



AN OVERVIEW OF ALTERNATIVE PROTEIN SOURCES FOR RUMINANTS IN THE TROPICAL AREA

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Abstract

Investigating alternative protein sources for ruminants in tropical regions is crucial due to distinct climatic conditions and limited resources. Scientists, acknowledging the challenges, are exploring locally accessible alternatives to develop cost-effective and sustainable protein supplements for ruminant nutrition, emphasizing the importance of understanding the nutritional composition for advancing livestock husbandry sustainability. The aim of this research is to elucidate alternative local protein sources for ruminants in tropical areas and assess their effectiveness in promoting sustainable production and enhancing ruminant nutrition. Protein feed sources are essential for providing amino acids to animals and producing animal products such as meat, milk, and wool. Inadequate protein in animal feed may have a detrimental impact on rumen fermentation and animal output. Therefore, alternative protein sources have received increased attention in order to replace the main protein source. The alternative protein sources in this review were divided into five categories: agricultural biomass, fodder shrubs, biological protein and plant weeds, insect protein and agro-industry biomass. Furthermore, plant secondary compounds found in alternative protein sources might be used to modulate rumen fermentation and improve animal performance. By focusing on regional sources of protein, feed costs are reduced while availability is raised. Based on this analysis, it can be concluded that there are a number of alternative protein sources that might be used in place of primary protein sources and that they could support ruminant production in the face of several animal feed crises. However, the potential use of any protein source should be assessed in light of the country's availability and animal production practices.

Key words: animal nutrition, protein feed, ruminant, soybean meal, tropical area

In contemplating various global scenarios, it is crucial to account for the unpredictable nature of current agricultural production. This unpredictability exacerbates the challenges faced by developing nations, where the scarcity of affordable ruminant animal feed intensifies overall feed insecurity (Suntara et al., 2023). Addressing the complexities of ruminant-based food production involves navigating multiple contemporary issues. These encompass the imperative to meet the demands of a growing global population, ensuring food security, combating environmental pollution, and addressing the escalating impacts of climate change (Halmemies-Beauchet-Filleau et al., 2018). A noteworthy critique of the animal production industry revolves around the concept of food-feed competition, which entails diverting human-edible sources to ruminants and utilizing arable land for animal feed production rather than directly cultivating human-edible food (Global Market Insights, Inc., 2022). The escalating interest in biofuel production adds to the competitive demand for arable land, intensifying the challenges faced by the industry.

Due to their high fiber and low protein values, forages are typically of poor nutritional value in the tropics, which lowers the efficiency of animal production. Thus,

both temperate and tropical regions look for local protein sources. Additionally, soybean meal (SBM) is a major source of dietary protein in the majority of intensive livestock production systems (Toral et al., 2022). This reliance is of special significance due to the limitations on the use of meat meal and its analogs in ruminant feeding in Europe, which promotes the search for new protein-rich feeds (Lamminen et al., 2019).

When compared to conventional feeds, factors like feed value, animal production responses, and feed costs help determine whether using alternative feeds for ruminants is feasible. The global alternative protein market for livestock feeds is estimated to be valued at \$3.96 billion in 2023, experiencing a compound annual growth rate (CAGR) of 7.5%. According to the increasing number of ruminant animals, human consumption, and rivalry of feed to other sections, alternative protein consumption will rise to US\$ 8.2 billion by 2033, accounting for around 7.5% of the world market (Global Market Insights, Inc., 2022).

Therefore, the investigation of alternative protein sources for ruminants is critical in tropical regions owing to the distinctive climatic conditions and limited availability of resources. To address the unique challenges

tropical environments present in the development of cost-effective and sustainable protein supplements for ruminant nutrition, scientists are examining locally accessible alternatives (Halmemies-Beauchet-Filleau et al., 2018). It is imperative to comprehend the nutritional composition and appropriateness of these substitutes in order to advance the sustainability and effectiveness of livestock husbandry in tropical regions (Wanapat, 2001).

The article explores a comprehensive range of subtopics related to agricultural biomass, fodder shrubs, biological proteins, plant weeds, insect protein and agro-industry biomass. Within the agricultural biomass category, the discussion delves into cassava hay, cassava/cassarea, yeast-fermented cassava chips (YEFECAP), winged bean tubers, highlighting their significance in the broader context. The fodder shrubs section intricately covers *flemingia*, *Leucaena leucocephala*, Brazilian spinach, mulberry tree, *Sesbania* spp., moringa, and *jatropha*s, offering a background on each and their relevance. Biological proteins and plant weeds are addressed with a focus on single-cell protein, microalgae, seaweeds, and duckweeds, elucidating their importance. The insect protein segment explores *Gryllus bimaculatus* and black soldier fly larvae (BSFL) (*Hermetia illucens*), providing necessary context. Lastly, agro-industry biomass encompasses cassava pulp fermented by yeast waste (CSYW), cassava pulp fermented by *Lactobacillus* bacteria (LAB), citric acid fermented by yeast waste (CWYW), and red yeast fermented by tofu, elucidating their roles and connections within the broader framework. This review article aims to provide a comprehensive understanding of the diverse applications of alternative local protein sources for ruminants in tropical areas and assess their effectiveness in promoting sustainable production and enhancing ruminant nutrition.

Agricultural biomass

Cassava hay

Table 1 presents the chemical composition of agricultural biomass suitable for ruminant feed in tropical regions. A tropical annual tuber crop called cassava (*Manihot esculenta* Crantz) was assessed nutritionally as a potential source of protein for ruminant nutrition (Prachumchai et al., 2022). Harvesting the foliage begins three months after planting and continues every two months until the crop is a year old for cassava biomass planting for dried leaf and hay (Morgan and Choct, 2016). Increased soil fertility and more fodder are two benefits of intercropping cassava with leguminous plants like cowpea (*Vigna unculata*) and wild tamarind (*Leucaena leucocephala*). Hay or cassava leaves are low in hydrocyanic acid (HCN) and contain 20–25% CP in dry matter (Wanapat, 2003; Li et al., 2023). Cassava leaf and hay have the potential to be good sources of protein, according to Wanapat (2003). In order to achieve this, additional biomass was harvested throughout the year in addition to the leaf or whole crop that was harvested at an early stage of development (Li et al., 2023). According to reports, the yield of cassava hay varies from 2 to 8 tons per hectare depending on the

variety, cultivation techniques, and fertilizer used (Wanapat, 2001). The use of cassava in integrated farming systems has been proposed (Preston, 2001). Hay and cassava leaves had low fiber levels but relatively high protein levels. Additionally, methionine and threonine concentrations were comparable to those found in SBM (Preston, 2001). In feeding trials with cattle, high levels of dry matter (DM) intake (3.2% of body weight [BW]) and high DM digestibility (71% of DM) were found when animals were fed with cassava hay (Wanapat, 2001). Adding cassava hay at a rate of 1–2 kg/head/d to the diet of dairy cows could significantly reduce the amount of concentrate they need while also improving the yield and fat composition of their milk (Wanapat, 2001). Additionally, adding cassava hay to the diet of dairy cows may raise milk thiocyanate levels, potentially enhancing milk quality and storage, especially in smallholder dairy farming (Promkot and Wanapat, 2009). It has also been demonstrated that the condensed tannins in cassava hay have the potential to lower gastrointestinal nematodes and thus function as an anthelmintic agent (Wanapat and Kang, 2000). Given its high protein content, cassava hay is a good source of multiple nutrients for animals. It can also be processed along with its leaves and some nutrient enrichment by grinding, chopping, or pelletizing. Cassava has the potential to improve tropical livestock production systems' productivity and profitability overall (Wanapat et al., 2000).

Cassava/cassarea

In recent efforts at developing new products, cassava chips as an energy source, urea as a non-protein nitrogen (NPN), and cassava leaf/hay as a rumen bypass protein have all been used. Two new cassava-based products, cassarea and cassaya, have been created. Cassarea was created with the following ingredients: cassava chips 83.6% + urea 13.4% and 3% tallow (cassarea I, 40% CP); cassava chips 80.2% + urea 16.8% and 3% tallow (cassarea II, 50% CP). Using a nylon bag technique, cassarea's rumen degradability of CP was assessed, and it was discovered to be between 46.2 and 56.7%. It was discovered through additional testing with cassarea II (40% CP) that it could be used to supplement lactating cow rations in place of SBM, but the addition of a rumen bypass protein like cottonseed meal should also be looked into. The ingredient list for the product known as cassaya (30% CP) included chopped whole cassava crop hay (85%), SBM (5%), cassava chips (5%), urea (2%), tallow (2%), and sulfur (1%), which were all combined with water, put through a pellet press, and then dried in the sun to 85% dry mass. When cassaya was used as a protein source in lactating dairy cows, rumen fermentation was promoted, milk yield and composition improved, and there was a financial benefit. Nevertheless, initiatives should be taken to boost cassaya production.

Yeast-fermented cassava chips (YEFECAP)

Cassava is a tropical and subtropical crop that is widely grown (Wanapat et al., 2011). Its root is a good

source of rumen fermentable carbohydrate and has been used in ruminants with NPN, particularly urea. While dietary yeasts, particularly *Saccharomyces cerevisiae*, have been widely used as ruminant feed because they contain useful nutrients for ruminants, particularly a high lysine composition (8.0 g/100 g of protein). Yeast products benefit ruminants by increasing dry matter (DM) intake and overall animal performance. Boonnop et al. (2009) and Polyorach et al. (2013) reported that yeast-fermented cassava chip (YEFECAP) was prepared, and the CP of cassava was increased from 3.4 to 32.5% CP in the YEFECAP. According to Boonnop et al. (2009), YEFECAP increases CP content from 3.4 to 30.4% when compared to the non-fermented group. The high CP could be due to the effect of urea in the 250 mL nutrient solution [urea (48 g) and molasses (24 g)].

Boonnop et al. (2010) conducted further research on the impacts of YEFECAP as a protein source replacement for SBM in concentrate and found that YEFECAP could totally replace SBM in terms of rumen fermentation efficiency and nutritional digestibilities in beef cattle. When compared to the SBM group, the supplementation of YEFECAP led to a significantly higher rumen bacterial and fungal zoospore population as well as a decreased protozoal population (Wanapat et al., 2011). Additionally, dairy cows' milk yield, milk fat, and milk protein levels increased when SBM was replaced by YEFECAP at 100% in a concentrate diet (Wanapat et al., 2011). YEFECAP could therefore be a different source of protein for ruminant feed.

Winged bean tubers

A rare tropical legume found in ruminant diets is the winged bean (*Psophocarpus tetragonolobus*). Thailand is one of the tropical nations where this plant, a valuable tropical legume vegetable with great nutritional value, may be produced (Suntara et al., 2022, 2023). The edible winged bean's pods, seeds, leaves, blooms, and tuberous roots may be found in all of Thailand's provinces (WB). On the chemical composition of this plant, there was, however, very little information. The chemical composition of the tuber was unknown until Sriwichai et al. (2021) showed that it might be utilized as an alternative animal feed. A dual-purpose species with a big tuber was also discovered in this investigation, albeit it is not widely utilized as animal feed. It was therefore suggested that it be utilized as a cassava substitute so that farmers may benefit from it, which would eventually result in large-scale production for animal feed.

With their excellent nutritional content, winged bean tubers (WBT) may be employed in feed formulations in place of cassava. In addition to having a high calorie content (3.82 Mcal/kg), comparable to that of cassava chips (4.04 Mcal/kg), WBT also contains a high protein content (19% DM), ether extract that ranges from 0.2 to 1.0 DM, and neutral detergent fiber (NDF) that ranges from 15 to 30% DM (Suntara et al., 2022, 2023). In Thailand, winged bean tuber production output has reached 15.5

t/ha (Sriwichai et al., 2021). Based on *in vitro* results, WBT containing 19–21% CP could be successfully incorporated into ruminant diets (Suntara et al., 2022, 2023; Srichompoo et al., 2024). Unnawong et al. (2023) also suggested steaming WBT to improve feed efficiency, decrease degradability, and preserve rumen pH, making it a more practical option to cassava chip as it includes more starch and protein. However, additional analyses of *in vivo* tests are required to confirm the findings.

Fodder shrubs

Flemingia

Fodder trees and shrubs are associated with a range of livelihoods, encompassing nitrogen-fixing in soil, provision of firewood, fencing materials, and serving as sources for both human foods and animal feeds. Among these, tropical *Flemingia* stands out as a traditional fodder shrub with significant potential to be utilized as feedstuff. The biomass yield of *Flemingia* (*Flemingia macrophylla* (Willd.) Merr.) can vary based on factors such as soil fertility, climate, and management practices. Under optimal conditions, it has the potential to produce substantial biomass, offering a valuable forage source for livestock. Actual biomass yields per hectare may vary, and incorporating fertilizer can enhance productivity. Banful et al. (2000) reported that, initially, the biomass yield of *F. macrophylla* was 12 t/ha. However, over six subsequent cuttings between August 1992 and August 1994, the yield increased to 35 t/ha. *Flemingia* has condensed tannins in the range of 2.4% to 3.3% of dry matter and protein in the range of 16.9% to 23.7% CP (Viennasay and Wanapat, 2020; Phesatcha et al., 2021). This finding suggests that condensed tannin is a natural supplement for ionophores to be replaced in order to enhance ruminal fermentation when compared to the other species studied. Ruminants' rumen fermentation can be changed by condensed tannins from *Flemingia*. In addition, tannins act as antagonists of protein digestion in the rumen by forming complexes with dietary proteins, reducing their availability for microbial degradation (Viennasay and Wanapat, 2020). The incorporation of *Flemingia macrophylla*, a tannin-rich forage, offers a potential solution as the tannin-protein complexes formed during digestion can enhance the production of bypass protein, allowing for a more efficient utilization of dietary proteins in the small intestine of ruminants (Kang et al., 2017). After four months of regrowth, *Flemingia* leaf was cut, dried in the sun, and pulverized to provide a supplement powder for beef cattle (Kang et al., 2017).

According to Kang et al. (2017), adding *Flemingia* hay meal (FMH) could improve *in vitro* fermentation and reduce methane (CH₄) production. Moreover, FMH supplementation in dairy steers increased ruminal fermentation, particularly propionate, and influenced the digestibility of nutrients, according to Phesatcha et al. (2016). According to Phesatcha et al. (2021), 100% FMH replacement in the concentrate mixture improved the efficiency of rumen fermentation, the digestibility of

nutrients, the bacterial population, the generation of microbial protein, and the production of CH₄ in beef calves fed rice straw. Flemingia hay meal supplementation at 150 g/cow/d dramatically increased milk production and rumen fermentation efficiency in lactating dairy cows (Phesatcha et al., 2021).

Leucaena

Leucaena (*Leucaena leucocephala*), a perennial leguminous tree or shrub offering substantial nutritional benefits for cattle, features deep roots and serves as a valuable feed resource for ruminants. It is tasty, nutritious, resistant to drought, and long-living. Banful et al. (2000) observed that in the first harvest, the biomass yield of *L. leucocephala* was elevated at 18 t/ha. However, across six successive cuttings spanning from August 1992 to August 1994, the biomass production of *L. leucocephala* increased to 27 t/ha. Leucaena is re-

garded as a significant source of protein for ruminants in tropical and subtropical areas. At 22.8–25.9% CP in dried leaf and 31.1% CP in seeds, it has a high protein content (De Angelis et al., 2021; Table 2). Leucaena leaf pellets (LLP; the pellet mixture of leucaena leaf powder, urea, and some binding agent) were tested on buffalo fed on rice straw (Hung et al., 2013). It has been demonstrated that adding LLP at a dosage of 450 g/d can enhance rumen ecology by enhancing nitrogen-retention and microbial N supply (Hung et al., 2013). Ensiling leucaena for two weeks and substituting 60% of rice straw significantly enhanced the microbial population in the rumen of dairy steers, as observed in the study by Nguyen et al. (2017). According to Idan et al. (2023), leucaena leaves could be used as a supplement to inferior straws to increase the output of small ruminants, particularly during the long dry seasons in the tropics.

Table 1. Chemical composition of agricultural biomass possible to use for ruminant feed in the tropical area

Item*	DM, %	CP, % DM	OM, % DM	EE, % DM	NDF, % DM	ADF, % DM	CT, % DM	Sources
Cassava								
cassava hay	86.3	25.0	87.5	6.2	44.3	30.3	3.9	Wanapat (2003)
dried cassava leaf	90.0	20–30.0	90.0	5.9	29.6	24.1	4.3	Wanapat (2003)
cassava leaf	43.0	19.8	96.6	7.9	–	–	–	Morgan and Choct (2006)
cassava root	39.2	2.6	70.2	0.9	–	–	–	Morgan and Choct (2006)
cassarea I	90.7	40.0	–	3.4	11.5	4.4	–	Wanapat and Kang (2015)
cassarea II	91.9	50.0	–	3.3	11.1	4.3	–	Wanapat and Kang (2015)
cassaya	86.3	30	–	7.2	38.3	26.6	3.3	Wanapat and Kang (2015)
yeast-fermented cassava chips (YEFECAP)	85.3	30.4	97.6	5.8	7.3	5.8	–	Boonnop et al. (2009)
winged bean tubers	43.5	18.9	96.3	0.4	16.8	5.4	–	Suntara et al. (2023) Srichompoo et al. (2024)

*DM = dry matter; CP = crude protein; OM = organic matter; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; CT = condensed tannin.

Table 2. Chemical composition of fodder shrubs possible to use for ruminant feed in the tropical area

Item*	DM, %	CP, % DM	OM, % DM	EE, % DM	NDF, % DM	ADF, % DM	CT, % DM	Sources
1	2	3	4	5	6	7	8	9
<i>Flemingia macrophylla</i>								
dried leaf	84.6	25.6	92.3	–	53.1	31.2	5.6	Phesatcha et al. (2021)
silage	25.6	18.1	95.6	–	47.5	37.1	10.2	Viennasay and Wanapat (2020)
<i>Leucaena leucocephala</i>								
dried leaf	86.2	22.8–25.9	91–93.6	4.7	17.4	12.8	10.2–21.0	De Angelis et al. (2021)
silage	30.3	25.1	90.9	–	36.2	21.4		Giang et al. (2016)
seeds	94.8	31.1	95.5	5.6	–	–	0.8	De Angelis et al. (2021)
Brazilian spinach								
leaf	12.9	21.2	97.6	–	41.7	21.0	–	Somma et al. (2021)
leaf-stalk	16.8	14.9	95.7	–	49.7	21.3		Somma et al. (2021)
mulberry tree								
leaf	19.8	22.3	86.4	3.5	31.1	18.3	–	Vu et al. (2011)

Table 2 – contd.

1	2	3	4	5	6	7	8	9
<i>Sesbania sesban</i>								
leaf	26.7	29.7	99.2	–	17.4	13.1	12.1	Debela et al. (2011)
twigs	30.4	19.4	88.6	–	47.9	40.1	5.1	Debela et al. (2011)
whole forage	19.3	22.8	91.7	–	23.8	20.2	10.9	Debela et al. (2011)
green pods	22.5	24.8	96.5	–	49.4	40.7	0.8	Debela et al. (2011)
<i>Sesbania grandiflora</i> seed pod	92.1	21.7	68.6	–	54.1	44.5	10.8	Unnawong et al. (2020 a)
<i>Moringa oleifera</i>								
fodder leaf	7.8	21.7	88	6.5	8.0	6.0	–	Nouman et al. (2014)
fodder stems	8.0	8.7	87.5	2.0	28.0	21.0	–	Nouman et al. (2014)
tree leaf	8.6	23.5	85.5	3.0	13.5	11.0	–	Nouman et al. (2014)
<i>Jatrophas</i>								
dried leaf	86.3	19.5	91.5	2.0	–	–	0.3	Esonu et al. (2020)
seeds	95.0	29.4	95.1	46.2	–	–	8.5	Ugbogu et al. (2014)
kernel meal	90.2	61.8	91.6	–	9.7	4.8	–	Oskoueian et al. (2011)

*DM = dry matter; CP = crude protein; OM = organic matter; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; CT = condensed tannin.

Brazilian spinach

Brazilian spinach (*Alternanthera sissoo*), widely cultivated in tropical regions with its roots in Brazil, serves as an excellent protein supplement for ruminants. Moreover, Brazilian spinach contains phytonutrient compounds, specifically flavonoids, which could offer alternative utility for this plant. Sommai et al. (2021) observed that the yield of Brazilian spinach was notably influenced by the maturity ages after plantation and regrowth. As the maturity ages after planting and regrowth increased, there was a significant increase in yield. Specifically, the Brazilian spinach yield after 60 days of planting reached 18 t/ha, and after 160 days of regrowth, the yield increased to 25 t/ha. According to Manurung et al. (2017), the flavonoid content of tabat barito was strongly impacted by maturation ages. Flavonoids have the capacity to influence the rumen microbiota, resulting in alterations in food digestion and metabolism. The flavonoids in alfalfa have been demonstrated to increase the number of cellulolytic bacteria (*Butyrivibrio fibrisolvens*) in dairy cows (Zhan et al., 2017; Li et al., 2022). Importantly, flavonoids not only have the ability to change rumen fermentation dynamics, but they also favor beneficial microbes such as *Megasphaera elsdenii* (lactate-utilizing bacteria), which can therefore have a positive influence on animal performance (Li et al., 2022). According to Sommai et al. (2021), the flavonoid content of the leaf peaked at 15 days after planting (82.16 g/kg DM), but the CP content ranged from 21 to 25% DM. Thus, Brazilian spinach can be used as a ruminant protein source. Moreover, supplementation with flavonoid extract from Brazilian spinach enhanced *in vitro* total volatile fatty acid (VFA) and C3 concentrations considerably while decreasing CH₄ generation and protozoal population (Sommai et al., 2021). More research is needed to execute this in dairy cows, test CH₄ levels, and gather more relevant

information on the methanogenic archaea of flavonoids in Brazilian spinach.

Mulberry tree

Mulberry trees are useful for a variety of things; their fruits may be eaten and used to make wine, while the leaves can be utilized as fodder because of their abundant biomass, high levels of CP, and important phytonutrients. There are over 80 species of *Morus* on the globe; four of these, *M. albus*, *M. atropurpurea*, *M. multicaulis*, and *M. bombycis*, are mostly cultivated (Hassan et al., 2020). *M. albus* and *M. indica* are commonly used in animal feed, whereas paper mulberry (*Broussonetia papyrifera*) is gaining popularity due to its antioxidant potential. Mulberry displays adaptability to diverse agro-climatic conditions, thriving in various settings such as mountains, plains, irrigated areas, and challenging environments like humid and semi-arid lands. The average yield of leaf and stem biomass for forage purposes ranges from 8 to 52 tons/hectare/year, with this substantial variation attributed to factors including mulberry species, agro-climatic conditions, geographical location, soil type, and harvesting techniques (Hassan et al., 2020; Rohela et al., 2020). The commonly found mulberry species, *Morus alba* and *M. indica*, are capable of producing leaf biomass in the range of approximately 25 to 30 tons/ha/year, with a shorter harvesting interval of about 9 to 10 weeks, owing to their ease of propagation and excellent growth characteristics (Sánchez, 2002).

Mulberry leaves have excellent CP content ranging from 22.3% to 27.3%, 40.1% nitrogen-free extract, and 3.5% to 5.1% ether extract, while containing 31.1% and 18.35% of NDF and ADF, respectively (Vu et al., 2011; Hassan et al., 2020). Additionally, it includes micronutrients like vitamins C, D, and B₁, beta-carotene, iron, and zinc, as well as other macrominerals like calcium,

magnesium, potassium, and phosphorus. There were also reports of palmitic (26.38% and 25.99%), linolenic (34.97% and 37.57%), and linoleic acids (14.76% and 16.05%) (Hassan et al., 2020). Furthermore, He et al. (2020) found that mulberry leaf biomass contained a total flavonoid range of 34–58.42 mg/g which might benefit rumen fermentation efficiency. However, anti-nutritional elements such as phytate (451–488 mg/kg), tannins (5.32–5.78 mg/kg), cyanide (1.01–2.14 mg/kg), and oxalates (183 mg/100 g) on a DM basis have been discovered in these fodder shrubs and require to be removed before being utilized further (Hassan et al., 2020).

There are several previous studies that have incorporated mulberry tree into animal feed in different forms, such as dried leaf form, silage leaf form, pellet leaf form, or supplementation of flavonoid extract. There is a high potential use of mulberry leaves in terms of modulation of the rumen, blood metabolites, antioxidants, immunomodulatory effects, health benefits, milk production, and meat quality in ruminants. Mulberry leaf powder (MLP) at 15% of the feed might help with blood metabolites and meat quality in Hu lambs that are being fattened (Ouyang et al., 2020). The ensilage of mulberry leaf has been applied in order to preserve the feed resources available year-round. According to Wang and Luo (2021), mulberry leaf silage might serve as an effective feed ingredient or a bioactive pharmaceutical that enhances lambs' health. Lambs with improved antioxidant and immunomodulatory properties have a changed rumen microbial community as a result of mulberry leaf silage. In accordance with Li et al. (2022), feeding 5–10% mulberry leaf silage to dairy cows can alter the rumen microbiome and the fermentation process, as well as enhance the quantity of fiber-digestion, propionic acid production, and milk fat-related microbes, resulting in higher milk supply. Furthermore, Xiong et al. (2021) discovered that using 40% paper mulberry silage in the diet enhanced the growth efficiency and meat fatty acid composition of Hu lambs, perhaps leading to a relatively high level of unsaturated fatty acids in *longissimus dorsi* muscle. Tan et al. (2012) recognized the formation of pelleted mulberry leaf and indicated that mulberry leaf pellets (MUP) might be employed as a protein source. Since its improvement in dry matter intake, $\text{NH}_3\text{-N}$ concentrations, viable total bacteria, amylolytic bacteria, proteolytic bacteria, cellulolytic bacteria, and microbial protein synthesis in Thai native beef cattle, the authors advocate supplementing up to 600 g/head/d (Tan et al., 2012).

Furthermore, flavonoid extract from mulberry leaf has been determined by Li et al. (2022), who showed that adding 45 g/d of flavonoid to buffalo feed is a suitable dosage for improving rumen fermentation and bacteria for improved performance in hot and humid climates.

Sesbania spp.

Sesbania, a member of the legume family, has been the object of study in alternative fodder shrubs for ruminant feed (Unnawong et al., 2021 a, b). There are several

species considering using leaves as animal feed: *Sesbania sesban*, *Sesbania grandiflora*, *Sesbania aculeata*, and *Sesbania rostrata*, while some species can produce pods containing high levels of tannin or saponin, which may act as rumen enhancers. *S. sesban* has demonstrated the capability to produce 10 to 20 tons per hectare of fuelwood when cultivated as standalone fallows and up to 2 tons per hectare in relay intercropping scenarios (Sileshi et al., 2000). The crude protein (CP) content in leaves, twigs, whole forage, and green pods was 29.7%, 19.4%, 22.8%, and 24.8% DM, respectively (Debela et al., 2011; Table 2). Furthermore, condensed tannin content of 10.9% and 21.1% DM was found in leaves and whole forage, respectively. The inclusion of *Sesbania sesban* leaves up to 25% in goat diets to replace concentrate mixtures improved the growth performance and economics of goat production (Gholve et al., 2021). Similarly, Tekliye et al. (2018) found that 400 g/day of dried *Sesbania sesban* leaf supplementation increased apparent nutritional digestibility, average daily gain, feed conversion efficiency, and carcass characteristics for growing Farta sheep. In addition, Islam et al. (2021) demonstrated that the cows offered *Sesbania* in an amount equal to 25% of their required DM intake per day had a significantly higher average daily milk yield by 25% than that of the unsupplemented group. Perennial *Sesbania* forage for ruminants appears to be used most economically advantageously and safely as a protein supplement to poor-quality roughages like agricultural wastes or dried grasses.

Sesbania pod is also a rich source of various essential minerals, including sodium, potassium, phosphorus, and iron, which play crucial roles in providing essential nutritional elements (Islam et al., 2021). In addition, high condensed tannins (10.87%) and crude saponins (16.20%) have been found in the pod of *S. grandiflora*, which might be beneficial for rumen fermentation (Unnawong et al., 2021 a). An *in vitro* study revealed that the ruminal CH_4 concentration was reduced by 44.12% when 0.6% *S. grandiflora* pod meal was added after 8 h of incubation (Unnawong et al., 2021 a). Similarly, in Thai native beef cattle supplemented with *S. grandiflora* pod meal at 0.6% dry matter intake, not only was CH_4 production reduced, but nitrogen utilization was also enhanced (Unnawong et al., 2021 b).

Moringa

Moringa (*Moringa oleifera*), also known as the miracle tree and native to India, is one of the potential tree fodders (Leitanthem et al., 2023). For the moringa crop, Sánchez et al. (2006) observed that the optimal planting density was 250,000 plants per hectare. This resulted in the production of 80 t and 17 t/ha of fresh and dry biomass annually, with harvesting occurring every 40–75 days (Newton et al., 2006; Sánchez et al., 2006). Its leaves include a significant amount of vitamin C (188–279 mg/100 g), calcium (2.65%), and phosphorus (0.304 g/100 g), and CP (29.40%) (Padayachee and Baijnath, 2020). Furthermore, rumen bypass protein, with

its essential amino acid content, constitutes 47% of the protein in *M. oleifera* leaves (Leitanthem et al., 2023). *M. oleifera* leaves have many advantages over other plants when used as an animal feed additive in tropical regions because they can withstand cold and drought stress, are readily palatable and digested by animals, and have antinutritional qualities (El-Badawi et al., 2023). *Moringa oleifera* is helpful as a feed supplement due to the alkaloids, polyphenols, and polysaccharides it contains. The use of *M. oleifera* leaves for animal feed in ruminants was described by Nouman et al. (2014), which improved microbial protein production in the rumen. *M. oleifera* leaves' effects on the productivity and quality of milk produced by cows and goats have been the subject of numerous studies (Kekana et al., 2020). According to Al-Juhaimi et al. (2020), *M. oleifera* leaves enhance goats' immune systems and oxidative state using bioactive composites. The addition of *M. oleifera* leaves improved the Nubian goats' feed intake, ruminal fermentation, and milk quality (Kholif et al., 2018).

Additionally, feeding 50 g of powdered *Moringa oleifera* leaves to milking buffaloes increased their capacity for total antioxidants, milk production, milk profile, and nutrient digestion (El-Badawi et al., 2023).

Jatrophas

Jatrophas (*Jatropha curcas* L.) are small, drought-resistant trees or shrubs that are commonly cultivated across tropical and subtropical areas of the world. They are native to the American tropics. More than 175 species make up the *Jatropha* genus, with *Jatropha curcas* being one of the most researched species for animal feeding. Arunyanark et al. (2023) observed variations in wood fresh weight, leaf fresh weight, and total fresh weight of *jatropha* between the first and second years, with mean values over the two-year period recorded at 32.11, 6.34, and 38.46 t/ha, respectively. Notably, there was a significant disparity in leaf dry weight between the two years, with the first year showing a higher value of 1.74 t/ha compared to the second year's 1.09 t/ha. *Jatropha curcas* seed annual yield was 2.2 t/ha, as reported by Lama et al. (2018). *Jatropha* is an intriguing biofuel crop due to its high 46.2% ether extract (EE) (Ugbogu et al., 2014), and its defatted kernel by-product, or *jatropha* kernel meal, is a great protein material that contains CP at 61.8% DM (Oskoueian et al., 2011; Table 2). Esonu et al. (2020) noted that dried leaves of *jatropha* contain 19.5% CP, while they contain a low level of tannin at 0.3% DM (Table 2). Lysine is lacking in *jatropha* protein compared to soya bean protein, while other important AA are more abundant such as, leucine or arginine (Makkar et al., 2012). The majority of *jatropha* species can be efficiently detoxified, although they are very poisonous to ruminants and monogastrics (1 to 3 mg/g kernel meal; Makkar et al., 2012). It is essential to fully detoxify in order to prevent animal deaths (Elangovan et al., 2013). Unless these are inactivated through heat treatment and the addition of the phytase enzyme, *jatropha*'s use, particularly for monogastrics, may be restricted because it contains high levels of

antinutrient substances such as trypsin inhibitors, lectins, and phytate. When thoroughly detoxified, the replacement of *jatropha* for soya beans did not affect the sheep and goats' DM intake or average daily gain (Patil et al., 2017). Despite the high yield potential, the spread is largely constrained by the inconsistent yields of the present cultivars.

Biological proteins and plant weeds

Single-cell protein

Yeast, bacteria, fungi, or microalgae can provide single-cell proteins (SCP), namely microbial cells. Those microbes could use a variety of readily available wastes and feedstocks as carbon sources, nutrients, and energy for growth in order to generate biomass that is high in protein. Table 3 showcases the intriguing chemical compositions of various yeast products, including yeast cells, yeast extract, yeast hydrolysate, and yeast cell wall, boasting an impressive CP content ranging from 14.7% to 62.5% on a dry matter basis. Single-cell proteins contain a varying CP depending on the culture, species, and strains used, but follow a similar pattern to that of soya bean expeller, claim Lindberg et al. (2016). The key restrictions are high nucleic acid content (bacteria and yeasts > fungus > microalgae; 60 to 120, 70 to 100, and 30 to 80 g/kg DM, respectively) and cost-effective mass production and harvesting. If these microorganisms are grown on contaminated and polluted substrates, they become especially problematic (Nasseri et al., 2011; Lindberg et al., 2016). The rumen breaks down dietary nucleic acids and their derivatives very quickly, and some of the byproducts can be used as sources of carbon and nitrogen for bacterial growth (McAllan, 1982). However, the availability of N in nucleic acids is not as high as it is in actual protein or ammonia. The following are the essential steps in producing SCPs: (1) establishing the medium; (2) conducting fermentation or photosynthesis; (3) harvesting; and (4) downstream processing, including cell disruption, cell disruption washing, and protein extraction and purification (Ravindra, 2000). The SCP idea was first put forth during World War I, primarily as human food (Lindberg et al., 2016). However, compared to more conventional diets and feeds, the main reason SCP has not yet been widely adopted commercially is probably because of its higher manufacturing costs as well as the challenges associated with effective and affordable cell recovery. Examples of well-established processes include the cultivation of yeasts *Candida lipolytica* and *Candida tropicalis* on alkanes as a substrate (product called Toprina), bacteria *Methylophilus methylotrophus* on CH₄, filamentous fungi *Peacilomyces variotii* on sulphite-based spent liquor from the forest industry sidestream (Pekilo), and yeast *Kluveromyces marxianus* on whey (Nasseri et al., 2011). The factors that could increase the popularity and viability of the SCP concept in the future include the requirement to intensify resource and nutrient consumption as well as the sharp price increases brought on by the potential for protein scarcity (Lindberg et al., 2016).

Table 3. Chemical composition of biological proteins and plant weeds possible to use for ruminant feed in the tropical area

Item*	DM, %	CP, % DM	OM, % DM	EE, % DM	Lys	Met	CT, % DM	Sources
Yeast								
<i>S. cerevisiae</i> cell	95.1	44.8	94.8	1.6	4.5	1.1	–	Patterson et al. (2023)
<i>Candida utilis</i> cell	94.8	52.0	91.6	0.16	4.8	0.8	–	Patterson et al. (2023)
yeast extract	95.2	62.5	90.5	0.1	4.3	0.9	–	Podpora et al. (2016)
yeast hydrolysate	43.0	52.5	86.8	2.3	3.4	1.4	–	Yang et al. (2020)
yeast cell wall	95.4	14.7	94.2	9.2	–	–	–	Lin et al. (2020)
Microalgae								
<i>Spirulina platensis</i>	92.9	47.0	92.4	36.4	–	–	–	Ramírez-Rodrigues et al. (2021)
<i>Chlorella vulgaris</i>	97.8	43.9	90.1	9.7	5.3	1.1	–	Cabrita et al. (2022)
Seaweeds								
<i>Padina gymnospora</i>	89.3	77.7	9.9	1.2	–	–	–	Nazarudin et al. (2022)
<i>Scenedesmus quadricauda</i>	23.8	34.6	74.3	0.6	–	–	–	Arguelles (2018)
<i>Macrocystis pyrifera</i>	–	17.5	66.3	0.4	–	–	–	Mansilla and Ávila (2011)
<i>Palmaria palmata</i>	–	11.2	87.7	1.1	–	–	–	Lafeuille et al. (2023)
<i>Saccharina latissimi</i>	92.4	1.1	53.5	–	–	–	–	Stokvis et al. (2021)
<i>Laminaria digitata</i>	94.0	1.2	75.9	–	–	–	–	Stokvis et al. (2021)
<i>Laminaria ochroleuca</i>	19.7	9.7	73.7	0.5	–	–	–	Maia et al. (2016)
<i>Gracilaria vermiculophylla</i>	–	16.7	72.2	0.3	–	–	–	Maia et al. (2016)
<i>Gigartina</i> sp.	21.2	11.9	64.2	0.3	–	–	–	Maia et al. (2016)
<i>Ulva lactuca</i>	86.1	2.3	63.7	–	–	–	–	
Duckweeds								
<i>Lemna minuta</i>	95.3	17.5	85.8	2.9	–	–	0.9	Demann et al. (2023)
<i>Spirodela polyrrhiza</i>	89.7	24.6	82.3	2.2	–	–	0.28	Demann et al. (2023)
<i>Lemna obscura</i>	93.4	37.0	5.6	6.5	–	–	0.30	Demann et al. (2023)

*DM = dry matter; CP = crude protein; OM = organic matter; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; Lys = lysine (g/100 g N); Met = methionine (g/100 g N).

Microalgae

Microalgae are a diverse group of simple or complex multicellular or unicellular microorganisms with a variety of dietary compositions. There are numerous potential uses for microalgae as animal feed. Table 3 presents the intriguing chemical composition of microalgae potentially suitable for ruminant feed in tropical regions, showcasing a notable high CP content ranging from 43.9% to 47.0% (Ramírez-Rodrigues et al., 2021; Cabrita et al., 2022). Ovine or bovine milk fat can be improved for human consumption by using lipid-rich species to increase the content of trans-11 18:1, cis-9, trans-11 18:2, and n-3. One such species is the 22:6n-3-enriched *Schizochytrium* sp. Supplementing with algal 22:6n-3 increased the n-3 content of ruminant meat, but no changes in CH₄ production were noticed (Moate et al., 2013). Consequently, standard protein or energy feeds can be replaced with microalgae or defatted microalgae residues high in CP (such as *Spirulina platensis* and *Chlorella vulgaris*). Although it can vary greatly between species, microalgal AA con-

tent typically compares favorably to SBM and rapeseed meal (Luke, 2018). However, microalgal protein is frequently lower in histidine than rapeseed and soya bean meal, the first AA that reduces milk yield on plant silage and grain-diets. Several microalgae species have been shown to have higher protein degradability than rapeseed (Costa et al., 2016), soya bean, and cottonseed meals, but this may be modified by microalgae growing and harvesting circumstances. Depending on the composition of the microalgae, an excess of algae or an algae removal fat by-product might have a detrimental effect on ruminant feed consumption in contrast to traditional protein or starch feedstuffs (Lamminen et al., 2017). Feed processing, such as pelleting, has the potential to increase the palatability of microalgae. The content of protein in microalgae may be slightly lower than that of rapeseed meal, but comparable to soya bean protein, based on the fact that microalgae had no effect on milk yield compared to rapeseed meal, but in late lactation cows led to declining milk protein (Lamminen et al., 2017). Provid-

ing energy for feed drying, preservation, and transportation could be reduced by producing microalgae locally on-farm in ponds or closed photoreactors linked to ruminant water systems. This would make microalgae culture a feasible idea for large-scale farming in the future. In order to encourage the production of bacterial cells in the rumen and feed digestion, microalgae have actually been successively given by drinking water to cattle fed grasses of low quality (Panjaitan et al., 2015). Additionally, the future replacement of fossil fuels with depleting stocks of renewable biofuels derived from microalgae has great potential. Although the cost of making biofuels from microalgae is not yet competitive with that of fossil fuels, it may soon become so with the development of new technologies and potential government incentives, enabling the large-scale production of defatted microalgae waste for cattle.

Seaweeds

Complex multicellular creatures known as seaweeds develop in salt water or a marine environment's littoral zone. They can have a wide variety of sizes, colors, forms, and compositions. Fresh seaweed must be consumed right away or kept in some other way, such as drying or ensiling, because it contains a significant amount of water (70.0 to 90% DM). The lower CP content of brown algae (Phaeophyceae) (up to 14.0 vs. 50.0 and 30.0% DM, respectively) makes them less nutritious than red (Rhodophyceae) and green algae (Chlorophyceae). Seasonal changes in the protein composition of marine seaweeds have little effect on *in situ* rumen degradable proteins, which have a significant level of intrinsic variability amongst algae species (24% to 51% of CP; Tayyab et al., 2016). Table 3 reveals the intriguing chemical composition of seaweeds, showcasing a diverse range of crude protein levels spanning from 1.1% to a remarkable 77.7%, alongside notable observations of high ash content.

Sulfur amino acids like methionine and cysteine, as well as lysine and tryptophan, are frequently identified as amino acids with limitations in seaweed protein (Galland-Irmouli et al., 1999). This compositional pattern exhibits variability across different seaweed species. Notably, red algae commonly face deficiencies in leucine and isoleucine, while brown algae often lack methionine, cysteine, and lysine as essential amino acids (Cian et al., 2014). Seaweeds are rich in certain complex carbohydrates but have a modest cellulose content (approximately 40 g/kg DM) (e.g., alginate, laminarin, and fucoidan). Seaweed consumption may be gradually increased to allow rumen microorganisms to adapt and use these chemicals (Makkar et al., 2016). Due to the high concentration of heavy metals and minerals in seaweeds from seawater and the fact that seaweeds have a higher ash content than land plants, which require regular inspection, seaweeds have a lower gross energy value than land plants (Van der Spiegel et al., 2013; Makkar et al., 2016). According to a current publication on seaweed by Makkar et al. (2016),

some species (such as *Macrocystis pyrifera*, *Palmaria palmatata*, *Laminaria digitata*, and *Ulva lactuca*) may be able to help ruminants meet their protein and energy needs, while others may consist of a variety of biological components that may act as prebiotics to improve animal growth as well as health (e.g., *Ascophyllum nodosum*). On the basis of the basal diet, another seaweed variety has additionally demonstrated capacity to reduce ruminal enteric CH₄ *in vitro* (Maia et al., 2016). Seaweeds can be grown or harvested in the wild to reduce nutrient loading and combat eutrophication processes. Seaweeds are used as animal feed (Lindberg et al., 2016). Unfortunately, excessive wild harvesting has harmed the equilibrium of coastal habitats (Makkar et al., 2016). Moreover, greater seaweed planting would encourage enhanced synthesis of bromoform, a metabolic byproduct of seaweeds that contributes to the ozone layer's deterioration (Carpenter and Liss, 2000).

Duckweeds

The four genera of monocotyledonous, tiny, floating plants known as duckweeds belong to the botanical family Lemnaceae (*Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella*). Duckweeds are widespread, but they thrive in still water between 17.5°C and 30°C, where they can increase their biomass by 50% every two days. Consequently, duckweed represents a potential new source of nutrients for herbivores all around the planet. A diverse array of duckweed species have been documented to exhibit substantial CP levels, ranging from 17.5% to 37.0% (Demann et al., 2023; see Table 3 for details). Duckweed in ruminants has only been the subject of a few small investigations (Van der Spiegel et al., 2013). Duckweed is generally well-tolerated in both fresh and dried forms, providing animals with ample protein and other nutrients without negatively affecting their performance (Zetina-Cordoba et al., 2013). Comparing it to SBM and rapeseed expeller, duckweed protein has substantially lower contents of the important amino acids histidine, methionine, and lysine, which may be what limits the production responses. According to Heuzé and Tran (2015), calculations of the rumen degradable protein (RUP) range significantly from 50% to 80%. Although duckweed has a minor content of lignin (5.7% DM; Heuzé and Tran, 2015 a), it includes considerable levels of ash and NDF. The use of duckweed for animals may be limited, nevertheless, due to its high oxalic acid level (Van der Spiegel et al., 2013). Similar to the production of microalgae, local on-farm cultivation of duckweed, for instance, in ponds, may be a workable notion for the future production of ruminant feed. By recovering nutrients from field runoffs, manure, and graywater, duckweeds can support farm-level circular economy initiatives, and ruminant-based food production systems should have less of an environmental impact. Due to its extremely rapid growth, duckweed can be collected and provided to ruminants in fresh form (Van Krimpen et al., 2013). Fresh duckweed is fed, which reduces the price of drying and preserving it on the farm. Duckweed may be harvested

easily and mechanically since its particles are larger than those of microalgae.

Insect protein

The need for alternative protein-rich meals is driven by intensive ruminant production's heavy reliance on SBM and the harm this commodity causes to the environment. Alternatives to the plant proteins that are often provided to animals, such as insects, have been proposed (Shah et al., 2022). Due to their ease of adaptation to various national environments, role in the bioconversion of waste, and reduced need for land, insects are more environmentally friendly sources of nutritional protein for livestock than SBM (Makkar, 2018).

In many countries – if not all of the world – insects are now acknowledged as a significant potential source of new sustainable raw materials for animal diets. Some animal species consume primary insects as part of their natural diets, which provide animal feed with the necessary nutrients and amino acid (AA) profiles (Makkar, 2018). Large-scale insect production offers numerous ecological advantages. These include a decreased demand for land, the ability to produce 1 kg of protein in a smaller footprint, a reduction in competition between feed and food resources, and the capacity to convert organic waste materials or by-products into high-value protein products for insects (Shah et al., 2022). The Food and Agriculture Organization (FAO) (2009) has extensively discussed “edible insects,” emphasizing the strengths and issues underpinning research gaps and the potential to scale-up production and engage all stakeholders. Various species of insects have been found to boast an impressive CP, spanning a wide range from 27.5% to 61.2% (Table 4) (Ahmed et al., 2021; Khonkhaeng et al., 2022; Lu et al., 2022). Numerous insects have been utilized as animal feed, with the most promising being *Gryllus bimaculatus* and the BSFL, among others.

Nevertheless, within the European Union (EU), restrictions on the utilization of insects in the diet of ruminant animals arise due to legislative limitations and apprehensions over adherence to food safety regulations. Comprehensive evaluations are required to verify adherence to EU rules due to the strict laws governing the

sourcing, processing, and incorporation of insect-derived products in animal feed (Renna et al., 2023).

Gryllus bimaculatus

A species of cricket belonging to the Gryllinae sub-family is known by the name *Gryllus bimaculatus*. The potential insect species *G. bimaculatus* has an acceptable amino acid composition (lysine, threonine, and tryptophan) and a high CP content (beyond 52.4–61.7% DM) (Ahmed et al., 2021; Khonkhaeng et al., 2022; Table 4). Insects contain significant amounts of fatty acids (FA), particularly unsaturated FA, in addition to the nutritional value derived from high protein and AA concentrations. All of these benefits have boosted interest in using insects as a feed item for animals, particularly ruminants.

Additionally, Ahmed et al. (2021) demonstrated that replacing SBM with insects at a 25% inclusion rate in concentrate feed significantly enhanced ($P < 0.01$) *in vitro* fermentation, leading to reduced CH_4 production while maintaining degradability. Nevertheless, other findings were comparable to SBM treatments because of insects' high CP, AA profiles, and FA (14% to 26%, primarily unsaturated FA, omega 6 and 3). Additionally, *G. bimaculatus* meal was shown to be a 100% effective replacement for SBM as a protein source in a concentrate diet by Phesatcha et al. (2022), leading to an increase in propionate production in the rumen, a decrease in protozoan population, and a decrease in CH_4 production. The decrease in CH_4 production observed in these insects could be attributed to their elevated fat content. It is widely acknowledged that introducing fat, irrespective of its origin, into the ruminant diet results in a reduction in CH_4 production (Ahmed et al., 2021). Furthermore, the presence of chitin and chitosan in insects has demonstrated the capability to influence rumen fermentation, promoting a shift towards lower acetate and higher propionate levels, consequently lowering CH_4 production and the population of methanogens (Jayanegara et al., 2017). Furthermore, Khonkhaeng et al. (2022) show that the combination of *G. bimaculatus* and a tannin source (*S. grandiflora*) increased protein consumption by lowering $\text{NH}_3\text{-N}$ levels in the rumen and boosting rumen undegradable protein. As a result, it is prudent to investigate insect protein as a viable feed source in the future.

Table 4. Chemical composition of insect proteins possible to use for ruminant feed in the tropical area

Item*	DM, %	CP, % DM	OM, % DM	EE, % DM	NDF, % DM	ADF, % DM	Chitin, % DM	Sources
Insect species								
<i>Gryllus bimaculatus</i> meal	95.3	58.5	92.0	27.8	–	–	–	Khonkhaeng et al. (2022)
<i>Gryllus bimaculatus</i>	95.5	56.5	94.8	15.8	37.6	24.2	20.8	Ahmed et al. (2021)
<i>Acheta domesticus</i>	95.5	61.2	94.6	14.6	39.3	17.2	14.6	Ahmed et al. (2021)
<i>Brachytrupes portentosus</i>	96.1	53.3	95.1	22.2	40.3	17.3	12.4	Ahmed et al. (2021)
<i>Bombyx mori</i>	96.5	52.4	94.6	26.7	40.3	24.2	9.8	Ahmed et al. (2021)
Black soldier fly larvae (BSFL)								
full-fat	–	27.5	93.4	51.5	–	–	3.8	Lu et al. (2022)
defatted	–	55.4	91.9	9.8	–	–	7.2	Lu et al. (2022)

*DM = dry matter; CP = crude protein; OM = organic matter; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Black soldier fly (Hermetia illucens) larvae (BSFL)

Recent studies have examined BSFL as a promising source of protein for animal feed, both for monogastrics and ruminants. It is notable for its great content of CP and beneficial amino acid contents (Fukuda et al., 2022). Due to their nutrient profile, which includes 27.5%–55.4% CP, 51.5% fat (Lu et al., 2022), scalability, and favorable feed efficiency with *in vitro* protein digestibility of 66.0–68.7%, BSFL have the potential for large-scale utilization in feed applications (Lock et al., 2016; Jayanegara et al., 2017; English et al., 2021; Fukuda et al., 2022). According to Jayanegara et al. (2017), BSFL has more than 40% protein in its body on a dry matter basis. Aside from its high protein content, BSFL has the additional benefit of growing quickly on a variety of organic substrates and having no negative effects on the environment or human health.

According to Jayanegara et al. (2017), substituting BSFL for SBM in ruminant diets results in lower nutritional value *in vitro* but has the advantage of lowering ruminal CH₄ emission. The first *in vivo* feeding trial evaluated the suitability of BSFL as a protein supplement for cattle. In the United States, Fukuda et al. (2022) studied the effects of feeding BSFL to beef cows as a protein supplement and found that the animals' consumption improved, promoting weight gain. The production of rumen NH₃-N, volatile fatty acid synthesis, or rumen pH were not adversely affected by the addition of insect meal.

Despite its effectiveness, BSFL contains a significant quantity of chitin, which may limit its usage as animal feed. Chitin is a N-acetylglucosamine polymer that is commonly found in insects. The chitin concentration of BSFL was reported to range between 3.8 and 7.2% (Lu et al., 2022; Table 4). Numerous insect species, most notably the BSFL, have high fractions of neutral detergent insoluble crude protein, which suggests that chitin is insoluble in neutral detergent solution (Jayanegara et al., 2020). It has been suggested that the chitin found in BSFL be removed, at least partially, in order to improve its nutritional value.

Agro-industry biomass*Cassava pulp fermented by yeast waste (CSYW)*

Cassava pulp (CS), the residue of the processing of starch from cassava roots, can have detrimental impacts on the environment. As a result, the starch industry has

taken steps to eliminate it or find substitutes. Utilizing CS as animal feed is one approach to resolving this problem (Dagaew et al., 2022). However, CS is abundant in fiber and low in crude protein, thus several methods have been devised to increase its nutritional value. Yeast (*Saccharomyces cerevisiae*) is a probiotic source that aids in rumen fermentation. Nonetheless, *S. cerevisiae* products can be costly; therefore, alternative yeast sources should be examined. *S. cerevisiae* produces yeast waste (YW) as a byproduct of the fermentation of molasses and sugarcane juice to generate ethanol. YW is produced all year and contains 60–70% yeast living cells and 30–35% CP (Daz et al., 2017). Dagaew et al. (2021) showed that CS fermented with yeast waste (CSYW) may enrich CP from 2% to 53.7% when compared to the non-fermented group (Table 5). To make this medium solution, combine 24 g of molasses and 50 g of urea in 100 mL of distillation water, resulting in an increase in nitrogen concentration in the product. Recent research found that replacing SBM in a concentrated feed with CSYW enhanced ruminal propionic acid (C3) concentration in Thai native beef cattle (Dagaew et al., 2022). In their study involving dairy cows, Dagaew et al. (2023) observed that incorporating CSYW in concentrate diets could yield similar results to SBM-based diets. Furthermore, they found that cows consuming the CSYW-based diet exhibited higher total intake and milk protein levels compared to those fed the SBM-based diet. The cost of feed, contaminants, and risks related to improper waste disposal are all decreased when CSYW is used as a protein source in diets up to 100%.

Cassava pulp fermented by Lactobacillus bacteria (LAB)

In the context of augmenting CS quality in animal diets, various approaches have been explored, including both physical, chemical, and physicochemical methods. As previously discussed, yeast waste represents a viable avenue for improvement. Expanding on this theme, researchers have delved into the application of homofermentative strains of LAB, such as *Lactobacillus casei* strain TH14, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus*, to further enhance CS quality (Pholsen et al., 2016). Notably, the study by Pholsen et al. underscored the exceptional lactate-acid production capability of strain TH14, positioning it as a potent silage inoculant that outperforms other strains in improving silage quality.

Table 5. Chemical composition of agro-industry biomass possible to use for ruminant feed in the tropical area

Item*	DM, %	CP, % DM	OM, % DM	EE, % DM	NDF, % DM	ADF, % DM	Sources
Cassava pulp fermented by yeast waste	34.9	53.7	89.7	–	24.3	11.3	Dagaew et al. (2021)
Cassava pulp fermented by <i>Lactobacillus</i> bacteria	16.5	25.9	97.3	2.5	43.8	24.6	Pongsub et al. (2022)
Citric acid fermented by yeast waste	88.2	53.5	89.4	–	40.2	29.4	Suriyapha et al. (2024)
Red yeast fermented by tofu	11.2	38.4	95.4	–	14.3	8.3	Maungthipmalai et al. (2019)

*DM = dry matter; CP = crude protein; OM = organic matter; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Moreover, the integration of LAB into the fermentation process has demonstrated significant improvements in CS quality. Pongsub et al. (2022) reported that fermenting CS with 4% urea, 4% molasses, and *Lactobacillus casei* TH14 resulted in a remarkable increase in CP levels from 2.0 to 29.5% (Table 5). This not only elevates the nutritional profile but also enhances overall CS quality, presenting a promising alternative protein source for ruminants. It is worth noting that while these studies have shed light on silage improvements, further research is needed, particularly in exploring *in vitro* techniques for ruminant feed.

Citric acid fermented by yeast waste (CWYW)

Citric waste, the residue of citric acid production, is produced by fermenting rice, corn, cassava, or CS with *Aspergillus niger*. The citric waste still includes a large amount of fiber (86.1% DM of NDF and 19.7% DM of acid detergent fiber (ADF), respectively) and yet has a nutritional value of 3.0–7.0% of CP (Suriyapha et al., 2022 a). By fermenting with yeast waste, citric waste has boosted CP content, raising CP from 11.0 to 53.5% (Table 5; Suriyapha et al., 2024). Citric waste, yeast waste, brown sugar, and urea were used to make CWYW (Suriyapha et al., 2021). Citric acid fermented by yeast waste can be used in place of SBM as a source of protein in beef cattle without having an unfavorable effect on feed consumption, rumen fermentation characteristics, or blood metabolites (Suriyapha et al., 2022 a). Suriyapha et al. (2022 b) also showed that top-dressed CWYW at 0.4% of BW was equivalent with SBM top-dressing and had higher CP digestibility, ruminal ammonia-nitrogen ($\text{NH}_3\text{-N}$), blood urea-nitrogen (BUN), and milk urea-nitrogen (MUN) while having reduced somatic cell count in milk. They did advise that additional long-term studies (covering the complete 305-day lactation) be conducted in order to understand how production responses in tropical lactating dairy cows will change. Recently, Kanakai et al. (2023) developed CWYW pellets to address feed storage issues and enhance digestibility despite rumen undegradable protein. In beef cattle, supplementing CWYW pellets with 4% or 6% of DM per day increases CP intake, digestibility, rumen bacterial population, and propionate concentration.

Red yeast fermented by tofu

A by-product of the industrial production of tofu and soy milk, tofu waste is interesting to use as feed due to its accessibility and affordability in regions where soybeans are cultivated. Moreover, tofu waste has significant nutritional value, particularly in terms of amino acid profile. However, the protein level is insufficient on its own to serve as the primary source of protein in a concentrate diet. Hence, increasing its protein content is quite intriguing. Yeast, a single-cell protein, is frequently utilized to increase the nutritional value of agricultural and industrial fermentation products. Wet soybean waste fermented using yeast culture, according to Maungthipmalai et

al. (2019), might boost the protein content from 18.1 to 38.4%. Yeast cells have the capacity to produce microbial protein as part of their metabolic activities. During the process of fermentation, yeast consumes sugars and carbohydrates in the feed, resulting in a growth of microbial biomass (Dagaew et al., 2022). The yeast cells in this microbial biomass create proteins, which contribute to the total protein content of the fermented feed (Suriyapha et al., 2021). In addition, there is a different type of yeast called *Rhodotorula* sp., which produces the red pigments of the carotenoids group, which are precursors to vitamin A and are helpful for ruminants' health. Red yeast is also a good source of protein.

Tofu fermented with *Rhodotorula rubra* TISTR5134 combined with molasses and urea could be a 100% replacement of SBM in a concentrate diet without having a negative impact on concentrate intakes, digestibility, carcasses, or meat quality, according to Somboonchai et al. (2022). According to this study, fermented products might be employed as a protein feed to substitute SBM in the concentrate diet by 50%, which could reduce the cost of feed. To learn more about the advantages of carotene found in red yeast on carcass quality and meat quality, however, longer-term research should be done.

Conclusion and recommendations

Protein is essential for ruminant animals to maintain and increase productivity. Protein deficiency causes reduced productivity in animals by reducing rumen fermentation efficiency and harming rumen microorganisms. As the price of primary protein sources, particularly SBM, has increased, new protein alternatives have been introduced to satisfy the shortage. Agricultural biomass, agro-industry biomass, fodder shrubs, biological protein, plant weeds, and insect protein are effective alternative protein sources in ruminant diets. Furthermore, certain alternative protein sources not only deliver amino acids but also phytonutrient compounds, which have the potential to control the rumen and mitigate CH_4 generation. Additional research into improving these protein sources, such as increasing volume production, extracting essential components, and evaluating them in large herds of animals, could be considered.

Data availability

No data was used for the research described in the article.

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Conflicts of interest

The author declares no conflicts of interest.

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