Feature Article

Are insects a solution for feeding ruminants? Legislation, scientific evidence, and future challenges

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Implications

- Insects are promising alternatives to conventional protein and fat sources for ruminants.
- Legislation is more restrictive for the use of protein-rich insect meals than for insect oils.
- Insect meals seem to have lower in vitro digestibility than conventional plant meals.
- Insect products may modulate the ruminal environment (e.g., CH_4 emissions and biohydrogenation).

Key words: animal performance, digestibility, insect oil, processed-animal-protein

Introduction

The growing interest of the scientific community on the inclusion of insect-derived products in ruminant diets is leading to a boost of research papers on this topic. Even though in absolute terms the increase in the number of published papers is limited—because limited is also the literature on this topic—the scientific production in 2022 equals the scientific production of the former 19 years (about 10 papers published in each above-mentioned time-period). The reason is that insect-derived products are considered not only promising but also sustainable feed ingredients, thanks to the ability of insects to successfully convert relatively low-value agri-food waste into high-quality proteins and fats. The insect crude protein content varies, according to insect stage and processing technology, from 7.5% to 91% (dry matter—DM—basis), while the crude fat values range between 46% and 64% (DM basis) (Finke and Oonincx, 2023). Moreover, their production is characterized by limited environmental footprint. A generalization of their use in ruminant farming could contribute to limit its impact and to move towards a Circular Economy model (Gasco et al., 2020). However, this requires that reality meets expectations.

On this basis, in this review we provide information about the state-of-the-art on the use of insects in the nutrition of domestic ruminants, including an overview of the current legislation worldwide and the hitherto known effects of insect-derived products on nutrient digestibility, animal performance, and ruminal biohydrogenation (BH). Following the identified differences in legislative frameworks and the potential application of insect products, information will be provided by dividing them into protein-rich feedstuffs, lipid-rich products (differentiating full-fat meals and free oils), and other insect-derived ingredients for ruminant diets.

Overview of the Legislation on the Use of Insects as Ruminant Feedstuffs

A major obstacle to evaluating the inclusion of insect-derived products in ruminant diets is represented by the legislation on the potential risk of mad cow disease (Bovine Spongiform Encephalopathy; Renna et al., 2022a). Insect meals are classified as processed-animal-proteins (PAPs) and prohibition on the use currently applies in most high-income countries (e.g., European countries, Japan, and China; Figure 1);

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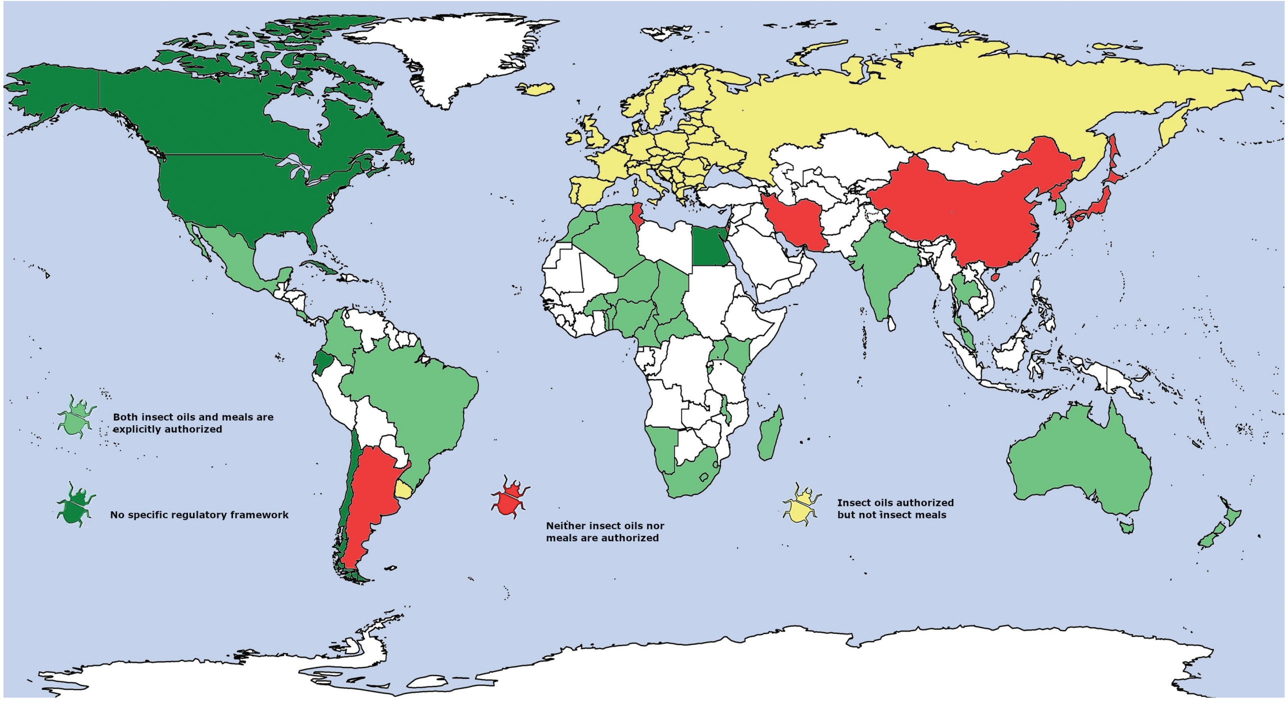

Figure 1. Current worldwide legislation framework on the use of insects as feed for ruminants.

contrarily, developing and emergent regions usually lack specific legislation on this issue (Lähteenmäki-Uutela et al., 2017). For example, in different countries in the Americas, only the prohibition of meat meal, blood meal, bone meal, and mammalian offal as raw materials for ruminant feeding was found. At the same time, no explicit ban or authorization was found on the use of insect proteins for the processing, marketing, and use of animal feed (Lähteenmäki-Uutela et al., 2017).

Under the European Union (EU) Law, insects, and their derived products—excluding live insects—that are intended to be used in animal feed are explicitly considered "animal by-products". This qualification entails a number of obligations for producers, as defined in Regulation No 1069/2009 and its implementing Regulation No 142/2011—also known as the "EU animal by-products legislation" (IPIFF, 2022). It is promising that EU laws on the use of insects as feedstuffs for monogastrics have been updated in recent years and, since July 2017, it is permitted in aquaculture, and in April 2021, the Standing Committee on Plants, Animals, Food, and Feed approved it for poultry and pig as well. The Regulation No 2001/999 (Annex IV), as amended by Regulation No 2017/893 (Annex X) and Regulation No 2021/1925, allows insect PAPs from several species [namely, black soldier fly (*Hermetia illucens* L.), common housefly (*Musca domestica* L.), yellow mealworm (*Tenebrio molitor* L.), lesser mealworm (*Alphitobius diaperinus* Panzer), house cricket (*Acheta domesticus* L.), banded cricket (*Gryllodes sigillatus* Walker), field cricket (*Grillus assimilis*), and silkworm (*Bombyx mori* L.)] in aquaculture, poultry, and pig feeding, as part of the legislation on animal by-products). On a positive note, although the EU prohibits the use of insect-derived PAPs for ruminants, no restriction applies for insect oils.

Despite these regional variations in the legal rules governing the use of insects as feed, a clear global interest exists among researchers and feed manufacturers in fostering innovation and research in this sector. In the near future, this may contribute, as occurred for monogastrics, to promote legislative changes that favor the use of insects in ruminant feeding worldwide.

Insect Meals as Protein-rich Feedstuffs

In vitro evaluation

Insect meals are considered as promising alternatives to plant proteins commonly used as ruminant feedstuffs (e.g., soybean meal; SBM). As for monogastrics, the first available studies on this topic focused on full-fat insect meals and were conducted in vitro (Table 1). In animal nutrition research, in vitro methodologies allow decreasing the length and cost of experiments, the number of animals used as well as the total time of their use. Of course, information obtained in vivo is the most reliable and therefore in vitro studies are commonly used for preliminary testing, prior to conduct large in vivo trials.

In an in vitro trial with bovine, Jayanegara et al. (2017a) examined the potential of full-fat *H. illucens* larvae meals to substitute 50–100% of SBM, the most common protein-rich feed for ruminants, using a 60:40 forage-to-concentrate (F:C) diet as the incubation substrate. The high crude protein (CP) and ether extract (EE) contents of *H. illucens* larvae meal suggested a suitable use to substitute SBM but decreases in in vitro organic and dry matter digestibility (IVOMD and IVDMD, respectively) were found compared to SBM. Using cannulated sheep as inoculum donors, Renna et al. (2022b) investigated the in vitro

Table 1. Diets containing insect-derived products compared to control diets: impact on nutrient digestibility and ruminal fermentation parameters

Ruminant species	Insect species	Insect form	Inclusion level, %	IVOMD	IVDMD	Intake	IDNDN	Total VFA	Ac:Pr	pH	NH_3-N	Reference
Bovine	HI1	larvae meal	20	🟥	🟥			⬜		🟩	🟥	Jayanegara et al. (2017a)
	HI2	larvae meal	20	🟥	🟥			⬜		🟩	🟥	
	HI1	larvae meal	40	🟥	🟥			⬜		🟩	🟥	
	HI2	larvae meal	40	🟥	🟥			🟥		🟩	🟥	
Bovine	HI	larvae oil	5	⬜	⬜			⬜		⬜	⬜	Jayanegara et al. (2020)
	OS	larvae oil	5	🟥	⬜			⬜		⬜	⬜	
	TM	larvae oil	5	🟥	🟥			⬜		⬜	⬜	
	ZM	larvae oil	5	🟥	🟥			⬜		⬜	⬜	
	GB	nymph oil	5	🟥	🟥			⬜		⬜	⬜	
Bovine	HI	larvae oil	1	⬜	🟥			⬜		⬜	⬜	Jayanegara et al. (2021a)
			2	🟥	🟥			⬜		⬜	🟩	
			3	🟥	🟥			⬜		⬜	🟩	
			4	🟥	🟥			⬜		⬜	🟩	
			5	🟥	🟥			⬜		⬜	🟩	
Bovine	ACD	adult meal	10	⬜	⬜			⬜		🟩	🟩	Ahmed et al. (2021)
	BP	adult meal	10	⬜	⬜			⬜		🟩	🟩	
	GB	adult meal	10	⬜	⬜			⬜		🟩	⬜	
	BM	pupae meal	10	⬜	⬜			⬜		🟩	🟩	
Bovine (beef)[#]	HI	larvae meal	36			⬜						Fukuda et al. (2022)
Bovine	GB	adult meal[*]	4-16		🟩			⬜	⬜	🟩		Phesatcha et al. (2022)
Bovine	HI	larvae meal	20	🟥	🟥			🟥	⬜	🟥		Mulianda et al. (2020)
		larvae meal, chemical defatting	20	⬜	⬜			🟩		⬜	🟩	
		larvae meal, mechanical defatting	20	⬜	🟥			🟥		⬜	🟥	
Bovine (crossbred cattle)[#,†]	BM	defatted pupae meal	1.4			⬜		⬜		⬜	⬜	Rashmi et al. (2022)
			2.7			⬜		🟥		⬜	🟥	
			4.1			⬜		⬜		⬜	🟥	
Ovine	HI	larvae oil	2		⬜			⬜	⬜	⬜	⬜	Hervás et al. (2022)
	ACD	adult oil	2		⬜			⬜	⬜	⬜	⬜	
	BM	pupae oil	2		⬜			⬜	⬜	⬜	⬜	
Ovine	ALD	larvae meal	100	🟥				🟥	🟩	🟩	🟥	Renna et al. (2022b)
	HI	larvae meal	100	🟥				🟥	🟩	🟩	🟥	
	MD	larvae meal	100	🟥				🟥	🟩	🟩	🟥	
	TM	larvae meal	100	🟥				🟥	⬜	🟩	🟥	
	BL	subadult meal	100	🟥				🟥	🟩	🟩	🟥	
	ACD	adult meal	100	🟥				🟥	🟩	🟩	🟥	
	GB	adult meal	100	🟥				🟥	⬜	🟩	🟥	
	GS	adult meal	100	🟥				🟥	🟩	🟩	🟥	
Ovine	TM	larvae meal	100				🟩					Toral et al. (2022)
	ZM	larvae meal	100				⬜					
	ALD	larvae meal	100				⬜					
	ACD	adult meal	100				⬜					
Caprine (post-weaning)[#]	GB	adult meal	15			⬜	⬜	⬜			⬜	Astuti et al. (2019)
			30			⬜		⬜			⬜	

Compared with controls: 🟩 Increase ($P < 0.05$); ⬜ No significant change; 🟥 Decrease ($P < 0.05$).

Abbreviations: IVOMD, in vitro organic matter digestibility; IVDMD, in vitro dry matter digestibility; IDNDN, in vitro intestinal digestibility of the non-degraded nitrogen; VFA, volatile fatty acids; Ac:Pr, acetate:propionate ratio; HI1 and HI2, *Hermetia illucens* larvae age 1 week and 2 weeks, respectively; OS, *Oecophylla smaragdina*; TM, *Tenebrio molitor*; ZM, *Zophobas* morio; GB, *Gryllus bimaculatus*; ACD, *Acheta domesticus*; BP, *Brachytrupes portentosus*; BM, *Bombyx mori*; ALD, *Alphitobius diaperinus*; MD, *Musca domestica*; BL, *Blatta lateralis*; GS, *Grylloides sigillatus*.

[*] Global effect of the addition of 4 incremental levels of GB meal. Two forage:concentrate ratios (F:C 60:40 and 40:60) were tested, but the effect of the interaction F:C × GB level was not significant.

[#] In vivo trials.

[†] Values were compared with fermentations after 8h.

effects of eight insect meals (for species of origin, see Table 1) on rumen fermentation characteristics. Consistent with Jayanegara et al. (2017a), Renna et al. (2022b) observed poor IVOMD for the insect meals, except for *Blatta lateralis*, compared to reference plant meals (i.e., soybean, sunflower, and rapeseed meals). Nevertheless, low inclusion levels of insects may help avoiding the depression of digestibility parameters, as suggested by Ahmed et al. (2021) after replacing 25% of SBM (≈10% of total diet) with insect meals obtained from *A. domesticus*, *Brachytrupes portentosus*, and *Gryllus bimaculatus* adults, and from *B. mori* pupae. None of them caused adverse effects on nutrient digestibility when incubated in vitro with rumen inoculum from cows.

Full-fat insect meals are characterized by high EE and chitin contents, which may contribute to explain detrimental effects on nutrient digestibility, especially on structural carbohydrates, since the presence of high amounts of unsaturated lipids can be toxic for ruminal cellulolytic microbiota (Jayanegara et al., 2017a). Moreover, Jayanegara et al. (2017a) hypothesized that chitin, a complex polysaccharide naturally present in insect exoskeletons, might contribute to impair nutrient digestibility, being nondegradable by ruminal microbiota. However, a subsequent study by the same research team showed that lowering the chitin content in *Gryllus assimilis* did not improve its overall digestibility characteristics (Jayanegara et al., 2017b), suggesting a minor role of chitin on overall digestibility. Renna et al. (2022b) also found that *B. lateralis* had the highest chitin content among the tested full-fat insect meals, but it displayed the most promising results in terms of fermentation. These results would support that chitin is degradable to certain extent in the rumen (Fadel El-Seed et al., 2003).

As mentioned above, a major reason for the expectations placed on insects as innovative feeds is their high protein content. To properly assess protein digestibility in ruminant species, it is necessary to evaluate the extents of ruminal protein degradation and of intestinal digestibility of nondegraded protein in the rumen. Dietary proteins that reach the rumen are largely degraded to ammonia-nitrogen (NH_3-N), a source of nitrogen for ruminal microbial growth. However, due to the high cost of protein-rich feeds and the need to minimize nitrogen losses, there is high interest in using proteins that by-pass the rumen, and reach the small intestine, where they are absorbed and favor animal performance (Putri et al., 2019). Robles Jimenez et al. (2022a) evaluated the use of larvae meals from *H. illucens*, *T. molitor*, and *Notonecta spp*., reporting similar in vitro degradable protein contents with that of SBM (≈60%) and higher when compared to fish meal (19%). Ruminal degradation of SBM proteins has been well characterized, whereas information on protein fractions and protein degradation rate of insect meals is still very scant. Renna et al. (2022b) observed lower ruminal ammonia content when comparing the use of various full-fat insect meals and plant meals, consistent with a previous comparison between *H. illucens* larvae meal and SBM (Jayanegara et al., 2017a). Such result would be advantageous in ruminant nutrition provided that intestinal digestibility of nondegraded protein in the rumen is high. This latter parameter was estimated by Toral et al. (2022) in *T. molitor*, *Zophobas morio* Fabricius, and *A. diaperinus* larvae, and *A. domesticus* adults, focusing on nitrogen disappearance, rather than in CP, since a misleading value could be obtained with the use of the conventional nitrogen-to-protein conversion factor (*Kp*) of 6.25. It should be highlighted that, in insects, the classical CP analysis with the Kjeldahl methodology may lead to an overestimation of the real CP content by including part of the nitrogen embedded in chitin (Janssen et al., 2017). Taking this into consideration, Toral et al. (2022) observed high (>64%) in vitro intestinal digestibility of nondegraded nitrogen for tested insects, with no difference relative to SBM, except for a higher intestinal digestibility in *T. molitor*.

In vivo evaluation

The literature on the use of insect-derived products in vivo is limited to only one or two papers on each ruminant species.

Astuti et al. (2019) published one of the first in vivo trials on this topic, evaluating the inclusion of cricket meal in rations destined to preweaning and postweaning goat kids. Feeding a milk replacer containing full-fat adult *Gryllus bimaculatus* meal revealed no negative effects on physiological parameters in goat kids, which showed higher nutrient intake, comparable final body weight, and average daily gain, but lower feed efficiency, relative to the group fed goat milk. Compared with artificial milk replacers, goat milk helps the transfer of immunity from mothers to kids, which could affect animal performance. In postweaning kids fed a 30:70 F:C diet, the inclusion of 15% and 30% cricket meal in the concentrate (replacing 50% and 100% of SBM respectively), showed encouraging results, with no negative effects on DM and nutrient intakes, average daily gain, feed efficiency, and rumen fermentation profiles.

In the ovine, Robles-Jimenez et al. (2022b) evaluated in vivo the effects of including *T. molitor* meal in the diet of growing lambs, compared to SBM and fishmeal. While feed intake and nitrogen intake were not impaired by the protein source, insect meal, and fishmeal decreased DM and organic matter digestibility, and nitrogen retention relative to SBM. In dairy ewes, however, Robles-Jimenez et al. (2022c) found that replacing SBM by *Notonecta spp.* in the diet increased milk production (up to 60%), fat-corrected milk and fat-protein corrected milk (Figure 2).

Finally, in the bovine, Fukuda et al. (2022) investigated the effects of full-fat *H. illucens* larvae meal, compared to conventional protein sources (cottonseed and SBM), as the main protein source in beef steers consuming low-quality forages. The authors focused their attention on feed intake and digestion performance, reporting no depression of the overall digestibility or impairments in ruminal fermentation. However, feeding the insect meal decreased the intake of organic matter from roughages, relative to the use of vegetable meals.

Defatting as a tool to enhance digestibility

Research aimed at improving the technological processes to obtain insect meals is moving alongside with investigations on their inclusion in animal diets. Manufacturers have increased the production of defatted insect meals, which allow increasing protein concentration (Mishyna et al., 2021). Moreover, defatted meals have better stability during storage, due to reduced risk of lipid oxidation and rancidity.

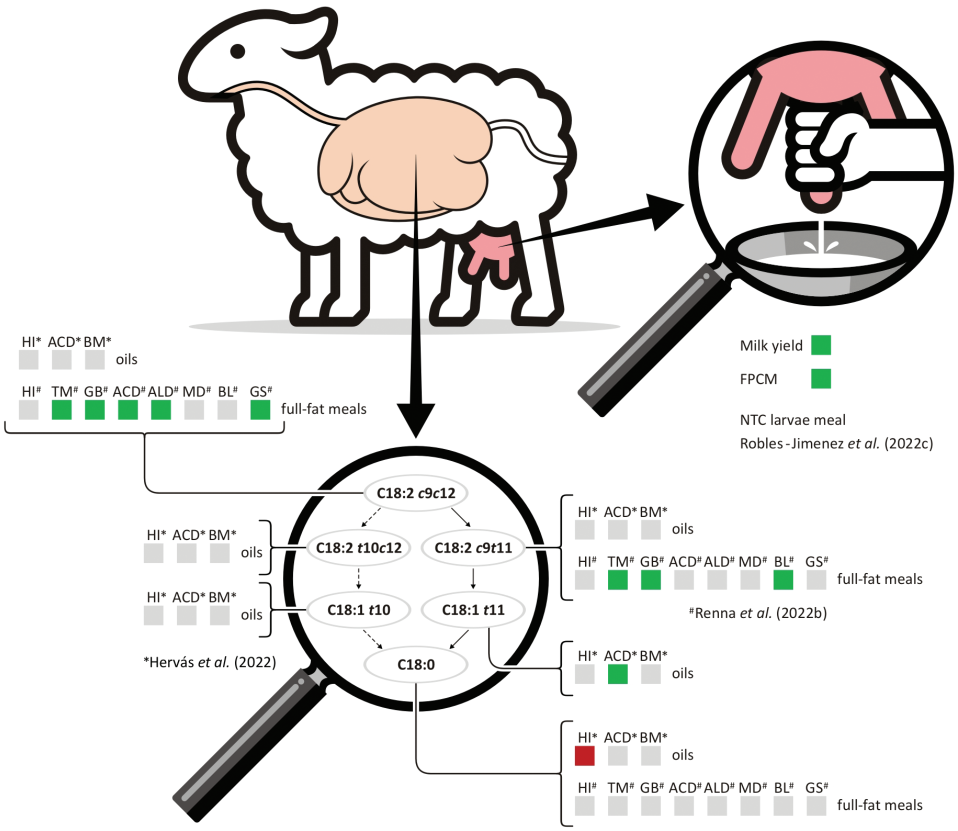

Figure 2. Diets containing insect-derived products compared to control diets: impact on dairy performance and major pathways of ruminal biohydrogenation of linoleic acid. Compared with controls: 🟩 increase ($P < 0.05$); ⬜ no significant change; 🟥 decrease ($P < 0.05$). Abbreviations: ACD, *Acheta domesticus*; ALD, *Alphitobius diaperinus*; BL, *Blatta lateralis*; BM, *Bombyx mori*; FPCM, fat-protein corrected milk yield; GB, *Gryllus bimaculatus*; GS, *Grylloides sigillatus*; HI, *Hermetia illucens*; MD, *Musca domestica*; NTC, *Notonecta spp*.; TM *Tenebrio molitor*.

However, we are aware of only three reports—two in vitro (Mulianda et al., 2020; Phesatcha et al., 2022) and one in vivo (Rashmi et al., 2022) – on the inclusion of defatted insect meals in the diet of ruminants. Interestingly, Phesatcha et al. (2022) reported that replacing SBM by defatted cricket meal improved ruminal fermentation by enhancing DM degradability and reducing methane production and acetate:propionate ratio. Mulianda et al. (2020) suggested that defatting of black soldier fly meal, in particular by chemical means, improved its nutritional value. Moreover, Rashmi et al. (2022) replaced 10%, 20%, and 30% of SBM by defatted silkworm pupae meal in the diet of crossbred cattle, also with positive results. Indeed, indirect comparisons with the use of full-fat insect meals (Jayanegara et al., 2017a; Renna et al., 2022b) would suggest a greater overall digestibility of defatted silkworm, which may be included up to 30% into rations for cattle without impairments of rumen fermentation patterns and nutrient utilization (Rashmi et al., 2022). Because ruminant diets are usually characterized by low lipid contents, defatting insect meals might represent an advantage for developing innovative and sustainable protein-rich sources for ruminants.

Insects as a Source of Lipids

Full-fat insect meals

As mentioned above, besides their high protein content, full-fat insect meals are also rich sources of lipids, with a high variability in terms of aliphatic chain length and unsaturation level. Therefore, when testing these meals in ruminant nutrition, it is fundamental to investigate the effects on ruminal BH of fatty acids. In this regard, some of the full-fat insect meals examined by Renna et al. (2022b) were rich in polyunsaturated fatty acids (PUFA), which remained partly unaffected by ruminal BH. It could be speculated that the high EE and chitin content of PUFA-rich insects (*A. domesticus*, *T. molitor*, and *G. sigillatus*) might have inhibited the microbial populations involved in BH, raising interest in the study of the use of full-fat insect meals to improve the fatty acid profile of ruminant-derived products, such as milk and meat (Figure 2).

Additionally, some full-fat insect meals, and especially those obtained from *H. illucens* larvae, are rich in medium-chain saturated fatty acids, such as lauric acid (C12:0), which can have antibacterial and antimethanogenic effects (Jayanegara et al., 2017a; Astuti and Komalasari, 2020; Ahmed et al., 2021).

Surprisingly, a potential to mitigate enteric methane production (up to −22.7%) was also observed in vitro by Phesatcha et al. (2022) using defatted adult *G. bimaculatus* meal, which was linked to a reduction of ruminal protozoa numbers (Table 2). Different mechanisms may therefore explain the potential antimethanogenic effects of insect-derived products, which warrants further research. In any event, these first promising results would suggest that insects may strategically be included in ruminant diets with the aim of reducing greenhouse gas emissions, further lessening the environmental impact of livestock activities. No less important are the implications of lowering CH_4 production on animal performance and feed efficiency, given the role of enteric methane emissions as an energy loss for the ruminant.

Insect oils

Oils represent a major product after defatting whole insects, whether they are intended for animal feed or for human consumption. Overall, the purpose of adding insect oils to ruminant diets is threefold: 1) to increase the energy density of the ration, 2) to potentially mitigate enteric methane emissions, and 3) to modulate ruminal BH to obtain dairy and meat products with a more favorable fatty acid profile for human health.

In terms of digestibility, diet supplementation with insect oils showed similar results to those obtained with full-fat insect meals. Jayanegara et al. (2021a) tested the in vitro effects of increasing levels of *H. illucens* larvae oil (1–5% of the diet), reporting a dose-dependent linear decrease in IVDMD and IVOMD. Despite an unexpected increase in ammonia concentration and protozoa population counts in rumen fluid, *H. illucens* oil decreased methanogenesis in a dose-dependent manner, with significant effects with inclusion levels equal or higher than 4%. The same general trend was also reported by Thirumalaisamy et al. (2020), using increasing levels of *B. mori* oil (0.5–5% diet), and in a previous study by Jayanegara et al. (2020), who tested the in vitro effects of the addition of 5% of insect oils with different unsaturation degree (species of origin are reported in Table 1). Interestingly, Thirumalaisamy et al. (2020) showed stronger antimethanogenic effects in high-forage substrates, whereas Jayanegara et al. (2020) reported no interaction between effect of insect oil and basal diet composition (70:30 or 30:70 F:C ratio) on enteric methane production. Overall, these studies suggest a promising potential to alleviate this energy loss and decrease the environmental impact of livestock.

Diet supplementation with PUFA-rich vegetable oils (e.g., soybean and linseed oils) has widely been examined as a tool to manipulate the nutritional quality of meat and milk fat. In this regard, Hervás et al. (2022) compared the in vitro BH of oils from *H. illucens*, *B. mori*, and *A. domesticus* (rich in lauric, α-linolenic, and linoleic acid, respectively) and soybean (rich in linoleic acid). In the absence of detrimental effects on ruminal fermentation, results supported that insect oils modulated ruminal BH, with results that were directly linked to their unsaturation degree. Specifically, *A. domesticus* oil was identified as the most interesting practical alternative to soybean oil, favoring the accumulation of the potentially health-promoting vaccenic acid (*t*11-18:1) without a shift towards the production of *t*10-containing fatty acids (Figure 2). These latter have been associated with undesirable effects on animal performance and human health.

Other Insect-derived Ingredients in Ruminant Feeding: Chitin and Chitosan

Few studies investigated the potential role of chitin, extracted from insect exoskeleton, and chitosan, obtained through chitin deacetylation, as feed additives for the mitigation of ruminal methane emissions and BH modulation. Regarding the first effect, Anggraeni et al. (2022) reported that chitosan can modify the ruminal environment by lowering bacteria and protozoal counts, feed digestibility, and the acetate:propionate ratio. Even if most ruminal methanogens are hydrogenotrophic, the above-mentioned effects on volatile fatty acids might strengthen the methane mitigation potential of chitosan, as few methanogens defined as “acetoclastic” (e.g., *Methanosarcina barkeri*) use acetate as an electron acceptor to produce CH_4. In an in vitro study, Haryati et al. (2019) observed that the addition of 1–2% of chitin or chitosan from *H. illucens* to a 60:40 F:C diet caused a dose-dependent decrease in gas production.

As a feed additive, chitosan may also inhibit the ruminal BH of unsaturated fatty acids. Jayanegara et al. (2021b) integrated results from published in vitro trials using chitosan from crustaceans at concentrations between 50 and 138 g/kg diet DM, and reported an increase in the ruminal accumulation of potentially health-promoting fatty acids (e.g., rumenic and *trans*-vaccenic acids), which could be subsequently available for mammary and adipose tissue uptake. However, Hervás et al. (2022) observed that chitosan from *H. illucens* at 30 g/kg DM did not modify the fatty acid profile of in vitro ruminal digesta, either when used alone or in combination with 20 g/kg DM of soybean, *H. illucens*, *A. domesticus*, or *B. mori* oils. The lack of effects on odd- and branched chain fatty acids, mostly derived from microbial synthesis, suggests that chitosan did not significantly alter ruminal microbiota. Further research is required to unravel whether these inconsistent effects of chitosans may be explained by their composition (e.g., species of origin and deacetylation degree), the experimental conditions (e.g., incubation time and basal diet composition), or other confounding factors.

Conclusions

Research on the use of insects in ruminant feeding is just taking its first steps and, although results obtained so far are mostly promising, available data are still scarce. In addition, these nutritional strategies are still subjected to several legislative limitations worldwide. Therefore, a major research effort is needed in coming years to achieve a detailed nutritional evaluation of insect-derived products (i.e., full-fat and defatted meals, oils, and other ingredients) and define specific recommended

Table 2. Diets containingi insect-derived products compared to control diets: impact on total gas, CO_2 and CH_4 productions, and ruminal protozoal count.

Ruminant species	Insect species	Insect form	Inclusion level, %	Total gas production	CO2	CH4	Protozoal count	Reference
Bovine	HI1	larvae meal	20	🟥		🟥		Jayanegara et al. (2017a)
	HI2	larvae meal	20	🟥		🟥		
	HI1	larvae meal	40	🟥		🟥		
	HI2	larvae meal	40	🟥		🟥		
Bovine	HI	larvae oil	5	🟥		🟥		Jayanegara et al. (2020)
	OS	larvae oil	5	⬜		⬜		
	TM	larvae oil	5	⬜		🟥		
	ZM	larvae oil	5	⬜		🟥		
	GB	nymph oil	5	⬜		🟥		
Bovine	HI	larvae oil	1	⬜		⬜	⬜	Jayanegara et al. (2021a)
			2	⬜		⬜	⬜	
			3	⬜		⬜	🟩	
			4	⬜		🟥	🟩	
			5	⬜		🟥	🟩	
Bovine	BM	pupae oil[*]	0.5	⬜		⬜	⬜	Thirumalaisamy et al. (2020)
			1	⬜		⬜	⬜	
			2	⬜		🟥	⬜	
			4	🟥		🟥	🟥	
			5	🟥		🟥	🟥	
	BM	pupae oil[#]	0.5	⬜		⬜	⬜	
			1	⬜		🟥	⬜	
			2	⬜		🟥	⬜	
			4	🟥		🟥	🟥	
			5	🟥		🟥	🟥	
	BM	pupae oil[†]	0.5	⬜		⬜	⬜	
			1	⬜		🟥	🟥	
			2	⬜		🟥	🟥	
			4	🟥		🟥	🟥	
			5	🟥		🟥	🟥	
	BM	pupae oil[‡]	0.5	⬜		⬜	⬜	
			1	⬜		🟥	🟥	
			2	⬜		🟥	🟥	
			4	🟥		🟥	🟥	
			5	🟥		🟥	🟥	
Bovine	ACD	adult meal	10	⬜	⬜	⬜		Ahmed et al. (2021)
	BP	adult meal	10	⬜	⬜	⬜		
	GB	adult meal	10	🟥	🟩	🟥		
	BM	pupae meal	10	⬜	⬜	⬜		
Bovine	HI	larvae meal	20			⬜		Mulianda et al. (2020)
		larvae meal, chemical defatting	20	🟩		⬜		
		larvae meal, mechanical defatting	20	⬜		⬜		
Bovine	GB	adult meal[‡‡]	4-16	🟩		🟥	🟥	Phesatcha et al. (2022)
Ovine	HI	larvae oil	2	⬜				Hervás et al. (2022)
	ACD	adult oil	2	⬜				
	BM	pupae oil	2	⬜				
Ovine	ALD	larvae meal	100	🟥		🟥		Renna et al. (2022b)
	HI	larvae meal	100	🟥		🟥		
	MD	larvae meal	100	🟥		🟥		
	TM	larvae meal	100	🟥		🟥		
	BL	subadult meal	100	🟥		🟥		
	ACD	adult meal	100	🟥		🟥		
	GB	adult meal	100	🟥		🟥		
	GS	adult meal	100	🟥		🟥		

Compared with controls: 🟩 Increase ($p < 0.05$) ⬜ No significant change 🟥 Decrease ($p < 0.05$).

Abbreviations: HI1 and HI2, *Hermetia illucens* larvae age 1 week and 2 weeks, respectively; OS, *Oecophylla smaragdina*; TM *Tenebrio molitor*; ZM, *Zophobas morio*; GB, *Gryllus bimaculatus*; ACD, *Acheta domesticus*; BP, *Brachytrupes portentosus*; BM, *Bombyx mori*; ALD, *Alphitobius diaperinus*; MD, *Musca domestica*; BL, *Blatta lateralis*; GS, *Grylloides sigillatus*.

[*] 70:30 forage:concentrate (F:C) ratio.

[#] 60:40 F:C ratio.

[†] 50:50 F:C ratio.

[‡] 40:60 F:C ratio.

[‡‡] Global effect of the addition of 4 incremental levels of GB meal. Two F:C ratios (60:40 and 40:60) were tested, but the effect of the interaction F:C × GB level was not significant.

levels of inclusion for ruminants, taking into account the large variations in insect composition due to species and production systems. This information is essential to formulate balanced diets and to rule out detrimental effects on animal performance, for which the possible differences between ruminant species and production systems must also be considered. As a next step, it would be advisable to evaluate the use of insect products in commercial diets for ruminants, taking into account their economic and environmental impact on different farming systems. Finally, the safety of both insect-derived products and of the substrate on which insects are grown should be thoroughly considered prior to establish a new livestock sector based on insects as feed. Progress in this direction should also be accompanied by the transfer of research output to lawmakers as a way to foster a regulation update worldwide.

Further Directions for Research on Insects as Ruminant Feedstuffs

- The lack of data on best inclusion levels of insect-derived products must be addressed.
- Information on the use of defatted insect meals, particularly in vivo, should be improved.
- Palatability of insect meals and oils needs to be assessed.
- Technologies to protect insect fatty acids from biohydrogenation need to be implemented.
- Safety data are required to confirm or reject a role of insects as prion vectors.
- The transfer of research output to lawmakers may foster a regulation update worldwide.

Conflict of interest statement. None declared.

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