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FEED CONVERSION RATE, GROWTH PERFORMANCE AND BIOLOGICAL EFFECTS OF AZOLLA ON POULTRY AND LIVESTOCK: A SYSTEMATIC REVIEW

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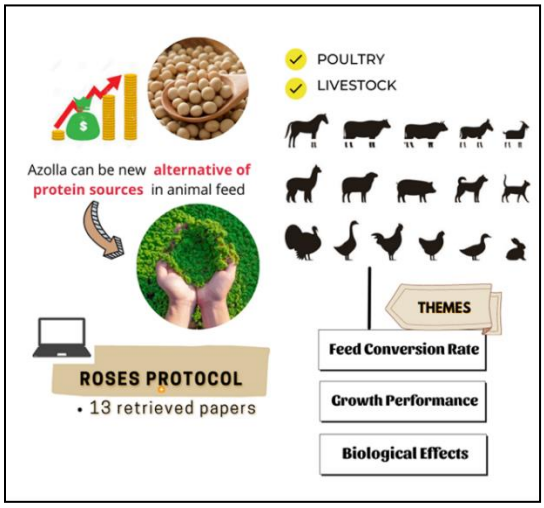
Graphical abstract



Abstract

The rapid rise in soybean prices has necessitated a potential replacement protein source for animal feed. Azolla is a Salviniaceae duckweed with valuable properties that have captivated the public. Its abundance reduces feed costs, which account for the majority of overall production costs. The research on Azolla is extensive, but the efficiency as a potential feed for livestock and poultry is limited. Thus, there is an urgent need to study the efficacy of Azolla as feed materials. A systematic literature review was conducted to collect and analyze information on Azolla as feed materials. Following the Report Standards for Systematic Evidence Synthesis (ROSES) protocol, 13 studies (years 2000-2021) were extracted and reviewed from Scopus, Web of Science, and PubMed. Three aspects were primarily highlighted to review the efficacy of using Azolla as feed: (1) feed conversion rate, (2) growth performance, and (3) biological effects on poultry and livestock. This study revealed that the efficacy and proportion of Azolla in feed vary by animal type. The findings offer greater information on current research directions on this subject.

Keywords: Azolla, livestock feed, feed conversion rate, growth efficiency, biological effect

Abstrak

Kadar peningkatan harga kacang soya yang mendadak memerlukan penggantian sumber protein yang berpotensi dijadikan makanan haiwan. Azolla merupakan tumbuhan akuatik dari Salviniaceae yang mempunyai ciri-ciri yang menarik perhatian awam. Kepadatan bilangan Azolla dapat mengurangkan kos makanan yang merupakan sebahagian besar daripada keseluruhan kos pengeluaran. Kajian yang meluas telah dijalankan ke atas Azolla tetapi keberkesanannya sebagai makanan untuk ayamitik dan ternakan adalah terhad. Justeru, pentingnya untuk mengkaji keberkesanan Azolla sebagai bahan makanan. Analisis Literatur Sistematik (SLR) telah dijalankan untuk mengumpul dan menganalisa maklumat berkaitan. Menggunakan protokol ROSES, 13 kajian (tahun 2000-2021 Scopus, Web of Science dan PubMed telah diekstrak dan diulas. Tiga aspek utama telah ditekankan untuk mengkaji keberkesanan Azolla sebagai sumber makanan: (1) kadar konversi makanan, (2) kadar pertumbuhan dan (3) kesan biologikal ke atas ayamitik dan ternakan. Kajian ini membuktikan

bahawa keberkesanan dan perkadaran Azolla sebagai sumber makanan berbeza mengikut jenis haiwan. Dapatan kajian ini dapat menyumbangkan informasi yang besar ke atas kajian semasa berkaitan topik ini.

Kata kunci: Azolla, makanan ternakan, kadar konversi makanan, kadar pertumbuhan, kesan biologikal

1.0 INTRODUCTION

According to OECD and FAO, global meat protein consumption is projected to increase 14% by 2030, compared to the base period average of 2018-2020 [1]. Backed by this data, world chicken meat consumption is projected to hit 15.1 kg/capita in 2029. On the other hand, beef and pork meat are forecasted to reach 6.3 and 11.8 kg/capita, respectively [2]. Population growth is the primary reason why meat consumption is expected to rise 30% in Africa, 18% in Asia and Pacific, and 13% in Latin America, while it rises just 0.4% in Europe and 9% in North America [1]. This emphasizes the significance of the poultry and livestock sectors in meeting market demands

Poultry and livestock are two meat production methods that supply meat to consumers. There is a distinction between poultry and livestock, despite appearing to have the same definition. Domesticated birds raised for meat or eggs are referred to as poultry. In contrast, domestic animals raised in an agricultural setting to provide resources like meat, eggs, milk, fur, cloth, and wool are livestock. These poultry and livestock consume diets consisting primarily of a few essential ingredients, such as imported soybeans and corn as their protein sources. Since soybeans and corn account for 70% of the industry production costs [3], several studies have been conducted to find better feed replacements.

Azolla is a Salviniaceae floating fern with a small branching stem and roots that dangle in the water. There are various Azolla species including *Azolla pinnata*, *Azolla filiculoides*, and *Azolla cristata*. *A. pinnata* is one of the Azolla that grows in Asia's tropical climates. Simultaneously, most of the *A. filiculoides* can be found in temperate climates throughout Europe [4]. Azolla ferns are notable for containing nitrogen-fixing cyanobacteria, *Nostoc azollae*, in their leaf cavities [4]. Nitrogen fixation by *N. azollae* is 0.09 mg N h^{-1} per gram dry biomass higher than Rhizobia's fixation in soybean root nodules [4]. Azolla's greater nitrogen fixation has led to numerous studies on its uses as a biofertilizer and bioremediation. Considering its superior amino acid profile compared to soybeans, Azolla has been extensively researched as a sustainable and cost-effective protein source for poultry and livestock. Azolla is currently covered in databases as a feed material for broilers and aquaculture, with few studies focusing exclusively on the plant's biomass productivity [4].

Figure 1 Example of *Azolla pinnata* cultivation for livestock feed [5]

"Green Gold" is a term that dictates Azolla as an economical feed due to its ease of cultivation, as shown in Figure 1 [5]. Azolla crop may generate 540-720 kg of protein monthly [6]. Furthermore, the Azolla's low lignin level, which aids digestion, increases its value. Azolla was considered a promising alternative because of its high protein content, which was estimated to be ranged from 21.0% to 25.8% [5-7]. Besides, Azolla contained a higher proportion of all essential amino acids than soybean, except for histidine [4].

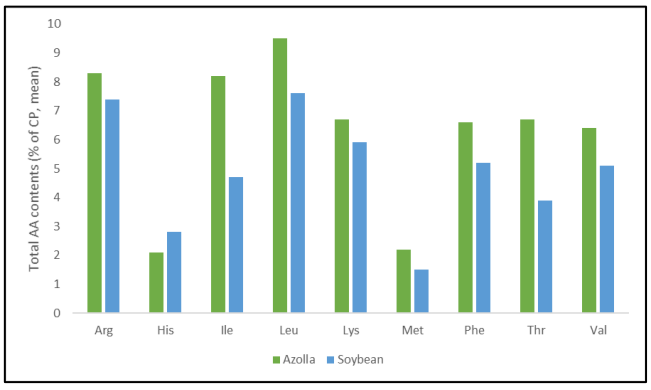


Figure 2 Average amino acid profile in Azolla species compared to average soybean adapted from [4, 8]

In addition, it also comprises numerous macronutrients, including calcium, which is required for bone formation and optimal nerve and muscle function [9]. It also contains magnesium, an essential cofactor in many metabolic processes, and potassium, which helps maintain the osmotic concentration between cells and interstitial fluid [9]. Azolla is significant in animal feed because it contains minerals including vitamin A (precursor beta-carotene) and B12 [5] which are necessary for animals' metabolic processes [9]. These facts proposed that Azolla could be used as an alternative protein source for various poultry and livestock animals.

Based on the leading databases, most of the research on Azolla focuses on the use of the plant as a fish feed substitute in aquaculture [10]. It appears that there are only a few research papers available that address multiple aspects of Azolla's effectiveness in poultry and livestock. However, they are rarely compared to each other. Given the scarcity of information, implementing a systematic literature review (SLR) on Azolla and animal feeds could provide a comprehensive overview of previous studies that a conventional literature review paper would not. Therefore, this review aims to assist researchers in evaluating and comparing the efficacy of Azolla in a variety of poultry and livestock species, thereby increasing the rigor and scope of relevant literature.

According to this systematic literature review, the research question "How does the use of Azolla in feeds affect poultry and livestock" was answered in a systematic approach. A rigorous search strategy was used to obtain publications concerning the keywords covered in this study. The findings were defined and categorized according to several aspects that were critical in assessing Azolla's impacts on their feedstock. This SLR enables researchers to compare the feed conversion rate, growth performance, and biological effects that influence poultry and livestock to the previous studies.

2.0 METHODOLOGY

2.1 Review Protocol

The validity of evidence about the topic was determined using the Report Standards for Systematic Evidence Synthesis (ROSES) protocol. ROSES was chosen as the review protocol over PRISMA and QUORUM because it is explicitly tailored for environmental research [11]. To begin, the author proposed a research question to provide insight on the topics. The author used ROSES Systematic Maps to direct the search strategy, which began with identification, screening, eligibility, and quality appraisal.

2.2 Research Question

Amidst the interest in Azolla as a potential universal feedstock alternative, only a few studies demonstrated Azolla's potential as poultry or livestock feeds. Besides, the efficacy of the Azolla feed in poultry and livestock is frequently discussed. The author discovered that most of Azolla studies focused on biofertilizers and bioremediation. Hence, there is an urge to study the efficacy of Azolla in poultry or livestock for future references. Therefore, the review aims to search through Azolla-related databases to gather as much information as possible from previous studies to identify study gaps. The research question that guides the navigation is "How does the use of Azolla in feeds affect poultry and livestock?".

2.3 Searching Strategy

A well-designed search strategy is a critical component of a systematic review because it influences search efficiency. Hence, several key aspects led by the ROSES protocol were addressed to retrieve quality search results. Boolean operators like OR, AND, and NOT were used to connect words to broaden or narrow a search. Figure 3 depicted the search strategy flow, starting with identification and ending with quality appraisal and the number of articles screened, following the ROSES protocols.

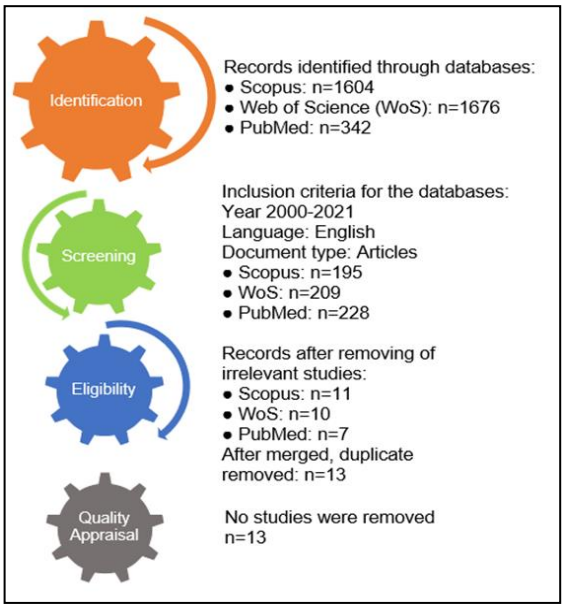


Figure 3 Systematic diagram of the review protocol (n represent the number of studies after each step)

2.3.1 *Identification*

The author begins the identification process on March 15, 2021, using three leading databases: Scopus, Web of Science (WoS), and PubMed. The databases are subscribed by the institution affiliated with all authors, the library of Universiti Teknologi MARA. Scopus and WoS are databases incorporating extensive and enriched data from scientific literature from a broad range of studies. The author also considered PubMed

as one of the databases to provide a better perspective of the review on the biological effects. To identify the most relevant information, the TITLE-ABS-KEY in Scopus and TOPICS in WoS search strings were used to retrieve only results that included any or all keywords related to the focus topic. To broaden the search, the author used possible general terms in extracting the title, abstract, and keywords to obtain all relevant information. The broad keywords were used to retrieve as many articles as possible relevant to the topics, ensuring that no related publications were omitted. After a few trials, the identification method in SCOPUS, WoS, and PubMed generated 1604, 1676, and 342 results, respectively, as tabulated in Table 1.

Table 1 Search string for the identification process

Databases	No. of articles (n)	Search String
Scopus	1604	Title-Abs-Key; azolla OR azolla AND pinnata
Web of Science (WoS)	1676	TOPICS= (azolla OR azolla AND pinnata)
PubMed	342	Azolla

2.3.2 *Screening*

As per the review protocol, the search results were then screened with selected inclusion and exclusion criteria to narrow the search. To address the consistent results, all inclusion and exclusion criteria for all databases were the same. Due to scarcity of information, the author chose to use a broader range for the publication year, from 2000 to the latest (2020). The author decided not to limit the publication to any country. The research article is the only document type included in this process since it is documented as original research and a primary source. Finally, only papers written in English were screened to ensure that the findings were standardized. The author extracted 195 articles from Scopus, 210 from WoS, and 228 from PubMed using the inclusion and exclusion criteria summarized in Table 2.

Table 2 Summary of the inclusion and exclusion criteria

	Inclusion	Exclusion
Publication Year	2000 to latest	Before year 2000
Language	English	Other than English
Country	All	No exclusion
Document type	Article	Review, Books, Chapter, Conference Paper

2.3.3 *Eligibility*

The authors then decided to carry on with the eligibility check. To ensure that only valid studies were selected, the author began skimming through the title and abstract. A few results that did not apply to the studies were excluded, such as the use of Azolla in biofuels, rice production, and nitrogen fixation. This study emphasizes poultry and livestock; hence some papers that discussed Azolla feeds in aquaculture were also excluded. As a result, both Scopus and WoS have yielded 11 and 10 articles respectively, while 7 articles were retrieved from PubMed. After merging the papers and removing duplicates, only 13 articles remained for data extraction.

2.3.4 *Quality Appraisal*

The articles were evaluated using a quality appraisal method to guarantee the quality of the literature reviews. The analysis is then categorized as being of high, moderate, or low quality. No findings were withdrawn as a result of these evaluations.

2.4 Data Extraction

In synthesizing the data, the authors construct a table to extract all the findings from the publications in an organized form. The table is divided into columns for title, aims, findings, and conclusion. Summary for each article and studies that focused on similar aspects were grouped. Only then will the authors assess and identify the common themes in the studies to review.

3.0 RESULTS AND DISCUSSION

3.1 Background of Studies Reviewed

This study discovered 13 full articles to acquire details on the thematic analysis. The authors have identified three key aspects that focus on Azolla as a sustainable feed for poultry and livestock that will be addressed and discussed further in this review. Even though the year of publication selections is extensive, there are still a limited number of studies on Azolla as a sustainable feed, which urges more studies to be done on this topic. The oldest article reported was published in 2007. A single relevant article is reported in each of the years 2007, 2010, 2014, and 2017. Most of the studies were published after 2014, with two articles in 2015, 2016, and 2018. Recently, three articles were published in year 2020. Although the search strategy included all countries, only a few countries are reported in the reviewed themes. This systematic review thus provides the knowledge of research carried out by various countries.

According to Scopus and Web of Science, three publications were reported in two different countries each. A broiler chicken study [12] was categorized in Egypt and the United States, while another study [13] was classified in Japan and Bangladesh. A study of sows [14] was classified in Belgium and Colombia. The other three articles, on the other hand, were published in Nigeria, Indonesia, and Malaysia. As predicted,

India has the most publications on Azolla, with 7 articles reported there, indicating that there have been substantial studies there. This may be attributed to the proliferation of Azolla populations in their countries. According to the Food and Agriculture Organization of the United Nations, agriculture and its associated sectors are the primary source of revenue in India [15]. It was also mentioned that 70% of rural households still rely primarily on the agriculture sector for a living [15]. Considering that Azolla is easy to cultivate and economical, this may be one of the factors due to the growing interest in Azolla research in India. Globally, the current status of Azolla as inclusion in feed for animal husbandry is still insignificant, except in India. As most of the papers retrieved were from India, it is evident that they are aware of the benefits of Azolla as feed for their livestock. However, in other countries, the use of Azolla as feed remains to be acknowledged.

Besides, this systematic review paper also analyses the effect of Azolla in various types of poultry and livestock. Since it includes a wide range of species, this systematic review may include a wealth of information and might be helpful for future studies. Many experiments are focused on poultry rather than livestock. The majority of the poultry surveyed were chickens, including broilers [12, 13, 16], layers [3, 17], Chabro [18], and Kampung chickens [19]. Aside from that, turkeys [20] and broiler ducks [21] were analysed. Sows [14], white Yorkshire pigs [22], and buffaloes [6] were the only livestock that was tested. Broiler chickens are the most well-known poultry that several experts have extensively researched. This might be because the broiler industry has expanded to meet customer requirements for low-cost chicken meat.

3.2 Themes

This SLR study examined three main aspects of Azolla's effectiveness in poultry and livestock: feed conversion rate, growth performance, and biological effects. According to this review, each article refers to at least one theme. However, four studies [12, 17, 20, 23] addressed all the three themes since the findings are looking at various scopes.

3.2.1 Feed Conversion Rate (FCR)

Eight articles focused on the feed conversion rate (FCR) for various poultry and livestock species. The articles discussed the results of the FCR study conducted on broiler chickens [12, 13, 23], broiler ducks [21], turkeys [20], layer chickens [3], pullets [17], and kampung chickens [19]. The articles compare the FCR of control feed and Azolla-including feed. Feed costs are often the most significant spending factor in animal husbandry. As a result, accurate metrics are needed to assess the degree of output obtained from a feed. The feed conversion rate is an example of such an indicator since it represents the efficacy of a feed strategy. The lower FCR values indicate a high-quality feed. Various outcomes were seen with regards to the impact of Azolla on FCR.

In a study on turkeys [20], it was discovered that the choice-feeding group had a slightly better overall FCR ($p < 0.05$) than the 5% Azolla-feeding group. According to the findings, turkeys fed with basal diets and a choice-feeding group would have a lower FCR with no adverse consequences. Shukla *et al.* [20] reported that lower FCR in the choice-feeding group treatment is due to an underlying mechanism in which the birds' gastrointestinal system can choose the optimal feed for them. The evidence is aligned with the findings in the broiler chicken study by Abdelatty *et al.* (2020) which stated that the inclusion of Azolla in broiler chicken feed resulted in a linear increase in FCR with the percentage inclusion of 0%, 5%, and 10% Azolla respectively. The control group, which received a basic soybean-corn diet, had the lowest FCR, which is 1.58.

On the other hand, Samad *et al*. (2020) found that the addition of Azolla in broiler chicken feed ingredients proved the opposite. Even though there were no major differences in FCR ($p > 0.05$), the cumulative mean values demonstrated that the FCR of the treatment groups (5%, 10%, and 15% Azolla) was 0.01 lower compared to the control group. The authors reported that consuming 15% Azolla results in 15.2% to 30.3% of total protein [23]. Hence, the FCR decreases as Azolla intake increases. Aside from that, in a layer chicken study by Ara *et al*., (2018), the FCR of chicks fed a diet with Azolla instead of groundnut cake showed no significant ($p < 0.05$) difference among the different groups, including the control. However, better FCR has been observed in the group served 5% Azolla in their diet, which had the lowest FCR relative to the others.

The FCR determined by using Azolla in the White Pekin Broiler duck's basal diets [21], on the other hand, showed a difference. Both Azolla-fed groups had a lower value in the fifth week compared to the control. The 10% Azolla group had a significantly ($p \leq 0.01$) lower value than the other group treatments by the sixth week. The study reported that the 10% Azolla group had the highest feed efficiency, followed by the 5% Azolla group and the control group [21]. The FCR values in an analysis of growing pullets [17] did not follow any striking trend. However, the FCR of all birds feeding Azolla meal (AZM) in their diets was better than that of birds fed a control diet. The lowest FCR is 8.38 when 15% of Azolla has been included, followed by 9.33 when 5% of Azolla is included. In an analysis of kampung chickens [19], it was discovered that the 20% Azolla-feeding group had a lower calculated FCR than the control group.

When the analysis was conducted, there is inconsistency regarding the effects of FCR on the animals. Most of the studies found that including Azolla in the animal feed increases FCR value, lowers the FCR value, or has no apparent changes from the control. Even though the FCR of feed without Azolla is lower, and therefore more efficient, there are still costs to consider. It has been shown that incorporating 20%

Azolla will lower feed costs and improve economic performance [19]. The addition of 20% Azolla to Kampung Chicken decreased feed costs by up to IDR 1200/kg and increased revenue without affecting the chicken's appearance [19]. Lastly, it was supported by a study that found that the inclusion of 5% Azolla in the broiler chicken diet resulted in the lowest FCR, production expense and was proven profitable compared to other diets [13]. As a result, Azolla feed has the potential to be more economical.

According to prior research, a few variables contribute to the substantial variations in FCR. This may be because of the variety of Azolla strains utilised in the research. For example, two studies [12, 13] using *A. pinnata* agreed that including 5% *A. pinnata* might help improve FCR in broiler chickens. However, the other study by [23] did not clarify the Azolla strains included in their research. Besides, the type of species compared may also influence the FCR discrepancies. To compare, two research employing the same Azolla species *(A. pinnata)* revealed different results. In a research on broiler chickens [12], 5% Azolla was shown to have the lowest FCR, whereas, on pullet [17], 15% Azolla had the lowest FCR.

However, the overall findings show that the Azolla treatment group had a better FCR, with various inclusion percentages for different types of poultry. The amount of Azolla included in the diets of broiler chickens (5-15%), broiler ducks (5-10%), turkeys (5%), layer chickens (5-20%) [3], pullets (5-15%), and kampung chickens (20%). Table 3 provides a summary of studies evaluating the effects of FCR on various poultry and livestock.

Table 3 Summary in Feed Conversion Rate (FCR)

	Author/s [ref]	Species	Azolla Inclusion (%)	Findings
1	Shukla *et al.*, 2018 [20]	Turkeys	5%	The choice-feeding group had a substantially better FCR than the other two experimental groups
2	Abdelatty *et al.*, 2020 [12]	Broiler chickens	5 -10%	FCR increased linearly with the addition of Azolla Leaf Meal (ALM)
3	Samad *et al.*, 2020 [23]	Broiler chickens	5-15%	No significant differences in FCR. FCR in Azolla treatment groups is better than the control group
4	Ara *et al.*, 2018 [3]	Layer chickens	5-20%	No significant differences in FCR. The 5% Azolla has the lowest FCR.
5	Acharya *et al.*, 2015 [21]	Broiler ducks	5-10%	FCR value of 10% Azolla is lower than the control.
6	Alalade *et al.*, 2007 [17]	Pullets	5-15%	No significant differences in FCR. 15% Azolla with the lowest FCR.
7	Setiadi *et al.*, 2016 [19]	Kampung chickens	20%	FCR value of 20% Azolla is lower than the control.
8	Islam and Nishibori, 2017 [13]	Broiler chickens	5-7%	FCR value of 5% Azolla is lower than the control.

3.2.2 Growth Performance

One of the most important aspects to consider when evaluating the effectiveness of feeds is growth performance. Of the 13 articles, eight studies the poultry and livestock growth performance based on body weight (BW) and body weight gain (BWG). Six studies show how Azolla can contribute to an increase in the growth performance of the animals. However, two studies have shown that adding Azolla causes less growth than control after a certain percentage of Azolla is included.

Acharya *et al.* [21] investigate whether adding fresh Azolla to the diet of White Pekin broilers would improve their growth rate. Ducklings aged 108 days were subjected to a two-week adaptation span in the study. Absolute gains were determined by subtracting the second week's initial body weight from subsequent weeks' final body weights [21]. The study discovered that body weights did not vary significantly ($p \geq 0.05$) between groups in any given week as they increased gradually with increasing percentage (0%, 5%, 10%) of Azolla included. However, with 1271.67 g of average weekly body weights, group 3, which consisted of basal diet including 10% fresh Azolla of basal diet, showed significantly ($p \leq 0.01$) higher than the other groups in the fifth week. According to Acharya *et al.* [21], the essential nutrients present in Azolla, which are rich in amino acids and many growth promoter intermediaries, minerals such as calcium, phosphorous, magnesium, potassium, iron, and copper, are the contributing factors to the animals' growth.

Samad *et al.* [23] found that broiler chickens fed with 15% Azolla gained the highest body weight. Initially, 200 day-old chicks were fed with allocated diets and treated to the same level of maintenance, including access to water, food, spacing, and lighting [23]. However, the inclusion of 5% and 10% Azolla in the broiler chickens feed resulted in lower body weight and weight gain than control. Results from this study showed that the inclusion of Azolla must reach a

certain amount before it can improve the growth performance of some poultry or livestock. The authors stated that the changes in weight growth are seen at various levels of Azolla spp. maybe attributed to variances in the species and stage of maturity of the plants utilised, which can affect the nutritional content [23]. While the further analysis may be needed to determine the optimal proportion, another study suggested that the necessary amino acids and minerals found in Azolla support broiler chicken weight increase [21]. Azolla also offers carotene and biopolymers, which aid in the growth of animal production and health. [19] also found that the kampung chicken fed with 20% Azolla gained final body weight than the control, indicating that Azolla could supply important amino acids for the birds' development.

Shukla *et al.* [20] published a related study that looked at the growth performance of growing turkeys. During the 8-16 weeks period, there was no significant variation in body weight gain between the treatment groups. An analysis of growing pullets [17] yielded similar findings, as no major differences in body weight gain were found. However, the addition of 5% AZM with 6.5 g differences resulted in a slight increase in body weight gain of the developing pullets. In contrast, Azolla was used to compare the effect of adding multivitamins and acidifiers to the common diets of broiler chickens on their growth efficiency [13]. The chickens gain the most body weight when 1 mL multivitamins and 1 mL acidifier/liter water are added. To support the findings, they found that multivitamins include essential vitamins and minerals, while acidifiers reduce the formation of toxic effects, resulting in improved digestibility and weight gain [13].

Abdelatty *et al*. [12] reported that adding Azolla at 5% and 10% improved the broiler's body weight gain as compared to control. They stated that the inclusion of ALM can activate the critical muscle protein synthesis regulator p70S6 kinase, which contributes to the significant increases in the broiler chicken's growth performance. The ribosomal protein S6 kinase (p70S6K) is known to regulate cell development. When ALM was added to the basic corn-soybean meal, the analysis found a ($p = 0.06$) increase in the body weight of the birds [12]. They also looked at the expression of total p70S6 kinase and phosphorylated p70S6 kinase-a in the pectoral muscle of the broiler chickens. They found that the phosphorylated p70S6 kinase-a increased linearly with increasing amounts of ALM (5% and 10%) in the diet. The study was well documented. It also well acknowledges the significance of Leucine in Azolla. Leucine, which is abundant in Azolla included in the diet, is a known activator of mammalian targets of rapamycin (mTOR) in mammals. It helps in the activation of the major muscle protein synthesis regulator p70S6 kinase in broiler chickens [12].

However, it was demonstrated that the inclusion of 5% and 7% Azolla in the meals resulted in lower live weight growth of the broiler chickens compared to the control [13]. The term "live weight" refers to the weight of an animal before it is killed. Similar results were observed in layer chicken [3], where increasing inclusion of Azolla at 10%, 15%, and 20% resulted in decreased body weight, which may be dependent on the levels of Neutral Detergent Fibre (NDF) in Azolla meal, limiting factors for its efficient use. According to a study, NDF consists of cellulose and lignin, which are not digestible by simple-stomached animals [24]. This reduces digestible energy intake beyond what a simple-stomached animal can adjust for, limiting animal growth [24]. Table 4 summarises the main evidence recorded to evaluate the effectiveness of Azolla in poultry growth performance.

It has been discovered that most of the research; 8 out of 13 research studied the impact of Azolla inclusion by analysing the feed conversion rate and growth performance of the animal. The author discovered a correlation between FCR and animal growth efficiency after conducting the thematic analysis. Lower FCR values could improve the growth performance of the animals. Most research has shown that including Azolla resulted in increased FCR and decreased BW and BWG. However, it may vary in response to changes in the percentage or over time. For example, Ara *et al*., [3] reported that a higher Azolla percentage could reduce the BWG of the broiler ducks. Thus, 5% Azolla would be the optimum quantity for broiler ducks since it also has the highest crude protein percentage, the highest BWG, and the lowest FCR when compared to the other Azolla treatments. As a result, it is critical to identify the optimum quantity of Azolla to include in the animals' diets. Indeed, using the highest possible proportion of Azolla in the feed would optimize the economic advantages, particularly for farmers.

Table 4 Summary in Growth Performance

	Author/s [ref]	Species	Findings
1	Acharya *et al*., 2015 [21]	Broiler ducks	10% of Azolla showed the highest BW in the fifth week
2	Samad *et al*., 2020 [23]	Broiler chickens	15% of Azolla resulted in the highest BW and BWG.
3	Setiadi *et al*., 2016 [19]	Kampung chickens	20% of Azolla shows average higher in BW compared to control
4	Shukla *et al*., 2018 [20]	Turkeys	No significant differences in BWG
5	Alalade *et al*., 2007 [17]	Pullets	5% Azolla resulted in a non-statistically increase in BWG compared to the control
6	Islam and Nishibori, 2017 [13]	Broiler chickens	Azolla treatment group have lower live weight compared to the addition of multivitamin and acidifier
7	Ara *et al*., 2018 [3]	Layer chickens	Increasing inclusion of Azolla (5%, 10%, 15%, 20%) resulted in decreasing BW
8	Abdelatty *et al*., 2020 [12]	Broiler chickens	The addition of 5% and 10% Azolla improved the BWG.

3.2.3 Biological Effects

In deciding whether using Azolla in the feed will yield healthy and high-quality animals, studying the consequences of biological effects of Azolla inclusion in poultry and livestock is needed. This review highlights several biological effects, such as digestibility, haematological, blood biochemicals, and immunity of the poultry and livestock.

Digestibility is a key factor for assessing the nutritional content of the animal feed. This is because Azolla has polyphenolic tannins instead of lignin which may reduce digestibility [4]. Skrabanja *et al.* [25] studied the protein-polyphenol interactions and discovered that polyphenols' potential for binding to different proteins varies, affecting protein digestion and absorption. Three studies discussed the digestibility of the animals due to the Azolla inclusion in their diet. One study focused on sows [14], while the other focused on broiler chickens [12, 23]. According to Leterme *et al.* [14], Azolla incorporated in sow diets at a rate of 150 g kg^{-1} Dry Matter (DM) does not inhibit digestive processes. However, increasing the dosage of Azolla up to 300 g kg^{-1} substantially reduces digestibility. They utilized two factors to calculate the effect of Azolla inclusion in sows, such as Gross Energy (GE) and Ileal Protein Digestibility. The GE digestibility derived from diets containing 150 and 300 g kg^{-1} was 0.75 and 0.05 for Azolla. In Ileal Protein Digestibility, 150 g kg^{-1} resulted in a 12% drop in digestibility, but when 300 g Azolla was added, the decrease was more significant. Considering both measures, the results indicate that Azolla might be added up to 0.15 kg of the sow's diet without harmful consequences.

In comparison with broiler chickens by Abdellaty *et al.* [12], there was a linear spike in DM, organic matter, crude protein (CP), and ether extract digestibility when 5-10% of Azolla was included [12]. However, this research does not fully describe the relationship between ALM and nutrient digestion efficiency. An attempt to examine the relationship between ALM and intestinal tract histopathology and morphometry is suggested. Another study by Samad *et al.* [23] also found that the addition of *Azolla* spp. in broiler feed increased the volume of CP and CF digestibility. The authors back up their findings by suggesting that broilers could easily digest the CF in *Azolla* spp. since the size which is 1-2.5 cm was acceptable for their consumption. According to Brouwer *et al*. [4], *A. filiculoides* are more suitable for protein feed than *A. pinnata* because they have greater protein contents and fewer polyphenols when grown at ambient CO_2. Besides digestibility, the study also reviewed the haematological consequences of Azolla inclusion in poultry and livestock. It is important to check the blood profiles of the poultry or livestock that have been fed with Azolla to ensure that they are healthy. Three reported studies reviewed the haematological effects on the animals. Several parameters such as Packed cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC), White blood cell (WBC), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), and Mean corpuscular volume (MCV) were examined for the haematological test.

Cherryl *et al.* [22] performed a study on white Yorkshire pigs in which the haematological values were reported at different periods, such as at the start, during, and end of the experiment for all treatment categories. The finding indicates no significant variations between the initial and final haematological values. This implies that the blood values are more or less similar to the reference values, implying that using Azolla in pig diets does not affect white Yorkshire pigs [22]. Similarly, the haematological profile of the Chabro chicken was unaffected by any treatment [18]. However, there is an increase in heterophils, lymphocytes, and eosinophils in the 5% and 7.5% Azolla groups than in control [18]. Regardless, the authors stated that the values of the haematological parameters reported in their studies were within normal limits. Alalade *et al.* [17] found that the PCV, RBC, Hb, and WBC levels obtained are within the normal range and have no adverse effects on the growing pullets. However, this study does not fully acknowledge the relevance of the haematological values concerning the influence on the pullets.

In addition to haematological characteristics, some researchers looked at blood biochemicals to determine the index of the organism's physiological, pathological, and nutritional status [17]. Shukla *et al.* [20] reported no substantial variation in blood biochemicals except for plasma uric acid between the control group fed with a basal diet and the Azolla-based diet in growing turkeys. However, the authors explained that the decreased plasma uric acid in 5% Azolla was related to improved protein utilization in the diet. Likewise, all blood biochemicals, such as glucose, creatinine, cholesterol, total protein, albumin, uric acid, and triglycerides, were shown to be consistent in a Chabro chicken report [18]. The relevance of the blood biological parameters is well documented. Thus, the findings were generally acknowledged.

In terms of immunity, Chichilichi *et al*. [16] examined the effect of partially supplementing commercial broilers with sun-dried Azolla as a protein source on their immunity status. The result presented that the antibody titers against sheep red blood cells (SRBC) were substantially higher in 10% of Azolla treated with enzymes than other treated groups [16]. Furthermore, the greater positive immune response found in this study may be attributed to the availability of vital nutrients in Azolla. The evidence presented by the authors supported the conclusion that is replacing soybean with 10% Azolla, along with enzyme supplementation in the diet, results in an improved immunological response in broiler chickens. Cellulase, xylanase, pectinase, and phytase were the enzyme utilized in the research [16]. However, the interaction of enzymes with the SRBC response was not acknowledged by the authors.

Aside from the biological aspects discussed, there is also information on the animals' milk production.

Kerketta *et al*. [6] performed research on lactating buffaloes' milk production. Over six fortnights, the average milk yield (kg/d) was slightly higher ($P < 0.05$) with the addition of 2 kg Azolla (7.4 ± 0.08) than with standard feed (6.5 ± 0.13). According to the findings, Azolla can be used as a possible alternative feed supplement for lactating buffaloes to increase milk productivity since it is a good source of protein, particularly in areas where green fodder is limited [6]. Table 5 presents the key findings on the effect of various biological factors.

Table 5 Summary in Biological Effects

No	Author/s	Poultry species	Findings
	Digestibility		
1	Leterme *et al.*, 2010	Sows	Azolla at 150 g kg^{-1} DM in sows' diet does not significantly affect digestion. However, increasing the Azolla dose to 300 g kg^{-1} reduces digestibility.
2	Abdellaty *et al.*, 2020	Broiler chickens	The inclusion of 5% and 10% Azolla in the diet may enhance digestibility compared to the control diet.
3	Samad *et al.*, 2020	Broiler chickens	10% or 15% Azolla resulted in a significant enhancement in nutritional digestibility compared to a control group.
	Haematology		
4	Cherryl *et al.*, 2014	White Yorkshire Pigs	There is no statistically significant difference in the mean values of PCV, Hb, RBC, WBC, and MCHC.
5	Mishra *et al.*, 2016	Chabro chicken	Heterophils and lymphocytes were greater in 5% and 7.5% of Azolla, respectively, whereas eosinophils were higher in 7.5% of Azolla.
6	Alalade *et al.*, 2007	Pulllets	PCV, RBC, Hb, and WBC values were consistent among Azolla treatments.
	Blood Biochemicals		
7	Shukla *et al.,* 2018	Turkeys	No significant differences among the blood biochemicals parameters except for lower plasma uric acid in 5% of Azolla.
8	Mishra *et al.*, 2016	Chabro chickens	Blood biochemicals values were reported within the normal range
	Immunity		
9	Chichilichi *et al.*, 2015	Broiler chickens	SRBC response was improved in 10% Azolla with enzyme supplementation.
	Milk Production		
10	Kerketta *et al.*, 2020	Buffaloes	The average milk production of buffaloes treated with Azolla was substantially greater.

Additionally, this study addressed several factors that should be emphasized while analysing the animals' biological impacts. Among the animals listed, three major digestive systems were identified: monogastric, avian, and ruminant. Most animals studied under this theme have avian digestive systems, such as chickens, ducks, and turkeys. Monogastric animals such as sows and White Yorkshire pigs have a single-compartment stomach. In addition, only one ruminant was discussed in the theme, which focused on the buffaloes' milk production. However, no research is conducted on the haematological, blood biochemistry, digestibility, or immunity impacts on ruminants. As a result, there is a knowledge gap about Azolla's biological effects on the ruminant species. To determine the effectiveness of incorporating Azolla in the animal's diet, it is essential to compare the biological effects on animals, particularly their digestibility, as their digestive system is unique.

The results provided important information, such as the fact that various types of poultry and livestock need varying quantities of Azolla, which may be added to the feed. This is done to avoid any adverse effects that may occur to the animals. For example, the sows [14] can only tolerate a dose of Azolla in their diet of up to 0.15 kg. Otherwise, it may harm its digestion. As a result, additional studies on the main reasons contributing to the restriction of Azolla usage need to be conducted to better understand the circumstance.

This comprehensive analysis may offer valuable input to researchers looking to expand the use of Azolla in the poultry and livestock industry. Furthermore, since Azolla could be one of the keys to lowering feed costs in this industry, this study could be one of the inputs for gaining in-depth information. The results demonstrate how Azolla could be beneficial to animals in a variety of ways.

4.0 RECOMMENDATION AND CONCLUSION

In general, 13 articles related to the topic were extracted for this systematic literature review using the ROSES protocol. Reviewing how the addition of Azolla in feed might influence the FCR, growth performance and biological effects of poultry and livestock showed

that Azolla could serve as a promising alternative to conventional protein sources in the feed. The significance of this systematic study indicates that Azolla has the potential to affect the animals in a couple of areas, including reducing FCR, improving growth performance, and influencing a variety of biological effects.

As the use of Azolla in feed has the potential to reduce feed costs and enhance the performance of the animals, suggestions are required to guarantee a substantial improvement in the use of Azolla in feed for future research. In previous research, tannins are shown to possibly reduce digestibility if they attach to proteins when poultry and livestock consume Azolla biomass [4]. To overcome this limitation, the amount of tannins contents might be reduced and improved by fermentation using effective microbes such as Lactic Acid Bacteria (LAB). Since other plants such as soybeans used this technique to enhance the nutritional content, fermentation of Azolla may thus be recommended.

Besides, Azolla has promising results, perhaps reducing the cost of using it as an alternative protein source for animal feed. Prior to implementation, it is critical to consider the FCR, the animals' growth performance, and the biological consequences. The percentage of Azolla that is safe to feed the species must be determined since exceeding the recommended quantity of Azolla could make the animals more prone to bad development and digestion problems. The reasons why Azolla cannot be used as a complete portion of the feed may be resolved as research gaps in the future. This study could serve as a benchmark to prove that certain animals need a certain amount of Azolla in their diet to prevent adverse consequences in their productivity.

To summarise, the use of Azolla biomass for feed production may offer more environmental and economic benefits to farmers, but it is not yet widely spread in many countries. Deeper insights study on Azolla for feed application may be undertaken, since Azolla may offer a lower FCR, improved growth performance, and improved animal biological consequences. Several elements of Azolla's productivity as a partial feed substitute in poultry and livestock should be explored for application in future research.

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