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Impacts of red clover and sainfoin silages on the performance, nutrient utilization and milk fatty acids profile of ruminants: A meta-analysis

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Abstract

Inclusion of plants rich in secondary metabolites into grass ensiling offers multiple benefits for ruminants, from improving productive performance to health-promoting effects as well as helping to reduce environment pollution. The present meta-analysis summarizes the dietary inclusion levels of red clover silage (RCS) and sainfoin silages (SS) as well as the types of silages fed to dairy cows and small ruminants. A total of 37 in vivo studies (26 articles in dairy cows and 11 articles in small ruminants) were aggregated after being strictly selected using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A mixed model methodology was used to examine our objectives. This method declares the subject 'study' as random effects and 'inclusion level' as fixed effects. Results indicated that RCS proportion was not associated with nutrient digestibility except for a quadratic effect ($p < 0.05$) on neutral detergent fibre digestibility. Higher RCS inclusion linearly increased ($p < 0.05$) nitrogen (N) intake but had no effect on dairy cows' production. Increasing RCS proportion altered milk fatty acid profile where the concentration of conjugated linolenic acid (CLA), C18:3 α-linolenic acid (ALA) and C18:0 linearly increased ($p < 0.01$). In small ruminants, SS proportion had no relationship with nutrient digestibility, N metabolism and growth performance ($p > 0.05$). However, a combination of dietary RCS + SS resulted in significantly higher ($p < 0.05$) CLA and ALA concentration in cow milk and average daily gain (ADG) in small ruminants compared to diets composed from either grass silage or alfalfa silage. Altogether, this meta-analysis highlights the synergistic effects of a combination of SS + RCS inclusion in improving milk fatty acids (FA) profile of dairy cows and ADG of small ruminants.

KEYWORDS

bioactive legume, fatty acid profile, growth performance, ruminant, silage

1 | INTRODUCTION

Inclusion of legume plants silage rich in functional properties to a grass-silage-based diet is a widespread practice in ruminants. In a typical grass diet, imbalance between energy and protein becomes one major limitation leading to the poor nitrogen (N) use efficiency (NUE) (Lee et al., 2019). In addition to improving NUE, numerous studies have reported that offering mixture legume-grass silages (GSs) can significantly increase feed efficiency and animal performance, reduce enteric methane emission and biohydrogenation (BH) of polyunsaturated fatty acids (PUFAs) in the rumen with potential health benefits. Red clover (*Trifolium pratense*) and sainfoin (*Onobrychis viciifolia*) have gained an attractive interest among legume species as a sustainable nutritional approach to improve productivity, promote animal health, and reduce N pollution to the environment because they are high in bioactive compounds (Campidonico et al., 2016; Huyen et al., 2020; Luciano et al., 2019; Niderkorn et al., 2019).

Red clover contains a considerable amount of polyphenol oxidase (PPO), an enzyme that can form a protein-bound phenol (PBP) via quinones catalyzation. This mechanism helps to preserve protein deterioration during ensiling to protect protein from rumen degradation to be available postruminally (Copani et al., 2014; Lee et al., 2014). This enzyme also exhibits a potential to protect PUFA from rumen BH via inhibition of plant's lipases activity (Gadeyne et al., 2015). Similarly, sainfoin is rich in condensed tannins, a secondary metabolite with a high ability to form tannin-protein complexes and suppress key bacteria involved in the last step of BH in the rumen in turns, increase N metabolism, PUFA transfer efficiency to animal products, as well as decrease CH_4 production (Huyen et al., 2020; Jayanegara et al., 2012; Irawan, Noviandi, et al., 2020).

There have been increasing evidences that red clover silage (RCS) incorporation in grass-silage diets boosted sheep performance due to increasing nutrient intake and utilization (Niderkorn et al., 2019), and improved milk FA profile in dairy cows by partly reducing rumen BH (Lee et al., 2019; Vanhatalo et al., 2006; Schulz, Westreicher-Kristen, Molkentin, et al., 2018). Similarly, some benefits have also been attributed to sainfoin silage (SS) inclusion, such as reducing CH_4 emission and ruminal C18:3n-3 BH while improving milk fatty acids (FA) profile in dairy cows (Girard, Dohme-Meier, Silacci, et al., 2016; Huyen et al., 2016, 2020), increase feed value (Aufrère et al., 2013) and nutrient digestibility (Wang et al., 2007). Moreover, there were some reports suggesting that RCS and SS synergistically improve voluntary intake, FA profile and oxidative stability in animal products (Campidonico et al., 2016; Copani et al., 2016; Luciano et al., 2019; Niderkorn et al., 2019).

By contrast, recent studies in dairy cows suggest that increasing RCS proportion do neither improve NUE nor alter beneficial FA (Broderick, 2018; Ineichen et al., 2019). Inter-studies variances and conflicting results are often reported in feeding studies due to many confounding factors. Such factors can be associated with the composition of forage in the diets and their chemical composition which can affect their nutrient and bioactive compound profile (Ineichen et al., 2019). Under in vitro experiment, lipid-protective mechanism was confirmed but it did not occur when co-ensiled with ryegrass (Van Ranst et al., 2013), meaning that co-ensiling with different type of grass should be further clarified. In regard to the degree of protein binding, there is also evidence that higher proportion of PPO in the RCS did not always linearly increase protein protection (Lee et al., 2019). On the other hand, inclusion sainfoin in grass-silage diets resulted in lower ADG in sheep (Copani et al., 2016).

Such inconsistency warrants a research synthesis with respect to depict the relationship between the proportion of RCS and/or SS inclusion on the ruminants' performance using valuable information from available publications. Meta-analysis is recognized as a powerful statistical method to quantitatively review by integrating and contrasting data from primary studies published (Sauvant et al., 2008; St-Pierre, 2001). Therefore, the objective of the present study was to estimate the effect of increasing dietary proportion of RCS and/or SS silages on the productive performance of ruminants. In addition, different legume species are often used within the existing studies that may contribute to the discrepancies of the results. To account for the effects of these confounding variables, this study also compared the effect of types of legumes included in the diets as categorical data on the response variables included in this meta-analysis.

2 | MATERIALS AND METHODS

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this study was conducted using generated data from published experiments without directly used animals.

2.1 | Literature search

Searching for articles was conducted on Web of Science (www.webofknowledge.com) and Scopus (www.scopus.com) databases. These platforms were used because they contain high-quality standard peer-reviewed publications. Articles related to RCS were obtained with keywords 'red clover', 'silage', and 'ruminant' while 'sainfoin', 'silage', and 'ruminant' keywords were used to identify articles related to SS utilization in ruminants. In the initial screening, we found 899 titles for RCS and SS.

These articles were subjected to selection analysis following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline to select papers that best match with the study purpose (Liberati et al., 2009). At this stage, the article had to: (1) be published in English language; (2) concurrently report the utilization of either RCS or SS and its proportion as a treatment and other forages-based silages as a control; (3) be conducted in an in vivo experiments using dairy, beef cattle or small ruminants (lamb, sheep and goat); (4) report replication unit and response variable with its variance (i.e., SD and SE). Review articles, in vitro experiments, and duplicate papers were excluded from the database. Articles without control diet were also disregarded. The selection procedure of the articles is shown in Figure 1.

Following these inclusion criteria, a total of 37 papers consisting of 26 articles with RCS studies and 11 papers with SS studies were selected to be integrated into a database. Within these articles, there were six containing both RCS and SS. As the RCS and SS have distinct characteristics, data of the respective legumes were extracted and analyzed separately. In this context, RCS database contained 29 studies conducted in dairy cows and three studies in beef cattle. We decided to exclude studies with beef cattle because the experimental unit was too small with very few variables of interest. Thus, 26 studies comprising 44 experiments evaluating RCS on dairy cows were finally analyzed (Table 1). In addition, most of the studies using SS were conducted in small ruminants with only three studies conducted in beef and dairy cows. Therefore, we also excluded the data of sainfoin on beef cattle and focus to discuss the effect of SS-based diets on small ruminants. Finally, there are 11 studies containing 13 experiments reporting SS inclusion in small ruminants as listed in Table 2.

2.2 | Database extraction

The selected articles from RCS and SS studies were extracted to a Microsoft Excel spreadsheet. Information on the first author, year of publication, the number of replications, forage species composition, inclusion level in the diet and chemical composition of the diet was classified. In addition to the basic information, response variables for RC category from each article were inputted with respective variance (SD, SE) for the following variables: digestibility (dry matter, DMD; organic matter, OMD; neutral detergent fibre, NDFD; acid detergent fibre, ADFD; and total N, ND), nitrogen metabolism (N intake, faecal N, urinary N, milk N), rumen fermentation characteristics, DM intake (DMI), milk yield, adjusted milk yield, feed efficiency (milk/DMI), milk composition and fatty acids profile of the milk. For sainfoin category, response variables included were nutrient digestibility, nitrogen metabolism, rumen fermentation profile, average daily gain (ADG), DMI and feed conversion ratio. In some articles, data were visualized

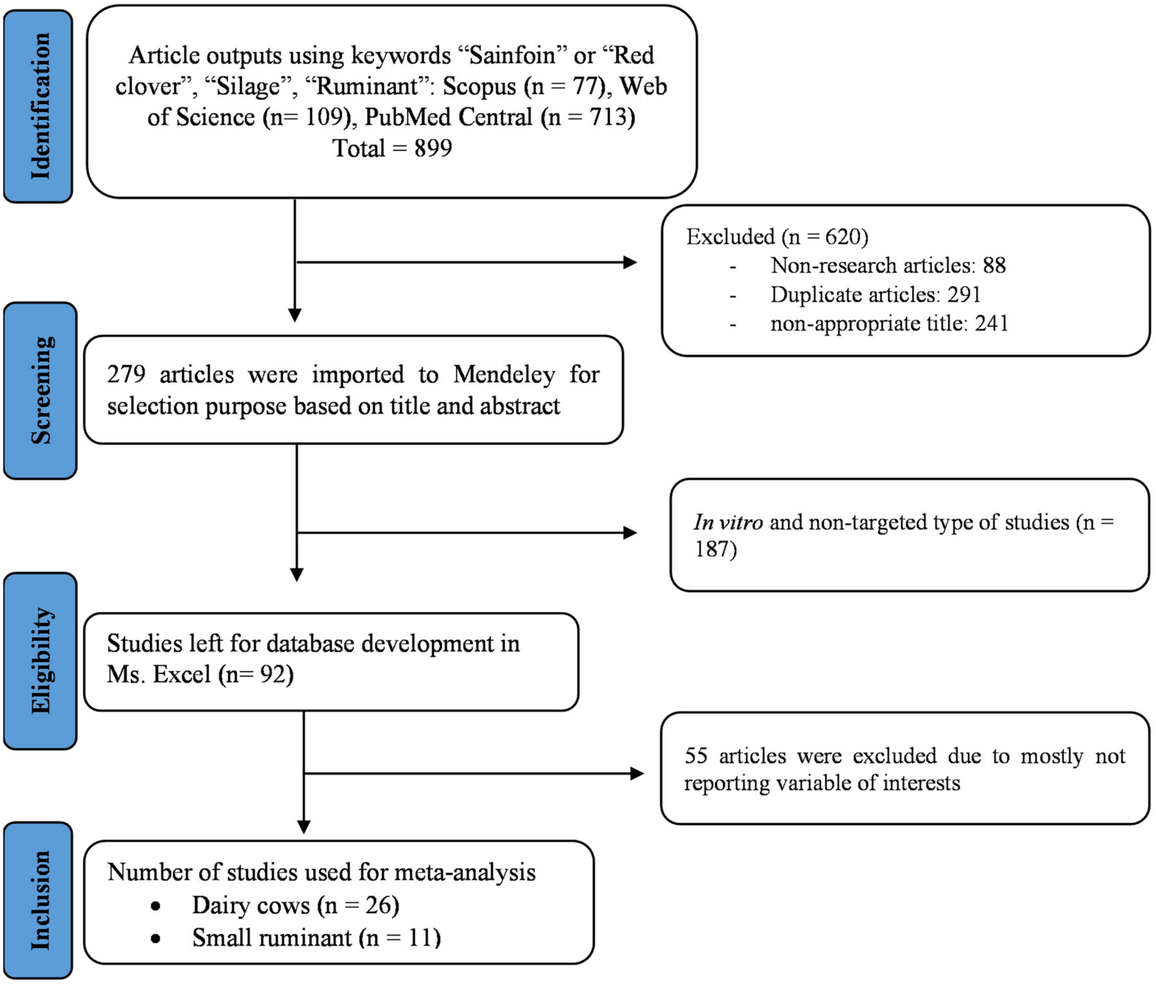

FIGURE 1 Flow chart of the article selection procedure. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Studies included in the meta-analysis of red clover inclusion in grass silage diet on dairy cows.

Study	Reference	Country	Ensiled forage composition	Red clover inclusion (%)	Forage to concentrate ratio	Type of diet
1	Johnston et al. (2020)	Ireland, UK	Perennial ryegrass + red clover	0–50	50:50	TMR silage
2	Ineichen et al. (2019)	Switzerland	Corn, plantain, ryegrass, red clover	0–63	76:14	TMR silage
3	Schulz, Westreicher-Kristen, Molkentin, et al. (2018)	Germany	Corn, red clover	0–60	75:25	TMR silage
4	Broderick (2018)	USA	Alfalfa, red clover	0–47.4	67:33	TMR silage
5	Schulz, Westreicher-Kristen, Knappstein, et al. (2018)	Germany	Corn, red clover	0–60	75:25	TMR silage
6	Leduc et al. (2017)	Canada	Alfalfa, red clover	0–37.8	50:50	TMR silage
7	Johansen et al. (2017)	Denmark	Ryegrass, festulolium, tall fescue, ryegrass, red clover, white clover	0–100	70:30	TMR silage
8	Gidlund et al. (2017)	Sweden	Grass, red clover	0–45	60:40	TMR silage
9	Campidonico et al. (2016)	France	Timothy, sainfoin, red clover	0–50	0.00	Forage-based silage
10	Ouellet & Chiquette (2016)	Canada	Birdfoot trefoil, alfalfa, sainfoin, red clover	0–100	0.00	Forage-based silage
11	Moorby et al. (2016)	Wales, UK	Corn, red clover	0–67.5	75:25	TMR silage
12	Berthiaume et al. (2015)	Canada	Red clover, timothy, tall fescue	0–50	0.00	Forage-based silage
13	Halmemies-Beauchet-Filleau et al. (2014)	Finland	Timothy, meadow fescue, red clover	0–60	60:40	TMR silage
14	Lee et al. (2014)	Wales, UK	Cockfoot, ryegrass, red clover	0–100	0.00	Forage-based silage
15	Halmemies-Beauchet-Filleau et al. (2013)	Finland	Timothy, ryegrass, red clover	0–60	60:40	TMR silage
16	Hymes-Fecht et al. (2013)	USA	Alfalfa, red clover, birdfoot trifoil	0–50	75:25	TMR silage
17	Dewhurst et al. (2010)	Ireland, UK	Ryegrass, corn, red clover	0–100	75:25	TMR silage
18	Vanhatalo et al. (2009)	Finland	Timothy, meadow fescue, red clover	0–100	65:35	TMR silage
19	Moorby et al. (2009)	Wales, UK	Ryegrass, red clover	0–100	76:14	TMR silage
20	Moorby et al. (2008)	Wales, UK	Ryegrass, red clover	0–100	76:14	TMR silage
21	van Dorland et al. (2007)	Switzerland	Ryegrass, red clover, white clover	0–30	0.00	Forage-based silage
22	Broderick et al. (2007)	USA	Alfalfa, red clover, corn	0–54	60:40	TMR silage
23	Brito et al. (2007)	USA	Alfalfa, corn, red clover	0–51	50:50	TMR silage
24	Broderick et al. (2001)	USA	Alfalfa, red clover, ryegrass	0–60	60:40	TMR silage

TABLE 1 (Continued)

Study	Reference	Country	Ensiled forage composition	Red clover inclusion (%)	Forage to concentrate ratio	Type of diet
25	Broderick et al. (2000)	USA	Alfalfa, red clover	0–65	65:35	TMR silage
26	Hoffman et al. (1997)	USA	Alfalfa, red clover	0–58	58:42	TMR silage

Abbreviation: TMR, total mixed ration.

TABLE 2 Studies included in the meta-analysis of sainfoin inclusion in grass silage diet on small ruminants.

Study	Reference	Country	Ensiled forage composition	Sainfoin inclusion (%)	Concentrate to forage ratio	Type of diet
1	Huyen et al. (2020)	The Netherlands	Corn, grass, sainfoin	0–35	30:70	TMR silage
2	Niderkorn et al. (2019)	France	Timothy, ryegrass, sainfoin, red clover	0–50	0	Forage-based silage
3	Luciano et al. (2019)	Italy	Timothy, ryegrass, sainfoin, red clover	0–100	0	Forage-based silage
4	Copani et al. (2016)	France	Timothy, ryegrass, sainfoin, red clover	0–50	0	Forage-based silage
5	Campidonico et al. (2016)	France	Timothy, sainfoin, red clover	0–50	0	Forage-based silage
6	Girard, Dohme-Meier, Silacci, et al. (2016)	Switzerland	Birdfoot trefoil, alfalfa, sainfoin, red clover	0–100	7.0:93	TMR silage
7	Bouchard et al. (2015)	Canada	Alfalfa, sainfoin	0–100	0	Forage-based silage
8	Theodoridou et al. (2012)	France	Sainfoin	0–100	0	Forage-based silage
9	Wang et al. (2007)	Canada	Alfalfa, sainfoin	0–100	0	Forage-based silage
10	Scharenberg, Arrigo, Gutzwiller, Wyss, et al. (2007)	Switzerland	Grass-clover, sainfoin	0–100	0	Forage-based silage
11	Fraser et al. (2000)	Wales, UK	Sainfoin, red clover	0–100	0	Forage-based silage

Abbreviation: TMR, total mixed ration.

as a figure of graphic. To deal with such graphical data, WebPlotDigitizer (https://apps.automeris.io/wpd/) was employed to obtain the value of the variables.

2.3 | Statistical analysis

Statistical method used for the present meta-analysis was linear mixed model methodology introduced by St-Pierre (2001) and Sauvant et al. (2008) as the most widely used method of meta-analysis study in the area of animal sciences. The model recognizes the study as random effects where the RC or sainfoin-related factors were set as fixed effects. Prior to analysis, all data units were transformed into the same units of measurement. Moreover, variables with small sample size were disregarded because it may decrease the statistical power. The estimation of the influence of RC or sainfoin inclusion level on the response variables was accomplished by the following statistical model:

$$Y_{ij} = B0 + B1X_{ij} + B2X_{ij}2 + s_i + B_iX_{ij} + e_{ij},$$

where Y_{ij} is the the estimated output from the variable X as a continuous variable in the study i, $B0$ the overall intercept of the experiments (fixed effect), $B1$ the linear regression model of Y coefficient on X (fixed effect), B2 the quadratic regression model of Y coefficient X (fixed effect), X_{ij} the value of the continuous predictor variable X (levels of RCS or SS inclusion in the diets), s_i the random effect model of experiment i, b_i the random effect model of experiment i on the coefficient of regression of Y on X in experiment i and e_{ij} the residual error. The 'study' variable was declared in the CLASS statement because it does not contain numerical information.

Additionally, data were weighted by n replication in each study where an unstructured variance–covariance matrix (type = UN) was also performed at the random effect part of the model in order to avoid a positive correlation between the intercepts and slope (Irawan, Hidayat, et al., 2020; Jayanegara et al., 2012). Models of statistics used were p value, root mean square errors (RMSE) and Akaike information criterion (AIC). The AIC value was used to select the better-fit models when quadratic value was found to be significant. When quadratic term was not significant, the model was transformed back to the linear model (Patra, 2014) where quadratic term was not stated in the table. Significance was declared at p value of ≤0.05 while there is a tendency to be significant if the p value obtained is between 0.05 and 0.10.

Furthermore, using different types of forages ensiled, categorical covariate analysis was performed to identify the influence of RCS, SS or their combination (SS + RCS) in comparison to the control group on the response variables as previously described (Irawan, Hidayat, et al., 2020; Irawan et al., 2021). Given that in many studies control treatments were either grass or alfalfa, those were denoted separately as GS and alfalfa silage (AS). This analysis was performed using mixed model effects on SAS statistical software following the mathematical model:

$$Y_{ij} = \mu + s_i + \tau_j + S\tau_{ij} + e_{ij},$$

where Y_{ij} is the the estimated output for the dependent variable Y, μ the overall mean of the category, S_i the random effect of i study, τ_j the fixed effect of the j level, $S\tau_{ij}$ the random effect interaction between i study and the j level, and e_{ij} the unexplained residual error. A significant effect was declared at $p < 0.05$ or a tend to be significant if p value is between 0.05 and 0.10. The least-square means of the categories were compared to Tukey's test.

3 | RESULTS

3.1 | Characteristics of studies

This meta-analysis includes 37 studies that were published between 1997 and 2020. The studies were all conducted in the regions of Europe (48.9%), United Kingdom (18.9%) and North America (32.4%). In dairy cows, most included studies were indoor feeding studies where total mixed ration (TMR) with silage were the most common type of diets, representing 80% of the studies while the rest of studies used 100% forage-based silage. In contrast, nine out of 11 studies in small ruminants used forage-based silages as a basal diet and only two studies fed the animals with TMR-based diets. The dietary inclusion levels of RCS in dairy cows and SS in small ruminants varied between 30% and 100%. The RCS and SS mostly either replaced AS or substituted to forage-based silages such as perennial or annual ryegrass, tall fescue, timothy, birdsfoot trefoil and festulolium.

3.2 | Meta-regression analysis of red clover inclusion on response variable

The effects of RCS inclusion levels on the performance of dairy cows across studies are presented in Table 3. According to the estimated model, increasing RCS proportion in the diets significantly influenced the NDF digestibility in a curvilinear pattern ($p < 0.05$) but it was not associated with OM, ADF and N digestibility ($p > 0.10$). There were strong evidences that higher RCS proportion quadratically affected N intake and linearly increased urinary N and faecal N outputs ($p < 0.05$) while milk N concentration was not changed ($p > 0.05$). Increasing proportion of RCS was associated to an increase pH value and quadratically influenced acetate (A) concentration ($p < 0.05$). However, no effects were observed on NH_3-N, a total of volatile fatty acids (VFAs) concentration, propionate (P), butyrate (B), and A to P ratio ($p > 0.10$) when RC inclusion increased. There were also no effects of elevating RC silage proportion on DMI and milk yield of dairy cows ($p > 0.10$). However, milk fat composition decreased ($P < 0.01$) as RCS inclusion increased while milk protein content linearly increased ($p < 0.01$). Increasing RCS proportion in the diets, on the other hand, positively increased feed efficiency ($p < 0.01$) as expressed in milk/DMI in a linear pattern. Additionally, the concentration of C18:3 alpha linolenic acid (ALA) and conjugated

TABLE 3 Regression models of dairy cows' performance as influenced by inclusion level of red clover silage.

Response variable	*n*	Model	Parameter estimates				Model statistics		
			Intercept	SE $_{Intercept}$	Slope	SE $_{Slope}$	*p* value	RMSE	AIC
Digestibility, g/kg DM									
Organic matter	70	L	706.7	10.08	0.09	0.369	0.798	44.18	620
Neutral detergent fibre	65	L	560.4	25.76	1.46	0.761	0.061	97.31	646
		Q			−0.017	0.008	<0.05		646
Acid detergent fibre	43	L	525.0	38.09	1.81	1.225	0.153	114.8	394
Nitrogen (N)	44	L	645.9	11.97	−0.68	0.470	0.157	48.47	454
N partitioning (g/day)									
N intake	35	L	375	31.0	3.03	0.800	<0.01	0.852	96.4
		Q			−0.02	0.010	<0.05		96.4
Urinary N	37	L	123	12.3	0.70	0.400	<0.05	0.393	47
Faecal N	31	L	122	10.1	0.10	0.300	<0.01	0.331	39
Milk N	23	L	132	7.34	0.40	0.281	0.172	19.02	202
Ruminal fermentation									
pH	27	L	6.49	0.06	0.006	0.003	<0.05	0.204	21.2
NH_3-N, µg/mL	11	L	7.91	0.36	0.07	0.061	0.302	0.971	51.3
VFA, mM	23	L	91.77	5.61	0.08	0.136	0.567	13.37	180
Acetate (A), %	27	L	64.77	1.10	0.23	0.090	<0.05	6.314	0.18
		Q			−0.002	0.001	<0.05		
Propionate (P), %	27	L	19.49	0.60	0.05	0.035	0.207	2.037	137
Butyrate (B), %	27	L	11.67	0.67	−0.06	0.028	0.052	11.50	130
A:P ratio	27	L	3.48	0.15	0.008	0.011	0.449	0.606	80.1
Cows' performance									
DMI, kg/d	99	L	15.92	5.919	0.104	0.120	0.388	27.72	801
Milk yield	94	L	29.28	0.939	0.03	0.028	0.264	4.443	478
ECM, kg/d	37	L	32.99	0.665	−0.03	0.029	0.285	2.201	176
Fat %	94	L	4.03	0.081	−0.004	0.002	<0.01	0.241	42
Protein %	86	L	3.28	0.051	0.003	0.001	<0.05	0.218	−51.5
Lactose %	71	L	4.71	0.019	0.002	0.001	<0.01	4.727	−128
		Q			−0.0002	7.19E-03	<0.01		−128
Milk/DMI	47	L	1.42	0.037	0.0009	0.001	0.418	0.128	−38.1
Milk fatty acids composition (g/100 g FA)									
C18:0	27	L	31.51	16.45	0.16	0.066	<0.05	34.12	192
C18:1 t-9	12	L	1.07	0.487	0.01	0.014	0.475	0.801	41.8
C18:1 c-9	27	L	64.67	35.325	0.24	0.185	0.209	71.91	239
C18:2 LA	19	L	5.85	3.025	0.006	0.026	0.829	6.14	102
C18:3 ALA	8	L	0.53	3.234	0.31	0.113	0.052	3.823	53.4
CLA	27	L	1.56	1.148	0.05	0.022	<0.05	2.539	124

Abbreviations: AIC, Akaike information criterion; CLA, conjugated linolenic acid; DMI, dry matter intake; ECM, energy corrected milk; FA, fatty acids; L, linear pattern; n, sample size; Q, quadratic pattern; RMSE, root mean square errors; VFA, volatile fatty acids.

linoleic acid (CLA) in milk linearly increased ($p < 0.05$) due to increasing of dietary RCS.

3.3 | Meta-regression analysis of sainfoin inclusion on response variables

Evaluation of elevating dietary SS proportion had no association on DM, OM, NDF and N digestibility in small ruminants ($p > 0.10$, Table 4). There were also no correlations on N balance parameters such as N intake, faecal N and urinary N with dietary SS level ($p > 0.10$). Increasing SS inclusion quadratically ($p < 0.05$) affected acetate proportion (%) and linearly increased ($p < 0.05$) propionate level (%) and A:P ratio where NH_3-N and total VFA were not influenced ($p > 0.10$).

3.4 | Effect of types of silages on response variables

In the categorical analysis, the effects of different types of silages including GS, AS, SS, RCS and a combination of SS + RCS on response variables are presented in Table 5. Incorporation of SS, RCS or SS + RCS decreased ($p < 0.05$) the digestibility of DM, OM, NDF and ADF compared to GS while SS had lower ($p < 0.05$) N digestibility compared to other categories. When compared to GS, legume silages (AS, SS, RCS, SS + RCS) had higher ($p < 0.05$) N intake. In addition to that, SS + RCS inclusion had higher ($p < 0.05$) urinary N and faecal N excretion where SS resulted a lowest ($p < 0.05$) N output especially in faeces than any other silage groups. Milk N concentration did not differ between silages. Analysis on the ruminal fermentation profile showed that a combination of SS + RCS resulted in higher ($p < 0.05$) NH_3-N concentration compared to GS and RCS or SS alone. The SS + RCS group was also able to increase ($p < 0.05$) A and P molars concentration (%) when compared to GS and RCS alone but it was not different to AS and SS groups for acetate and AS for both acetate and propionate concentration. As a consequence of higher gap of A and P in the SS group, this was being significantly higher ($p < 0.05$) for A:P ratio than those of AS and SS + RCS but had no difference to GS.

In regard to the productive performance and its component, it was shown from Table 5 that feeding dairy cows with SS or RCS alone had no effect on milk production parameter in comparison to

TABLE 4 Regression models of small ruminants' performance as influenced by inclusion level of sainfoin silage.

			Parameter estimates				Model statistics		
Response variable	n	Model	Intercept	$SE_{Intercept}$	Slope	SE_{Slope}	*p*-value	RMSE	AIC
Digestibility, g/kg DM									
Dry matter	12	L	677.7	27.92	0.185	0.731	0.807	43.94	123
Organic matter	14	L	673.8	24.65	0.66	0.832	0.454	58.34	144
Neutral detergent fibre	12	L	606.4	64.68	−0.21	2.015	0.917	122.71	139
Nitrogen (N)	17	L	572.1	131.6	2.36	3.728	0.546	253.26	179
N partitioning (g/day)									
N intake	12	L	20.8	2.01	−0.003	0.013	0.846	0.432	44.4
Urinary N	12	L	8.9	1.00	−0.001	0.007	0.994	0.393	31.8
Faecal N	12	L	6.5	1.00	0.002	0.004	0.599	0.177	22.9
Ruminal fermentation									
NH_3-N, µg/mL	9	L	18.7	3.09	0.07	0.138	0.610	5.648	68.4
VFA, mM	11	L	105.7	7.94	0.61	0.338	0.132	17.57	97.5
Acetate (A), %	9	L	71.2	0.77	−0.14	0.037	<0.05	2.443	52.1
		Q			0.001	0.000	<0.05		52.1
Propionate (P), %	9	L	17.9	0.82	0.11	0.024	<0.01	2.320	48.1
Butyrate (B), %	9	L	7.4	0.58	0.004	0.017	0.813	0.879	43.7
A:P ratio	9	L	3.9	0.28	0.03	0.007	<0.05	0.566	32.1
Small ruminants' performance									
DMI, g/d	17	L	640	28.3	−0.07	0.129	0.564	6.676	125
ADG, g/d	14	L	170.7	38.5	−0.39	1.128	0.738	19.23	148

Abbreviations: AIC, Akaike information criterion; ADG, average daily gain; DMI, dry matter intake; L, linear pattern; n, sample size; Q, quadratic pattern; RMSE, root mean square errors; VFA, volatile fatty acids.

TABLE 5 Effect of sainfoin, red clover silages and their combination on the performance of ruminants.

Response variable	*n*	Silage categories GS	AS	SS	RCS	SS + RCS	SEM	*p* value
Digestibility, g/kg								
DM	72	721.9a	645.8b	677.2b	662.4b	663.3b	6.367	<0.001
OM	88	723.3a	661.2b	640.7bc	691.9b	678.2b	6.187	<0.001
NDF	81	652.1a	486.7c	458.0c	586.8b	565.8b	12.30	<0.001
ADF	51	539.6a	456.4b	465.9ab	512.6a	436.9b	24.19	0.045
N	60	604.8a	631.8a	417.6b	612.5a	598.9a	17.98	0.004
Nitrogen partitioning (g/kg $BW^{0.75}$)								
N intake	49	2.94ab	3.59a	3.72a	3.61a	3.96a	0.194	0.001
Urinary N	47	1.16b	1.38b	0.45c	1.31b	1.52a	0.064	0.001
Faecal N	44	1.02b	1.58a	1.09b	1.32a	1.47a	0.067	0.001
Milk N, g/d	25	113.2	-	99.06	116.1	137.6	8.168	0.247
Ruminal fermentation								
pH	34	6.52	6.47	6.43	6.61	6.66	0.033	0.303
NH_3-N, µg/mL	20	10.43b	16.25a	6.89c	12.72b	13.67a	1.608	0.007
VFA, mM	34	93.99	97.10	105.02	100.08	102.04	2.762	0.366
Acetate (A), %	36	67.23ab	69.08a	74.47a	61.37b	69.60a	0.934	0.001
Propionate (P), %	36	17.73b	21.06a	16.81b	17.86b	21.66a	0.369	<0.001
Butyrate (B), %	36	10.45	10.19	9.96	9.14	9.40	0.417	0.313
A:P ratio	36	4.01a	3.22c	4.41a	3.57b	3.13c	0.099	<0.001
Small ruminants' performance								
DMI, g/d	114	612.6b	-	623.4b	698.5a	695.3a	43.65	0.015
ADG, g/d	14	148.5ab	146.5ab	128.1b	190.3a	203.7a	16.17	0.040
Feed efficiency	11	0.60	0.56	0.55	0.59	0.54	0.191	0.376
Dairy cows' performance								
DMI, kg/d	99	15.59b	16.58ab	-	15.76b	18.47a	28.82	0.005
Milk yield, kg/d	94	27.67b	30.79a	29.12b	29.29b	31.04a	0.466	0.041
ECM, kg/d	41	31.82	32.27	31.72	31.49	-	0.424	0.658
Fat, %	94	4.15a	4.00b	4.08a	3.95b	3.79b	0.040	0.002
Protein, %	86	3.35a	3.23b	3.23b	3.19b	3.29b	0.025	<0.001
Lactose, %	71	4.71	4.73	4.71	4.75	-	0.013	0.129
Milk/DMI	35	1.52	1.42	-	1.44	-	0.021	0.107
Milk FA composition. g/100 g FA								
18:0	31	27.14bc	27.55bc	27.13bc	29.01b	40.68a	5.679	<0.001
18:1 t-9	16	0.80	0.67	0.74	0.89	0.97	0.188	0.973
18:1 c-9	31	53.43	52.35	52.48	60.92	56.92	12.03	0.499
18:2 LA	23	4.54	4.35	4.43	4.59	5.43	1.180	0.940
18:3 ALA	12	0.99bc	0.94bc	1.10bc	3.71b	8.98a	0.956	0.038
CLA	31	1.38c	1.45bc	1.44bc	1.89b	6.31a	0.422	<0.001

Note: Means within a row with different letters (a, b, c) differ ($p < 0.05$).

Abbreviations: AS, alfalfa silage; AIC, Akaike information criterion; CLA, conjugated linolenic acid; DMI, dry matter intake; ECM, energy corrected milk; FA, fatty acids; GS, grass-based silage; L, linear pattern; n, sample size; Q, quadratic pattern; RMSE, root mean square errors; RCS, silage with red clover inclusion; SS + RCS, silage containing sainfoin and red clover; SS, silage with sainfoin inclusion; VFA, volatile fatty acids.

GS and was lower ($p < 0.05$) when compared to AS. On the other hand, a combination of dietary SS + RCS had a significantly higher ($p < 0.05$) milk production than those of GS, SS and RCS. However, such combination had lower ($p < 0.05$) milk fat and protein composition compared to GS while lactose concentration and feed efficiency among treatment groups were similar. There were contradictory results on SS + RCS group because it did not only significantly increase ($p < 0.05$) the CLA and ALA contents while also increasing the C18:0 concentration of the milk ($p < 0.01$). Other FA such as 18:1 t-9, 18:1 c-9 and 18:2 LA were similar among groups. Feeding SS in small ruminants also failed to improve their productive performance as the DMI and ADG variables were not changed.

4 | DISCUSSION

4.1 | Dairy cows' performance

The results of the present meta-analysis indicated that under various circumstances, increasing the proportion of RC silage at a certain level either to replace legume-based silage or grass-based silage can improve NDF digestibility. However, high inclusion of RCS might negatively affect NDF digestibility. These results might be attributed to the variance of forage species used as comparisons. Theoretically, higher NDF digestibility is achieved for forage with a higher rate of fibre fermentation in the rumen which can be associated with, for example, plant maturity and lignification of the plants (Dewhurst, 2013; Schulz, Westreicher-Kristen, Molkentin, et al., 2018). Since the baseline used as a control in this meta-regression analysis was either grass or legume, further subgroup analysis may help to confirm the effect.

Table 5 suggested that feeding dairy cows with silage containing RC and SS or their combination had lower DM, OM and NDF digestibility compared to GS, in particular SS with the lowest NDF and N digestibility. This can be partly explained from the chemical composition of SS in which more fibrous than that of RC and some legume species such as alfalfa thus resulting in lower fermentation rate of SS particle in the rumen (Copani et al., 2016; Niderkorn et al., 2019). Previous in vitro studies corroborated this finding whereas sainfoin had lower gas production and total VFA production compared to alfalfa and red clover (Calabrò et al., 2012; Grosse Brinkhaus et al., 2017) and lower in vitro dry matter degradability compared to timothy grass (Copani et al., 2015). Other studies, in addition, suggested that CT had a substantial role to decrease fibre digestibility of SS because it is able to bind nutrient polymer including fibre and protein when giving at high proportion (Scharenberg, Arrigo, Gutzwiller, Wyss, et al., 2007; Randby et al., 2012). Protecting CP from rumen metabolism by CT from sainfoin is favourable to increase true protein availability or bypass protein (Copani et al., 2016). Therefore, although N digestibility decreased, this phenomenon did not adversely influence milk yield and energy-corrected milk (ECM) in dairy cows (milk yield) as shown in Table 5.

It is noteworthy that RCS inclusion is more favourable when supplemented to grass-based silage compared to AS. Indeed, RCS and AS resulted in higher ruminal NH_3-N production because they are major source of N by nature when compared to GS. However, RCS and AS have comparable N excretion, indicating that RCS provide higher protection effectiveness against rumen proteolysis than that of AS, indicating that RCS is more beneficial than AS in terms of better NUE. This result was supported by other authors who reported that alfalfa exhibited higher proteolysis compared to RC silage (Broderick, 2018; Li et al., 2018). An in vitro study also demonstrated that RC had a more favourable protein fraction profile and promote more B_3 vitamin production than alfalfa (Grosse Brinkhaus et al., 2017). Thus, replacing AS with RCS silage is a favourable alternative because it can promote more environmentally friendly outputs. In addition, sufficient N source is important for rumen microbial synthesis. As legume species typically contain higher N and more fermentable, diets containing RCS, SS + RCS or AS resulted in higher propionate synthesis which is an important precursor for energy in ruminants to support the optimal production performance (Irawan, Noviandi, et al., 2020).

One interesting reason to include RCS in the diets is to increase NUE and PUFA concentration in milk due to the effective roles of PPO in preventing rumen protein degradation and BH. In this study, higher RCS proportion in the diets had linear correlation with the increase of both N intake and N outputs via urine and faecal (Table 3), indicating that no positive effect can be drawn in terms of increasing NUE by offering RC in the grass-based diet or it can be speculated that the desirable effect on NUE improvement is conditional and level-dependent. Previously, a number of studies reported similar finding where increasing RCS level reduced NUE both in GS diets and TMR silage-based diets (Gidlund et al., 2017; Halmemies-Beauchet-Filleau et al., 2014; Ineichen et al., 2019; Moorby et al., 2009). Although no improvement was observed, feeding GS most likely led to N deficiency and then further resulted in lower milk production. This was evident in the present meta-analysis showing that a combination of SS + RCS inclusion in the diet increased milk yield than dairy cows fed only GS (Table 5). Improvement of milk yield in the SS + RCS group is most probably due to the increase of N supply from the diet although the milk/DMI was similar among groups.

Dairy cows offered GS and sainfoin had superior milk fat content than other groups (AS and SS + RC). Although previous studies reported contrary findings where RCS inclusion enhanced milk fat content (Schulz, Westreicher-Kristen, Knappstein, et al., 2018), others also reported that replacing maize silage with RCS did not change milk fat (Cheng et al., 2011; Dewhurst et al., 2010; Moorby et al., 2016). These discrepancies may be associated with chemical composition of diets especially related to N-energy balance. Also, higher milk fat percentage is associated with acetic acid molar production as a major precursor for milk fat synthesis which can be linked to the fact that grass with lower fermentable carbohydrate is most likely to produce higher acetate proportion (Steinshamn 2010). Lowering milk protein concentration in this study was in agreement with previous studies replacing GS with RCS (Halmemies-Beauchet-Filleau et al., 2013; Vanhatalo et al., 2009) while another study reported that substituting RCS to GS did not influence protein content of the milk (Arvidsson et al., 2012). The decrease of milk protein concentration may be associated with the lower of amino acids availability as a result of decreasing the microbial protein supply (Halmemies-Beauchet-Filleau et al., 2014).

Moreover, dairy cows also received more benefit by consuming diets containing RCS because it promoted to increase PUFA concentration in milk especially CLA content in comparison to GS. However, combining SS + RCS seemed to be more consistent to increase such beneficial milk FA than those of diets substituted alone with either RCS or SS as can be seen that this group resulted in significantly higher C18:3 ALA and CLA contents (Table 5). On the other hand, this study also found that C18:0 concomitantly increased with SS + RCS group which is somewhat undesirable. Numerous health benefits have been associated to diets containing plants containing bioactive compounds in terms of enriching beneficial PUFA including RCS. Our results agreed with most studies using SS or RCS to be substituted in grass-type silages which resulted in positive alteration of PUFA contents in milk (Gadeyne et al., 2015; Ineichen et al., 2019; Leduc et al., 2017; Moorby et al., 2016; Schulz, Westreicher-Kristen, Molkentin, et al., 2018). It was also reported that replacing maize silage with incremental RCS proportions increased the milk stearic acid (SA) concentration (Schulz, Westreicher-Kristen, Molkentin, et al., 2018), which needs further exploration in this area.

The approach of this study was to evaluate the protective effects of bioactive compounds of SS and RCS, we expected that diets with these legume species would transfer higher beneficial PUFA to the milk. Generally speaking, the milk FA profile is reflected from FA composition of the diets (Schulz, Westreicher-Kristen, Molkentin, et al., 2018). Major portions of unsaturated FA in the diets undergo an extensive BH process (isomerization and desaturation) to form saturated fatty acids in milk (Shingfield et al., 2010). At this point, secondary metabolites such as PPO and CT play a substantial role to inhibit these transformation processes, particularly of the last step ruminal BH act by rumen microbes (Irawan et al., 2021; Niderkorn & Jayanegara, 2020). Condensed tannins present in sainfoin, in respective to the BH pathway, help to reduce the saturation of PUFA by two possible ways: directly suppressing microorganisms that caused the PUFA saturation and/or indirectly protect the PUFA from rumen biohydrogenation which can lead to increase the accumulation of PUFA especially CLAs in the small intestine and finally in the milk (Girard, Dohme-Meier, Wechsler, et al., 2016; Huyen et al., 2020). In addition, PPO from RCS silage can also help to control plant lipolysis in the rumen as it catalyzes ortho-quinones to react with cellular nucleophiles which further form a phenol-bound protein (PBP) complex (Lee et al., 2014). Although it has not been fully elucidated, the available theory suggested that linking PUFA protection with the PBP complex is possible through the lipid-glycerol entrapment with the PBP which is a physical barrier representation to microbial lipases (Lee et al., 2014; Van Ranst et al., 2013). In relation to the ortho-quinones, moreover, it is possible to protect the lipid-glycerol since the lipids have nucleophilic binding sites potential which then further protect the PUFA from rumen microbial biohydrogenation (Gadeyne et al., 2015). Thus, it is clear that feeding sainfoin and red clover would synergistically increase the beneficial PUFAs content as confirmed from the present study that feeding SS + RC had more than doubled CLA and C18:3 ALA contents (Table 5).

4.2 | Small ruminants' performance

Inclusion of sainfoin to grass-base silage for small ruminants had no effect on nutrient digestibility, nitrogen metabolism and growth performance. Many reports have indicated that sainfoin did not significantly contribute to the voluntary intake when offered to lambs despite it contains higher quality nutrients than GS (Copani et al., 2016; Frutos et al., 2004; Scharenberg, Arrigo, Gutzwiller, Wyss, et al., 2007). When compared to AS, no benefit was observed in terms of voluntary intake and nutrient utilization (Fraser et al., 2000). As sainfoin contains moderate to high percentage of CT, it may affect palatability and also reduce nutrient digestibility due to its binding ability effect (Scharenberg, Arrigo, Gutzwiller, Soliva, et al., 2007). Numerous in vitro studies also demonstrated that sainfoin had lower gas production, degradable DM and OM and VFA production (Copani et al., 2015; Grosse Brinkhaus et al., 2017; Wang et al., 2022) and also NDF digestibility (Zhang et al., 2021) compared to alfalfa and grass.

In addition, no benefit was recorded on feed efficiency parameter by feeding sainfoin or red clover to GS. However, the inclusion RCS alone or together with sainfoin in GS had a greater effect at the ADG of small ruminants in comparison with GS, SS and AS. This can be related to higher N supply in RCS-based diets as also observed in this study. Although there is no difference in N use efficiency, higher N supply can promote better protein synthesis that can be used for gain by the animals. Most recent studies supported the current finding whereas silage containing SS and RCS increased ADG of lambs by 29.8% compared to pure timothy silage thus promote higher growth rates and carcass percentage (Copani et al., 2016; Niderkorn et al., 2020).

5 | CONCLUSIONS

The present meta-analysis highlights that elevating RCS proportion into GS-based diet for dairy cows had no substantial effect on nutrient digestion and fermentation parameters except for pH due to the buffering capacity of RCS. However, incremental RCS inclusion markedly increased N intake, giving a reason for the improvement of ADG in small ruminants but was not able to enhance dairy production. RCS shows a noticeable improvement of milk fatty acids profile as indicated by the linear increase of CLA and C18:3 ALA when RCS proportion increases in the diet. In small ruminants, increasing SS inclusion is not able to improve production performance. However, a combination of RCS and SS into the diets of dairy cows and small ruminants shows synergistic effects in improving milk FA profile of dairy cows and ADG of small ruminants. Thus, establishing a more diverse legume rich in various bioactive compounds such as red clover and sainfoin in the diets of ruminants is promising to help improving ruminants' production, nitrogen use efficiency, and healthier product quality.

AUTHOR CONTRIBUTIONS

Agung Irawan: Database development, statistical analysis, writing the original manuscript. **Vincent Niderkorn**: Conceptualization, data collection, data validation, writing—review & editing. **Anuraga Jayanegara**: Conceptualization, methodology, data validation, writing—review & editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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