


Review

Exploring the Applications of *Lemna minor* in Animal Feed: A Review Assisted by Artificial Intelligence

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Abstract: The work aims to apply cheap and widely accessible tools based on artificial intelligence to analyze, group, and categorize a large amount of available research literature (from a massive bibliographic search) on the use of *Lemna minor* for animal feed, not only comprehensively and objectively, but also in a more effective and less time-consuming way. In addition, a comprehensive and critical summary was conducted to highlight recent applications of *L. minor* in animal feed. The Scopus database was used for the original bibliographic search. Then, a newly developed online and freely available tool called “Jupyter Notebook on Google Colab” was applied to cluster the large volume of bibliographic data (1432 papers) obtained in the basic search, which allowed their reduction until only 148 papers. These papers were reviewed in a traditional way obtaining relevant information about *L. minor* production, nutritional value, composition, and its application as animal feed. In this sense, the most successful applications were for fish and poultry feeding, reaching levels of inclusion of 15–20% in fish and 5–15% in poultry. It is of great interest because of the expected increase in prices of conventional sources of protein for animal feed.

Keywords: aquatic plants; feeding; *Lemna* spp.; bibliographic research; artificial intelligence



Academic Editor: Wojciech Kolanowski

Received: 24 April 2025

Revised: 11 June 2025

Accepted: 15 June 2025

Published: 16 June 2025

Citation: Bethancourt-Dalmasí, H.; Viuda-Martos, M.; Lucas-González, R.; Borrás, F.; Fernández-López, J. Exploring the Applications of *Lemna minor* in Animal Feed: A Review Assisted by Artificial Intelligence. *Appl. Sci.* **2025**, *15*, 6732. <https://doi.org/10.3390/app15126732>

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1. Introduction

The search for new protein sources for animal feed is increasingly urgent due to the rising world population, projected to reach 9.7 billion by 2050 [1]. This demand for animal protein creates challenges such as resource overuse, greenhouse gas emissions, and strain on ecosystems. The competition for limited resources like land, water, and crops has led to rising prices for conventional protein sources, including soybean meal and fishmeal [2–4]. Additionally, traditional sources, such as soy, are associated with deforestation [5].

Several strategies are being explored to mitigate competition for resources, including efficient agricultural practices and sustainable technologies that use alternative protein sources in animal feed. These approaches aim to meet the nutritional needs of animals while protecting environmental health, often by sourcing local, fast-growing materials [2,6–8]. Aquatic plants (mainly *Lemna* spp.) are a promising option, as they are high in protein, grow

20–30 times faster than traditional crops, and can recycle 97% of water during their growth and harvesting. Additionally, they absorb carbon dioxide, enhancing their environmental benefits [4,7,9].

Lemna spp. is an aquatic plant commonly known as duckweed or water lentil. *Lemna* spp. is used as a general term to refer to various species of the genus *Lemna* when the specific species are not identified. It is an angiosperm aquatic plant belonging to the family Lemnaceae. Its vegetative body corresponds to a thalloid form, i.e., in which the stem and leaves are indistinguishable. It consists of a single oval or oval-obovate thallus (with a slightly succulent texture and smooth margins), a green structure, and a single thin, white root. This genus consists of 12 species (*Lemna aequinoctiales*, *Lemna disperma*, *Lemna gibba*, *Lemna minor*, *Lemna japonica*, *Lemna minuta*, *Lemna obscura*, *Lemna perpusill*, *Lemna tenera*, *Lemna trisulca*, *Lemna turionifera*, and *Lemna valdiviana*) [10] whose differences in size, shape and reproductive strategies contribute to their adaptability to different aquatic environments. Of these, *Lemna minor* is the most common and well-known [9,10]. Its size is very small, reaching 2 to 4 mm in length and 2 mm in width, being recognized as one of the smallest angiosperm species in the plant kingdom [8,9].

Lemna minor is a small, fast-growing floating plant that thrives in dense clusters in still or slow-moving waters rich in nutrients. It has been reported that the growth rate of *L. minor* is 0.355–0.390 g per day (in the laboratory) and 0.144–0.860 g per day (in the field). In general, maximum *L. minor* biomass is achieved after 14–28 days after inoculation. They can grow in both fresh and brackish water. Its distribution is universal; these plants are found across a variety of geographical and climatic regions, although they do not thrive in arid deserts or permafrost areas [10,11]. They flourish best in tropical and subtropical zones, where they are commonly found in lakes and lagoons.

Lemna minor can become a source of feed ingredients because it is a highly productive biomass resource that is available, cheap, easy to obtain, and not polluting [8,12,13]. Alternative high-quality feed must be easy to cultivate and not require extensive land. It also has an interesting nutritional value in terms of protein and carbohydrates for animal feed [4,7]. *L. minor* can achieve protein levels up to 38% of its biomass (dry), with a complete essential amino acid composition. In addition, *L. minor* also contains a high level of crude fiber (17–20%) [9,13,14]. However, to ensure that the use of *Lemna* spp. in animal feed can be applied at an industrial level, researchers focus on obtaining high biomass yields (such as media growth and the control of some environmental parameters such as salinity, temperature, and light intensity, among others) and continuous processing technologies (harvesting, drying, and mixing, among others) should be implemented. In any case, its implementation as a way to take advantage of a local and sustainable resource (accessible, cheap, and nutritious) for animal feed on small farms (local farmers) should not be underestimated.

Furthermore, *L. minor* has shown great potential in other interesting fields that fall under the umbrella of environmentally friendly production and processing technologies. Notably, it has applications in wastewater treatment [10], energy production as a biomass source [11], and the creation of natural fertilizers [15,16]. Additionally, it serves as a valuable model for studies in biology, ecology, and genetics [17], and has potential pharmaceutical applications in neuropharmacology and immunostimulation [18].

Artificial intelligence (AI) holds immense potential across various fields, including scientific research, and continues to evolve rapidly. Regardless of the research field, one key area where AI tools can be particularly valuable is conducting literature reviews on specific topics [19]. The volume of research papers published in academic journals has surged dramatically (Journal Citation Reports, 2024). This overwhelming growth makes reviewing literature an increasingly challenging task, often surpassing the cognitive limits of human

capability. In this complex and information-heavy landscape, AI offers a powerful solution to streamline the scientific literature review process, which is also a crucial first step in starting any research project. Natural Language Processing (NLP) AI tools can be used to analyze and categorize documents of data collected in a review process to facilitate this time-consuming task.

The advantages of employing various Natural Language Processing (NLP) tools during the review process are substantial and evident [20,21]. Numerous studies have explored the different stages of this process, and several online platforms are available to aid researchers in this intricate task. For example, PubMed's 'Similar Articles' feature and other AI-powered literature search engines leverage natural language processing (NLP) to recommend related articles based on a paper's content, streamlining the discovery of relevant literature. Additionally, specialized software like VOSviewer[®], integrated with platforms such as Web of Science, enables comprehensive database searches and visualizations, widely applied in review-type studies, including in agri-food science and technology. However, despite advancements in NLP algorithms and tools, there remains a gap in studies applying NLP specifically to conduct systematic reviews in certain domains, such as agri-food science. Furthermore, many NLP-based tools lack transparency and user interactivity, limiting their ability to guide the review process effectively compared to more established tools like VOSviewer[®].

The objective was to utilize affordable and easily accessible AI-driven methods to examine, categorize, and group a substantial volume of existing research (obtained from an extensive bibliographic search) focusing on the application of *Lemna minor* as animal feed. This approach aimed to be not only thorough and unbiased but also more efficient and less time-consuming. In addition, a detailed and critical summary was conducted to highlight current applications of *L. minor* in animal feed.

2. Methodology

The bibliographic database used to carry out a bibliographic search was Scopus, which is the most relevant to the specific studied topic. The selected keywords were "Lemna" and "minor", limiting the search field to "title", "abstract", and "keywords" and adding a date range of 2013–2025 and as a type of document "article" and "review". The number of documents obtained from this basic search was 1342.

After that, a newly developed online and freely available tool called Jupyter Notebook on Google Colab (Table S1) was used to cluster the large volume of bibliographic data obtained in the basic search. This tool is innovative, objective, user-friendly, customizable, and adaptable to any topic. Furthermore, has been recently applied in the field of agri-food scientific literature review reporting its effectiveness, great usefulness, and accuracy [19,22].

To summarize, the program operates through a series of stages, encompassing a general examination of the dataset, the incorporation of necessary libraries, data characterization, data arrangement, data refinement, text preparation, text analysis, grouping using K-means, identifying the ideal cluster count, automated labeling based on cluster centroids, visualizing the clusters, studying cluster distribution across recent years, and formulating conclusions. This instrument can also provide supplementary significant information about the groupings, such as their interconnections, their closeness, and their evolution over time, among other features. Consequently, the 1342 articles from the basic search (Scopus) were processed and examined utilizing this novel AI method, and notably, the entire procedure was completed in merely 200.12 s. The subsequent phase mirrored the conventional scientific review methodology, specifically, a thorough evaluation of each paper identified as pertinent in the preceding stage to pinpoint crucial elements for the present review.

3. Results of the Bibliographic Literature Analysis Assisted by Artificial Intelligence Tools

3.1. Text Processing and Visualization

The AI program applied begins to deliver valuable information for analysis from the initial text pre-processing phase. From the analysis of the language provided by the 1342 papers, the following information about the most frequent words was obtained (Figure 1).

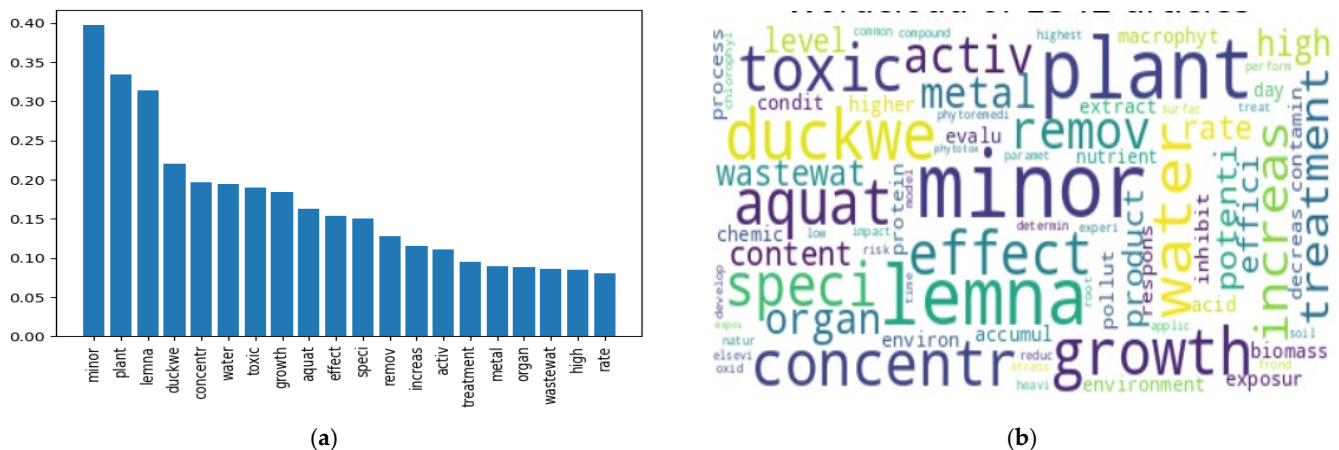


Figure 1. (a) Frequency bar chart of the 20 most common words, and (b) word cloud of the 75 most common words (the higher the size of the words, the higher their frequency), from the 1342 papers about *Lemna minor*.

3.2. Text Clustering

Following this, the articles were grouped based on the examination of their textual content using linguistic similarities, employing the K-means algorithm—a straightforward and widely recognized unsupervised clustering technique [23]. This algorithm computes the separation between data points (here, article abstracts) and assembles nearby points, suggesting their resemblance. A desirable cluster is characterized by shorter distances among its members compared to the distances between distinct clusters. Furthermore, identifying the most suitable number of clusters is crucial for gathering information.

To achieve this, two approaches were utilized: the elbow technique and the average silhouette method. The elbow technique computes the within-cluster sum of squares (SSE: the total of the squared distances between each data point and its assigned cluster's center). Nevertheless, there's a point where increasing the number of clusters (K) doesn't substantially reduce the SSE, and the rate of reduction starts to diminish. This point is typically referred to as the elbow point (the inflection point on the graph) and is often used as a sign of the suitable number of clusters [24].

The average silhouette score method calculates silhouette values that reflect how alike the language of the items is to their assigned topic cluster in comparison to other clusters. A greater average silhouette score across all articles suggests that the clustering effectively groups similar articles together [25]. The two methods employed enabled us to ascertain the optimal number of clusters (19 clusters; Figure 2), which represents the most fitting way to structure and categorize the articles according to their content.

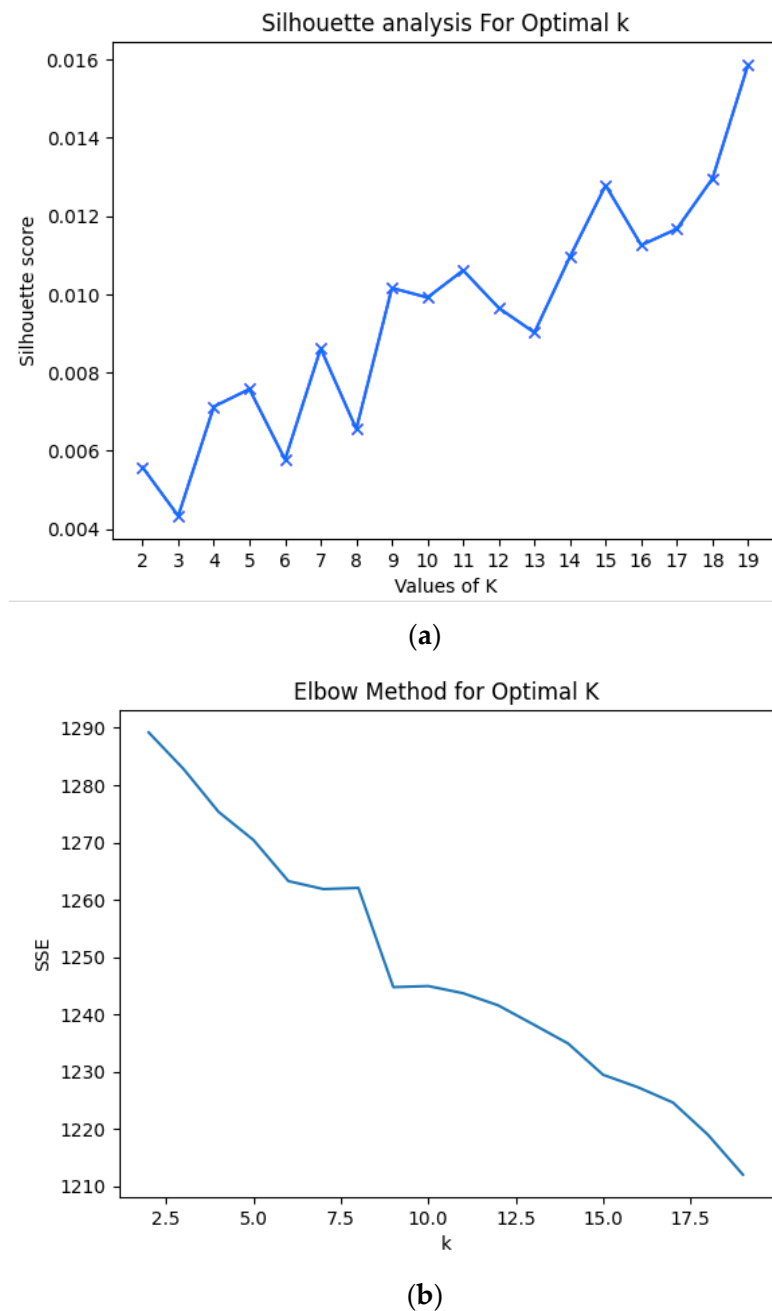


Figure 2. (a) Silhouette and (b) elbow graphs for the determination of the optimal cluster number from the *Lemna minor* dataset.

The K-means algorithm allows us to identify which abstract belongs to each cluster and so, to quantify the number of papers included in each of the 19 clusters (numbered from 0 to 18) obtained (Figure 3a). A cluster is stronger, the greater the number of papers it contains.

The program allows us to visualize the relationship among the obtained clusters (19) in a 2D scatter plot using a Principal Component Analysis (PCA) (Figure 3b). In this scenario, PC1 represents the axis where the data points (including documents, words, and sentences) are most dispersed. This indicates that PC1 is the most significant feature for explaining the variability within the dataset. On the other hand, PC2 captures the variability in the data that PC1 does not, offering an additional dimension for distinguishing the data. As can be seen in this figure, there are clusters whose points show a high dispersion (i.e., clusters 2 or 8) from their centroid (black point) and others whose points are very close to it (i.e.,

clusters 3, 15, or 16). In addition, some clusters are close to others (i.e., cluster 15 is close to cluster 16) but separated by a considerable distance in other cases (i.e., clusters 11 and 10 are separated by a long distance), indicating that each one includes different terms and information.

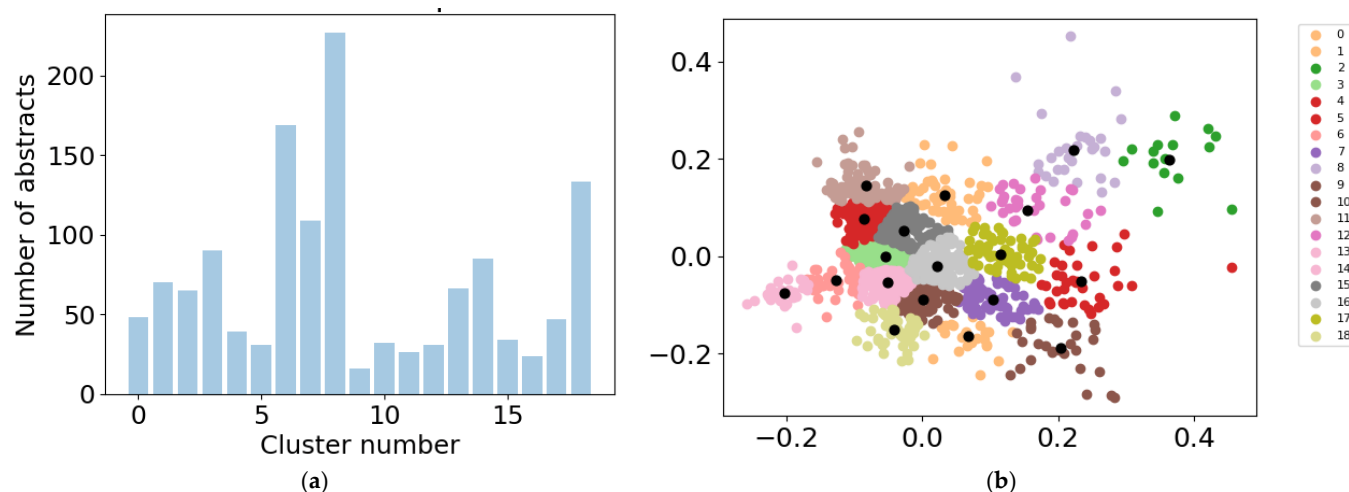


Figure 3. (a) Distribution of papers in the 19 clusters obtained. (b) Scatter plot of the Principal Components Analysis results for the 1342 articles included in the datasheet of *Lemna minor*. Each cluster is represented by a different color; the black dot represents the centroid of each cluster.

An idea of the evolution of interest in each of the clusters over the last years (2013–2024) can be seen in Figure 4.

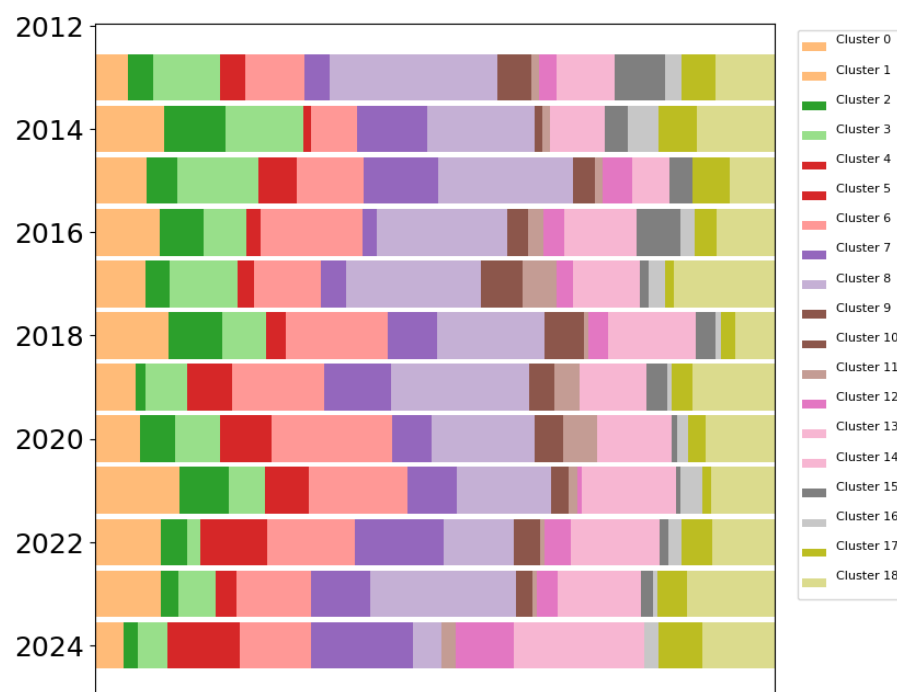


Figure 4. Distribution graph of 19 clusters (0–18) of articles on “*Lemna minor*” by year (2013–2024).

3.3. Text Analysis of Each Cluster

The program gives us information about the top words in each of the 19 clusters obtained in the form of their frequency figure bars (Figure 5) and word clouds, the same as reported in Section 3.1.

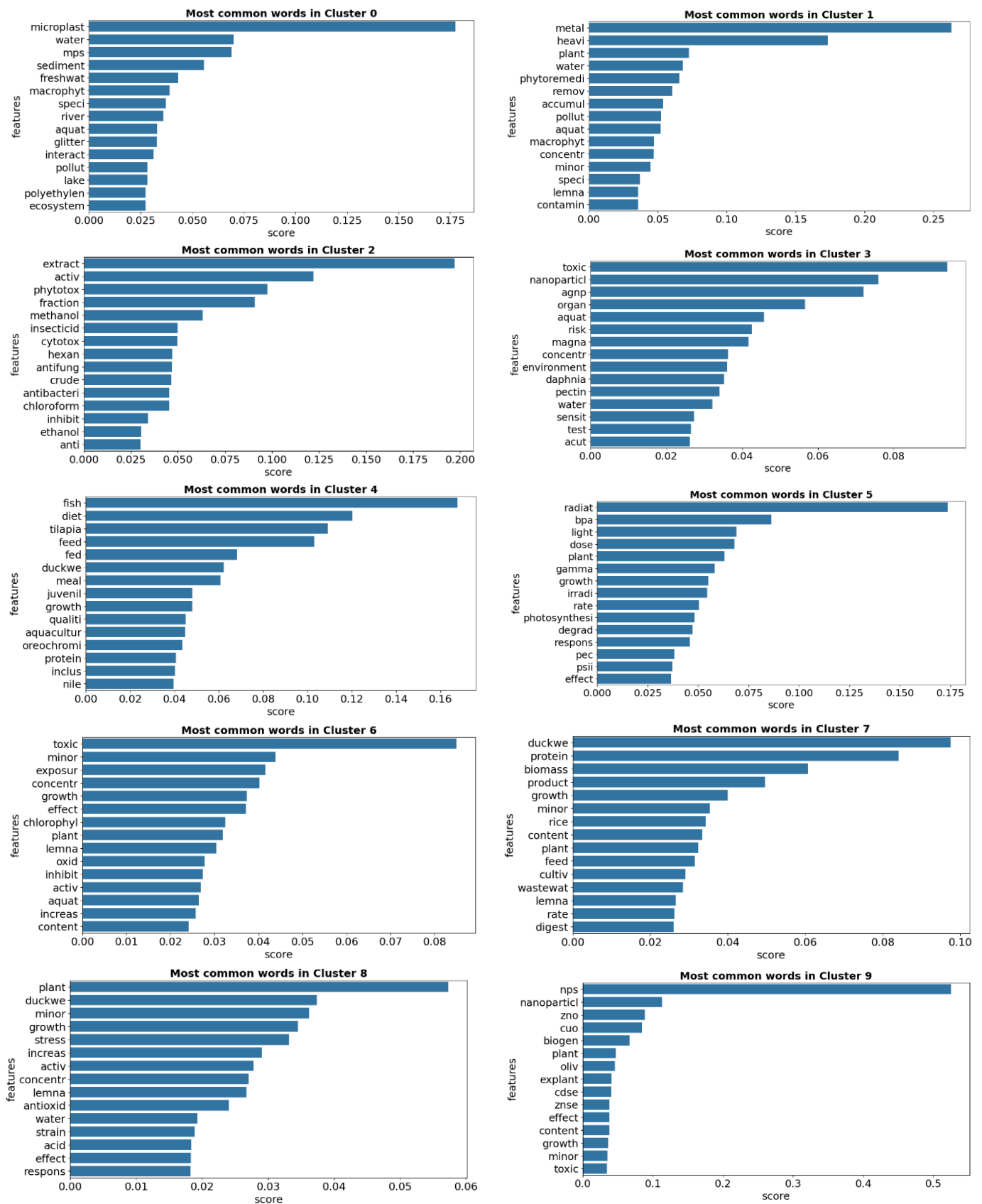


Figure 5. Cont.

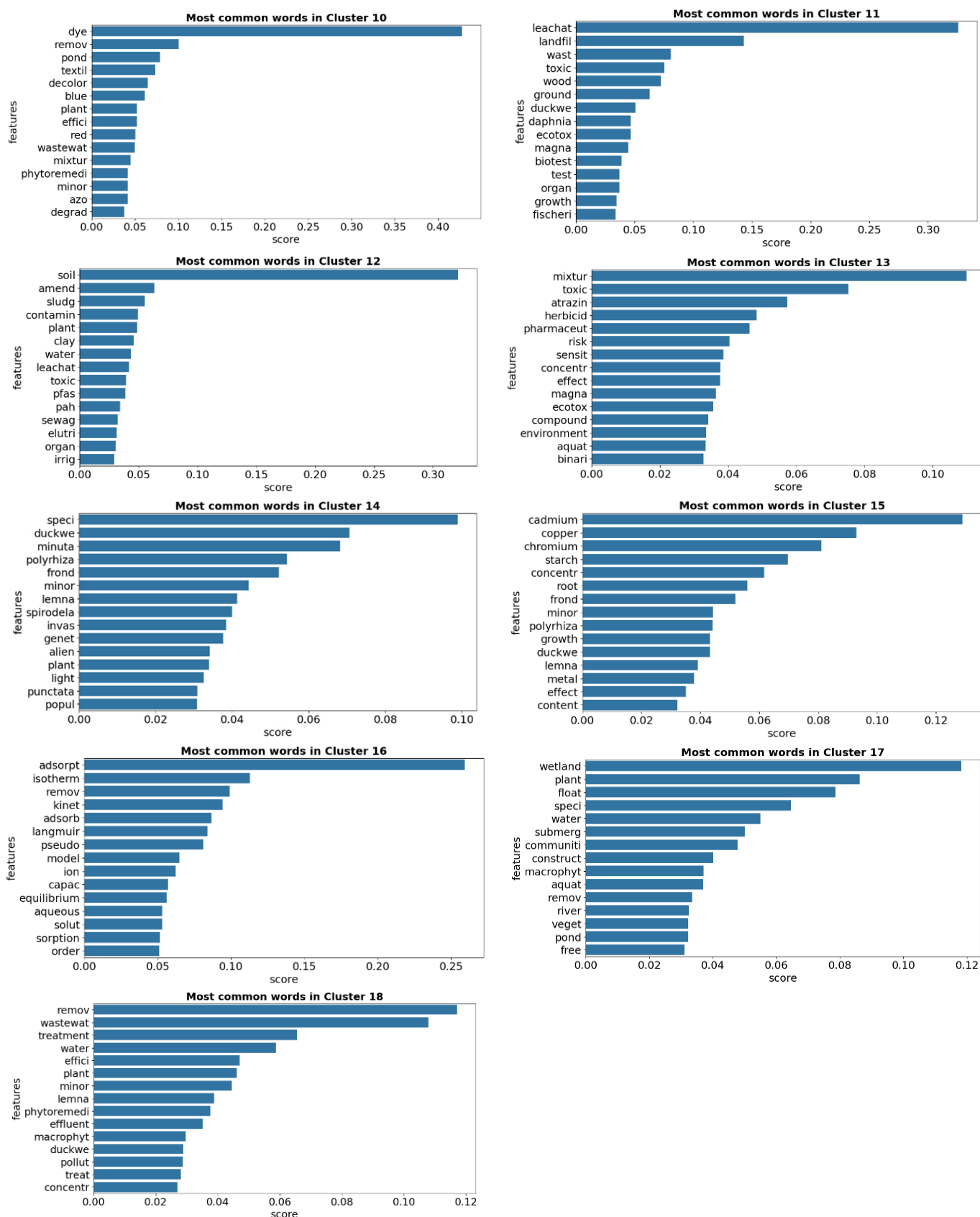


Figure 5. Figure bars of the 19 clusters (numbered from 0 to 18) with the 15 top words (highest frequency appearance).

From the analysis of the keywords of each cluster, looking for those related to “growth”, “diet”, “biomass”, “feed”, “protein”, “crude” or “fish”, 4 clusters (2, 4, 7 and 8) were selected

are relevant. The rest of the clusters were more related to other applications of *Lemna minor*, such as phytoremediation, bioaccumulation of contaminants, microplastics or nanoparticles, irradiation, antioxidants, and antimicrobials. Looking at the selected clusters in Figure 3b, clusters 2 and 8 are side by side but show a large dispersion, while clusters 4 and 7 are more compact, i.e., all points are very close to their centroids.

The four selected clusters now totaled 440 papers, achieving a reduction of 67% from the total initial papers (1342). However, 440 papers are still too many for a traditional review, so the clustering process was repeated on these 440 papers.

In this second round, using only the 440 papers previously selected, the program gives us the word clouds of all of them. In Figure 6 it can be observed that all of them seem to be more related to the real objective of this review (use of *Lemna minor* as feed) than the initial word cloud (Figure 1).

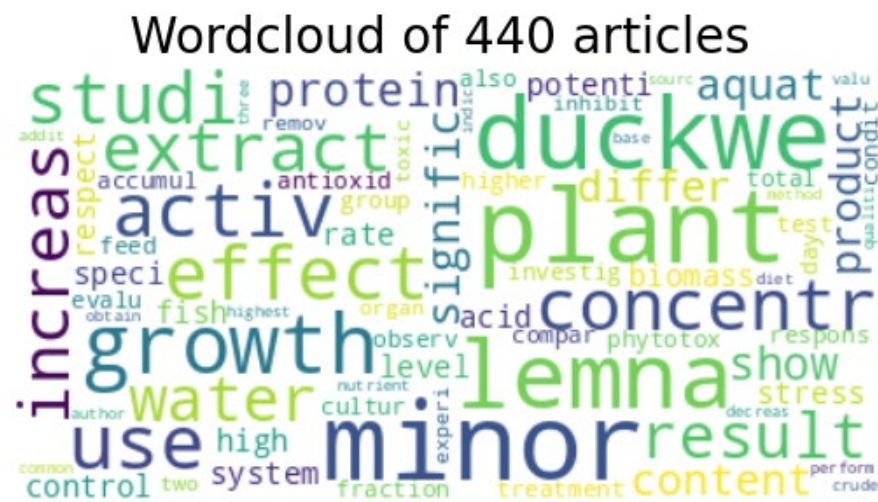


Figure 6. Word cloud obtained in the second round of the analysis.

In this case, 9 subclusters were obtained and from the analysis of their keywords (following the same criteria applied for the text analysis in the first round), clusters 4 (46 papers) and 6 (64 papers) were selected as relevant for this review (Figure 7). Therefore, 89% of the reduction was obtained in this second analysis, but 92% with respect to the initial numbers of papers.



Figure 7. Word cloud of the two clusters, numbers 4 (a) and 6 (b) selected in the second-round analysis.

The 147 papers were reviewed using the traditional procedure. However, after carefully assessing all of them, some were still discarded due to a lack of relevance to the topic, and therefore, they do not appear in the article's references.

4. *Lemna minor* and Its Production

Lemna minor is an aquatic plant belonging to the *Lemna* ceae family and is commonly found in lakes and lagoons in tropical and subtropical regions. *L. minor* exhibits rapid growth, which has motivated the development of research for its cultivation. *L. minor* can be cultivated outdoors in artificial lakes over the ground (Figure 8) and indoors in plastic tanks [26]. As a floating plant, it can absorb dissolved nutrients from water for its growth. Several types of fertilization have been tested for *L. minor*.



Figure 8. *Lemna minor* cultivated in artificial pond outdoors.

It has been reported that with organic fertilization, *L. minor* grew similarly as inorganic fertilization, with no significant difference. However, the protein, lipids, and ashes levels were higher in *L. minor* with organic fertilization than inorganic [2]. Another study showed that better results as far as growth and crude protein content (37.8%) were obtained when a molar ratio of 75% nitrate-N to 25% ammonium-N, with a dilution of 50%, was achieved [27].

L. minor was successfully cultivated with effluents from aquaculture [28]. With a fertilization level of 2 g/L, better results were obtained when goat manure was used, compared to chicken manure or organic fertilizer. This is attributed to the levels of nitrates (1.99 ppm), ammonium (2.69 ppm), and available phosphate (7.71 ppm) in the water that was fertilized with goat manure [6]. Low levels of salinity in water are recommended for *L. minor* cultivation. Salinity levels higher than 2 g NaCl/L caused a reduction in lipids and minerals (Fe, Mn, and Zn) in the proximate composition of *L. minor*. Protein levels were reduced with a salinity of 4 g NaCl/L or higher [29,30].

In cultivation under artificial light, white LED lights with a daily photoperiod of 16:8 (dark:bright) contributed to maximizing the growth of *L. minor* in a system where *L. minor* was cultivated with effluents of red tilapia (*Oreochromis* spp.) [31]. Due to its rapid growth in tanks under controlled conditions, it is considered appropriate to collect *L. minor* twice a week [8].

Special attention should be placed on food safety because *L. minor* may contain heavy metals and antinutrients. Due to this, it has been suggested that its cultivation is under controlled conditions [9]. This fact is relevant because *L. minor* can be successfully grown with organic fertilizers and effluents of animal origin [32,33].

If *L. minor* is exposed to high levels of cadmium in water, it may transfer cadmium to fish that eat it [34]. On the other hand, it has been demonstrated that *L. minor* produced with pig effluents contains levels of cadmium, arsenic, and plumb below the recommended limits in Europe for animal feed [35]. Even so, the production of *L. minor* using organic fertilizers should be done under strict control. *L. minor* had higher levels of nitrates following the increase in macronutrients in the water where it was cultivated, reaching 530 mg NO₃/kg

in medium with concentrated fertilization. This is a safe level for human consumption, considering the recommended limits by the European Commission (2000 mg NO₃/kg), but it is potentially risky for ruminants. In pigs and fish, the risk of toxicity due to nitrates is considered very low [35]. Therefore, the cultivation conditions can be controlled, and the amount of *L. minor* in feed for different species.

5. Nutritional Value and Composition of *Lemna minor*

Lemna minor can contain high levels of crude protein (CP). This makes it attractive as a substitute for traditional sources of protein such as soybean meal and fish meal. Some authors have reported mean values of 17.5, 20.33, and 22.5 g/100 g of CP in *L. minor* on a dry weight base [7,12,14]. But also as high as 31 g/100 g CP for *L. minor* cultivated in water with pH between 7 and 8, and up to 42.8 g/100 g CP when fertilized with animal manure [33,36]. Table 1 shows a summary of the nutritional value (proximate composition and macro-minerals) of *L. minor* with data from different authors and countries. Table 2 shows a summary of the main microminerals in *L. minor*.

Table 1. Summary on proximate composition and some macro-minerals of *Lemna minor* for animal feed.

Country	Crude Protein (%)	Crude Fiber (%)	Ether Extract (%)	Ash	NFE ¹	Ca (mg/kg)	P (mg/kg)	Mg (mg/kg)	Ref
Indonesia	23.24	11.53	3.43	13.23	-	1.3	1.3	-	[37]
India	36.07	-	8.45	21.41	-	-	-	-	[2]
Indonesia	23.47	29.92	3.99	23.60	19.02	-	-	-	[38]
Indonesia	29.86	13.22	3.80	14.00	41.24	-	-	-	[39]
India	36.47	-	7.39	21.72	-	-	-	-	[40]
India	20.33	18.06	3.10	30.35	-	2.80	1.10	-	[14]
Pakistan	32.66	-	7.33	-	-	0.015	-	0.034	[30]
Italy ¹	25.38	10.80	2.79	8.26	-	-	-	-	[41]
Italy	28.13	15.20	5.10	16.40	-	-	-	-	[3]
Nigeria	41.08	1.25	2.18	8.63	-	-	-	-	[42]
Nigeria	29.96	27.10	2.83	14.63	-	0.12	0.68	-	[43]
Saint Lucia	41.10	29.20	1.15	8.64	-	-	-	-	[42]
Pakistan	30.30	-	7.34	-	-	0.032	-	0.29	[8]
Iran	22.55	8.31	2.85	19.58	46.76	1.42	0.55	0.48	[12]
China	17.5	29.7	3.4	16.6	-	-	-	-	[44]
Bangladesh	28.90	-	-	-	-	0.029	-	0.029	[36]
Kenia	35.34	5.35	8.36	13.67	-	-	-	-	[45]

Notes: Results given on a dry weight (DW) basis. ¹ A mixture of *L. minor* and *L. gibba* was analyzed.

Table 2. Summary of micro-minerals (mg/kg) in *Lemna minor* for animal feed.

Cu	I	Mn	Z	Fe	Mo	Co	F	Cr
<2.5–5.82	0.4–53	230–333	20–106	53–543	<0.5	1.68	543	1–26.5

Based on: Jaimes et al. [46], Sosa et al. [47], and Witkowska et al. [48].

It was reported that *L. minor* has a higher true digestibility of protein (79%) than corn (62%), but lower than the digestibility of soybean meal (88–90%) [49]. For a mixture of *L. minor* and *L. minuta* a standardized ileal digestibility of crude protein in broiler chicken of 40.2 g/100 g was reported. But its level of tannins (0.29 g/100 g) suggests that it is not an optimized biomass for broiler chicken [7].

Traditional protein sources such as soybean meal and fishmeal are higher in total crude protein content than *L. minor*. Fishmeal may contain between 56.5 g/100 g and 72.0 g/100 g CP, while soybean meal may contain between 36.6 and 52.7 g/100 g, depending on its quality. Fishmeal is often considered costly, and its use is limited in poultry diets because more than 10% of fishmeal in the diet can add a fishy taste to the meat. It is recommended that the levels of some essential amino acids, including methionine, lysine, and cysteine, be analyzed in animal diets, and appropriately balanced for the species being fed [41–43].

The amino acids leucine, isoleucine, and valine predominate in *L. minor* constituting 48.67% of total essential amino acids in *L. minor*, while glutamic acid represents 25.87% of non-essential amino acids [2]. Another study reported that in its crude protein, *L. minor* contained more of the following amino acids: leucine (6.41%), arginine (4.56%), lysine (4.49%), phenylalanine (4.25%), tryptophan (3.89%), and isoleucine (3.62%) [3]. A summary of the amino acid content of *L. minor* (% protein in dry mass) is presented in Table 3.

Table 3. Amino acid profile (g/100 g crude protein) of *L. minor*.

Amino Acids	Miltko et al. [50]	Basnet et al. [51]	Jaimes et al. [46]	Chakrabarti et al. [2]
histidine	1.18	2.06	1.50	0.89
serine	3.84	4.76	4.10	2.35
arginine	3.94	8.14	4.80	3.06
glycine	3.90	5.46	4.60	2.86
aspartic acid	7.90	18.97	8.20	3.71
glutamic acid	9.41	11.08	9.80	6.43
threonine	3.42	4.46	4.00	1.92
alanine	4.88	5.34	5.10	2.88
proline	3.47	4.06	3.80	1.25
lysine	3.99	5.90	5.00	2.68
tyrosine	2.09	-	3.10	1.91
valine	4.40	5.46	4.60	2.66
isoleucine	3.33	4.29	3.70	2.04
leucine	5.78	8.42	7.30	4.13
phenylalanine	3.34	-	4.40	2.57
cysteine	1.12	-	0.90	0.38
methionine	1.37	-	1.60	0.86
tryptophan	1.35	-	-	0.37

An experiment with a diet for broiler chicken that contained 50% *L. minor* reported a reduced ileal digestibility of amino acids, which suggests that the composition of the diet should be optimized to improve the digestibility of protein in general [7]. This reduced digestibility may be caused by a very high level of *L. minor* inclusion in the diet. A mixture of *L. minor* and *L. gibba* contained the following vitamins: beta-carotene, 14.5–29.4 mg/kg, Retinol, <700 IU/kg, Tiamine, <0.15 mg/kg, Riboflavin,

0.47–1.24 mg/kg, Niacin, 5.87–9.54 mg/kg, Pantothenic acid 0.80–1.47 mg/kg, Pyridoxine, 0.58–0.95 mg/kg, Biotin, 89.3–109.0 µg/kg, Folate, 233–338 µg/kg, Cyanocobalamin, <2.5–16.3 µg/kg, Ascorbic acid, <5–16.1 mg/kg, alpha-Tocopherol, 7.5–18.0 mg/kg, Phylloquinone, 216–452 µg/kg [41].

Concerning fatty acids, a study reported that *L. minor* cultivated in exterior conditions had more polyunsaturated fatty acids (37.13 g/100 g) and less saturated fatty acids (33.75%), compared to *L. minor* cultivated indoors (21.96 and 41.63 g/100 g, respectively) [26]. Miltko et al. [50] reported that the ratio of omega n-6 polyunsaturated fatty acids (PUFAs) to omega n-3 PUFAs of *L. minor* cultivated in the natural environment in ponds is 0.844, which is considered more favorable than the ratio of omega n-6/n-3 of *L. minor* cultivated under artificial conditions in laboratories, which was 2.86.

Other studies have reported the presence of some anti-nutritional compounds such as oxalate and phytate in *L. minor*, which should be controlled and reduced, previously to its application in animal feed, if it were necessary [47]. On the other hand, *L. minor* is also a rich source of bioactive compounds such as carotenoids and phenolic compounds (mainly flavonoids, phenolic acids, and glucosinolates) which are responsible for its antioxidant activity [47].

6. Use of *Lemna minor* in Animal Feed

Most of the literature that has been published on *L. minor* as feed, focuses on feeding fish and poultry, although there are some studies on its usage in diets for ruminants and pigs. In areas where the climate does not allow continual grazing, *L. minor* could be used to feed cattle. Production costs of *L. minor* should be considered and compared to other sources of forage. In addition, it can be used not only as a staple diet for animal feed but also as a dried supplement (protein concentrates for example) [47].

6.1. *Lemna minor* in Aquaculture

Lemna minor is interesting in aquaculture because fish may naturally eat *Lemna* spp. in lakes where it appears in the wild. Aquaculture farmers have considered the need to substitute fishmeal with another ingredient, so diverse studies have been made feeding *L. minor* to fish [2]. In Table 4 a summary of studies where *L. minor* has been used as feed in aquaculture is presented, with its mode of application and results.

Some studies where levels of inclusion of *L. minor* between 15% and 30% was used (through live grazing, dried and pelleted forms) showed positive results as far as growth rate and feed conversion, without a negative impact on fish survival [4]. Similarly, an inclusion of 30% of *L. minor* (dried) in diets of rohu fish (*Labeo rohita*) substituting fishmeal did not affect growth in fish [40].

Lemna minor has been used to feed colossoma fish (*Colossoma macropomum*) in Nigeria. When 50% of soybean meal was substituted with *L. minor* (dried), the best results in weight were achieved. But when 100% of soybean meal was replaced with *L. minor*, the weight of the fish was very low compared to the control group [13].

Herawati et al. [38] reported better levels of weight of biomass, feed utilization efficiency, and protein efficiency ratio with a low inclusion of 2.5% of *L. minor* (dried and fermented with probiotic microorganisms) in the diet of tilapia fish (*Oreochromis niloticus*). In another study, the inclusion of 15% and 20% of *lemna* in diets of Nile tilapia cultivated in freshwater incremented the proportions of omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) in muscle without adverse effects on fish for human consumption, which allowed decreasing the utilization of fish meal and fish oil in tilapia diets [45].

This same level of inclusion of *L. minor* (dried) in carp diets (*Cyprinus carpio*) significantly improved the final weight of fish and improved levels of protein, essential amino

acids, and lipid content, including n-3 long-chain polyunsaturated fatty acid (LC-PUFA) in carp fish [52].

A study with barramundi fish (*Lates calcarifer*) showed good results in relation to protein efficiency ratio when a fermented *L. minor* was included in the diet at a level of 35% [39]. With juvenile catfish (*Clarias gariepinus*) *L. minor* inclusion of 40% of the diet produced optimal results in growth when compared with lower inclusion levels and the control group [42].

There was no significant difference in the weight of tilapia that were fed 3 times a day with commercial feed and eating 0.5% of their bodyweight in fresh *L. minor* daily compared to the control group (without *L. minor*) [53]. Another study reported that up to 20% inclusion of *L. minor* substituting traditional sources of protein in the diet of rainbow trout had no adverse effects on the growth of fish [3].

Table 4. Summary of the use of *Lemna minor* as feed in aquaculture.

Country	Fish Species	Inclusion Levels of <i>L. minor</i> in the Diet	Feed Strategy	Results	Recommended Inclusion Level in the Diet	Ref.
India	<i>Labeo rohita</i>	22.1%	Dried <i>L. minor</i> was combined with fishmeal at 1:1 ratio.	The level of inclusion did not affect the growth of the fish ($p < 0.05$)	up to 30%	[40]
Indonesia	<i>Oreochromis niloticus</i>	2.5%, 5% and 7.5%	Fermented <i>L. minor</i> as substitution of soybean meal	Greater level of biomass weight and feed utilization efficiency ($p < 0.05$).	2.5%	[38]
Indonesia	<i>Lates calcarifer</i>	15%, 25%, 35% and 45%	Fermented <i>L. minor</i> as substitution of fishmeal proteins	Good results in weight gain and protein efficiency ratio ($p < 0.05$).	35%	[39]
Italia	<i>Oncorhynchus mykiss</i>	10%, 20%, and 28%	Dried <i>L. minor</i> (flour) as substitution of traditional protein sources	No adverse effects in fish growth were observed ($p > 0.05$).	20%	[3]
Nigeria	<i>Clarias gariepinus</i>	20%, 40%, 60% and 80%	Ground <i>L. minor</i> as substitution of fish meal	Optimal growth results ($p < 0.05$) were obtained.	40%	[54]
India	<i>Cyprinus carpio</i>	5%, 10%, 15% and 20%	Part of the soybean meal, wheat flour, and sunflower oil was substituted by dry <i>L. minor</i> .	Increased levels of final weight, protein, essential amino acids, and lipid content ($p < 0.05$).	15–20%	[52]
Kenya	<i>Oreochromis niloticus</i>	5%, 10%, 15% and 20%	Fishmeal and fish oil were substituted by dry ground <i>L. minor</i> .	Increased levels of n-3 long-chain polyunsaturated fatty acids ($p < 0.05$).	15–20%	[45]
Taiwan	<i>Oreochromis</i> spp.	5% and 10%	Feed pellets were substituted by dry <i>L. minor</i>	Increased weight gain and protein efficiency ratio ($p < 0.05$).	5%	[31]
Nigeria	<i>Colossoma macropomum</i>	25%, 50%, 75% and 100%	Substitution of soybean meal with dry <i>L. minor</i> (flour) and visceral fish meal.	50% substitution of soybean meal with <i>L. minor</i> achieved greater weight gain ($p < 0.05$).	50% of total soybean meal	[13]

The inclusion of *Lemna minor* at a level of 20% of the diet is the most common formulation used in aquaculture [4]. Fish feed based on *Lemna minor* has been considered more ecological and sustainable than conventional feed. *L. minor* has also been offered to fish fresh (Figure 9), dried (Figure 10), and pelletized [4,55].



Figure 9. Fresh *Lemna minor* freshly harvested from artificial pond.



Figure 10. Dehydrated *Lemna minor*.

6.2. *Lemna minor* in Poultry Feed

Lemna spp. in the wild has been consumed by migratory birds and ducks. This has caused a surge in interest in having *L. minor* as an ingredient in broiler chicken, hens, and turkeys. Birds can eat fresh *L. minor*, as well as dried and ground, as an ingredient in their diet. *L. minor* is an attractive ingredient in poultry because it may substitute part of the traditional sources of protein in poultry feed. A study reported that offering fresh *L. minor ad libitum* to broiler chickens to which conventional feed was restricted in order to increase fresh *L. minor* consumption did not show any advantage [56]. However, other studies where *L. minor* was an ingredient in a formulated feed showed results that indicate some advantages. Broiler chicken evidenced that an inclusion, of *L. minor* at a level of 5% of the diet plus enzyme generated a similar final weight in chicken compared to the control group. However, it is considered that the fiber in *L. minor* is a limiting factor in chicken feed [14].

Lemna minor has been used to feed laying hens at a level of 15% of inclusion in the diet, substituting wheat gluten and soybean meal. The results were an improvement in egg yolk color and a hematoprotective effect in hens [12]. Feeding native hens with an inclusion of 20% *L. minor* in their diet did not yield any significant difference in weight gain and feed conversion when compared with the control group, even when the hens with the conventional diet had a higher average final weight [37].

Transgenic *L. minor*, which was modified to contain phytase, was used in poultry feed with initial results that appear to be beneficial, but similar to those with non-transgenic *L. minor* [57]. In Table 5 a summary is presented with experiments where *L. minor* was used as feed in poultry.

Table 5. Summary of the use of *Lemna minor* as poultry feed.

Country	Type of Poultry	Level of Inclusion of <i>L. minor</i> in the Diet	Feed Strategy	Results	Recommended Level of Inclusion in the Diet	Ref.
Iran	Laying hens	7.5% and 15%	Substitution of wheat gluten and soybean meal by dry <i>L. minor</i>	Improvement in the color of egg yolk and a hematoprotective effect ($p < 0.05$) in hens.	15%	[12]
India	Broiler chicken	5 and 10% with and without enzyme	Inclusion of <i>L. minor</i> (flour) plus enzyme	A final weight in chicken similar to the control group ($p > 0.05$)	5% plus enzyme	[14]
Indonesia	Native hens	10%, 20% and 30%	Conventional diets including <i>L. minor</i> were prepared	No differences ($p > 0.05$) with the control group in weight gain nor feed conversion.	20%	[37]
Vietnam	Broiler chicken	<i>Ad libitum</i>	Fresh <i>L. minor</i> offered <i>ad libitum</i> restricting de base diet of corn and soybean	Fresh <i>L. minor</i> showed no advantage ($p > 0.05$) when conventional feed was restricted.	-	[56]

6.3. *Lemna minor* in the Feeding of Other Terrestrial Animals

Although it is true that the main works related to the use of *L. minor* as feed have been made in fish and poultry, a few studies about its application in cows and pigs have also been published. For example, it was reported that including fresh and dehydrated *L. minor* in diets of cows that were fed with forage and supplement, improved the hematological condition (red cells) and antioxidant profile compared to the control group (5.24 vs. $4.78 \times 10^3 \text{ mm}^{-3}$ and 13.31 vs. $9.96 \text{ nmol mg}^{-1}$, respectively) [44]. *L. minor* has been used in pigs as a substitute for traditional feeds reporting a favorable effect on the body weight gains of pigs when fresh *L. minor* ($1.5 \text{ kg/day/animal}$) was consumed instead of potato tops, but when it was used as a dried substitute (10%) of sorghum and soybean meal, no significant differences in live weight gain and feed conversion rates were obtained [58]. Rojas et al. [59] studied the potential of using protein concentrates from *L. minor* as a protein source for young pigs, comparing its digestibility with that of other traditional protein sources (soybean meal and fish meal) concluding that there were no differences in terms of nutrient value and so that this concentrate could be included in their diet.

7. Other Uses of *Lemna minor*

In addition to its value as feed, *Lemna minor* could also be used for human consumption, for pharmacological applications, or even for water treatment. *L. minor*, as a source of protein, is considered a novel food, as well as mycoproteins, algae proteins, and bacterial proteins. Through a panel on nutrition and novel food, EFSA has informed that the consumption of a mix of *L. minor* and *L. gibba* is safe for health [60]. However, a previ-

ous article had expressed worries concerning the concentration of manganese found in *L. minor* and ruled that the level of trace elements and contaminants that *L. minor* may have depends on the cultivation conditions and the fertilization it receives [41]. Proteins have been extracted from *L. minor* for food products for human consumption. Advances in protein extraction have been made when combining the alkaline extraction method and ultrasound [9]. *L. minor* has been considered a good candidate for recombinant protein production for pharmacological purposes, and a transgenic *L. minor* for this purpose has been obtained [61,62]. In this sense, a phytase from transgenic *L. minor* has been proven favorable as an additive in experiments with laying hens [63]. *L. minor* may be also used in an aquaculture system with the intention of improving water quality. This is due to the fact that effluents of aquaculture can be treated with *L. minor* even in water recirculation systems, which favors water quality and fish development [64–67]. It was reported that the water where *L. minor* is combined with aquaculture had less ammonia, nitrite, nitrate, potassium, and phosphates [68]. But in lagoons that had abundant *Lemna* spp., higher fish mortality was reported, which was attributed to greater eutrophication and lower oxygen levels in water [69].

8. Conclusions

In the realm of literature review, as the volume of scientific data across various subjects continues to grow, traditional review methods are struggling to keep up. This situation highlights a significant opportunity for AI-powered tools to make a substantial impact. The artificial intelligence tool that has been used for this study (based on Natural language Processing) has allowed a rapid arrival to the most relevant articles for our study objective. Specifically, it has allowed a 95% reduction in the initial number of scientific contributions obtained from the Scopus database in less than 3.5 min. In different climates, including tropical, subtropical, and semiarid zones, *L. minor* has been considered an interesting option as a source of sustainable proteins for animal feed. Sustainability is considered one of the most valuable aspects in the decision to use raw materials in the food chain in general, and in animal feed in particular, in line with the UN Sustainable Development Goals.

The tendency found in published articles is that *L. minor* as feed is more applicable to aquaculture and poultry, although incipient but interesting works have been also done to explore this application in ruminants and pigs. These applications are based on the analysis of its nutritional value and diverse experiments in different countries. *Lemna minor* can be used fresh, dried, or processed into meals for supplementation and its nutritional value depends on it. Drying is the most common method to preserve it and make it easier to incorporate into feed formulations. From a nutritional standpoint, the most valuable aspect of *Lemna minor* is its high protein content and its balanced amino acid profile. In addition, its mineral, vitamins, polyunsaturated fatty acids, carotenoids, and phenolic compounds content contribute significantly to its nutritional value and potential health benefits (antioxidant).

The recommendation for the inclusion level of *L. minor* (dried) in fish diets depends on the fish species under cultivation, but the recommended level is from 5% to 10% of the diet. In poultry feed, the level of inclusion ranges from 5% to 15% of the diet. These limitations are mainly due to the presence of some anti-nutritional factors (oxalates, tannins, and phytates, which could be reduced by applying several processing methods) that could reduce its digestibility. In addition, its potential for heavy metal accumulation is also an important hazard issue that must be controlled.

9. Future Trends

The perspective of the utilization of *L. minor* in animal feed is that its utilization will increase according to the expected increase in prices of conventional sources of protein for animal feed. More innovative feed formulations that leverage the unique nutritional profile of *L. minor* potentially combining with other alternative proteins or functional ingredients should be developed. In this sense, new research to determine optimal inclusion rates and the effects of growth performance, feed efficiency, and product quality depending on the animal group for which it is intended is needed. Future research will also focus on identifying and mitigating some anti-nutritional factors through selective breeding, processing methods, or even specific feeding strategies to ensure optimal nutrient utilization and animal health.

But a massive use of *L. minor* will also depend on more efficient systems of continual production of *L. minor* (both cultivation and processing technologies), about which more publications are needed. Efficient production of *L. minor* in the future will contribute to its greater availability in feed.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app15126732/s1>, Table S1: Data, IA-based tools and results obtained during the review of *Lemna minor* in animal feed.

Author Contributions: Conceptualization, H.B.-D., J.F.-L. and F.B.; methodology, H.B.-D., J.F.-L., and F.B.; formal analysis, M.V.-M. and R.L.-G.; writing—original draft preparation, H.B.-D. and J.F.-L.; writing—review and editing, H.B.-D., J.F.-L., M.V.-M., R.L.-G. and F.B.; supervision, J.F.-L. and F.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: All data are available by request to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AI	Artificial Intelligence
NLP	Natural Language Processing
PCA	Principal Component Analysis
CP	Crude Protein
LC-PUFA	Long Chain-Polyunsaturated Fatty Acids

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