META-ANALYSIS OF RESPONSES OF SMALL RUMINANTS TO DIETARY PHYTOGENIC FEED ADDITIVE INTERVENTION

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Abstract. There are conflicting findings on the effect of phytogenic feed additives (PFA) on the productivity of small ruminants. The objective of this meta-analysis, therefore, was to evaluate the efficacy of PFA intervention in improving dry matter intake (DMI), ruminal fermentation, serum biochemical markers, and milk production in small ruminants. Studies on Scopus, Web of Science, ScienceDirect, Google Scholar, and PubMed were searched. Among the 852 articles retrieved, 14 were used for the analysis. Effect size was calculated using the random-effects model, and results were displayed as standardised mean differences (SMD) at 95% confidence intervals (CI). Results showed that methane production ($P = 0.007$), acetate production ($P = 0.030$), and acetate-to-propionate ratio ($P = 0.019$) were reduced by PFA with evidence of significant heterogeneity. In contrast, concentrations of total volatile fatty acids ($P = 0.046$) and propionate ($P = 0.039$) were increased by PFA intervention. Serum total proteins ($P = 0.009$) and serum glucose ($P = 0.028$) were increased, whereas serum urea was decreased ($P = 0.009$) by PFA intervention. Egger's test suggested no publication bias. Subgroup results showed that PFA intervention decreased methane production while increasing propionate production in sheep compared to goats. Meta-regression showed that moderators were significant predictors of the treatment effect on aspects of ruminal fermentation parameters and explained most of the heterogeneity in the effect of PFA intervention on ruminal fermentation parameters in small ruminants. These results suggest that PFA intervention enhanced ruminal fermentation and serum biochemical markers in small ruminants while reducing methane production.
Keywords: *phytogenics, ruminants, methane, milk yield, blood metabolites*

Introduction

Sheep and goat production plays an important role in improving food security and socio-economic well-being of rural and the peri-urban households in developing countries (Herrero et al., 2013). Under the smallholder production system, sheep and goats are fed agro-byproducts and underutilised browses (Bateki et al., 2019). According to Patra (2020) and Mizrahi et al. (2021), ruminants have the potential to convert low-quality fibre-rich materials to high-quality products (meat and milk), owing to the activities of microbes, which regulate fermentation processes in the rumen. However, the activities of these microbes during feed fermentation in the rumen result in various undesirable processes, such as excessive degradation of protein, methanogenesis, and the biohydrogenation of unsaturated fatty acids (Belanche et al., 2021). These undesirable activities not only result in the loss of dietary energy and poor protein utilisation but also exacerbate environmental issues by emitting methane gas and reducing the quality of meat and milk. Furthermore, the conversion of fibre-rich feed by rumen microbes to useful metabolites is also not efficient. Thus, ruminant nutritionists and researchers have long sought to overcome these problems by modifying rumen fermentation using nutritional approaches (Henderson et al., 2016; Candyrine et al., 2018; Ogbuewu and

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Mbajiorgu, 2023). Recent studies have focused on the use of probiotics and phytogenics (i.e., natural growth promoters derived from spices, essential oils, herbs, or secondary products of herbal origin) to enhance ruminal fermentation efficiency while reducing the environmental impact of ruminant production (Adegbeye et al., 2020; Faniyi et al., 2021; Singh et al., 2021; Singla et al., 2021; Hassan and Karsli, 2022; Ogbuewu and Mbajiorgu, 2022; Shilwant et al., 2023). Phytogenics are endowed with important bioactive compounds shown to have several biological and pharmacological effects, such as cholesterol-lowering, immunomodulation, anti-inflammatory, antimicrobial, antilipidemic, and antioxidant activity. Because of their antimicrobial effects, plants and plant-based products are added to ruminant feed in small amounts to modify rumen fermentation efficiency (Patra and Saxena, 2009). On the same hand, their antioxidant and immune-stimulating effects have also led to their use to enhance ruminant health and productivity (Olagaray and Bradford, 2019).

Inclusion of phytogenic feed additives (PFA) rich in phenolics and saponins at 10, 20, 30, and 40 g/kg in lactating goat diets increased total VFA and propionate production, reduced acetate-to-propionate ratio, and improved nutrient utilisation while reducing methane and ammonia production (Shilwant et al., 2023). Likewise, Al-Mamun et al. (2021) suggested that inclusion of pineapple, garlic leaf, moringa, and their mixture in the diet of mature sheep reduced methane production. These findings agree with Faniyi et al. (2021), who demonstrated that herbal mix has the potential to modify rumen fermentation and reduce methane emissions in small ruminants. On the other hand, Razo-Ortíz et al. (2022) reported that total VFA, acetate, propionate, butyrate, and acetate to propionate ratio were not affected by PFA intervention in sheep. Shilwant et al. (2023) demonstrated dietary PFA intervention at 10, 20, 30, and 40 g/kg diet in lactating beetal goats enhanced milk production but had no effect on blood metabolites. This finding is consistent with EL-Ghousein (2010), who discovered improved milk yield and components in Awassi ewes fed a basal diet supplemented with chamomile flowers or Nigella sativa seeds at 10 g/kg feed. In a similar study, EL-Ghousein (2010) found improved blood biochemical markers in Awassi ewes fed a basal diet supplemented with chamomile flowers or Nigella sativa seeds at 10 g/kg feed, which contrasted with the results of Shilwant et al. (2023). These discrepancies could be attributed to sex, quantity of PFA added to the diet, presentation form of PFA (extract vs powder), ruminant type (sheep vs goats), chemical composition of PFA used, and other factors that may affect ruminant health and productivity.

The use of meta-analysis to combine studies with conflicting findings to increase statistical power, identify reasons for inconsistent findings, create new insight, and discover knowledge gaps has in recent times gained attention in animal nutrition (Ogbuewu et al., 2020; Ogbuewu and Mbajiorgu, 2022). Presently, there are scanty publications on the meta-analysis of PFA intervention on ruminant health and productivity. Therefore, the objective of this meta-analysis was to ascertain the efficacy of PFA intervention in improving DMI, ruminal fermentation efficiency, serum biochemical markers, and milk production in small ruminants.

Materials and methods

Database selection, literature search, and eligibility criteria

Five bibliographic databases (Web of Science, Scopus, ScienceDirect, Google Scholar, and PubMed) were searched for original studies on the topic from October to

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December 2024. Studies were retrieved using the following keywords (small ruminants, sheep, goats, sheep and goats, lactating goats, lactating ewes, lactating small ruminants, herbs, spices, medicinal plants, herbal products, phytogenics, phytogenic feed additives, rumen fermentation, methanogenesis, methane production, methane emission, milk production, milk yield, milk components, blood parameters, blood metabolites, volatile fatty acids) combined with the Boolean operators (AND/OR), phrase, and wildcard searching. The reference section of retrieved studies was searched for other relevant studies. Studies were selected using the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) as updated by Page et al. (2021).

Studies were selected using the PICO framework as presented in *Table 1*. Inclusion criteria were (i) studies that assessed the effect of PFA intervention on health and productivity of sheep and goats; (ii) studies measured at least the following measured outcomes of interest: ruminal methane production, ruminal fermentation characteristics (pH, total volatile fatty acids (VFAs), acetate, propionate, butyrate, acetate-to-propionate ratio), DMI, milk production (milk yield, protein, lactose, or fat), blood metabolites (glucose, urea, triglycerides or cholesterol) in sheep and goats; (iii) the diets did not contain antibiotics or other production enhancing agents such as probiotics, prebiotics, immunomodulators, and among others; (iv) the experiment has a control treatment fed diet without PFA intervention, and (v) trial reported the mean of measured of the measured outcomes in each treatment group. The search conducted on the five bibliographic databases produced 852 studies. 783 published studies were excluded for appearing in two or more databases and studies not done in PFAs. An additional 50 studies were excluded after screening the title and abstract. Out of the 16 full-text articles remaining, five were excluded for not having a control group and not reported in outcomes of interest. Fourteen full-text articles met the predefined eligibility criteria and were used for the meta-analysis (*Figure 1*). The included articles were independently assessed for eligibility by all the authors and the controversy on whether to include a study or not in the present meta-analysis was resolved by consensus.

Table 1. *PICO (Population, Intervention, Comparators, and Outcomes) framework*

PICO	Search strategy	Exclusion criteria
Population	Small ruminants	Large ruminants
Intervention	Phytogenic feed additives (PFAs)	Not in PFAs
Comparators	Control group (without PFA supplementation)	
Outcomes	Ruminal methane production, ruminal fermentation characteristics, blood metabolites, milk yield, and milk production.	

Database development and statistical analysis

A database of 14 studies that met the inclusion criteria for meta-analysis was built as shown in *Table 2*. Data presented in graphical formats were extracted using WebplotDigitizer Version 4.5 (Rohatgi 2021). Data generated were analysed in Open Meta-analyst for Ecology and Evolution (OpenMEE) software (Wallace et al., 2016). Results were presented as SMD at 95% confidence interval (CI) and a random-effects model was used. The pooled results were categorised as small or low effect (0.2 |SMD| < 0.5); medium or moderate effect (0.5 |SMD| < 0.8); and large effect (|SMD| ≥ 0.8) following the method of Cohen (1992). Heterogeneity was calculated using the standard

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methods (Higgins et al., 2003). Statistical heterogeneity was examined using the standard method (Borenstein et al., 2010). Meta-regression analysis was also performed on the following studied covariates: ruminant type (sheep vs goats), breed, and PFA type considered a priori to influence the outcomes of the meta-analysis. Meta-regression analysis was not carried out on measured outcomes with <10 studies due to low statistical power (Ogbuewu and Mbajiorgu, 2022; Sierra-Galicia et al., 2023; Hernandez-García et al., 2024). Subgroup analysis by ruminant type (sheep vs goats) was done to determine its effect on the measured outcomes. However, subgroup analysis was not conducted for outcomes with < 3 comparisons because of low sample size (Koricheva et al., 2013; Ogbuewu et al., 2020). Publication bias, which is the propensity for research with positive or statistically significant outcomes to be published more often than studies with null or negative outcomes was assessed in this meta-analysis using the Egger's regression asymmetry test (Egger et al., 1997). All analyses were considered significant at a 5% probability level.

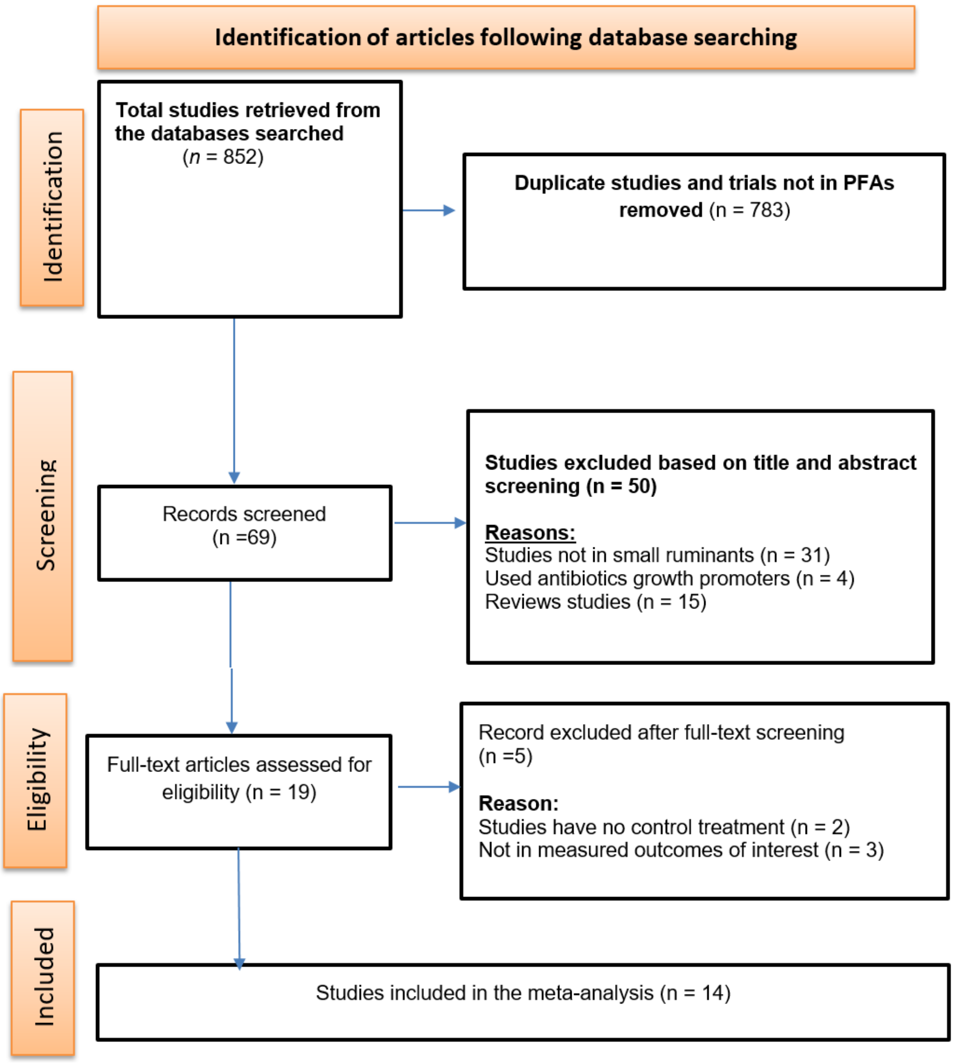

Figure 1. *Article selection steps for the study*

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Table 2. *Characteristics of studies used for the meta-analysis*

Study ID	Country	Continent	NT	BW* (kg)	Age (month)	Moderators		
						ST	**Breed**	PFA type
Faniyi et al. (2021)	Nigeria	Africa	7	-	-	Sheep	West African dwarf ewes	1
Chaturvedi et al. (2015)	India	Asia	11	-	-	Goats	-	2
Rabee et al. (2024)	Egypt	Africa	2	26.66	11-12	Goats	Shami male	3
Akanmu et al. (2020)	SA	Africa	8	-	-	Sheep	Merino	4
Marcos et al. (2019)	Spain	Europe	2	51.50	-	Goats	Dairy Murciano-Granadina	5
Al-Mamun et al. (2021)	Bangladesh	Asia	5	8.30	-	Sheep	Indigenous Bangladesh sheep	6
Rapetti et al. (2021)	Italy	Europe	3	48.50	-	Goats	Alpine goats	7
Jimenez et al. (2020)	Mexico	NA	3	12.25	3	Goats	Saanen goats	8
Dong et al. (2010)	China	Asia	4	45.00	-	Goats	Nanjiang Yellow goats	9
Wencelova et al. (2016)	SR	Europe	4	45.00	18	Sheep	Lacaune vs Suffolk	10
Shilwant et al. (2023)	India	Asia	5	-	-	Goat	Lactating Beetal goat	11
Razo-Ortíz et al. (2022)	Mexico	NA	4	23.52	-	Sheep	Hampshire x Suffolk	12
Abdelhamid et al. (2011)	Egypt	Africa	7	41.01	54	Goats	Lactating Zaraibi does	13
EL-Ghousein (2010)	Jordan	Asia	3	50.00	45.6	Sheep	Pregnant Awassi ewes	14

* - initial live body weight; NT – number of treatments; SA – South Africa; SR - Slovak Republic; ST – Small ruminant type, NA - North America

1- *Azadirachta indica*, *Moringa oleifera*, *Ocimum spp*, *Allium sativum*, *Zingiber officinale*, *Allium cepa*
2 - *Ocimum tenuiflorum, Curcuma longa, Phyllanthus emblica, Azadirachta indica, Clerodendrum phlomidis*
3 - Blends of *Zingiber officinale, Allium sativum, Artemisia vulgaris, Curcuma longa*
4 - *Aloe vera*, *Azadirachta indica*, *Moringa oleifera*, *Tithonia diversifolia, Jatropha curcas, Carica papaya*
5 - *Medicago sativa*
6 - Blends of *Ananas comosus* wastes, *Allium* sativum leaf, *Moringa oleifera*
7 - Whole *Linum usitatissimum*, *Cannabis sativa* seeds
8 - *Prunus virginiana* leaf
9 - *Medicago sativa* extract, *Artemisiae a*nnuae, herbal mix
10 - Herbal blend, *Helianthus annuus* L. seeds, herbal blend + *Helianthus annuus* L. seeds
11 - *Dolichos biflorus, Asparagus racemosus, Amoora rohituka, Punica granatum*
12 - *Chebulic myrobalan, Terminalia bellirica*, *Azadirachta indica*
13 - *Artemisia absinthium, Rosemarinus officinalis, Pimpinella anisum*
14 - *Matricaria chamomilla* flowers, *Nigella sativa* seeds

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Results

Study characteristics

Study selection process and characteristics of the 14 peer-reviewed journal articles used for the meta-analysis are shown in *Fig. 1* and *Table 2*, respectively. As shown in *Table 2*, studies used for the analysis were performed in 11 countries (Bangladesh, China, Egypt, India, Italy, Jordan, Mexico, Nigeria, Slovak Republic, South Africa, and Spain) that cut across four continents (Africa, Asia, Europe, and North America). Most of the publications used for the analysis were carried out in Asia (n = 5), followed by Africa (n = 4), and Europe (n = 3). The animals used for the study weighed between 8.30 and 51.50 kg.

Dry matter intake and ruminal fermentation characteristics

Data on the effects of PFA intervention on DMI and ruminal characteristics of small ruminants are presented in *Table 3*. Results showed that TVFA production was increased by PFA intervention. Results also revealed that DMI, rumen pH, and butyrate were not affected by PFA intervention in this meta-analysis. In contrast, PFA intervention reduced methane production (SMD = -0.12; -0.90, -0.66; P = 0.007) with evidence of significant heterogeneity (I^2 = 88%; P < 0.001). Proportion of ruminal acetate decreased (SMD = -0.10; -0.44, -0.24; P = 0.030), but proportion of ruminal propionate increased (SMD = 0.10; 0.31, 0.51; P = 0.039), which decreased the acetate-to-propionate ratio (SMD = -0.20; -0.68, -0.27; P = 0.019). The proportion of butyrate in the rumen was not significantly altered by PFA intervention.

Table 3. *Effect of PFA supplementation on DMI and ruminal characteristics of small ruminants*

Outcomes	n	SMD	95% CI		SE	p-val	Heterogeneity		ET
			Lower	Upper			I^2 (%)	p-val	p val
DMI kg/d	19	0.15	-0.20	0.50	0.18	0.404	0	0.834	0.602
MP kg/d	40	-0.12	-0.90	-0.66	0.40	0.007	89	< 0.001	0.091
TVFA mM	30	0.07	0.27	0.41	0.17	0.046	42	0.009	0.805
Acetate (A) mM	36	-0.10	-0.44	-0.24	0.18	0.030	51	< 0.001	0.213
Propionate (P) mM	36	0.10	0.31	0.51	0.21	0.039	64	< 0.001	0.050
A -to-P ratio	36	-0.20	-0.68	-0.27	0.24	0.019	72	< 0.001	0.132
Butyrate mM	35	0.16	-0.36	0.68	0.26	0.543	75	< 0.001	0.086
pH	25	0.01	-0.24	0.25	0.13	0.962	0	1.000	0.064

DMI, dry matter intake, MP, methane production; ET Egger test; SMD, standardised mean difference; SE, standard error; CI, confidence interval; p, probability; n number comparison; TVFA, total volatile fatty acids

Milk yield and milk components

The results of the influence of PFA intervention on milk yield and composition in sheep and goats are displayed in *Table 4*. Taking heterogeneity into consideration (I^2 = 60%; P = 0.004), results suggested that PFA intervention increased milk yield (SMD = 0.10; 0.38, 1.81; P = 0.003) in small ruminants. On the other hand, the concentrations of lactose, fat, and protein in the milk of sheep and goats fed diets supplemented with PFA were higher than those fed diets without PFA intervention.

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However, the differences observed were not significant. There was no evidence of significant heterogeneity across studies that evaluated the effect of PFA intervention on concentrations of protein (I^2 = 0%; P = 0.720), fat (I^2 = 35%; P = 0.113), and lactose (I^2 = 0%; P = 1.000) in the milk.

***Table 4.** Milk yield and components of small ruminants on PFA supplementation*

Outcomes	n	SMD	95% CI		SE	p-val	Heterogeneity		ET
			Lower	Upper			I^2 (%)	p-val	p val
Milk yield (kg/d)	12	1.10	0.38	1.81	0.37	0.003	60	0.004	0.0561
Milk fat g/kg	12	0.50	-0.03	1.03	0.27	0.064	35	0.113	0.214
Milk lactose g/kg	12	0.19	-0.21	0.59	0.20	0.358	0	1.000	0.235
Milk protein g/kg	12	0.32	-0.09	0.73	0.21	0.123	0	0.720	0.196

SMD, standardised mean difference; SE, standard error; ET Egger test; CI, confidence interval; p, probability; n, number comparison; I^2, Inconsistency index

Serum biochemical markers

The effects of PFA intervention on serum biochemical markers of sheep and goats are displayed in *Table 5*. In comparison to the control group, results suggested that serum total protein (SMD = 1.06; 0.26, 1.87; P = 0.009) and glucose (SMD = 0.09; 0.41, 0.59; P = 0.028) were higher in the PFA-supplemented group than those in the control group. In converse, serum urea levels were lower in the group offered PFA than in the control group (SMD = -0.99; -1.73, -0.25; P = 0.009). Concentrations of cholesterol and triglycerides in the serum of sheep and goats were not affected by PFA intervention. There is evidence of significant heterogeneity across studies that assessed the effect of PFA intervention on serum total protein, urea, cholesterol, and triglycerides in small ruminants.

***Table 5.** Effect of PFA supplementation on blood metabolites of small ruminants*

Outcomes	n	SMD	95% CI		SE	p-val	Heterogeneity		ET
			Lower	Upper			I^2 (%)	p-val	p val
Total protein g/dl	11	1.06	0.26	1.87	0.41	0.009	66	< 0.001	0.082
Glucose mg/dl	15	0.09	0.41	0.59	0.25	0.028	41	0.051	0.625
Urea mg/dl	15	-0.99	-1.73	-0.25	0.38	0.009	69	< 0.001	0.091
Cholesterol mg/dl	15	-0.45	-1.26	0.36	0.41	0.273	75	< 0.001	0.086
Triglycerides mg/dl	9	0.11	-0.93	1.14	0.53	0.843	77	< 0.001	0.214

SMD, standardised mean difference; ET Egger test; SE, standard error; CI, confidence interval; p, probability; n, number comparison; I^2, Inconsistency index

Subgroup analysis of ruminal fermentation markers

Table 6 summarises the results of restricted subgroup analyses of small ruminant types (sheep versus goats) on ruminal fermentation markers. Restricted subgroup analysis by ruminant types showed that PFA intervention reduced methane production in sheep but not in goats. Results showed that the proportions of acetate, propionate, and the acetate-to-propionate ratio in the rumen were not significantly affected by ruminant types. However, PFA intervention reduced ruminal acetate production and acetate-to-propionate ratio in sheep and goats. In contrast, PFA intervention increased propionate production in sheep but not in goats.

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Table 6. *Subgroup analysis of the effect of ruminant types on aspects of ruminal fermentation markers*

Outcomes	Subgroup	SMD	95% CI		SE	p-val
			Lower	Upper		
Methane production mL	Sheep	-1.33	-1.88	-0.79	0.28	< 0.001
	Goats	0.39	-0.62	1.40	0.52	0.453
Acetate (A) mM	Sheep	-0.77	-1.54	-0.02	0.39	0.041
	Goats	-0.40	-0.09	-0.71	0.16	0.012
Propionate (P) mM	Sheep	0.60	0.97	0.22	0.19	0.002
	Goats	0.54	-0.05	1.14	0.30	0.073
A-to-P ratio	Sheep	-0.38	-1.26	-0.50	0.45	0.401
	Goats	-0.12	-0.69	-0.44	0.29	0.676

SMD, standardised mean difference; CI, confidence interval; p, probability

Publication bias and meta-regression

Results as displayed in *Tables 3-5* revealed that Egger's regression asymmetry test was not significant in any of the measured outcomes, implying no publication bias. Significant heterogeneity was noticed in aspects of ruminal fermentation variables (methane production, total volatile fatty acid, acetate, propionate, acetate-to-propionate ratio, butyrate, pH), milk yield, and serum metabolite markers (total proteins, urea, cholesterol, and triglycerides) as shown in *Tables 3, 4,* and *5*, respectively. However, several researchers (Ogbuewu and Mbajiorgu, 2022; Sierra-Galicia et al., 2023; Hernandez-García et al., 2024) have recommended that meta-regression analysis should not be performed on measured outcomes with less than 10 studies because of poor statistical power. Therefore, meta-regression was done only on methane production, acetate, propionate, and the acetate-to-propionate ratio.

Table 7 revealed that there was a significant relationship between methane production and breeds ($P = 0.0001$). However, there was no relationship between methane production and the following moderators: small ruminant types ($P = 0.077$) and PFA type ($P = 0.906$). Breeds ($P < 0.001$) and ruminant types ($P = 0.005$) were predictors of the effect of PFA intervention on the proportion of acetate in the rumen.

Table 7. *Relationships between measured outcomes and studied moderators*

Items	Moderators	Q_M	df	p-val	R^2 (%)
Methane production mL	Breeds	30.02	8	0.0001	100
	Small ruminant type	3.13	1	0.077	5
	PFA type	22.1	32	0.906	0
Acetate (A) mM	Breeds	65.7	8	< 0.001	100
	Small ruminant type	7.79	1	0.005	20
	PFA type	21.3	31	0.903	0
Propionate (P) mM	Breeds	67.80	8	< 0.001	100
	Small ruminant type	6.11	1	0.013	17
	PFA type	97.2	31	< 0.001	100
A-to-P ratio	Breeds	55.2	8	< 0.001	91
	Small ruminant type	0.178	1	0.673	0
	PFA type	39.8	31	0.133	25

Q_M, coefficient of moderators; df, degree of freedom; R^2, the amount of heterogeneity accounted for

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In contrast, there was no statistical relationship between acetate and PFA type (P = 0.903). Breeds ($P < 0.001$), ruminant type (P = 0.013), and PFA type ($P < 0.001$) were predictors of treatment effects on the proportion of propionate in the rumen. A significant relationship existed between acetate-to-propionate ratio and breeds ($P < 0.001$). The results suggest that ruminant types (P = 0.003) and PFA types (P = 0.024) were not significant predictors of the acetate-to-propionate ratio.

Discussion

Phytogenic feed additives (PFAs) are good sources of important phytochemical compounds such as polyphenols, flavonoids, saponins, and tannins (Singh et al., 2020; Shilwant et al., 2023) and other important nutrients improve ruminant health and productivity (Shilwant et al., 2023; Rabee et al., 2024). The objective of the present study was to assess the effects of PFA intervention on ruminal fermentation characteristics, methane production, serum biochemical markers, milk production, and components in small ruminants using a meta-analysis method. The pooled analysis revealed that PFA intervention had no adverse effects on DMI in small ruminants. Razo Ortiz et al. (2020) found no significant difference in DMI of Hampshire x Suffolk lambs on polyherbal feed additives (0, 0.25, 0.375 and 0.5%), which supports the results of the present meta-analysis. In a similar experiment, other researchers (Redoy et al., 2020; Orzuna-Orzuna et al., 2021) found no significant changes in the DMI of small ruminants with dietary PFA intervention (0, 5 and 10 g herbal mixture/kg 0, 1, 2 and 3 g herbal mixture kg DM, respectively).

The results of the present study suggested that methane, one of the end products of microbial digestion in the rumen, was reduced by PFA intervention. Methane production in the rumen is reliant on the availability of hydrogen from anaerobic fermentation of ration by bacteria and protozoa, followed by the conversion of hydrogen and carbon dioxide to methane by methanogenic archaea. Although the exact mode of action of dietary PFA on methanogenesis is not clear. It is reported that phytochemicals such as saponins, tannins, polyphenols, and flavonoids improved feed efficiency and decreased methane production in ruminants by altering the biosynthesis of the cell membrane of archaea and hence inhibiting the proliferation of methanogens (Wallace et al., 2015; Rabee et al., 2020; Huang et al., 2021). The current meta-analysis showed that dietary PFA intervention reduced methane production in small ruminants, which agrees with the earlier findings of Faniyi et al. (2019) and Adegbeye et al. (2020) who reported that dietary PFA intervention (0 and 25% herbal mix and 10% essential oils) improved rumen microbial ecology, fermentation efficiency, and reduced methane production in ruminants.

The results suggest that rumen butyrate production and pH were not affected by PFA intervention in this study. On the other hand, PFA intervention significantly increased TVFA and propionate in the rumen. The results support the earlier view of Razo Ortiz et al. (2020) that PFA intervention at 0, 0.25, 0.375 and 0.5% proliferates the growth of propionate-producing bacteria, which increases TVFA production. The increased propionate production may have contributed to the decrease in methane production in the present meta-analysis given that propionate functions as a hydrogen sink, shifting hydrogen from methanogenesis to propionate synthesis (Adejoro et al., 2020). The significantly low acetate-to-propionate ratio in the PFA intervention group implies the enhanced ability of dietary PFA intervention to reduce acetate production in the rumen,

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an increase in propionate production in the rumen, or a shift from acetate to propionate. The significant decrease in the proportion of acetate in the PFA-supplemented group might be attributed to the inhibitory effect of several bioactive compounds present in PFA on acetate-producing bacteria or a decline in hydrogen production (Castro-Montoya et al., 2011; Adejoro et al., 2020).

Serum biochemical markers are used as an index of the nutritional quality of feed and feed material (Ogbuewu et al., 2015; Shilwant et al., 2023). The results revealed that PFA intervention increased total protein, indicating improved microbial protein synthesis. Urea (a waste product of amino acid breakdown) concentration was lower in the PFA group than in the control group, which could be attributed to improved protein utilisation efficiency. Dietary PFA intervention increased glucose levels in the serum, which was likely due to increased proportions of ruminal propionate, a precursor in glucose production. The significantly high serum glucose due to PFA intervention in this study seems to suggest that bioactive compounds in PFA intervention altered glucose metabolism in small ruminants. This finding is not consistent with others (Akanmu et al., 2020; Redoy et al., 2020), who noticed no significant difference in serum glucose in ruminants administered 50 mg herbal mix extract/kg. This disparity could be ascribed to differences in diet composition, type of PFA used, ruminant species, age, and sex that affect serum biochemical markers (Ogbuewu et al., 2015). Serum levels of cholesterol and triglyceride were not influenced, suggesting that PFA intervention did not affect lipid metabolism.

PFA intervention increased milk yield and had no effect on milk components (fat, lactose, and lactose) in lactating sheep and goats. Although the mechanism by which PFA intervention increases milk yield in small ruminants is not clear, however, the significantly high milk yield in the PFA-supplemented group could be linked to the ability of bioactive compounds in PFAs to enhance the growth of propionate-producing bacteria in the rumen, resulting in an increase in ruminal propionate and TVFA levels while decreasing ruminal acetate production. This finding agrees with Zhang et al. (2020), who reported that inclusion of propionic acid in cow diets improved milk production. Similar to the findings of the current meta-analysis, Abarghuei et al. (2013) and Shilwant et al. (2023) demonstrated that the addition of pomegranate peel extract (PPE) and composite plant extract (CPE) intervention at 400 ml/cow/d, 800 ml PPE/cow/d and 1200 ml PPE/cow/d) and 10, 20, 30, and 40 g CPE/kg, respectively ruminant improved milk production.

To date, no meta-analytical studies have explored the influence of ruminant type (sheep vs goats), breeds, and PFA type on methane production and rumen fermentation parameters in sheep and goats fed PFA-supplemented diets. The results of this study indicate that breeds are significant predictors of the effects of PFA intervention on ruminal methane production and proportions of acetate, propionate, and acetate-to-propionate ratio in sheep and goats. This observation disagrees with Duthie et al. (2017) who found similar methane production in crossbred Charolais and purebred Luing beef cattle fed concentrate-straw or silage-based diets. The observed variation in methane yield can be attributed to genetic differences. The current meta-analysis showed the small effect of ruminant type as a moderator for ruminal propionate and acetate production in sheep and goats, accounting for about 17 - 20% of the sources of variability across studies that assessed the impact of PFA intervention on propionate and acetate production. These results also showed that PFA type is a limiting factor in this study and explained 100% of the variability of ruminal propionate production. The effect of dosage, age, diet (mixed

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or concentrate), and presentation form (powder or extract) that may influence the response of small ruminants to PFA intervention was not analysed in this study due to low sample size. However, this warrants further investigation into the impact of dose level, preparation form, and age on ruminal methane production and fermentation parameters in small ruminants.

Conclusion

This meta-analysis showed that PFA intervention reduced methane production and the proportions of acetate and acetate-to-propionate ratio in the rumen. Results indicate that PFA intervention increased the concentrations of total volatile fatty acids and propionate in the rumen. Also, PFA intervention increased concentrations of serum total proteins and glucose while decreasing the level of serum urea. The subgroup results suggested that the magnitude of effect of PFA on methane production and propionate production was higher in sheep than in goats. Meta-regression found significant relationships between aspects of ruminal fermentation parameters (methane production, acetate, propionate, acetate-to-propionate ratio) and studied moderators (breeds, ruminant type, and type of PFA). Results showed the presence of heterogeneity across studies included in the meta-analysis, and studied moderators explained most of the sources of heterogeneity. Therefore, this meta-analysis suggests that PFA can improve aspects of ruminal fermentation efficiency and serum biochemical markers in small ruminants while reducing environmental burdens linked to methane production. However, more studies are needed to determine the dose levels of PFA and their blends that optimised DMI, ruminal fermentation, milk production, and serum biochemical markers in small ruminants. The effect of age as a moderator on the impact of PFA intervention on DMI, ruminal fermentation, milk production, and serum biochemical markers in small ruminants could be determined in the present study due to sample size, and future studies should be directed in this area.

Author contribution. CAM, IPO and MM conceptualised and designed the meta-analysis. CAM and IPO conducted literature search and analysed the data. IPO and MM wrote the draft. CAM edited the draft, whereas all authors read and approved the final manuscript.

Conflict of interest. CAM, IPO and MM have no conflict of interest to state.

Animal welfare statement. The authors adhered to the ethical policies of the journal, as noted on the journal's author guidelines page. This study is a meta-analysis and does not require ethics approval.

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