of soybean meal and other protein rich oilcakes may not be fully exploited with traditional enzymes, as structures such as galacto-oligosaccharides and pectins require specific α-galactosidase, mannanase and other hemicellulolytic enzymes. This study aimed to summarize, in a meta-analysis, the results from independently run randomized controlled studies in various global locations that evaluated the effect of two distinct multicarbohydrase formulations, in which an α-galactosidase was combined with either xylanase (AGX) or β-glucanase (AGB). Through meta-regression analysis, the mean difference effects of AGX and AGB supplementation on broiler final body weight (BW), feed conversion ratio (FCR) and nitrogen-corrected apparent metabolizable energy (AME_N) were calculated for each relevant study. Fixed and random-effects models were used to compute the standardized mean difference (SMD) and confidence intervals, with corrective actions taken to ensure compliance with publication bias and heterogeneity by the Egger test and the Cochran Q test. Adjusted models showed that the AGX supplementation increased broiler BW (SMD=+30 g; 95 % CI: 0.08_0.48; P = 0.006) and improved FCR (SMD=−0.01 g/g; 95 % CI: -0.51_-0.11; P = 0.002). A composite evaluation of independent studies showed increased AME_N in broilers supplemented with AGX (SMD=+58 kcal/kg; 95 % CI: 0.45_1.10; P < 0.001). Furthermore, meta-analysis confirms that AGB supplementation increased BW (SMD=+56 g; 95 % CI: 0.32_0.91; P < 0.001), improved FCR (SMD=−0.04 g/g; 95 % CI: −0.76_−0.20; P = 0.006) and increased AME_N (SMD=+49 kcal/kg; 95 % CI: 0.30_1.07; P < 0.001). Overall, this meta-analysis found that dietary supplementation of broiler diets with the multicarbohydrases containing α-galactosidase considered in this evaluation, improved broiler growth during rearing periods of 35 days or more. These improvements may be supported by increases in energy utilization, as found in this study.

Abbreviations: AME_N, nitrogen-corrected apparent metabolizable energy; ANF, anti-nutritional factors; BW, body weight; CI, confidence interval; FCR, feed conversion ratio; NSP, non-starch polysaccharides; RCT, randomized controlled trial; SBM, soybean meal; SD, standard deviation; SMD, standardized mean difference.

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1. Introduction

The use of exogenous carbohydrases in broiler feed formulations has been common-place for several decades adding value to raw materials by mitigating the negative effects associated with anti-nutritional non-starch polysaccharides (NSP) (Adeola and Cowieson, 2011). Enzyme formulations comprising of either xylanase or β-glucanases and/or their combination used to hydrolyse cellulosic and hemicellulosic polysaccharides, namely arabinoxylans and β-glucans derived from cereal grains (Cozannet et al., 2017) are most representative of commercially available exogenous enzymes (Paloheimo et al., 2010).

Protein-rich oilcakes, such as soybean meal (SBM) and rapeseed meal, can be differentiated from most cereals and grains typical in monogastric diets by the presence of NSP structures comprised of galactose, glucose and mannose sugars (e.g. galactomannans), as well as smaller galacto-oligosaccharides (e.g. raffinose, stachyose) and various pectin based heteropolysaccharides (Choct et al., 2010). Traditionally, α-galactosidase, mannanase and other broad spectrum hemicellulolytic enzymes have been used to degrade these substrates in an attempt to reduce potential anti-nutritional factors (ANF) in monogastric diets (Jackson, 2010) and improve digestibility of nutrients derived from carbohydrates (Bedford, 2002). To this effect α-galactosidase has been investigated with varying degrees of success. Kidd et al. (2001) showed that enzyme preparations containing primarily α-galactosidase improved the feed efficiency of broilers fed corn-SBM diets. Increasing nutrient digestibility subsequent to the inclusion of α-galactosidase in low energy diets formulated with non-dehulled SBM has also been shown (Zhang et al., 2010). However, supplementation of broiler diets containing 15 % toasted guar meal with either α-galactosidase or β-mannanase did not improve broiler performance (Reddy et al., 2018), despite there being a calculated residual galactomannan content of 5% in the diet.

In recent years, multienzyme complexes delivering a range of activities designed for more complete utilization of cereal and protein-rich oilcake derived substrates have been developed. Jasek et al. (2018) reported significant improvements in ileal nutrient digestibility in broilers supplemented with a multicarbohydrase containing α-galactosidase and xylanase (AGX). Another multicarbohydrase with European Union approval as a zootechnical feed additive for broilers containing α-galactosidase and β-glucanase (AGB), increased broiler growth performance and feed efficiency (European Food Safety Authority (EFSA, 2011). These findings may support a holistic enzyme supplementation strategy targeting both the insoluble NSP fraction from cereals, as well as the oligosaccharide fraction from SBM as substrates in the whole diet.

The development and optimization of multicarbohydrase enzyme formulations should be validated through the evaluation of their effectiveness in increasing the nitrogen-corrected apparent metabolizable energy (AME_N) of the diet and broiler body weight (BW) as well as improving feed conversion ratio (FCR) in successive randomized controlled trials (RCT) (Morris, 1999). Therefore, the objective of this study was to conduct a meta-analysis using an internal database of RCT to evaluate the efficacy of both AGX and AGB supplementation to broiler feed in improving AME_N of the diet, broiler BW and FCR.

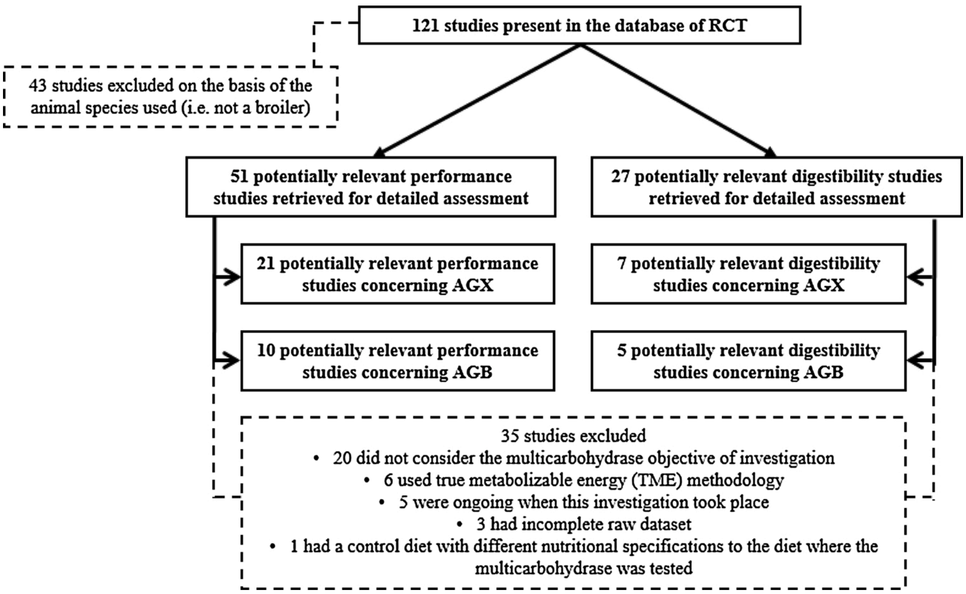

Fig. 1. Number of publications found in the methodical review of an internal database of randomized controlled trials (RCT) and number of studies selected for the meta-analysis evaluating the effect of a multicarbohydrase containing α-galactosidase and xylanase (AGX), and a multicarbohydrase containing α-galactosidase and β-glucanase (AGB) on the performance and energy digestibility of broilers.

2. Materials and methods

2.1. Selection of studies and description of data set

An internal database of independently conducted RCT was used to conduct a meta-analysis of results generated to date for multicarbohydrases containing α-galactosidase enzyme: 1) a commercially available multicarbohydrase containing α-galactosidase derived from *Saccharomyces cerevisiae* and other carbohydrases derived from *Aspergillus niger* and *Trichoderma citrinoviridae* (AGX, AlphaGal™ 280 P, Kerry Inc., WI, USA), and 2) another commercially available multicarbohydrase containing α-galactosidase derived

Table 1
Identification and characterization of the studies included in the meta-analysis.

Trial Code	Country Location	Year	Duration, days	Multicarbohydrase	Design	Animals	# TRT	N	N size	Feed Form
Performance studies										
US10-1	USA	2010	49	AGX	RCBD	Ross 708, straight run	5	10	45	pellet
US11-1	USA	2011	42	AGX	RCBD	Ross 308, straight run	8	8	50	pellet
IN12-1	India	2012	42	AGX	2 × 2 factorial	Cobb 400y, as hatched	4	4	50	mash
TH12-1	Thailand	2012	35	AGX	RCBD	Ross 308, males	5	10	25	pellet
TH13-1	Thailand	2013	35	AGX	RCBD	Ross 308, males	6	10	25	pellet
IN14-1	India	2014	42	AGX	RCBD	Cobb 400, males	6	13	25	mash
MX14-1	Mexico	2014	42	AGX	CRD	Ross 308, as hatched	4	14	50	mash
US14-1	USA	2014	42	AGX	RCBD	Cobb 500, males	5	12	35	pellet
US14-2	USA	2014	42	AGX	RCBD	Cobb 500, males	5	12	35	pellet
SA15-1	South Africa	2015	35	AGX	2 × 4 factorial	Cobb 500, males	8	12	24	pellet
US15-1	USA	2015	49	AGX	RCBD	n/a, males	6	12	45	pellet
US15-2	USA	2015	42	AGX	RCBD	Cobb 500, males	6	11	43	pellet
US16-3	USA	2016	42	AGX	RCBD	Cobb 700, males	5	9	10	mash
AR17-1	Argentina	2017	49	AGX	RCBD	Cobb 500, males	4	14	15	mash
BR17-1	Brasil	2017	42	AGX	CRD	Cobb 500, males	6	8	15	mash
MX17-1	Mexico	2017	49	AGX	RCBD	Ross 708, males	5	12	40	mash
US17-1	USA	2017	42	AGX	RCBD	Ross 708, males	6	10	25	pellet
US17-2	USA	2017	42	AGX	RCBD	Ross 708, males	6	12	30	mash
US17-3	USA	2017	42	AGX	RCBD	Ross 708, males	5	12	22	pellet
US17-4	USA	2017	49	AGX	RCBD	Cobb 500, males	6	10	36	pellet
US18-1	USA	2018	42	AGX	CRD	Ross 708, males	9	12	25	pellet
ES08-1	Spain	2008	42	AGB	CRD	Ross 308, males & females	4	12	22	mash
ES08-2	Spain	2008	42	AGB	CRD	Ross 308, males & females	4	12	22	mash
ES08-3	Spain	2008	42	AGB	CRD	Ross 308, males & females	4	12	22	mash
UK08-1	United Kingdom	2008	42	AGB	RCBD	Ross 308, males	4	12	40	mash
UK10-1	United Kingdom	2010	42	AGB	RCBD	Ross 308, males & females	3	10	40	mash
US11-2	USA	2011	42	AGB	RBCD	Cobb 500, males	5	12	20	mash
UK12-1	United Kingdom	2012	42	AGB	RCBD	Ross 308, males & females	6	8	40	mash
BE13-1	Belgium	2013	42	AGB	RCBD	Ross 308, males	5	8	28	mash
FR16-1	France	2016	42	AGB	RCBD	Ross 308, males	4	10	40	pellet
PL17-1	Poland	2017	42	AGB	RCBD	Ross 308, males	5	12	8	pellet
Digestibility Studies										
US16-1	USA	2016	21	AGX	RCBD	Cobb 500, males	4	18	8	mash
US16-2	USA	2016	21	AGX	RCBD	Cobb 500, males	6	15	8	pellet
US17-5	USA	2017	21	AGX	RCBD	Cobb 500, males	8	12	7	mash
US17-6	USA	2017	21	AGX	RCBD	Cobb 500, males	6	12	10	mash
US17-7	USA	2017	21	AGX	RCBD	Cobb 500, males	8	12	7	pellet
US18-1	USA	2018	21	AGX	RCBD	Cobb 500, males	8	12	7	pellet
US13-1	USA	2018	21	AGX	CRD	Ross 308, males	8	12	5	n/a
US10-1	USA	2010	20	AGB	RCBD	Cobb 500, males	8	8	4	n/a
UK14-1	United Kingdom	2014	21	AGB	RCBD	Ross 308, males	6	10	2	mash
UK15-1	United Kingdom	2015	21	AGB	RCBD	Ross 308, males	8	9	2	mash
PL17-1	Poland	2017	35	AGB	CRD	Ross 308, males	5	12	8	pellet
US17-18	USA	2017	21	AGB	RCBD	Cobb 500, males	8	12	6	mash

AGX, multicarbohydrase containing α-galactosidase and xylanase; AGB, multicarbohydrase containing α-galactosidase and β-glucanase; CRD, completely randomized design; RCBD, randomized complete block design.

Table 2

Summary of results from the meta-regression on the effect of supplementation with a multicarbohydrase containing α-galactosidase and xylanase (AGX; AlphaGal™ 280 P) in the original (Table 2a) and adjusted model (Table 2b) for final body weight (BW) and feed conversion ratio (FCR) of broilers.

2a)

	N	Original Unit Difference		Hedge's g		Publication Bias (Egger test)		Heterogeneity			Age effect	Change in effect			
												D 35-42		D 35-49	
		Mean	SD	Statistic	P	Statistic	P	I^2	Q	P	P	Statistic	P	Statistic	P
Original BW, kg	21	0.0450	0.06411	0.7582	<0.001	7.5983	<0.001	82.9 %	105.10	<0.001	0.660	0.1092	0.874	0.4947	0.534
Original FCR, kg/kg	21	−0.0249	0.04405	−0.7235	<0.001	−5.8037	<0.001	73.2 %	67.28	<0.001	0.014	−0.8327	0.125	−1.3332	0.033

2b)

	N	Original Unit Difference		Hedge's g		Publication Bias (Egger test)		Heterogeneity			Age effect	Change in effect			
												D 35−42		D 35−49	
		Mean	SD	Statistic	P	Statistic	P	I^2	Q	P	P	Statistic	P	Statistic	P
Adjusted BW, kg	18	0.0296	0.04374	0.2783	0.006	2.0223	0.060	4.1 %	15.63	0.407	0.417	−0.4022	0.157	−0.3125	0.341
Adjusted FCR, kg/kg	17	−0.0114	0.02383	−0.3128	0.002	−1.955	0.070	0.0 %	10.11	0.754	0.187	−0.4957	0.069	−0.4261	0.204

from *S. cerevisiae* and carbohydrases derived from *A. niger* (AGB, AGal-Pro® BL (4a17), Kerry Inc., Beloit, WI, USA). Each of these multicarbohydrases is recommended at a dose of 0.20 and 0.050 g/kg feed, respectively. AGX has a minimum α-galactosidase activity of 8 U/g (one α-galactosidase unit defined as the amount of enzyme that will produce one micromole of p-nitrophenol under the defined assay conditions) and 300 U/g of xylanase activity (one xylanase activity is defined as the amount of enzyme required to release one micromole or reducing sugar equivalents from arabinoxylan per minute under the defined assay conditions). AGB has a minimum α-galactosidase activity of 1000 U/g and 5700 U/g of β-glucanase activity (one β-glucanase unit defined as the amount of enzyme that will produce one milligram of reducing sugar (glucose equivalent) from β-glucan under the defined assay conditions). Therefore, AGX would deliver 1.6 U/kg and 60 U/kg of α-galactosidase and xylanase; whereas AGB would deliver 50 U/kg and 285 U/kg of α-galactosidase and β-glucanase, on a feed basis.

A methodical review was executed on an internal (or private) database with a total of 121 independently conducted studies, from various global locations and run under the supervision and expertise of personnel from multiple contract research organizations, between 2007 and 2018. The initial selection of studies was performed based on animal species investigated and only those applicable to broiler chickens were retained. Seventy-eight studies were maintained, of which 51 investigated the growth performance of broilers for a minimum period of 35 days, and 27 looked at the effect on energy digestibility. From these, only those experiments fulfilling the following criteria were assessed: 1) evaluated either of the relevant test products, namely AGX or AGB; 2) considered *in vivo* performance, including a control group with the same dietary composition as the treatment diet; 3) reported the BW and FCR of broilers OR determined AME_N of the diets; 4) raw data was available so that sample variation and duration of the study could be considered (i.e. standard deviation, (SD), sample size (N), respectively); and 5) the study was completed at the time of this assessment (Fig. 1). In studies where the growth performance was reported at intermediate time points between 35 days of age and the end of the evaluation period (typically 42 or 49 days), the impact of the duration of study on the change in treatment effect was also evaluated. Where several test product inclusion rates were used in individual studies, only the data corresponding to the manufacturer's recommended dose (0.2 g/kg or 0.05 g/kg for AGX and AGB, respectively) was included in the meta-analysis. This was done to avoid the over-weighting of a given study in the statistical model. The studies meeting the above mentioned criteria were coded to denote the country location for the experimental farm where the study took place (e.g. IN indicates India, US indicates USA) and the year in which the study was conducted (e.g. 14 relates to studies in 2014, 11 relates to studies in 2011), following by a number listing the studies in the same year (i.e. 1, 2, 3 etc). Table 1 provides specific information on the identification and characterization of each of the studies considered in the meta-analyses.

Reasons for omitting studies from the meta-analysis were: 1) test product did not comply with the description of composition defined as selection criteria (20 studies); 2) complete raw dataset not available (3 studies); 3) digestibility assessed as true metabolizable energy (6 studies); 4) control diet was different to formulation where the test product was included (1 study); and 5) studies were ongoing while this investigation took place (5 studies).

2.2. Statistical analysis

The mean difference between control and AGX or AGB using Hedge's *g* for pooling was considered in the calculation of a standardized mean difference (SMD), with consideration for the following classification for the effect size: small effect $0.2 \leq |g| < 0.5$; moderate effect: $0.5 \leq |g| < 0.8$; large effect: $|g| \geq 0.8$ (Hedges, 1981). The estimated effect sizes from the retained studies were visualized in forest plots. The summary effects across the studies were calculated using fixed and random effect models. The inverse weighting for pooling for between-study variance was used in random effect models. I^2, a heterogeneity measure to investigate common effect sizes and its dispersion among the studies used was performed (Huedo-Medina et al., 2006; Deeks et al., 2008; Borenstein et al., 2009). The Cochran's Q test for statistical heterogeneity analysed the statistical hypothesis that the true treatment effects (the effect size parameters) were the same in all the primary studies included in meta-analysis (Sutton et al., 2000). The Egger test for funnel asymmetry was applied to test for publication bias (Egger et al., 1997). Model selection was informed by removing 'biased' results systematically (i.e. one at a time) to determine the effect on the corresponding funnel plots as well as Egger's test. As the performance studies with AGX varied in duration, the effect of the test product was measured by meta-regression analysis using the mixed-effect model with final BW and FCR as outcomes. Where significant publication bias and heterogeneity of the data ($P < 0.05$) were found in the original meta-regression analysis, as highlighted by funnel plots and Egger's test, problematic studies were omitted, and the remainder studies conformed the adjusted model. Results presented in tabular format include both the original and adjusted meta-regression models, while forest plots are presented only for the adjusted model. Similarly, the change in terms of the original units of the measurement are presented, by applying the meta-analysis random weights for each study. Statistical analysis and visualization of SMD were performed using the *meta* package within the *RStudio* statistical program (RStudio, 2018).

3. Results

3.1. Effect of multicarbohydrases containing α-galactosidase enzyme on broiler performance

Analysis of the results from the dataset comprising the final 21 retained studies found a BW increase ($P = 0.001$; Table 2a) and FCR ($P < 0.001$) improvement in response to AGX supplementation. However, a significant publication bias ($P < 0.05$) and heterogeneity of the data set ($P < 0.05$) was also found. The I^2 index, representing the percentage of the variability in effect estimates due to heterogeneity ($P < 0.001$), was relatively large, suggesting also possible effects on BW from the varying study durations ($I^2 = 82.9$ %, $Q = 105.72$, $P < 0.001$). Similar heterogeneity was also characterised in the FCR dataset ($I^2 = 73.2$ %, $Q = 67.28$, $P < 0.001$).

Investigation of funnel plots and results from Egger's test found that 3 out of the 21 selected studies caused publication bias for final BW (MX14-1, US11-1 and MX17-1) and 4 studies out of the 21 RCT did likewise for FCR (IN14-1, US11-1, US10-1 and MX17-1). Consequently, these studies were omitted in the adjusted model to control for publication bias and heterogeneity ($P > 0.05$; Table 2b).

Results from the adjusted model indicated a significant effect of AGX on broiler final BW ($P = 0.006$; Table 2b) and overall FCR ($P = 0.002$). The meta-regression found that the response to AGX was not influenced by the age at completion of the study (e.g. 35, 42 or 49 days) for both BW ($P = 0.417$) or FCR ($P = 0.187$). This was also confirmed by a statistically insignificant effect of AGX on the change in effect size during the period from 35−42 days or 35−49 days ($P > 0.05$; Table 2b).

As a result, the meta-analysis for the adjusted model was considered independent of age at the end of the study. Significant increases in final BW of broilers supplemented with AGX were found in both fixed and random effect models when compared to the control group (SMD = 0.28; 95 % CI 0.08 to 0.48; $P = 0.006$; Fig. 2). The significant increase in terms of the original units of the measurement, using the meta-regression analysis random weights for each study, equated to 0.03 ± 0.044 kg/bird. A significant improvement in overall FCR was also found in broilers treated with AGX compared to the control group (SMD=−0.31, 95 % CI −0.51 to −0.11; $P = 0.002$; Fig. 3). The difference in overall FCR between the AGX and control broilers, was -0.011 ± 0.0238 kg feed/kg gain.

The data set pertaining to AGB included 10 individual RCT. The meta-analysis indicated that broiler BW increased ($P = 0.01$; Table 3a) and overall FCR was improved ($P = 0.004$) by dietary supplementation with AGB. Nonetheless, significant heterogeneity was observed across this dataset for both BW ($P = 0.004$; $I^2 = 63.2$ %) and FCR ($P = 0.004$; $I^2 = 62.9$ %). Egger's test indicated also significant publication bias, albeit only for overall FCR ($P = 0.043$). Funnel plots identified 2 outliers (UK10-1, FR16-1) out of the 10 selected RCT, contributing to the publication bias in the assessment of final BW, and therefore, both were omitted from the adjusted model. Furthermore, 1 study (US11-2) out of the 10 studies considered was eliminated from the adjusted FCR analysis. Since all studies were completed at the same age, a meta-analysis for the adjusted model was considered independent of the study duration (Table 3b).

Results indicated that AGB had a significant effect in increasing final BW when compared to the control group (SMD = 0.63, 95 % CI

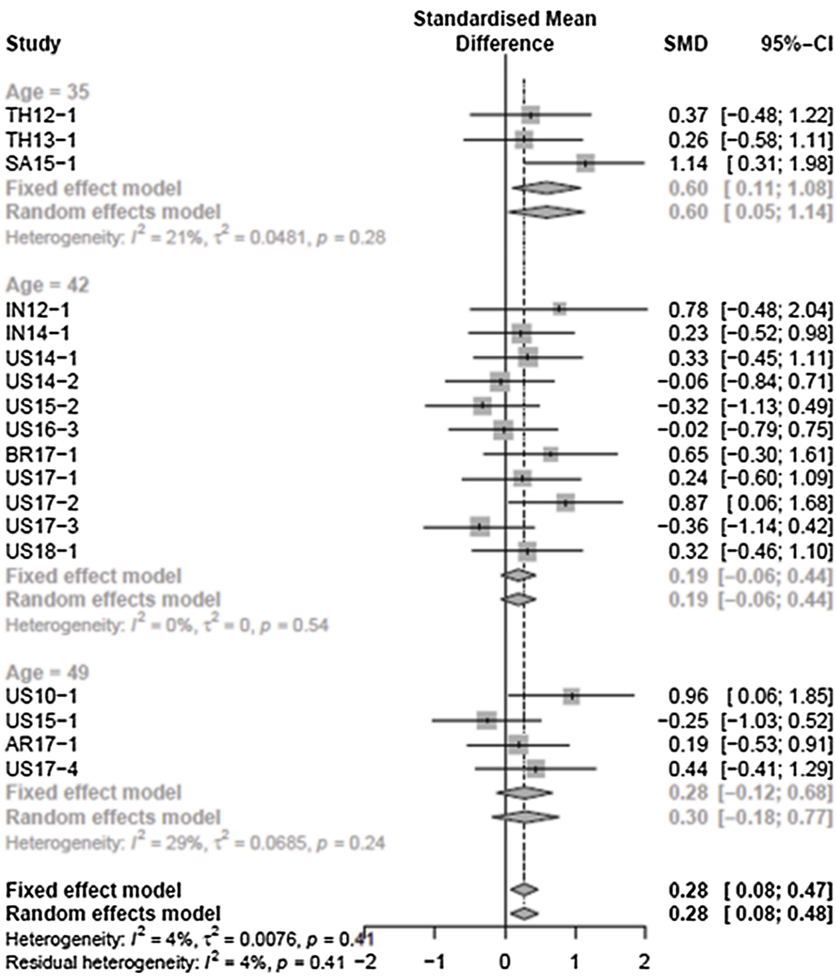

Fig. 2. Forest plot of 18 randomised, controlled experiments to study the effect of supplementation with a multicarbohydrase containing α-galactosidase and xylanase (AGX; AlphaGal™ 280 P) in final body weight (BW) of broilers.

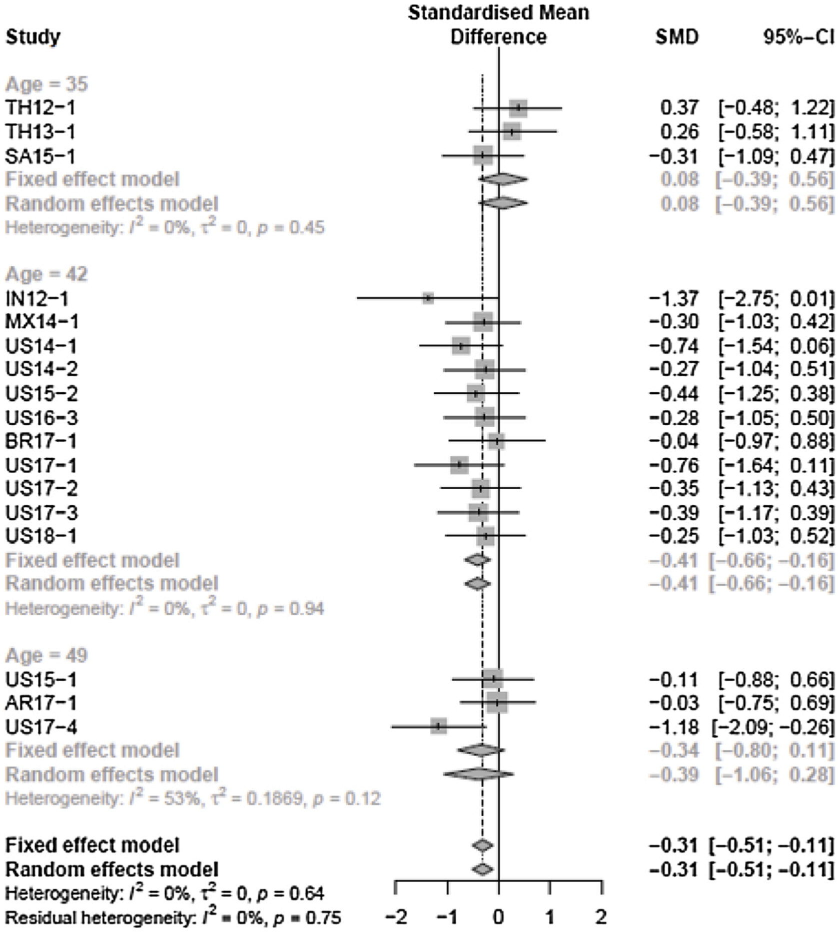

Fig. 3. Forest plot of 17 randomised, controlled experiments to study the effect of supplementation with a multicarbohydrase containing α-galactosidase and xylanase (AGX; AlphaGal™ 280 P) in overall feed conversion ratio (FCR) of broilers.

Table 3
Summary of results from the meta-analysis on the effect of supplementation with a multicarbohydrase containing α-galactosidase and β-glucanase (AGB; AGal-Pro® BL) in the original (Table 3a) and adjusted model (Table 3b) for final body weight (BW) and feed conversion ratio (FCR) of broilers.

3a)	N	Original Unit Difference		Hedge's g		Publication Bias (Egger test)		Heterogeneity		
		Mean	SD	Statistic	P	Statistic	P	I^2	Q	P
Original BW, kg	10	0.046	0.0507	0.5897	0.010	1.1874	0.269	63.2 %	24.45	0.004
Original FCR, kg/kg	10	−0.044	0.0463	−0.6605	0.004	2.4058	0.043	62.9 %	24.28	0.004

3b)	N	Original Unit Difference		Hedge's g		Publication Bias (Egger test)		Heterogeneity		
		Mean	SD	Statistic	P	Statistic	P	I^2	Q	P
Adjusted BW, kg	8	0.056	0.0460	0.6254	<0.001	1.2691	0.251	27.0 %	9.59	0.213
Adjusted FCR, kg/kg	9	−0.042	0.0492	−0.4916	0.006	−1.1547	0.286	35.5 %	12.37	0.135

0.28 to 0.97) (P < 0.001; Fig. 4). Average BW were 2.367 and 2.423 kg/bird in the control and AGB supplemented groups, respectively. Similarly, AGB improved broiler feed efficiency during the study period (SMD=-0.49, 95 % CI −0.84 to −0.14) (P = 0.006; Fig. 5). Overall FCR were 1.757 and 1.715 kg feed/kg gain in the control and AGB treated groups, respectively.

3.2. *Effect of multicarbohydrases containing α-galactosidase enzyme on AME_N*

Dietary supplementation with AGX, using the original data set of digestibility studies, increased AME_N compared to the control

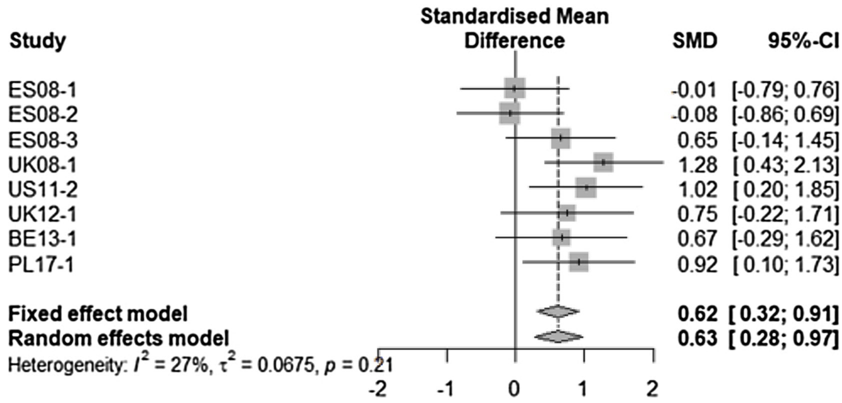

Fig. 4. Forest plot of 8 randomised, controlled experiments to study the effect of supplementation with a multicarbohydrase containing α-galactosidase and β-glucanase (AGB; AGal-Pro® BL) in body weight (BW) of broilers at 42 days of age.

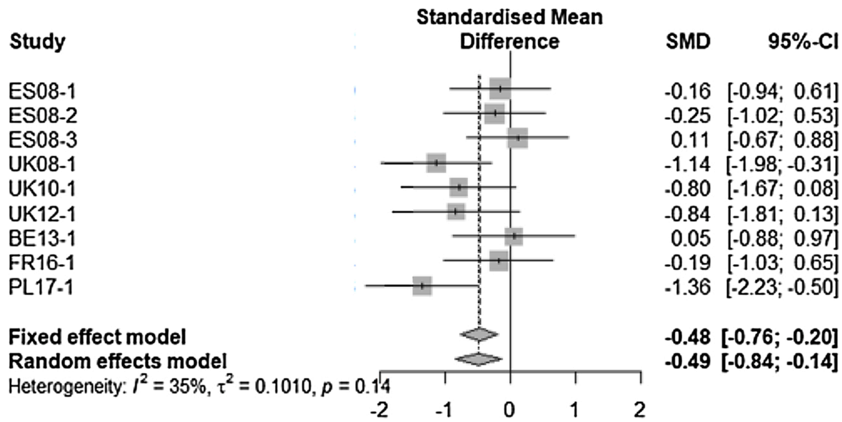

Fig. 5. Forest plot of 9 randomised, controlled experiments to study the effect of supplementation with a multicarbohydrase containing α-galactosidase and β-glucanase (AGB; AGal-Pro® BL) in feed conversion ratio (FCR) of broilers over a 42 days period.

group (P = 0.030; Table 4a). Funnel plots identified one outlier study (US16-1) out of the 7 selected, which contributed to a significant Egger's test value (P = 0.025) and likely heterogeneity, due to the relatively large I^2 (68.5 %; Q = 19.02; P = 0.004). When the model was adjusted to eliminate this data point (Table 4b), significant increases in the AME_N in broilers (SMD = 0.77, 95 % CI 0.38–1.17) (P < 0.001; Fig. 6) were found in response to AGX supplementation. Dietary AME_N was recorded as 3006 and 3064 kcal/kg in the control and AGX treated groups, respectively (Table 4b).

The original model investigating the effect of AGB comprised 5 independent studies (Table 4b) and the AME_N of broilers was found to increase in response to the AGB supplementation (SMD = 0.68, 95 % CI 0.30–1.07) (P < 0.001; Fig. 7). No adjustments were made to the model since publication bias measured by Egger's test was not statistically significant (P = 0.468; Table 4b) and data was compliant with heterogeneity requirements (I^2 = 0.0 %; Q = 3.00; P = 0.558). The average dietary AME_N in the control and AGB treated groups were quantified as 3002 and 3051 kcal/kg feed, correspondingly (Table 4b).

4. Discussion

This quantitative meta-analysis of data from RCT revealed that supplementation with multicarbohydrases containing α-galactosidase enzyme improved BW and feed efficiency in broilers. The positive effect of dietary supplementation with multicarbohydrases based on xylanase or β-glucanase and their mixtures, on broiler growth is widely reported (Cozannet et al., 2017; Dos Santos et al., 2017; Rios et al., 2017). However, this is to the authors' knowledge the first time that the benefits of multicarbohydrases containing α-galactosidase enzyme in broilers has been reported using a meta-analysis. The meta-analysis confirms a consistent positive impact of the investigated multicarbohydrases containing α-galactosidase enzyme on broiler growth performance, in line with findings from previous individual studies (Jasek et al., 2018; Llamas Moya et al., 2020).

The set of studies used in the evaluation of AGX varied in overall experimental duration (from 35 to 49 days) and in instances, they also differed regarding intermediate performance measurement points. Meta-regression and assessment of the age effect on the growth response of broilers to AGX supplementation indicated that age at point of measurement did not influence the effect of the enzyme

Table 4
Summary of results from the meta-analysis on the effect of supplementation with multicarbohydrases containing α-galactosidase and xylanase (AGX; AlphaGal™ 280 P) and α-galactosidase and β-glucanase (AGB; AGal-Pro® BL) in the original (Table 4a) and adjusted model (Table 4b) for nitrogen-corrected apparent metabolizable energy (AME_N) of broilers.

4a)

	N	Original Unit Difference		Hedge's g		Publication Bias (Egger test)		Heterogeneity		
		Mean	SD	Statistic	P	Statistic	P	I^2	Q	P
Multicarbohydrase containing α-galatactosidase and xylanase (AGX)										
Original AME_N, kcal/kg	7	36.92	62.039	0.5758	0.030	3.1725	0.025	68.5 %	19.02	0.004

4b)

	N	Original Unit Difference		Hedge's g		Publication Bias (Egger test)		Heterogeneity		
		Mean	SD	Statistic	P	Statistic	P	I^2	Q	P
Multicarbohydrase containing α-galatactosidase and xylanase (AGX)										
Adjusted AME_N, kcal/kg	6	58.32	40.877	0.7739	<0.001	0.8161	0.460	30.8 %	7.22	0.205
Multicarbohydrase containing α-galatactosidase and β-glucanase (AGB)										
AME_N, kcal/kg	5	48.51	16.272	0.6841	0.001	0.8288	0.468	0.0 %	3.00	0.558

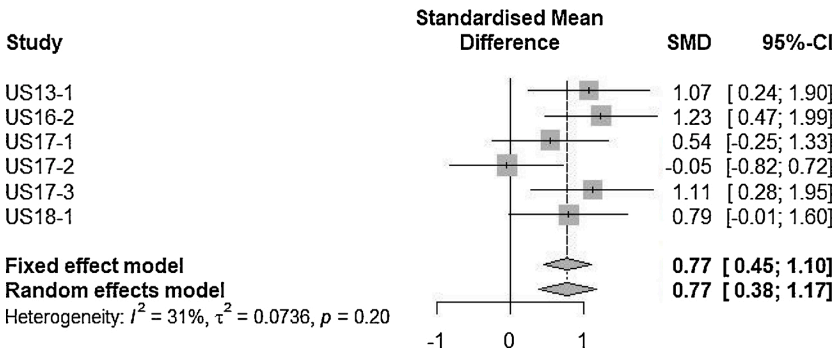

Fig. 6. Forest plot of 6 randomised, controlled experiments to study the effect of supplementation with a multicarbohydrase containing α-galactosidase and xylanase (AGX; AlphaGal™ 280 P) in nitrogen-corrected apparent metabolizable energy (AME_N) in broilers.

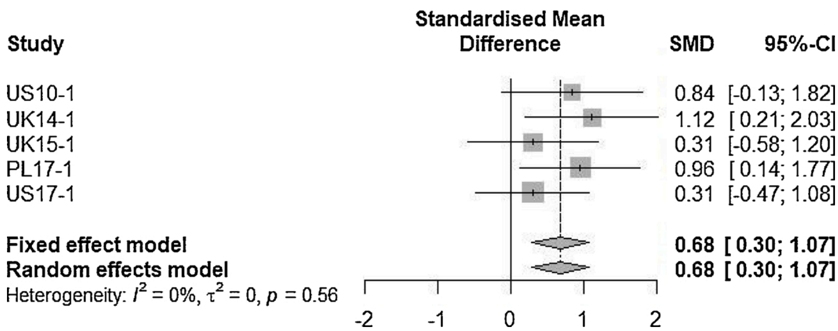

Fig. 7. Forest plot of 5 randomised, controlled experiments to study the effect of supplementation with a multicarbohydrase containing α-galactosidase and β-glucanase (AGB; AGal-Pro® BL) in nitrogen-corrected apparent metabolizable energy (AME_N) in broilers.

tested, during the age range evaluated. It has been reported in some instances that the efficacy of enzymes may be age dependent. A previous study found that supplementation with a cocktail of xylanase, amylase and protease increased nutrient retention to a greater extent in younger birds (Olukosi et al., 2007). However, results from the current study would suggest that the duration of studies evaluating AGX do not need to be greater than 35 days, as the benefit in BW and FCR of broilers is already seen at this age, and variations in the response are not expected with increasing age.

The benefits seen in broiler performance are likely reflective of improvements in nutrient digestibility. Solvent extraction and removal of soy-oligosaccharide ANF, such as raffinose and stachyose, has previously been reported to increase dietary AME_N in broilers (Leske and Coon, 1999). Furthermore, *in vivo* enzymatic hydrolysis of soluble arabinoxylans in wheat and corn has been shown to enhance nutrient absorption due to reduced viscosity in the bird's digesta (Gonzalez-Ortiz et al., 2016). Jasek et al. (2018) reported improvements in energy and amino acid digestibility in the ileum of broilers supplemented with AGX. Therefore, the improvements in nutrient digestibility with AGX and AGB supplementation, as indicated by increases in AME_N found here confirm the authors' working hypothesis using meta-analysis evaluation.

In practice, exogenous feed enzymes are formulated into diets with a corresponding matrix value to allow for the amount of nutrients that will be released when the enzyme is included in the diet (Barletta, 2010). Whilst the current study found an increase in AME_N due to AGX and AGB supplementation, it should be noted that this represents the magnitude of response in broilers at 21 days of age only and as such is not intended as guideline for the so called "matrix value". Other authors have suggested that the nutritional value of exogenous enzymes should be derived from empirical models, developed to quantify their potential for releasing nutrients (Plumstead and Cowieson, 2007), encompassing peculiarities in feed formulation and the average rearing period of broilers commercially (Rios et al., 2017). It is through practical experimentation, assessing the ability to counter the detrimental impact of nutrient dilution on growth performance over typical grow-out durations, that the authors from the current study propose as the most suitable method to determine the nutritional value of a given enzyme mixture for inclusion in the formulation matrix (Llamas-Moya et al., 2020).

In all the data sets and scenarios evaluated in this study and as reported by other authors (Cho et al., 2013; Blajman et al., 2014), various sources of publication bias and heterogeneity must be considered and corrected. It is accepted that funnel plot asymmetry can facilitate the detection of study effects contributing to publication bias (Egger et al., 1997), notwithstanding other factors that could explain these asymmetries (Peters et al., 2010). In the current study, subgroup analysis according to age did not remove this bias. There could be other contributing factors, outside the scope of the current meta-analysis, including correct dosing, particle size, ingredient inclusion in diets, diet storage conditions, enzyme stability and adequate recovery when pelleted feed was used, farm hygiene and overall health status of the animals. Therefore, the authors from this study acknowledge the presence of variation that could not be clarified (i.e. residual heterogeneity) by the factors included in the current study.

5. Conclusions

The results from this systematic review and meta-analysis support the efficacy of multicarbohydrases containing α-galactosidase enzyme in improving the growth performance of broilers over a minimum rearing period of 35 days. These improvements could be explained by increases in the energy utilization, which was found in the corresponding meta-analysis of digestibility studies. Appropriate correction measures to reduce the heterogeneity of the data set were considered, however, variation in the effect of the tested enzymes on broiler performance was not fully explained by age at conclusion of the studies. This highlights that other factors should be considered in future investigations, whereby dose level, ingredient selection and/or recoveries after pelleting should be included.

CRediT authorship contribution statement

S. Llamas-Moya: Conceptualization, Data curation, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing. **N.F. Higgins:** Writing - original draft, Writing - review & editing. **R. Adhikari:** Writing - original draft, Writing - review & editing. **P.G. Lawlor:** Writing - review & editing. **S. Lacey:** Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing - review & editing.

Declaration of Competing Interest

S. Llamas-Moya, N.F. Higgins and R. Adhikari declare that they are currently employed by Kerry Inc. and as such receive honorariums from this company for their function within this organization, which is beyond their specific contribution to this manuscript. P. Lawlor has no conflict of interest. S Lacey performed the statistical analysis for Kerry Inc in this study.

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