



Research article

The effects of herbal plant extract on the growth performance, blood parameters, nutrient digestibility and carcass quality of rabbits: A meta-analysis

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ABSTRACT

A meta-analysis was conducted to determine the effects of herbal plant extracts on the growth performance, blood parameters, nutrient digestibility and carcass quality of farmed rabbits. A dataset was created from 33 in vivo studies comprising 121 experimental units. Statistical meta-analysis was performed using a random-effects model and linear-mixed model meta-regression using R software (v. 4.3.0). Our results showed that although supplemental herbs did not affect average daily gain (ADG) and final body weight (BW), they reduced ($P < 0.01$) feed conversion ratio and mortality and increased the digestibility of dry matter (DM) ($P = 0.014$) and crude protein (CP) ($P = 0.018$). The herbal extracts also increased ($P = 0.037$) blood high-density lipoprotein (HDL) and decreased ($P = 0.004$) low-density lipoprotein (LDL). Immunoglobulin M (IgM) was elevated ($P = 0.009$) by herbal plant extract supplementation, although most blood components were unaffected. The inclusion of herbal plant extract up to 300 g/kg increased ($P = 0.011$) carcass percentage while the weight and percentage of other organs were unaffected. Subgroup meta-analysis further explained the different effect of the type of herbal plant extract. Moringa, olive oil, and pepper were more favourable to increase final BW compared to the other herbs. Interestingly, the majority of herbs showed efficacy in reducing mortality. A majority of the

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Table 1

Studies included in the meta-analysis of the effects of herbal plant extract on the growth performance, blood parameters, nutrient digestibility, and carcasses quality of rabbit [3–36].

No	References	Level given (g/kg)	Form	Type	Periods (d)	Strain of rabbit	Parameters Reported
1.	[27]	0–3	Powder	Turmeric (<i>Curcuma longa</i>)	63–84	Crossbreed	BWG, FCR, ADG, Mortality, ADFI, Carcass, slaughtered weight, kidney, liver, heart, DMD
2.	[28]	0–300	Powder	Cauli Flower (<i>Brassica oleracea</i>)	40–100	NZ White	BWG, FCR, ADG, ADFI, Lightness, Redness, Yellowness, DMD, CPD
3.	[8]	0–0.7	Liquid	Garlic (<i>Allium sativum</i>)	60–100	Californian	BWG, FCR, ADG, Mortality, ADFI, RBC, WBC, CPD
4.	[17]	0–1.4	Powder	Licorice (<i>Glycyrrhiza glabra</i>)	35–84	Not Reported	BWG, FCR, ADG, Mortality, ADFI, Carcass, slaughtered weight, thymus kidney, liver, heart, Lightness, Redness, Yellowness, DMD, CPD
5.	[22]	0–0.4	Powder	Purple Loosestrife (<i>Lythrum salicaria</i>)	35–84	160 Hycole	BWG, FCR, ADG, Mortality, ADFI, RBC, WBC, Lymphocyte, Haemoglobin, Total Protein, Cholesterol, Triglyceride, Albumin, kidney, liver, heart, Lightness, Redness, Yellowness, DMD, CPD
6.	[3]	0–5.5	Powder	Thyme (<i>Thymus vulgaris</i>) and Spirulina (<i>Spirulina Turpin</i>)	7–21	Coloured Dwarf	BWG, ADFI, slaughtered weight, DMD, CPD
7.	[14]	0–0.2	Liquid	Oregano (<i>Origanum vulgare</i>) and Rosemary (<i>Sabia rosmarinus</i>)	30–80	NZ White	Mortality, ADFI, Carcass, slaughtered weight, DMD, CPD,
8.	[15]	0–10	Powder	Silybum (<i>milk thistle</i>)	35–77	Pannon Large	Mortality, ADFI, Carcass, slaughtered weight, thymus kidney, liver, heart, Lightness, Redness, Yellowness,
9.	[4]	0–1	Liquid	Thyme (<i>Thymus vulgaris</i>)	30–63	V-line	BWG, FCR, ADG, Mortality, ADFI, RBC, WBC, Lymphocyte, Haemoglobin, Total Protein, Cholesterol, Triglyceride, Albumin, HDL, LDL, igG, IgM kidney, liver, heart, Carcass, slaughtered weight
10.	[26]	0–1	Powder	Bay Leaf (<i>Laurus nobilis</i>)	28–56	Crossbreed	FCR, ADG, Carcass, slaughtered weight, DMD, CPD
11.	[31]	0–1	Powder	Sage Leaf (<i>Salvia officinalis</i>)	48	Bianca Italiana	FCR, ADG, Mortality, Carcass, slaughtered weight, thymus kidney, liver, heart, Lightness, Redness, Yellowness, DMD, CPD
12.	[5]	0.1–1.5	Powder	Olive (<i>Olea europaea</i>) and Thyme (<i>Thymus vulgaris</i>)	28–60	Californian	BWG, FCR, ADG, Mortality, ADFI, Carcass, slaughtered weight, kidney, liver, heart,
13.	[7]	0–1.5	Liquid	Black Pepper (<i>Piper nigrum</i>)	50–91	NZ White	BWG, FCR, Mortality, RBC, WBC, Lymphocyte, Haemoglobin, total protein, albumin, HDL, LDL, igG, IgM, Carcass, slaughtered weight, kidney, liver, heart,
14.	[19]	0–2	Powder	Red Hot Pepper (<i>Capsicum frutescens</i>)	56	NZ White	BWG, FCR, ADG, Mortality, ADFI, RBC, WBC, Lymphocyte, Haemoglobin, HDL, LDL, igG, IgM, Carcass, slaughtered weight, kidney, liver, heart,
15.	[16]	0–0.9	Powder	Red Sage (<i>Salvia miltiorrhiza</i>)	35–63	Hyla	FCR, ADG, Mortality, ADFI
16.	[25]	0–0.02	Powder	Alligator Pepper (<i>Aframomum melegueta</i>)	56	NZ White	BWG, FCR, Mortality, ADFI, Carcass, slaughtered weight, kidney, liver, heart
17.	[24]	0–700	Powder	Drumstick tree (<i>Moringa oleifera</i>), Garlic (<i>Allium sativum</i>), Ginger (<i>Zingiber officinale</i>), and (<i>Piper nigrum</i>)	63–91	Not Reported	BWG, ADFI, ADG
18.	[13]	0–300	Powder	Garlic (<i>Allium sativum</i>)	Not reported	NZ White	RBC, WBC, Lymphocyte, Haemoglobin, Total Protein, Cholesterol, Albumin, igG, liver, heart,
19.	[6]	0–0.4	Powder	Oregano, pepper (<i>Capsicum</i>)	28–63	V-Line	BWG, FCR, Mortality, RBC, WBC, Lymphocyte, Glucose, Total Protein, Cholesterol, Albumin, igG thymus kidney, liver, heart
20.	[20]	0–0.75	Powder	Indian frankincense (<i>Boswellia serrata</i>)	0–42	NZ White	FCR, ADFI, RBC, WBC, Lymphocyte, Glucose, carcass, kidney, liver, heart,
21.	[29]	0–200	Powder	Wormwood (<i>Artemisia absinthium</i>)	35–119	NZ White	FCR, ADFI
22.	[10]	0–0.3	Powder	Pepper (<i>Capsicum</i>)	14–84	Crossbreed	BWG, FCR, ADFI, DMD, CPD.
23.	Zanouny and Elwan (2017)	0–0.2	Powder	Pepper (<i>Capsicum</i>)	32–56	NZ White	Total Protein, Albumin, Kidney, liver,
24.	[33]	0–0.01	Liquid	Lingzhi (<i>Ganoderma lucidum</i>)	80–102	SIKA	BWG, FCR, ADFI
25.	[9]	0–0.08	Liquid	<i>Rauvolfia vomitoria</i>	60–67	Not Reported	RBC, WBC, Lymphocyte, Total protein, Albumin,

(continued on next page)

Table 1 (continued)

No	References	Level given (g/kg)	Form	Type	Periods (d)	Strain of rabbit	Parameters Reported
26.	[12]	0–0.08	Liquid	<i>Rauvolfia vomitoria</i>	60–72	Not Reported	BWG, FCR, Mortality,
27.	[23]	0–0.05	Powder	Turmeric (<i>Curcuma longa</i>)	35–39	IRA	BWG, FCR, ADG, ADFI, Carcass, slaughtered weight, thymus, liver, CPD
28.	[32]	0–1.5	Powder	Clove (<i>Syzygium aromaticum</i>)	6–14	NZ white	ADFI, BWG, FCR Cholesterol, HDL, LDL, IgG, IgM, DMD, CPD
29.	Khaled and Qataf (2022)	0–0.04	Powder	Garlic (<i>Allium sativum</i>)	25–31	NZ white	Total Protein, Glucose, cholesterol, triglyceride, HDL, LDL.
30.	[35]	0–0.70	Powder	Pepper (<i>Capsicum annum</i>) Anise (<i>Pimpinella anisum</i>) Thyme (<i>Thymus vulgaris</i>) Mint (<i>Mentha spicata</i>) Garlic (<i>Allium sativum</i>) Rosemary (<i>Salvia rosmarinus</i>) Black cumin (<i>Nigella sativa</i>)	5–13	NZ white	FCR, BWG, ADFI, Carcass weight,
31.	Ragab et al. (2022)	0–0.06	Powder	Maca (<i>Lepidium meyenii</i>)	6–20	V-line	FCR, ADG, ADFI, RBC, WBC, Lymphocyte, Haemoglobin Total protein, glucose, triglyceride, albumin, HDL, LDL, carcass weight, kidney, liver, heart,
32.	[11]	0–1.0	Powder	Orange (Citrus)	6–14	NZ white	BWG, FCR, ADG, Mortality, ADFI, RBC, WBC, Lymphocyte, Glucose, Cholesterol, Triglyceride, HDL, LDL, Carcass, kidney, liver, heart, DPD, CPD
33.	[36]	0.-0.5	Powder	Rosemary (<i>Salvia rosmarinus</i>) Thyme (<i>Thymus vulgaris</i>) Black cumin (<i>Nigella sativa</i>) Fenugreek seeds (<i>Trigonella foenum-graecum</i>)	28–77	NZ white	ADG, ADFI, FCR, BWG, DMD, CPD, Carcass, slaughtered weight

ADFI – average daily feed intake; ADG – average daily gain; BWG – body weight gain; DMD – dry matter digestibility; CPD – crude protein digestibility; FCR – feed conversion ratio; HDL - high-density lipoprotein; LDL - low-density lipoprotein; IgG - Immunoglobulin G; IgM - immunoglobulin M; NZ – New Zealand; RBC – red blood cells; SW – slaughtered weight, WBC – white blood cells.

response variables in our meta-analysis showed no dose-response effect except for ADG, mortality, HDL, and LDL which were improved by herbs supplementation. The evidence from the perspective of both meta-analysis and meta-regression shows that the addition of herbs tends to positively affect the parameters for production performance and blood metabolites in farmed rabbits.

1. Introduction

Since their introduction in the 19th century as livestock animals, rabbits have been domesticated in European countries and worldwide. Rabbits farms have long been farmed for their meat and fur, with additional breeds introduced later, including New Zealand White, Chinchilla, Rex, V-line, and even crossbreeds. Rabbits are herbivorous animals with a digestive strategy that enables them to re-digest their soft faeces [1], known as caecotrophy. This results from the separation of digested particle feed eaten by the rabbit [1].

Herbs have many active biological properties that positively impact modern animal nutrition. From the outset, their use in the animal industry was instinctive. Ancient history records the beneficial effects of herbs, including antioxidant, anti-inflammatory, antimicrobial, and immune stimulating [2]. Until the advent of antibiotic growth promoters (AGPs) in the 20th century, herbs were the source of alternative feed additive treatment and a natural source additive [2]. However, the decreasing efficacy of synthetic drugs and the increasing contraindications of their usage have again made the use of natural feed additives [2]. Herbs have been utilised in various non-ruminant animals, including poultry, swine, equine, turkey, and rabbit [2].

Previous research findings into herbal plant extract have been inconsistent and contradictory. For instance, the use of thyme oil was non-significant to the growth performance of rabbits [3]. El-ghadid et al. [4], reported different results, where thyme oil improved the final body weight (BW) and reduced the feed conversion ratio (FCR) among rabbits. That study also reported a decrease of cholesterol, triglycerides, and LDL. Abdel-Wareth et al. [5] outlined that thyme oil tended to increase the feed intake and growth performance of

rabbits.

The first serious discussion and analyses of meta-analysis emerged during the 1970s, when the meta-analysis was developed to organise and synthesize the burgeoning literature in research. Meta-analysis was framed as a solution to the inconsistency of findings from multiple experiments at a certain level of generality by estimating an effect size from multiple studies. We hypothesize that herbs may have different roles and impacts when incorporated into the diets of rabbits. To assess the impacts of various herbs as a feed additive on rabbits, it is important to utilise systematic methods such as meta-analysis to obtain a robust conclusion. This study therefore employs a meta-analysis approach to determine the effects of herbs on the performance, blood parameters, nutrient digestibility and carcasses of domesticated rabbits.

2. Material and methods

2.1. Article search

Raw data were extracted from selected articles that reported the use of any herb in rabbits. Peer-reviewed publications were chosen and carefully evaluated following Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE') protocols. The following scientific platforms were chosen to search peer-reviewed published articles: Scopus (<https://www.sciencedirect.com/>); Web of Science (<https://mjl.clarivate.com/search-results>); PubMed Central (<https://pubmed.ncbi.nlm.nih.gov/>), Google Scholar (<https://scholar.google.com/>), and archive journal published in World Rabbit Science (<https://polipapers.upv.es/index.php/wrs>) using the keywords “herbs” AND “rabbits” accompanied by population terms including [(“growth performance” OR “nutrient digestibility” OR “serum biochemical” OR “carcase quality”). The review period was set as from 2011 to 2023. For each article examined, we also evaluated the reference lists to search for potentially relevant articles that may have been missed during the search.

2.2. Eligibility criteria

Articles were selected based on the following criteria: (1) written consistently in English, available as full text; reporting on the use of any herbs source in any breed and age of rabbit. (2) reported growth performance and nutrient digestibility or any additional parameters such as blood serum, and nutrient digestibility; (3) included information on the year published, level given, source, countries in which the experiment was conducted, the experimental period, and the strain of rabbit used. To facilitate the article evaluation, we used the PICOS (population, intervention, comparison, outcomes, and study selection) framework where the population was rabbits, the intervention was herbal plant extract supplementation in the diet, the comparison was a control diet without herbs, the main outcomes were growth performance parameters, blood parameters, and nutrient digestibility, and the study selection was peer-reviewed articles published between 2011 and 2023. A summary of the dataset is shown in [Table 2](#).

2.3. Article selection

The initial assessment contained, 412 peer-reviewed publications that used herbal plant extract on rabbits. A total of 117 peer-reviewed publications failed to meet the criteria due to non-relevant parameters, while 217 publications featured non-relevant herbal plant extracts. A further, 40 articles included non-relevant rabbits and 91 articles reported *in-vitro* studies. Meanwhile, 102 articles were too old. Next, after careful full-text evaluation, a further five articles were identified as having non-relevant parameters. This gave a final total of 33 eligible papers. The details for the study selection included in this meta-analysis are shown in [Fig. 1](#). A summary of the final dataset is presented in [Table 1](#).

After the articles were imported from scientific databases, four authors screened the titles and abstract lists. Three researchers were responsible for determining the final papers for inclusion. We excluded review articles, theses, dissertations, conference series, book chapters, and *in vitro* studies and articles that were not written in English. The articles were stored in Mendeley library. Finally, detailed information and data from the selected articles were tabulated in a spreadsheet with the following fields: references, year, level given, type of herbal plant extract, countries, strain of rabbit, and duration of experiment and outcomes. Peer-reviewed published articles containing graphical data and relevant figures were extracted and converted using an online tool, namely WebPlotDigitizer version 4.4.

After several steps of evaluation, the final dataset consisted of 33 *in vivo* studies comprising 121 experimental units. The peer-reviewed article that met the criteria are (Abdelnour et al., 2018; Abou-Kassem et al., 2021; Abdel-Wareth et al., 2018; 2019; Abd-

Table 2
PICOS criteria inclusion and exclusion of studies.

Parameters	Inclusion criteria	Exclusion criteria
Population	In vivo experiment; rabbit as a subject	Review studies and in-vitro studies
Intervention	Any herbs for rabbit	Non-herbs associated with rabbit
Comparison	Negative control without the intervention	No control group
Outcomes	Increasing of growth performance, without cause negative effect such as gizzard and liver. Improvement in carcase quality	–
Study design	Experimental-random studies	Review articles, theses, book chapters, <i>in vitro</i> , studies that not consistently being written in English

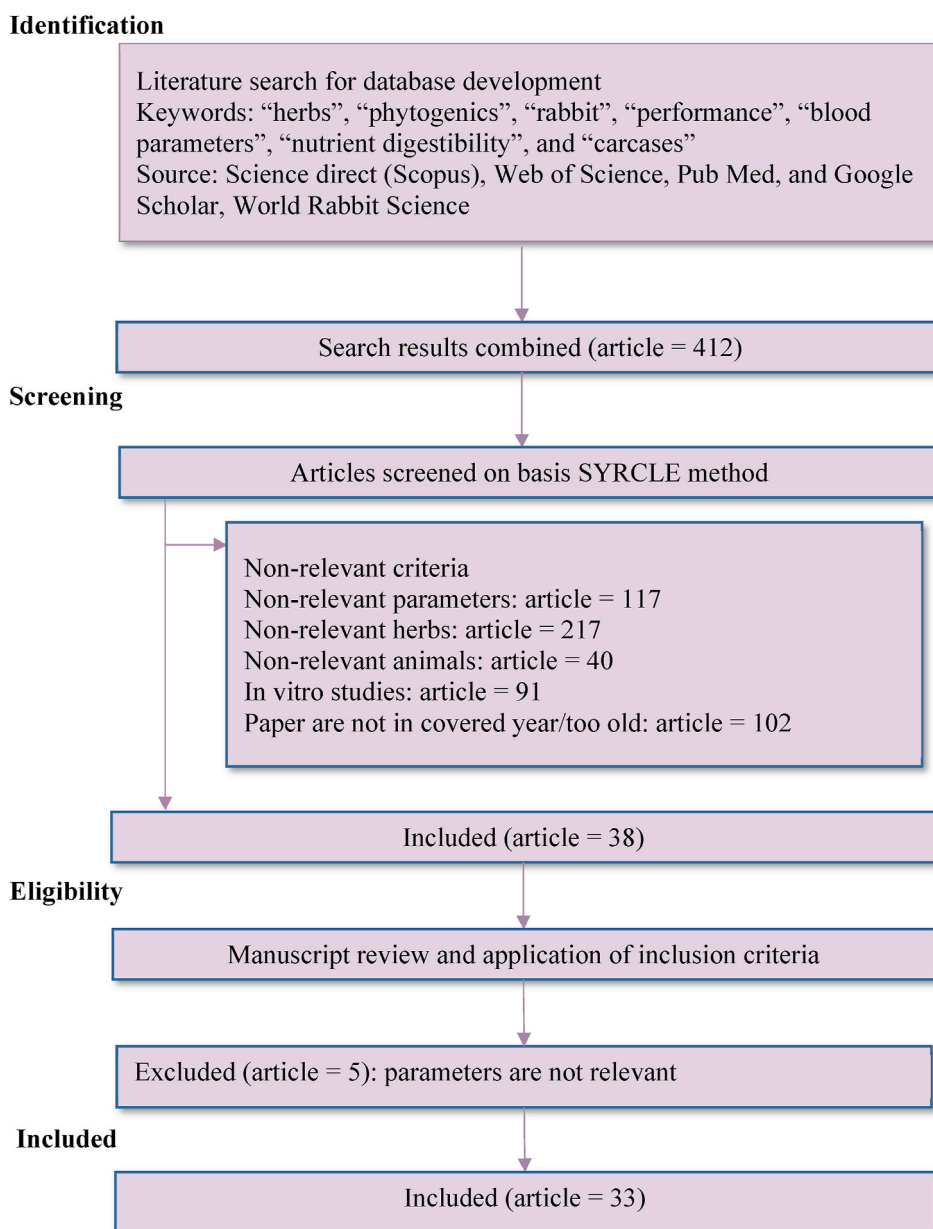


Fig. 1. Diagram flow of article selection in the meta-analysis using SYRCLE method.

El-Hady et al., 2014; Al-Sagheer et al. (2021); Adewale et al., 2021; Afolabi et al., 2019; Alagbe, 2021; Amaduruonye et al., 2017; Cardinali et al., 2015; Cullere et al., 2016; Dalle Zotte et al., 2013; 2020; Elghalid et al., 2020; Elwan et al., 2020; Elwardany et al. (2022); Ismail et al., 2019; Khaled and Qataf (2022); Kovitvadhi et al., 2016; Li et al., 2023; Mohammed et al., 2018; Olatunji et al., 2018; Palazzo et al., 2020; Peiretti et al., 2011; Perna et al., 2019; Popović et al., 2017; Ragab et al. (2022); Rotolo et al., 2013; Suliman et al., 2023; Trebušak et al., 2014; Wang et al., 2021; Zanouy and Elwan, 2017) [3–35].

2.4. Data analysis

Prior to the statistical meta-analysis, the data were transformed into similar units of measurement to enable direct analysis within a particular observation. Statistical analysis was conducted in R Studio (R program version 4.3.0; <https://www.r-project.org>) using lme4, lmerTest, and caret packages [36]. We performed a meta-analysis to assess the effect of various herbs on the response variables. To examine the effects of the different herbal plant extracts, we performed subgroup meta-analysis using the dmetar package [37]. The DerSimonian-Laird estimator was used to estimate the I^2 statistic which explains the total variance (%) across studies. The results of the

Table 3
Results of the meta-analysis and meta-regression.

Measured outcomes	n	Standardized Mean Difference (95% CI)				p-value	Meta-regression							
		Estimate	SE	Lower	Upper		Intercept	SE	Slope	SE	p-value	R ²	RMSE	AIC
BWG (g)	80	51.47	29.68	7.88	110.82	0.088	770.1	112.5	−0.147	0.23	0.530	0.044	339.4	1065
FCR	102	−0.28	0.08	−0.45	0.12	0.001	4.14	0.29	−0.002	0.00	0.337	0.034	1.25	210
ADG (g/h/d)	69	1.58	8.10	14.67	17.83	0.846	34.14	6.49	0.538	0.05	<0.001	0.604	11.25	725
Mortality (%)	69	−1.50	0.52	−2.53	0.46	0.005	3.22	1.56	−0.631	0.23	0.008	0.582	1.20	342
ADFI (g/h/d)	93	3.57	3.73	3.88	11.01	0.343	116.1	6.17	−0.022	0.03	0.402	0.001	60.2	840
DMD (%)	50	2.53	0.98	0.54	4.52	0.014	67.4	2.14	0.008	0.028	0.770	0.009	8.27	310
CPD (%)	50	2.81	1.08	0.54	5.08	0.018	76.7	2.45	0.134	0.679	0.846	0.012	10.2	146
RBC (10 ⁶ /μL)	36	58.08	90.05	126.69	242.84	0.524	482.3	476.8	−0.110	1.42	0.941	0.004	108.2	520
WBC (10 ³ /μL)	39	119.25	171.77	232.05	470.56	0.493	1085	1077	−0.150	2.78	0.957	0.032	19.9	620
Lymphocyte (%)	39	4.13	2.04	0.05	8.30	0.053	58.46	4.30	0.036	0.03	0.266	0.002	70.2	275
Haemoglobin (mg/dL)	31	1.18	0.40	−0.36	1.99	0.007	11.18	0.47	−0.002	0.01	0.589	0.002	8.05	103
Total Protein (g/dL)	40	112.85	80.68	52.15	277.85	0.172	6.39	0.20	0.008	0.00	0.979	0.367	6.33	581
Glucose (mg/dL)	26	8.74	8.86	9.87	27.35	0.337	128.7	10.08	−0.105	0.09	0.277	0.414	0.39	238
Cholesterol (mg/dL)	25	15.10	9.89	5.78	35.97	0.145	111.6	12.67	−0.063	0.12	0.603	0.093	0.30	234
Triglyceride (g/dL)	22	3.62	9.66	16.97	24.21	0.713	111.6	17.75	2.910	14.14	0.839	0.729	8.47	205
Albumin (g/dL)	32	44.65	45.82	50.14	139.44	0.340	3.76	0.08	0.002	0.00	0.978	0.394	10.3	421
HDL (mg/dL)	29	3.40	1.53	−0.22	6.59	0.037	54.09	8.28	4.280	1.09	0.001	0.586	14.5	191
LDL (mg/dL)	29	−21.38	6.55	−35.01	7.76	0.004	52.03	10.59	−18.11	5.27	0.003	0.843	0.07	253
IgG (mg/dL)	20	8.38	2.71	20.23	180.94	0.109	91.38	94.21	66.04	31.56	0.055	0.008	9.24	236
IgM (mg/dL)	17	8.38	2.71	−2.47	14.30	0.009	59.11	8.54	6.870	1.45	0.001	0.001	10.3	111
Carcase (%)	73	1.34	0.51	−0.32	2.37	0.011	58.36	1.47	0.003	0.02	0.844	0.009	68.9	372
SW (SW, g)	63	53.23	124.31	196.84	303.30	0.671	2040	171.4	−0.340	2.81	0.902	0.001	4.88	954
Thymus (g/kg)	17	−0.01	0.04	−0.10	0.07	0.733	1.23	0.41	−0.002	0.01	0.877	0.014	33.0	2.4
kidney (g/kg)	63	−0.02	0.06	−0.13	0.10	0.769	1.53	0.79	0.013	0.03	0.631	0.008	369.8	82
liver (g/kg)	70	−1.00	0.31	−1.63	0.37	0.002	7.83	2.95	−0.001	0.01	0.872	0.424	0.31	325
Heart (g/kg)	58	0.03	0.02	0.01	0.07	0.120	1.11	0.35	0.000	0.00	0.912	0.356	2.23	−42
Lightness	20	−0.40	0.44	−1.34	0.55	0.384	54.87	1.94	0.012	0.01	0.428	0.001	9.51	72
Redness	20	0.68	0.40	0.17	1.53	0.109	1.50	0.32	0.002	0.01	0.534	0.001	0.93	56
Yellowness	20	−0.46	0.19	−0.87	−0.06	0.028	3.85	1.68	0.000	0.00	0.228	0.391	1.85	47

ADFI – average daily feed intake; ADG – average daily gain; BWG – body weight gain; DMD – dry matter digestibility; CPD – crude protein digestibility; FCR – feed conversion ratio; HDL - high-density lipoprotein; LDL - low-density lipoprotein; IgG - Immunoglobulin G; IgM - immunoglobulin M; RBC – red blood cells; SW – slaughtered weight, WBC – white blood cells.

heterogeneity test indicated that all variables of interest had a high heterogeneity ($I^2 \geq 75\%$). Therefore, random effect model (REM) analysis was fitted to estimate the overall effects of the study interventions vs the control on the variable outcomes. By default, the Hedges' *g* effect size and its variance were used to estimate the standardised mean difference (SMD), weighted by the inverse-covariance matrix from the studies.

In addition, meta-regression was also performed to assess the relationships between the herbs inclusion levels and the response parameters. The meta-regression was based on linear mixed models (LMM) and was fitted using the following model:

$$\Delta Y_{ij} = \beta_0 + \beta_1 X_{ij} + \beta_2 X_{ij}^2 + (\beta_3 \times \beta_3 \dots \beta_n) X_{ij} \times s_i + b_i X + \varepsilon_{ij}$$

where ΔY_{ij} = estimated outcome of the dependent variable, β_0 = estimated intercept (fixed effect), β_1 = coefficient of linear regression of continuous predictor (fixed effect), β_2 = coefficient of the quadratic term of continuous predictor (fixed effect), X_{ij} = Herbs inclusion levels, the matrix of the continuous predictor variable, $\beta_3 \dots \beta_n$ = coefficient of the categorical variables, s_i = the random effect of the experiment, b_i = the random effect of experiment on the regression coefficient of Y on X, and ε_{ij} = the residual error at $\sim N(0, \sigma^2)$. The models used the inverse variance matrix as a weighting factor [38]. The RMSE and AIC were used as statistical parameters to select the best-fitted model [39]. Only the selected model for each response variable was shown in the results.

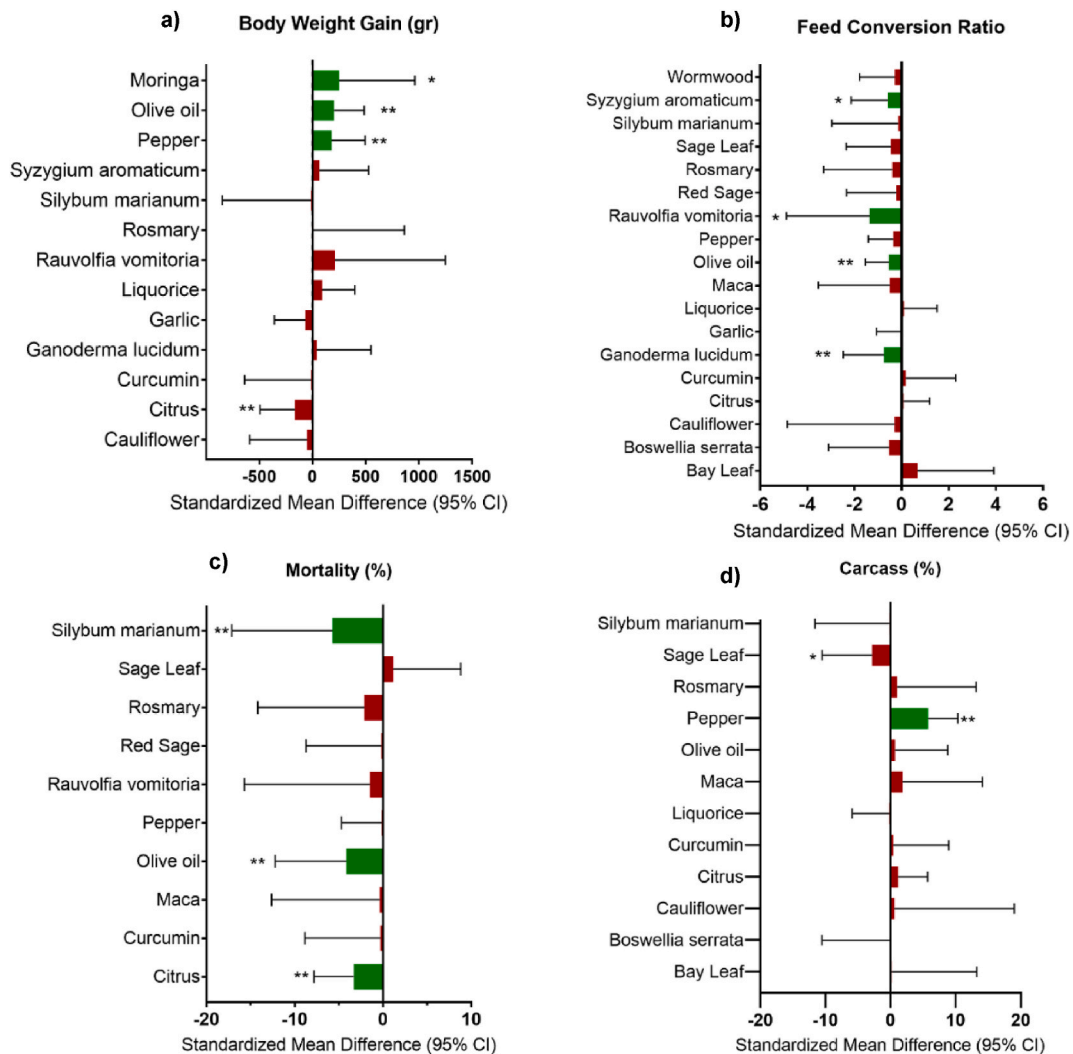


Fig. 2. Forest plot of subgroup herbal plant extract on the body weight gain (a), feed conversion ratio (b), mortality (c), and carcass (d) of broiler chickens. Forest plot of subgroup meta-analysis showing the 95% confidence intervals (lower – upper) of the standardized means difference (SMD) between the means of groups of different herbs (as covariates) on the body weight gain (upper left); feed conversion ratio (upper right); mortality (lower – left); and carcass (lower – right). The x-axis shows the SMD; central-dashed line represents the zero effect (SMD = 0) of dietary interventions; red-bar represent the overall effect while the specific symbols in each line represent the SMD (subgroup effect) of the specific group. Reduction effects are reflected when the SMDs are in the left of the central dashed-line and increasing effects are in opposite (to the right of the line). **symbol reflects the significance of the subgroup (P < 0.05).

3. Result

3.1. Growth performance

The results of LMM examining the effects of herbal plant extract on the growth performance, blood parameters, nutrients digestibility and carcass quality of rabbits are presented in Table 3. Our meta-analysis revealed that herbs supplementation reduced FCR ($P = 0.001$; 95% confidence interval (CI) = $-0.28 - 0.08$) and mortality ($P = 0.005$; 95% CI = $-1.50 - 0.46$). However, supplementation with herbal plant extracts did not ($P > 0.05$) affect the average daily gain (ADG) and average daily feed intake (ADFI; Table 3), due to variations among the types of herbs. In the meta-regression analysis, linear growth was observed for ADG in response to the increasing supplemental herbs ($P < 0.001$).

Forest plot (Fig. 2) summarised the subgroup meta-analysis based on the type of herbal plant extract on the growth performance of rabbits. Moringa, olive oil, and pepper increased ($P < 0.05$) the BWG of rabbits. *Silybum marianum*, olive oil, and citrus minimized the mortality as shown by the lower SMD. Moreover, several herbs, including *Syzygium aromaticum*, *Rauwolfia vomitoria*, olive oil, and *Ganoderma lucidum*, decreased ($P < 0.05$) FCR. Fig. 3 illustrates the supplementation forms, such as powder and liquid. Based on the formulation of herbal additives in the feed, the powder form yielded notably superior results in terms of BWG compared to the liquid form ($P < 0.05$). Furthermore, both the powder and liquid forms markedly reduced mortality rates and FCR ($P < 0.05$).

3.2. Blood biochemistry and immune parameters

The herbal plant extract did not affect blood biochemistry parameters including red blood count (RBC), white blood count (WBC), lymphocyte, total protein, glucose, cholesterol, triglycerides, and albumin concentrations but did lower ($P = 0.004$) the low-density lipoprotein (LDL) content while increasing ($P = 0.037$) the high-density lipoprotein (HDL) content of the blood. Immunoglobulin G (IgG) was unaffected while IgM increased significantly ($P = 0.009$) following the inclusion of herbal plant extract. The increase in HDL

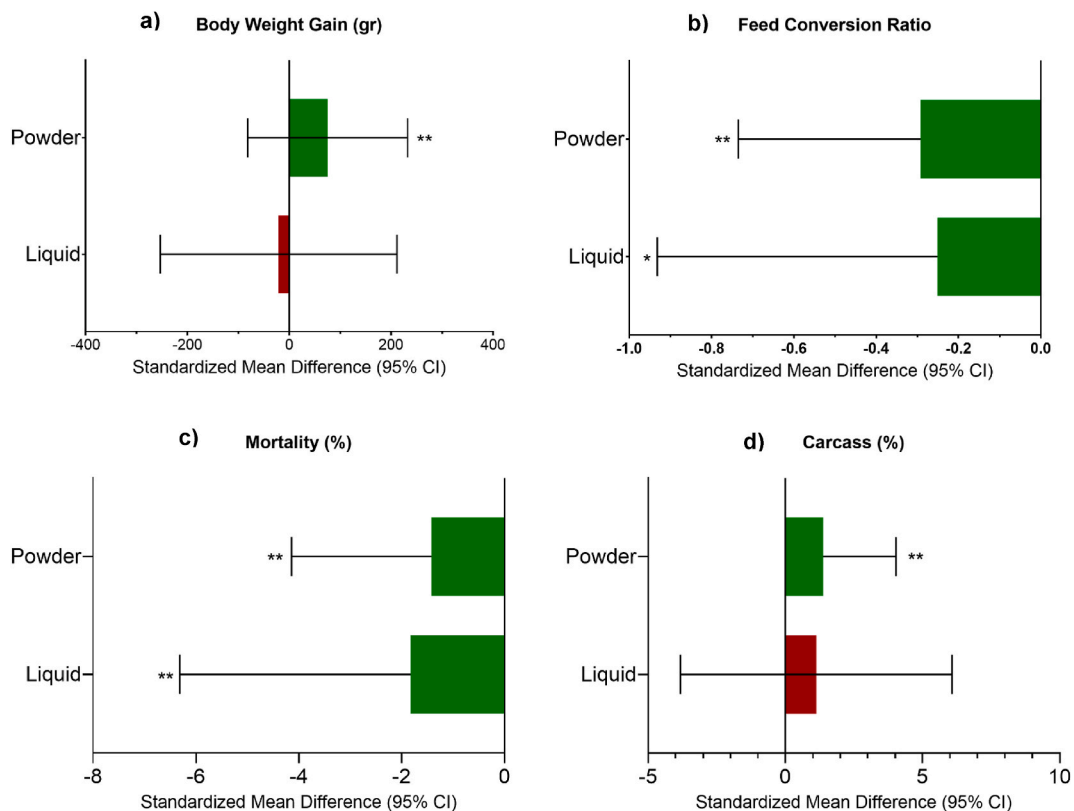


Fig. 3. Forest plot of subgroup form of herbal plant extract on the body weight gain (a), feed conversion ratio (b), mortality (c), and carcass (d) of broiler chickens. Forest plot of subgroup meta-analysis showing the 95% confidence intervals (lower – upper) of the standardized means difference (SMD) between the means of groups of different form of herbs (as covariates) on the body weight gain (upper left); feed conversion ratio (upper right); mortality (lower – left); and carcass (lower – right). The x-axis shows the SMD; central-dashed line represents the zero effect (SMD = 0) of dietary interventions; red-bar represent the overall effect while the specific symbols in each line represent the SMD (subgroup effect) of the specific group. Reduction effects are reflected when the SMDs are in the left of the central dashed-line and increasing effects are in opposite (to the right of the line). **symbol reflects the significance of the subgroup ($P < 0.05$).

and the decrease in LDL showed a linear pattern ($P < 0.05$) in response to the levels of dietary herbs supplementation. Similar observations of linear dose response effects ($P < 0.05$) were also found in IgG and IgM.

3.3. Carcase quality

The rabbits that received herbal plant extract in their diets showed a higher ($P = 0.011$) carcase percentage compared to those on the control diet. No major effect was found in the organ weight and carcase quality parameters. Among the various herbal supplements, pepper showed significant effects in augmenting the rabbit carcase percentage ($P < 0.05$). The rabbits that received herbal supplements in the powder form exhibited considerably higher carcase weight than those administered the liquid form ($P < 0.05$).

3.4. Nutrient digestibility

Our meta-analysis results confirmed that herbal plant extracts are had a minor impact on several of the parameters observed in rabbits. The rabbits that given herbal plant extract as a dietary additive showed an effect ($P = 0.014$) on dry matter digestibility (DMD) and crude protein digestibility ($P = 0.018$).

4. Discussion

After doing meta-analysis we found several effects on growth performance, blood serum parameters, organ weight, and carcase quality of rabbits. Later, after banning of the antibiotics growth promoters (AGPs), a lot of researchers started to seek the best alternative in order to fulfil replacement of AGPs by using plant and spices as an herb in the diet. Trying to guarantee satisfactory results such as growth performance, blood serum parameters, and carcase quality of rabbits. We hypothesised that herbs have specific biological pathways and bio-activation compounds with fermentation processes in the hindgut. Additionally, it was questioned whether increasing the level of herbs, either in powder or liquid form. In the end, the meta-regression was also conducted to look up the gap between duration of studies and source of herb use when selecting the published paper used in this section.

4.1. Growth performance

In terms of growth performance, Perna et al. [27] reported a lower effect of cauliflower powder on the New Zealand White (NZW) strain compared to the group. The current finding adds to the growing body of literature on BW Abdel-Wareth et al. [7], for example, showed significantly ($P < 0.001$) higher trend for garlic on final BW and average daily weight gain. Thus, herbs appear to offer benefits for mortality and a good health status. The improvement of final BW is in line with the reduction of the feed-to-gain ratio. The effect of using garlic may be due to the bioactive compound *allicin*, which has an important biological function and is an essential element for rabbit growth, controlling body energy balance, protein anabolism, and preventing oxidative thus boosting the immune system. Interestingly, Abdel-Wareth et al. [7] results showed that garlic reduced the negative effect of high temperature which is a stress factor for rabbits. However, the results do not explain the occurrence of these adverse effects.

Rabbits are more susceptible to death compared to other livestock, while temperature and environment are key factors when rearing them. The comfort zone for rabbits is 15°–20 °C [23]. Rabbits can become stressed at temperatures above the comfort zone which affects their physiological traits. Certain herbs contain bioactive compounds for example, *Moringa oleifera* contains flavonoids, ascorbic acid, phenolic, and carotenoid substances which act as natural oxidants. Garlic provides *allicin*, which plays a role in reducing lipid peroxidation [23]. The mechanism of garlic begins by scavenging reactive oxygen through the amplification of intercellular oxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. Ginger also acts as an antioxidant substance that mitigates and even prevents the regeneration of free-radicals.

Furthermore, Kovitvadhvi et al. [21] used purple loosestrife, the active components of which offered the potential of better flavor, which directly increased consumption among farmed rabbits. Mohammed et al. [23] noted that certain herbs led to a reduction in feed intake, which may indicate a pungent taste. Purple loosestrife thus provides substances known as pharmacokinetics that function as ant oxidative compounds. Moreover, Dalle Zotte et al. [15] hypothesised that thyme led to the improvement of certain micro biota in the gut. Since gut microbiota appears to contribute to virtually every aspect of the host's growth rather than development, it is unsurprising that a wide array of diseases and dysfunctions have been associated with an imbalance in either their composition, number, or habitat. Cardinali et al. [13] reported consistently different effects of oregano, and rosemary as herbal plant extracts among groups. The principal effect, however, was to stabilise hygiene and impact the intestinal microbiota by controlling pathogens. In addition, Abdelnour et al. [4] reported that pepper families display noteworthy antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. Surprisingly, alkaloids, terpenes, flavonoids, and glucosinolates were found to be the most effective bioactive compounds in plants, as reported by Elwan et al. [18].

4.2. Blood serum parameters

Our meta-analysis results found that herbs had various impacts on the blood parameters observed in rabbits. These differences may be attributable to the diverse chemical structures of the bioactive compounds in herbs, which could subsequently influence gut health and metabolism [34]. Bioactive compounds are secondary metabolites found in small quantities in specific herbs comprising mostly hydrophobic and poorly soluble compounds. There are several classes of bioactive compounds: 1) terpenes; 2) terpenoids; 3) alkaloids;

and 4) phenolic. To better understand the mechanism of the effects of herbs on blood serum parameters, Dalle Zotte et al. [16] compared several bioactive compounds that activate on liquorice: 1) triterpenes; 2) saponins; 3) flavonoids; 4) isoflavonoids; and 5) chalcones. The bioactive compounds in liquorice are responsible for its sweet taste. Liquorice is associated with increased blood pressure and excessive consumption can create a clinical picture similar to primary hyperaldosteronism. The mode by which these bioactive compounds are produced in liquorice is not fully understood. However, liquorice, as well as the increased use of herbs, is thought to inhibit angiotensin converting-enzyme (ACE) activity. Consequently, this bioactive compound acts as an inhibitory agent in blood circulation. Kovitvadhi et al. [21] demonstrated that purple loosestrife only increased the quantity of white blood cells. It was also remarked that echinacoside and chicoric acid played a role in inducing white blood cells. Regarding bioactive peptides, while the evidence shown in this review suggests a positive effect on blood pressure reduction, larger quantitative studies are required to draw a conclusive correlation. Nevertheless, the evidence to date is promising. The limited data available on bioactive compounds shows inconclusive and even conflicting evidence; thus, more studies are needed before any assumption is made.

Elghalid et al. [17] used herbs including carvacol, thymol, mentol, and propylene as evidence to demonstrate a decrease in total cholesterol, triglycerides and LDL. Surprisingly, the result also showed an increase in total HDL cholesterol and total antioxidant capacity in rabbits. Moreover, Elghalid et al. [17] reported the following evidence based on the mechanism, of herbs in blood circulation. Initially, herbs protect tissues from lipid peroxidation and reduce lipid activity in rabbits. Second, the effect supports the cardiovascular system via a protective influence with the additive supplementation. Third, the bioactive and phenolic compound in the herbs stimulated lipid metabolism in rabbit tissue by increasing the antioxidative enzymes and preventing the production of specific reactive oxygen species. This was followed by off-flavours derived from peroxidation of polyunsaturated fatty acids. Afterwards, LDL cholesterol was reduced via the stimulation of cellular cholesterol biosynthesis and a decrease in the intestinal absorption of cholesterol. The key to this activity was the ability of the polyphenolics and flavonoids in each herb to inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, which is a key regulatory enzyme in the cycle. Later, the receptor responsible for LDL cholesterol enhanced the removal, of LDL from blood circulation by decreasing the serum plasma concentration.

Abdelnour et al. [4] noted that black pepper could reduce total cholesterol, triglyceride, and LDL cholesterol as well as increase HDL. This may be attributable to a decrease in acetyl-CoA enzyme during the biosynthesis cycle. Hence, Abdelnour et al. [4] hypothesised that the addition of black pepper induced enzymatic activity in the transformation of cholesterol bile acid, which would subsequently be evident in the carcass. Furthermore, the data suggest that the utilization of herbs could support immune responses. Indeed, it is well-known that herbs have antioxidant properties that benefit immune system development. In addition, some plants or certain combinations of herbs in the diet can act as antioxidants by exerting superoxide scavenging activity or increasing superoxide dismutase activity in different tissue sites [40], due to their powerful immune properties. Furthermore, the suppressive effect of natural herbs on the immune response was closely associated with a reduction in the production of pro-inflammatory cytokines [41]. Nevertheless, studies in ruminants and poultry revealed that herbs could develop the immune-modulatory system [42,43]. Their use in feed additives could therefore be beneficial in supporting animal health.

4.3. Carcass quality

Our meta-analysis results showed that while herbs did not impact several parameters in carcass quality, they did significantly affect the carcass yellowness quality result. Reporting on the effects of herbal plant extracts on carcass quality has been scarce. Regarding meat quality, however, Abou-Kassem et al. [35] reported that a diet-supported by plant extracts and essential oils had no negative effects on meat quality characteristics or performance. Similarly, Elwardany et al. [38] demonstrated that, compared to control diets, rabbits fed diets containing medicinal and aromatic plants showed insignificant increases in the levels of protein, fat, and ash in their meat. Dalle Zotte et al. [16] stated that information on the use of liquorice in rabbits. Conversely, Kovitvadhi et al. [21] mentioned that bioactive compounds cause liver enlargement which is attributed to toxic substances especially when tannin levels are high. Hence, feeding livestock with plants rich in tannins or flavonoids may affect the carcass quality. Palazzo et al. [25] reported that flavonoids prevent lipid oxidation by reducing muscle cholesterol and the triglyceride content of carcasses. Wang et al. [3] mentioned that flavonoids have inhibitory effects on α -amylase and α -glucosidase enzymes. The activities of digestive enzymes such as α -amylase, sucrase, maltase, lipase, and trypsin are correlated with carcass quality results. Compared with the result from Peiretti et al. [26], the use of *curcuma longa* also makes no significant difference, although the authors did hypothesised that *curcuma longa* supports carcass trait parameters in rabbit tissue. However, Zounouy and Elwan [33] recommended the use of herbs to enhance rabbit liver and kidney function. In addition to antioxidant status in farmed rabbits. Meanwhile, Abd-El-Hady et al. [5] recommended that up to 300 g/kg of herbs can be used in rabbits with no adverse effects. The use of herbal plant extracts correlates with the production of healthy meat, which in turn concern the amount of saturated fatty acids (SFAs) needed to reduce and increase mono or polyunsaturated fatty acids (MUFA and PUFA) [26]. The beneficial effect will occur when healthy meat from farmed rabbits contains MUFA or PUFA. Peiretti et al. [26] reported that this can reduce atherogenic plaque in arteries. This aligns with Perna et al. [27], who agreed that the use of herbs in rabbits increases the PUFA and decreases the SFA content, respectively, in rabbit carcasses. However, they also play a role in preventing cardiovascular disease. Perna et al. [27] concurred that herbs are generally associated with antioxidants capacity and highlighted which, recommended flavonoids and phenolic acid as the major antioxidants of this bioactive. They also stated flavonoids can eliminate abdominal fat which is bound to glycine and taurine. In the next step, glycine and taurine are formed into bile salt and secreted to the duodenum where it is degraded by microbes.

4.4. Nutrient digestibility

According to Kovitvadhi et al. [21], the use of purple loosestrife showed a statistically significant difference in the digestibility of ether extract (EE) at the 0.4% level. Furthermore, giving 0.5% rosemary or thyme leaves meal 0.5% curcumin seed or 0.5% fenugreek seed meal to growing rabbits produced a significant increase of 9%–18% in the digestibility of DM, CP, crude fibre (CF), EE, and nitrogen free extract (NFE) [38]. Enhanced of nutrient digestibility was due to the presence of flavonoids namely carotenoid, ascorbic acid, and isothiocyanate which are potentially found in these herbs. These metabolite compounds stimulate gut activity by improving the digestibility of all nutrients [38]. Moreover, it has been hypothesised that certain enzymes help to maintain the poor digestibility of EE. This may benefit the target animal when the mode of action begins with α -amylase, trypsin and lipase that help to maintain the jejunal section of the intestine. In agreement with Wang et al. [3], the compounds in the herbs namely α -amylase, maltase, lipase, trypsin, and elastase significantly improved rabbit carcass quality. However, at an unknown level the α -amylase, sucrase, maltase, lipase, and trypsin enzymes may also reduce carcass quality. Dalle Zotte et al. [3] also mentioned that herbal plant extracts influence the EE result. They suggested that dry or powder forms were more effective than liquid. The inclusion level and type of fibre are the main factors that affect digestibility by stimulating the feed intake and mean retention in the caecum. Microbiota stimulation is then followed by the interaction of the principal constituents with digestive enzymes [15]. Wang et al. [15] result also suggests that the use of herbs as an additive may positively correlate with enhanced protein digestion. Additionally, specific herbs can improve the rumen environment, reduce pathogens, and increase antioxidant activity [15]. Adli et al. [1] reported that herbs may occasionally act as anti-nutrients, based on a sample such as lectins, polyphenols, anti-nutritional amino acids, saponins, cyanogen glycoside substances, protease inhibitors, and oxalate.

5. Conclusion

The empirical findings in this study provide a new understanding of the use of herbal plant extract on rabbits. Cumulatively, a variety of herbal plant extract can be beneficial for rabbits health and performance. Further study with an enhanced focus on the relationship between rabbit strain is therefore recommended.

CRedit authorship contribution statement

Danung Nur Adli: Writing – review & editing, Writing – original draft, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sugiharto Sugiharto:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Agung Irawan:** Writing – review & editing, Validation, Formal analysis, Data curation. **Yuli Arif Tribudi:** Writing – review & editing, Formal analysis, Data curation. **Syahputra Wibowo:** Writing – review & editing, Formal analysis, Data curation. **Amirul Faiz Mohd Azmi:** Writing – review & editing, Formal analysis, Data curation. **Osfar Sjojfan:** Writing – review & editing, Formal analysis, Data curation. **Anuraga Jayanegara:** Writing – review & editing, Validation, Supervision, Software, Methodology, Investigation, Conceptualization. **Heli Tistiana:** Writing – review & editing, Formal analysis, Data curation. **Teguh Wahyono:** Writing – review & editing, Formal analysis, Data curation. **Siska Aditya:** Writing – review & editing, Formal analysis, Data curation. **Mohammad Miftakhus Sholikin:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Sadarman Sadarman:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e25724>.

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